Does negative frequency-dependent selection maintain male polymorphism in the livebearing fish Xenophallus umbratilis?

▪ HONORS THESIS PROPOSAL ▪ COLLEGE OF LIFE SCIENCES

RESEARCH PURPOSE:

How genetic variation is maintained in the face of strong natural selection is an important problem in evolutionary biology. Selection should erode genetic diversity, leading to more and more homogeneous populations. Yet in nature, we commonly see high degrees of genetic variation, even for traits that are important for fitness. Negative frequency-dependent selection, a balancing selective force that favors traits when they are rare but not when they are common, is a mechanism proposed to maintain polymorphisms in a population. However, there is little empirical data to demonstrate how negative frequency-dependent selection sustains variation. Here, I propose to test the negative frequency-dependent selection hypothesis in the tropical fish *Xenophallus umbratilis*. This species is a bilaterally symmetrical organism that exhibits mating morphology that is asymmetrical, showing handedness in the male gonopodium (a mating structure use to inseminate females), which terminates with either a dextral or sinistral corkscrew. I test the hypothesis that in species like *Xenophallus umbratilis* where such asymmetrical morphologies exist, negative frequency-dependent selection is maintaining gonopodial variation within populations.

OVERVIEW AND BACKGROUND:

One of the most striking things about biological diversity is simply how much diversity there actually is. In this regard, natural selection is somewhat of a paradoxical force. Natural selection is known to contribute to the origin of new species, thus promoting diversity at the macro scale (Coyne and Ore 2004). Yet, natural selection and genetic drift (both mechanisms of evolution) tend to reduce genetic diversity within populations. How then does new genetic diversity arise, and what maintains this diversity in the face of selection and drift? New mutations can increase variation in populations by creating entirely new alleles, but mutations are rare, and beneficial mutations are even rarer (Fisher 2003, Kimura 1983). Recombination in sexual reproduction increases variation in offspring by assorting alleles that are already present. Variation can also be maintained in populations if selection varies over time and space (Kalske et al. 2016). While these mechanisms are both theoretically and empirically well established, there is an additional mechanism—negative frequency-dependent selection—that theoretically can maintain variation within populations (Sato and Kudoh 2017). The idea is simple. Traits are evolutionarily favored when they are rare but disfavored when they become common. Unfortunately, there is very little empirical work to evaluate this hypothesis [but see (Weir 2018, Gigord, Macnair and Smithson 2001)] and even less evidence that this process occurs in the wild (Yenni, Adler and Ernest 2012). Here, I propose a research project to test for negative frequency-dependent selection in the livebearing fish *Xenophallus umbratilis* found in northern Costa Rica.

I test the hypothesis that negative frequency-dependent selection maintains a known polymorphism for the male gonopodium within populations of *Xenophallus umbratilis*. The gonopodium is a modified anal fin used to transfer sperm to females (Evans, Pilastra, and Schlupp 2011)—males are either right handed or left handed for this trait, with the terminus of the gonopodium having either a sinistral or dextral twist. I predict that males with the rare morph will have higher fitness than those with the common morph because females will be more likely to avoid male harassment from males with the most common morph. I propose a combination of field collections, field experiments, and laboratory experiments to test the hypothesis that polymorphism for this trait is maintained by negative frequency dependent selection. My project will focus on three objectives. (1) Determining from field collections if populations in the wild vary in the ratio of left to right handed males. (2) By monitoring select populations over time, determine if the ratio of left to right handed males changes over time, oscillating between male morph types. (3) Using controlled laboratory experiments with live fish, determine if rare male morphs have a fitness advantage over common male morphs. Data from these three objectives will allow me to evaluate the general hypothesis of negative frequency-dependent selection in a wild population.

METHODS AND RESEARCH PLAN:

I will employ a three-pronged approach to test for negative frequency-dependent selection in *Xenophallus umbratilis*. Here I detail my research plan by describing each of these elements.

1) Quantifying Gonopodium Handedness in the Wild – The first step in understanding what process maintains polymorphism in *Xenophallus* is to quantify the degree of polymorphism in natural populations. Beginning in 1995, my advisor has been collecting and preserving fish specimens from Costa Rica. Earlier this year I had the opportunity to participate in some of this collecting. I have identified over 20 different populations of *Xenophallus* collections that are currently found in the Monte L. Bean Life Science Museum. I will sort adult males from these collections, and under a dissecting microscope count the number of adult males with left- and right-handed gonopodial morphs. This will allow me to assign a left:right ratio for each location. Of particular interest are locations where collections have been made at multiple points in time—if negative frequency-dependent selection is operating, these ratios should change over time at some predictable rate. Our expectation is that ratios will vary among locations. This work will also allow us to identify just how extreme these ratios can vary over time.

2) Male Pursuit Tests and Female Resistance Tests – An additional challenge is to find a mechanism to explain why rare male morphs should have an advantage over common male morphs. I hypothesize that females actively resist forced copulation mating attempts from males, a prediction consistent with observations in most livebearing fish species. I further predict that females can avoid forced copulation attempts from *Xenophallus* males by moving to the side where the asymmetrical gonopodium is ineffective at transferring sperm. I will test these predictions using two behavioral test assays. The first of these assays will focus on how each gonopodial morph influences male pursuit of a female. In this assay, I will quantify how much time a male spends on either side of a female as a function of his gonopodial morph. The

second assay is focused on female resistance to male pursuit. To see if mature females actively avoid mating, I will record how the female positions her body with respect to a male. I expect that females will orient themselves in a manner that will prevent males with the most common morph to successfully mate.

3) Tracking Handedness Ratios Over Time – A clear prediction of negative frequency-dependent selection is that rare phenotypes should increase in frequency over time until they become the majority phenotype, at which point they should decline over time. I will identify up to 10 locations in Costa Rica where we will track male morphs over time. This will include traveling to Costa Rica between 4 and 6 times over a single year and sampling male morphs. This period of sampling is based on our preliminary findings from one location sampled twice in 2018. My preliminary data from collections made in March 2018 and June 2018, showed that the majority of males sampled in March were right-handed, but only 3 months later over 90% of males sampled were left-handed.

LIMITATIONS:

My lab stock of *Xenophallus* came from a field population that was predominantly sinistral at the time they were collected. Without a sufficient number of dextral males in the lab, I am unable to get a complete picture of how gonopodial morphology might influence mating behaviors and how negative frequency-dependent selection favors rare morphs. Additionally, because male gonopodia end with a corkscrew, there is a possibility that the species as a whole may exhibit a lock-and-key mating system, wherein male and female genital morphology must match in order to facilitate fertilization. If females also exhibit genital asymmetry, I would also have to consider how variation in female morphology is driven by negative frequency-dependent selection.

CULMINATING EXPERIENCE AND EXPECTED PRODUCTS:

I will publish my findings in a peer-reviewed journal and present them at several scientific conferences (the Utah Conference of Undergraduate Research, Animal Behavior Meetings, and possibly the Evolution Meetings). As an undergrad, experience writing and publishing a peer-reviewed paper will be critical to set up my future success in graduate school. I am currently planning on studying evolutionary biology after I have completed my undergraduate degree, so studying something that is relatively unknown and not understood has promising implications for my future research in the field of evolution. The results from my project will also be used as preliminary data for a National Science Foundation grant to support a collaborative project working on the genomics basis of gonopodial morphology in *Xenophallus*.

Beyond publishing and presenting at meetings, I also hope to expand the influence of this work to include motivating/mentoring young students in Utah and to help develop teaching curriculum modules for Utah high school science teachers to incorporate evolutionary processes in the classroom. My faculty advisor's connections with the Utah Science Teachers Association can help me execute this plan.

TIMELINE:

I began work on this project during the Fall Semester of 2017, so I have gathered roughly half of the expected data for this project to date. Here I include what has been done with the project to-date as well as my plan to complete it this year:

- March 2018: Brought specimens back to BYU from Costa Rica (CR) to establish breeding colony
- June 2018: Scored male handedness in population in CR
- September 2018: IACUC approved to conduct behavioral experiment
- October 2018: Preliminary behavioral trials completed with specimens with sinistral phenotype
- November 2018: Attended Desert Fishes Council Meeting presenting data to-date; Poster received Best Student Project Award
- February 2019: Score specimens at various locations in CR; Collect specimens from CR (dextral phenotype); Attend Utah Conference for Undergraduate Research (oral presentation)
- March 2019: Analyze data; Prepare tables and figures for publication

- April 2019: Score specimens in CR
- September 2019: Begin writing manuscript for the Honors Thesis and submission for peer-reviewed journals
- November 2019: Submit thesis draft for Thesis Defense
- December 2019: Submit project manuscript for publishing in peer-reviewed journals

Honors deadline: ________

IACUC INFORMATION:

Breeding protocol number: 18-0602

PI: Jerald B. Johnson

Status: Approved

Research protocol number: 18-0803

PI: Jerald B. Johnson

Status: Approved

THESIS COMMITTEE QUALIFICATIONS:

<u>Jerry Johnson (Faculty Advisor):</u> Dr. Johnson is a professor in the Biology Department who has worked with Costa Rica's native fishes since 1995. Research in his lab focuses on the evolutionary diversification in the wild and how diversity emerges from varying ecological conditions. Many of the projects in his lab are centered on life histories, behavior, and morphology. This project analyzes how all three of these traits connect and might explain why *Xenophallus umbratilis* has such a unique morphology.

I have worked with Dr. Johnson since September of 2017 as a research assistant, student lab manager, and teaching assistant. Because he is my research mentor and already familiar with this research project, it is especially appropriate that Dr. Johnson also serve as the faculty advisor on my Thesis Committee. I meet with Dr. Johnson weekly to check my progress and plan out what needs to be done in the upcoming weeks and months. Dr. Johnson is an effective mentor for me because he encourages me to think questions through thoroughly and generate possible solutions on my own before offering suggestions and alternatives to fine-tune my ideas. This approach has been especially formative for me as a researcher, providing me with opportunities to develop critical thinking skills and learn how to do real, original science.

Bryon Adams (Faculty Reader): Dr. Adams is also a professor in the Biology department who studies evolution in Antarctica's invertebrate life including nematodes, rotifers, and tardigrades. His research uses morphometric and genetic data to describe and explain the relationships between these organisms on from trophic and phylogenetic perspectives.

I have taken several classes from Dr. Adams during my undergraduate experience and have also worked as a teaching assistant for his Bio 130 class. Dr. Adam's background in evolution makes him an exceptionally qualified faculty reader on this project. His insights and suggestions will help make my research and analyses thorough and complete.

Dr. Steven Peck is the Honors Coordinator for the Biology Department and is also on my Thesis Committee. Dr. Peck's insights will ensure that I meet the Honors Program requirements and that my thesis is publishable.

FUNDING:

Because this work relies on periodic surveys of several wild populations, I am requesting supplemental funds to cover the costs of travel (airfare, car rental), collection permits, and supplies for field work. I am also requesting funding for lab supplies like food, filters, and tanks to maintain breeding colonies in the lab. Estimates are listed below:

- Cost of airfare to go to Costa Rica – ($600-$1,000)
- Rental for vehicle – ($300)
- Collection permits for Costa Rica – ($100)
- Lab supplies, tanks, food – ($750)
- Total comes to $1,750 - $2,150

I am requesting $1,000 from the Honors Program to help cover some of these expenses, namely airfare. Remaining costs will be covered by my research advisor and funds awarded from a CURA grant.

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