Does Sympatry Reinforce the Behavioral Divergence of *Pseudacris feriarum* in South Carolina?

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Introduction

The United States (U.S.) houses many species of ***chorus frogs***, whose populations span across numerous regions. In North Carolina, populations of *Pseudacris feriarum* (*P. feriarum*) exist in allopatry, whereas populations of *P. feriarum* coexist in sympatry with *Pseudacris nigrita* (*P. nigrita*) and *Pseudacris brimleyi* (*P. brimleyi*) in South Carolina. The focus of this study is on the differences between the allo- and sympatric populations of *P. feriarum*:

- Previous studies have shown that the calls of male, sympatric *P. feriarum* have diverged from that allopatric populations through changes in pulse rate and number as a result of sympatry.
- This divergence is known to have been caused by the similarity between the calls of the frogs' respective allopatric populations and was continued to be reinforced as a means of decreasing the rate of hybridization between the two species, since hybrids would have lower fitness because their calls are not preferred by the females of either population, thereby lowering the survivability of the species.
- Although it is known why the divergence is being reinforced, there is still mystery surrounding the processes *by which* it is being reinforced, specifically by the females of the sympatric populations of *P. feriarum*.

The general purpose of this study is to determine how sympatry influences the preferences of female populations of *P. feriarum* and how these preferences drive the divergence of the males' calls, as well as to establish the neurological changes that occur in both demographics as a result of these divergences.

However, this research project has many smaller purposes within it, such as that of this research poster, which is to utilize a *particle swarm optimizer* in order to create a model of the neural circuits of the female population of *P. feriarum* to determine the effect of changes in the neural circuit on the responses and preferences of the females. Currently, the best model to determine this, is a four-neuron model in which each neuron receives either an excitatory or inhibitory signal, where different responses are dependent on how those signals are integrated into the cell. Figure 1 depicts this model well, with AMPA and $GABA_A$ receptors acting as excitatory and inhibitory signals, respectively, on the long interval counting (LINs), interval counting (ICNs), afferent, and relay neurons.

Methodology

The subject of the study was the samples of the *P. feriarum* populations from North and South Carolina. The study sought to measure and quantify the preferences of the female population through observation, as well as by viewing the neurological effects of the male calls through a series of tests.

In the male population, the pulse rates and numbers of their calls were measured through call analyses. These data were extracted from both allo- and sympatric populations of *P. feriarum*, which were collected during mating season, that were transported to research facilities at FSU.

Later on, individual male frogs were isolated and put into 16 sound-insulating boxes, where their calls were then recorded. After this, the recorded calls of both allo- and sympatric males were replayed—from opposite ends—to females that were laid within a shallow pool of water in order to determine which call they preferred.

This preference was determined by which of the two sides of the pool (which call) the female swam to and touched. Analysis on the collected data were done through a code on the MATLAB program. In addition to this, a series of genetic analyses were also performed on the frogs in order to determine the genotypic implications of sympatry.

However, in order to compile preliminary results, the behavioral data of populations from previous years of research were used to generate a neural circuit model where parameters were optimized to fit the data as a means of determining the different populations differed in the tunings of their neural circuits.

Results/Conclusion

Utilizing the data from previous years, preliminary results that may reflect those of the current study can be extrapolated in which a divergence in the preferences of female *P. feriarum* is observed.

These results are reflected in the differences between the combined activity of LINs and ICNs of the North (allopatric) and South Carolina (sympatric) populations, where the North Carolina population has more neural activity in response to the calls of *P. nigrita* and *P. brimleyi* than that of South Carolina, which shows little to no activity in response to those calls. Because of this, it can be concluded that this difference in activity arose as a result of the decreased reinforcement of hybridization in the sympatric population, since it adversely affects the fitness of the species.

Conversely, it is to be expected that the allopatric population of *P. feriarum* exhibits activity in response to interspecific calls because there is no imposed pressure to diminish the rate of hybridization in an allopatric population where no other species of chorus frogs are typically found.

However, because these results are preliminary and were gathered from past studies, no solid implications can be made without the collection and analysis of this year's data; therefore, research is still ongoing in order to determine the relation between sympatry and the divergence of the preferences of female *P. feriarum* and the calls of the male population.

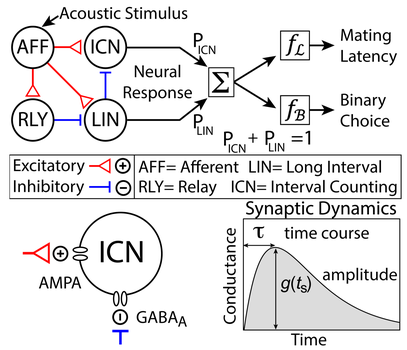

Figure 1: Diagram of the interactions and relationships between the long interval and interval counting neurons (LIN and ICN, respectively), as well as which neurons excite and inhibit others when activated.

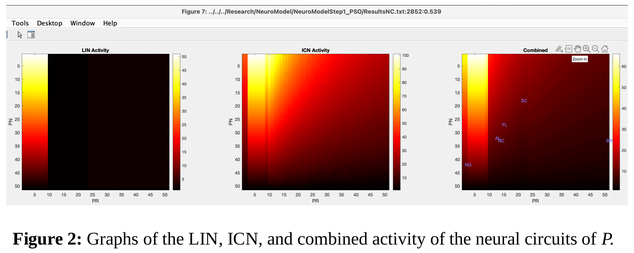

Figure 2: Graphs of the LIN, ICN, and combined activity of the neural circuits of *P. feriarum* in North Carolina with pulse number (PN) and pulse rate (PR) on the y- and x-axes, respectively.

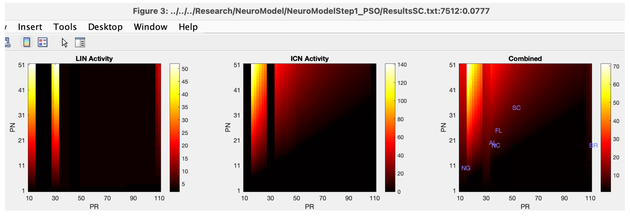

Figure 3: Graphs of the LIN, ICN, and combined activity of the neural circuits of *P. feriarum* in South Carolina with pulse number (PN) and pulse rate (PR) on the y- and x-axes, respectively.

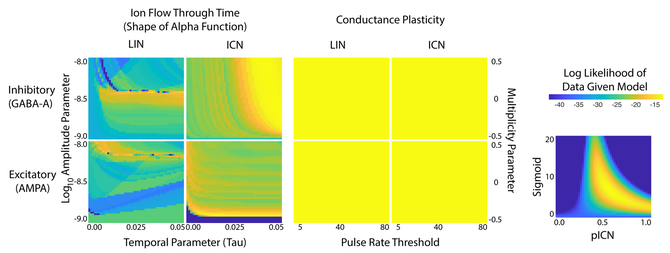

Figure 4: The graphic representation of the female frogs' preferences above shows the extent of the females' preferences when certain parameters are fixed and others are changed. In the diagram titled "Ion Flow Through Time," the top right quadrant reflects a gradual change in preference, whereas the bottom right quadrant depicts a finely tuned preference along a narrow line. Additionally, the diagram with sigmoid on the y-axis shows a stronger preference with higher sigmoid and a pICN value greater than or equal to 0.5

Discussion

As aforementioned in the results, based on data from previous years, the South Carolina population seems to exhibit a divergence in preference when compared to that of the allopatric population, which does not experience as many interspecific interactions.

Additionally, the main driving force of this divergence is the deterrence of hybridization in sympatric populations of *P. feriarum* as a means of maintaining the fitness of the species, which is not a selective pressure that is imposed on the allopatric populations of North Carolina. Furthermore, the divergence of the females' preferences is the primary pressure that leads to the change in the expression of the males' calls, since the sexual selection of certain behavioral phenotypes will eventually lead to the preference of said phenotypes (i.e., males with unselected expressions will become less fit, since their genes will not be passed onto the next generation).

However, some limitations of these findings are that they are not current in their scope; in other words, the data is not necessarily a proper reflection of ongoing trends in changes in preference and behavioral phenotypes because they are based on the data of previous years.

Because of this, future studies and research must still be done in order to determine the extent of the divergence between the allo- and sympatric populations of *P. feriarum* such as genetic and neurological analyses using histological tissue and neural circuit models, respectively, which are current components of the research project that will be explored in the future.

References

Garcia, T. S., Bredeweg, E. M., Urbina, J., & Ferrari, M. C. O. (2019). Evaluating Adaptive, Carry-Over, And Plastic Antipredator Responses Across A Temporal Gradient In Pacific Chorus Frogs. *Ecology (Durham)*, *100*(11), 1–11. https://doi.org/10.1002/ecy.2825

Lemmon, E. M., & Juenger, T. E. (2017). Geographic Variation In Hybridization Across A Reinforcement Contact Zone Of Chorus Frogs (Pseudacris). *Ecology And Evolution*, *7*(22), 9485–9502. https://doi.org/10.1002/ece3.3443

Malone, J. H., Ribado, J., & Lemmon, E. M. (2014). SENSORY DRIVE DOES NOT EXPLAIN REPRODUCTIVE CHARACTER DISPLACEMENT OF MALE ACOUSTIC SIGNALS IN THE UPLAND CHORUS FROG (PSEUDACRIS FERIARUM). *Evolution*, *68*(5), 1306–1319. https://doi.org/10.1111/evo.12366

Naud, R., Houtman, D., Rose, G.J., & Longtin, A. (2015). Counting on dis-inhibition: a circuit motif for interval counting and selectivity in the anuran auditory system. *J Neurophysiol*, *114*, 2804–2815. https://doi.org/10.1152/jn.00138.2015

Ospina, O. E., Lemmon, A. R., Dye, M., Zdyrski, C., Holland, S., Stribling, D., Kortyna, M. L., & Lemmon, E. M. (2021). Neurogenomic Divergence During Speciation By Reinforcement Of Mating Behaviors In Chorus Frogs (Pseudacris). *BMC Genomics*, *22*(1), 711–711. https://doi.org/10.1186/s12864-021-07995-3

Stewart, K. A., Austin, J. D., Zamudio, K. R., & Lougheed, S. C. (2016). Contact Zone Dynamics During Early Stages Of Speciation In A Chorus Frog (Pseudacris Crucifer). *Heredity*, *116*(2), 239–247. https://doi.org/10.1038/hdy.2015.96

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Figure 5: Image of an individual *P. feriarum*.