IDENTIFYING SPECIATION CANDIDATE GENES IN THE PSEUDACRIS GABA RECEPTOR

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INTRODUCTION

- *Pseudacris nigrita* and *Pseudacris ferarium* are known to have very loud and distinct mating calls, commonly described as a trilling sound that is often confused with insects
- The GABA alpha subunit, a ligand-gated ion channel, largely affects the organism's responsiveness, including their reaction to stimuli such as mating calls
- There was a similar previous study that tested for speciation in a similar manner, and they found that there was nucelotide variation, but not amino acid variation
- The purpose is to determine if there are certain nucleotide or amino acid variations among *Pseudacris* that are influencing mate preference, leading to sexually selective evolution
- We expect to see slight variation among the nucleotide sequences of the individuals, however it is not likely that there will be variation in the amino acid sequences seeing as this would critically affect other organismal functions

Figure 1. Photo of Pseudacris ferarium taken by Baran

METHODS

- Seventeen *Pseudacris* were collected and RNA samples were extracted from each
- Seven genes from the GABA-A receptor were sequenced from each of the individuals using Geneious Prime Software
- Regions were BLASTed to NCBI databases to identify regions of high similarity to known genes sequences in other species
- Both the nucleotide and amino acid sequences were tested for heterozygosity to determine genetic variation
- T-tests were run using R software to examine coverage differences between males & females and allopatric & sympatric individuals

RESULTS

- There were several sites of nucleotide variation among the 17 different samples, with up to 21 sites of heterozygosity in a single sequence
- None of these variations seemed to showed consistent trends between males and females samples or allopatric and sympatric samples
- There was little amino acid variation among the 17 samples
- P-values for coverage values showed that there were no significant differences indicating genetic variation
- We found no differences between populations or sexes in the GABA-A receptor gene expression levels

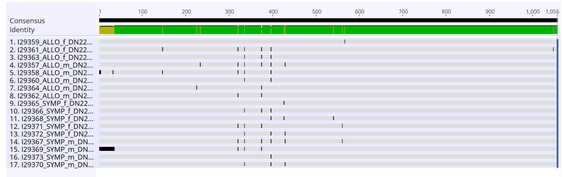

Figure 2. Gamma-aminobutyric acid receptor subunit gamma-3 nucleotidegamma nucleotide alignmentswith nucleotide variation across populations and sexes.

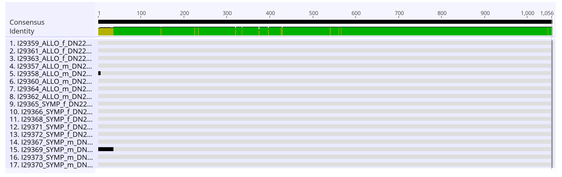

Figure 3. Gamma aminobutyric acid receptor subunit gamma-3 amino acidgamma-acid alignments displaying lack of amino acid variation across sexes and populations.

	Male vs. Female		Allopatric vs. Sympatric		Number of Heterozygous	Number of Heterozygous
Protein Name	*T-Statistic*	*P-Value*	*T-Statistic*	*P-Value*	Nucleotide Sites	Amino Acid Sites
Gamma-aminobutyric acid receptor subunit rho-1;^E	-0.9526769	0.585	1.39463	0.2729	5	0
Gamma-aminobutyric acid receptor subunit beta-4;^	-0.4013481	0.4024	-0.3008717	0.5437	4	0
Gamma-aminobutyric acid receptor subunit beta-3;^	-0.3434093	0.4664	-0.4423178	0.3467	0	0
Gamma-aminobutyric acid receptor subunit gamma-3;	-0.2117647	0.6587	-0.3723567	0.4544	8	0
Gamma-aminobutyric acid receptor subunit rho-3;^E	-0.711961	0.0735	-0.1429839	0.7279	21	0
Gamma-aminobutyric acid receptor-associated prote	-0.7675284	0.4654	0.7675284	0.4736	0	0
Gamma-aminobutyric acid receptor subunit gamma-2;	-0.3110147	0.464	-0.3587694	0.3908	17	0

Figure 4. T-Test results showing differences in coverage values between Males and Females as well as Allopatric and Sympatric individuals. Further outlines the amount of heterozygous sites between nucleotide and amino acid sequences

REFERENCES

Banker S.E., A. R. Lemmon, A. B. Hassinger, M. Dye, S. D. Holland, M. L. Kortyna, O. E. Ospina, H. Ralicki, E. Moriarty Lemmon, Hierarchical Hybrid Enrichment: Multitiered Genomic Data Collection Across Evolutionary Scales, With Application to Chorus Frogs (Pseudacris), Systematic Biology, Volume 69, Issue 4, July 2020, Pages 756–773, https://doi.org/10.1093/sysbio/syz074

Michaud, T. C. (1964). Vocal Variation In Two Species Of Chorus Frogs, Pseudacris Nigrita And Pseudacris Clarki, In Texas. Evolution, 18(3), 498–506. Https://Doi.Org/10.1111/J.1558-5646.1964.Tb01626.X

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

CONCLUSION

- There is no significant variation in protein expression, meaning that there is not significant differences in functional usage of the GABA-A receptor across different taxa
- Future research can be conducted on other GABA receptor locations, and in the AMPA and NMDA neurotransmitters
- Exploring how the brain processes auditory stimulus in chorus frogs helps us to understand sexual selection in the species, further allowing us to recognize genetic signs that help us to predict and understand evolution
- The effect of auditory stimulus on excitatory neurotransmitters in frogs can help us learn more about how to treat humans that suffer from hearing difficulty

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