Evolution of the GABA Receptor in Upland Chorus Frog (*Pseudacris ferarium)* and its Relationship to Mating Preferences and Speciation

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Introduction

Goal: to determine if amino acid variation is the cause of the difference in mating preference across geography and sex

Why the Gaba receptor?

- GABA receptors are inhibitory neuroreceptors in the central nervous system which control communication between neurons. (Allen et al., 2022)
- GABA receptors play a key aspect in determining the rate at which the male *Pseudacris* (chorus frog) call signal is processed within the female's brain
- A difference in the GABA receptors or their protein subunits in the female's brain could correlate to a difference in the mating preference
- The possible difference in the female's mating preference could lead to speciation of sympatric and allopatric populations

Prior research:

- Our RNA sequence data are from a total of seventeen *Pseudacris* individuals, four males and five females from a sympatric Florida population and five males and three females from an allopatric Alabama population (Ospina, 2021)
- In response to hybridization, which occurs when different variations or species mate, male *Pseudacris ferarium* (chorus frogs) have evolved their pulse rate of their calls to distinguish themselves from other similar species, such as the *Pseudracris nigrita.* (Ospina, 2021)
- Females of *Pseudacris ferarium* have begun to no longer recognize males of their own species from the two different populations, thus causing speciation between *Pseudacris feriarum* from allopatric and sympatric populations

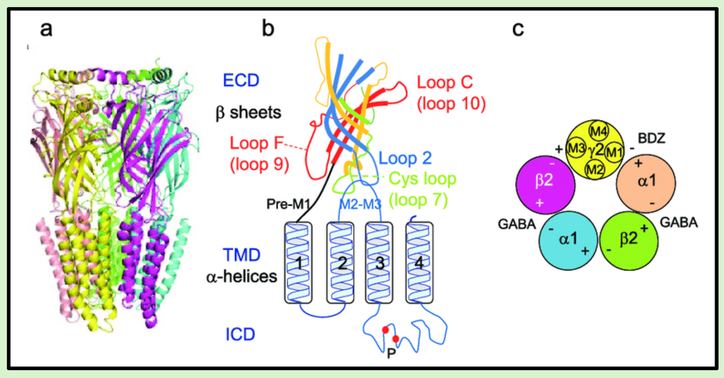


Figure 1 (above): 3-D modeling of $GABA_A$ receptor and its subunits

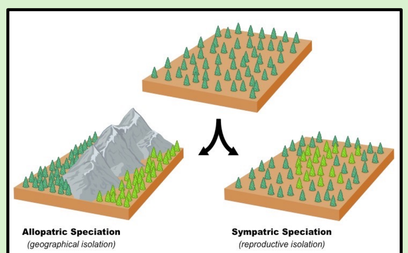
Image 1 (top-ight): P*seudacris Ferarium*

Figure 2 (bottom-right): Difference between allopatric and sympatric speciation.

top: a single population;

left: allopatric speciation

right: sympatric speciation



Methods

- Extract $GABA_A$ loci from RNAseq data of 17 *Pseudacris* individuals
- Align the same candidate gene from all individuals using Genius Prime software
- Compare the reads for nucleotide variation across the individuals
- Translate the nucleotide sequence to check for amino acid variation across individuals for each gene
- Extract the consensus sequence, the best representation of that gene from the 17 reads, from the RSCD Protein Data Bank
- Compare nucleotide and amino acid variation and the impact of geography and sex
- Compute expression coverage of each individual for each gene into the programming language, R, to compute the p values for allopatric vs. sympatric populations and male vs. female populations

Expected results

Possible Result 1:

If nucleotide variation affects GABA receptor function, then we expect to find amino acid level differences between allopatric and sympatric populations for the candidate genes.

Possible Result 2: (observed)

If amino acid differences are not observed, it could signal that there are differences in the level of gene expression or that there are other genes that underly the observed phenotypic differences between groups.

Conclusion and Future Direction

The lack of amino acid variation (Result 2) we found suggests other factors are at play, such as changes in the level of GABA gene expression, changes in quantity of GABA released from the presynaptic membrane, changes in release of GABA from glial cells, and the quantity of GABA receptors in the postsynaptic membrane.

The over arching goal of this project is to determine cellular-molecular factors in the female brain that may contribute to divergence of their mating preference and selection, and thus speciation. This could help us to better understand the auditory process in other animals, including humans, and auditory disorders.

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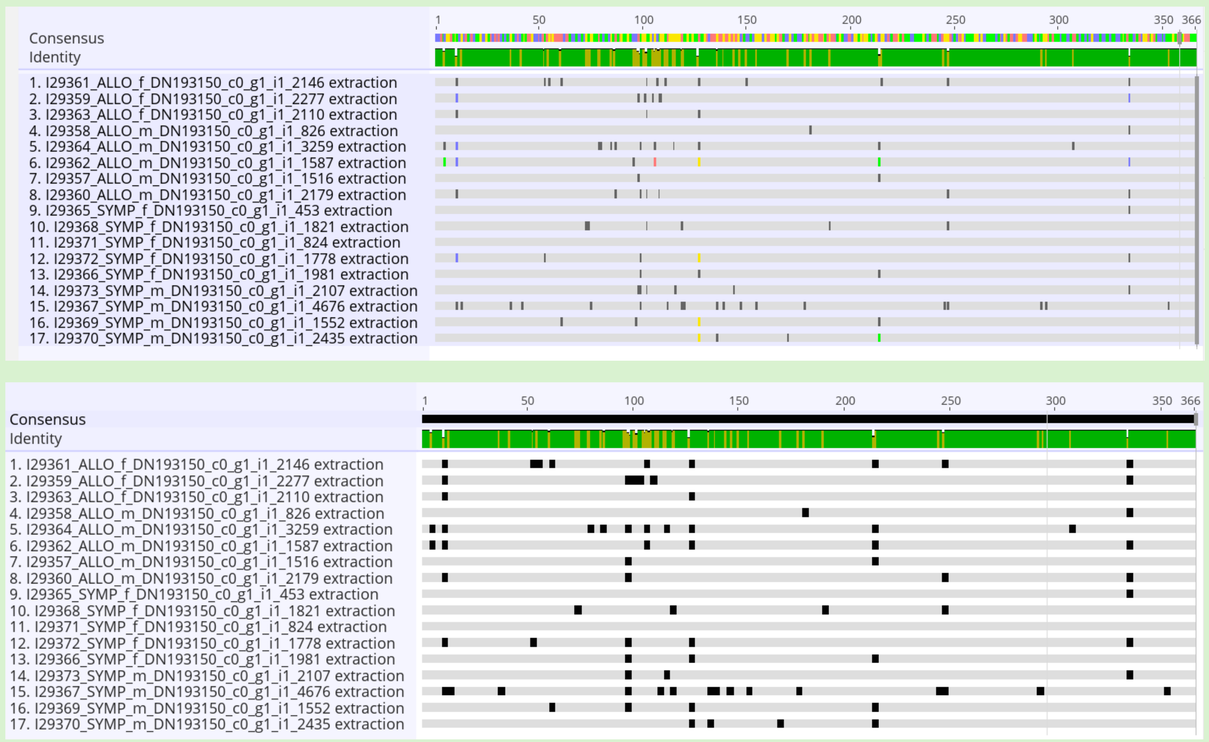

Image 2 (right): Marie holding a *Pseudacris feraroim*

Figure 3 (left): nucleotide (top) variation and amino acid variation (bottom), including heterozygous sites, among the 17 *Pseudacris* individuals for a selected region the $GABA_A$ receptor.

Note: this alignment has a substantial number of nucleotide and amino acid ambiguities

Figure 4 (below): chart showing variation and p values between different populations

Gene	Subunit	Number of nucleotide variation sites	Number of amino acid variation sites	P value (allopatric vs sympatric)	P value (male vs female)
DN9125_c0_g1_i1	Associated protein	71	0	0.5487	0.3492
DN24476_c0_g2_-12	Alpha-3	269	0	0.5452	0.5951
DN38368_c1_g1_01	Associated protein	7	0	0.2529	0.4813
DN43799_c0_gi_92	Alpha-6	609	0	0.2527	0.08942
DN193150_c0_g1_i1	Associated protein	52	0	0.6701	0.02953