The Evolution of the GABA Receptor in *Pseudacris* and its Contribution to Speciation

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Introduction

Why the GABA receptor?

• A mathematical model suggests that female chorus frogs may discriminate between pulsed calls by utilizing neuron systems that communicate through neurotransmitters (Naud et al., 2015).

•**GABA Receptors**: the predominant inhibitory neuroreceptors in the central nervous system which receive the amino acid GABA and control communication between neurons (Allen et al., 2022).

• Combinations of GABA receptors in *Pseudacris* (chorus frogs) determine female responses and preferences to male mating calls.

• A difference in GABA receptors or their protein subunits (focus: $GABA_A$) among populations could correspond to differences in female preferences to male calls, leading to speciation within *Pseudacris* populations.

• This project explores a new frontier for research: determining which genes are involved in animal behavior with respect to female choice for auditory signals with temporal components.

Prior Research:

• In previous experiments, a *Pseudacris* reference transcriptome was constructed using eight types of *Pseudacris* tissues (brain, eye, testis, liver, heart, lung, skin, leg muscle) from two adult allopatric male P. *feriarum* (Ospina, 2021).

• Seventeen *Pseudacris* individuals were RNA sequenced: five males and three females from an allopatric Alabama population and four males and five females from a sympatric Florida population (Ospina, 2021). Females in the allopatric and sympatric popuilations prefer different male signals. Reads from these individuals are the ones used in this current project.

• Reads from the RNA sequencing of the individuals were mapped to the reference transcriptome. Reads were then isolated from the mappings that corresponded to the $GABA_A$ receptor in order to determine candidate speciation ionotropic receptor genes.

• From the reference transcriptome annotations, a search was conducted for Gamma-aminobutyric acid ($GABA_A$) receptor subunits and associated proteins to isolate matches.

  • Transcripts that matched the specified annotation were selected.

• These transcripts were reduced down to a unique set of genes (sans isoforms).

• The next step is to focus in on various subunits of the $GABA_A$ receptor and analyze amino acid variation across the *Pseudacris* individuals to determine if amino acid variation is the cause of differences in mating call reception and selection.

Goal & Hypothesis:

• The **main goal** of this project is to determine if mate preference divergence across sex and geography occur due to amino acid variation.

• In this first step of a continuing project, we isolated the alpha1 subunit of the $GABA_A$ receptor to analyze amino acid variation.

• We **do not** expect to see variation in amino acids in important regions of the $GABA_A$ receptor because that variation would potentially alter other critical neurological functions.

• This project is necessary to rule out amino acid variation as the factor causing differences in mate call response and selection, a main driver in speciation.

Methods

• Selected the $GABA_A$ receptor subunit alpha-1, gene TRINITY_DN16293_c0_g1, to test for amino acid variation.

• Extracted reads from seventeen *Pseudacris* individuals (see Introduction for details) which mapped to the alpha1 subunit.

• Using Geneious Prime software, extracted the consensus sequence for each individual alignment.

• Aligned the seventeen consensus sequences and ordered them by sex or geographical similarities.

• Extracted and translated a selected nucleotide region which aligned to the amino acid sequence of the human alpha1 subunit of the $GABA_A$ receptor (RSCB Protein Data Bank).

• Compared nucleotide and amino acid variation in the selected region across the seventeen individuals to determine if the region displayed strong selection.

• Aligned the best translated consensus sequence (alpha1Ai1_129357) in the selected region with the corresponding region of the human alpha1 subunit.

• Compared this translated consensus sequence to the human alpha1 subunit amino acid sequence to determine sites of amino acid variation and similarity.

• Located these sites on the 3D human $GABA_A$ receptor alpha1 subunit model to determine placement of amino acid variation and similarity on the $GABA_A$ receptor.

β*

References

Allen, M. J., Sabir, S., & Sharma, S. (2022). GABA Receptor. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK526124/

GABRA1—Gamma-aminobutyric acid receptor subunit alpha-1—Homo sapiens (Human) | UniProtKB | UniProt. (n.d.). Retrieved February 24, 2023, from https://www.uniprot.org/uniprotkb/P14867/entry

Gupta, S., Alluri, R. K., Rose, G. J., & Bee, M. A. (2021). Neural basis of acoustic species recognition in a cryptic species complex. *Journal of Experimental Biology*, *224*(23), jeb243405. https://doi.org/10.1242/jeb.243405

Kasaragod, V. B., & Schindelin, H. (2019). Structure of Heteropentameric GABAA Receptors and Receptor-Anchoring Properties of Gephyrin. *Frontiers in Molecular Neuroscience*, *12*, 191. https://doi.org/10.3389/fnmol.2019.00191

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. *Journal of Neurophysiology*, *114*(5), 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. https://doi.org/10.1186/s12864-021-07995-3

RCSB Protein Data Bank. (n.d.). 3D View: 6D6T. *Human GABA-A Receptor Alpha1-Beta2-Gamma2 Subtype in Complex with GABA and Flumazenil, Conformation B*. Retrieved February 22, 2023, from https://www.rcsb.org/3d-view/6D6T

Rose, G. J. (2018). The numerical abilities of anurans and their neural correlates: Insights from neuroethological studies of acoustic communication. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*(1740), 20160512. https://doi.org/10.1098/rstb.2016.0512

Results

Result 1: Nucleotide variation, including heterozygous sites, was discovered among the seventeen aligned *Pseudacris* consensus sequences for the selected region (see **Figure 1**); however, there was no amino acid variation among the translated sequences (**see Figure 2**).

Result 2: When the most conserved, translated *Pseudacris* consensus sequence (an allopatric male) was mapped to the amino acid sequence for the human alpha1 subunit, there was very little amino acid variation between the sequences (see **Figure 5**). A few sites of variation are located across various regions of the alpha1 subunit, including a few notable variations near the GABA binding site (see **Figure 4**).

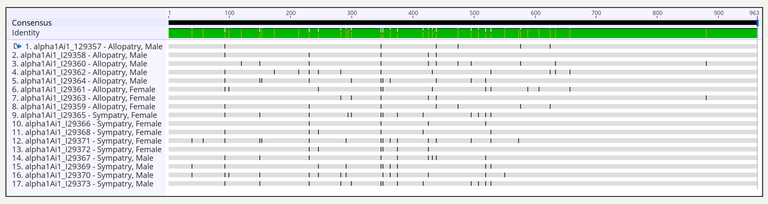

Figure 1 (left): Nucleotide variation, including heterozygous sites, among the 17 *Pseudacris* individuals for a selected region of the alpha1 subunit.

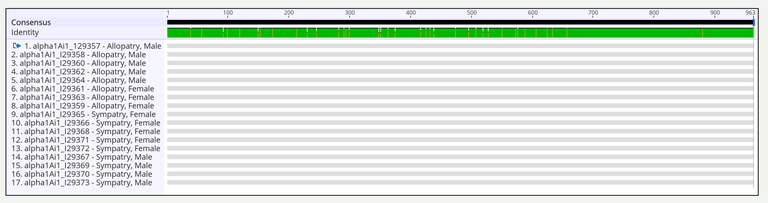

Figure 2 (left): Absence of amino acid variation across *Pseudacris* individuals for the same selected region of the alpha1 subunit.

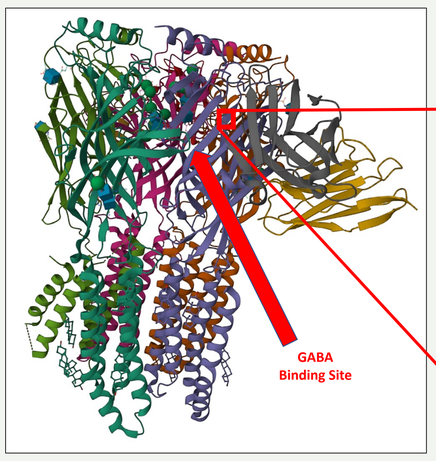

Figure 3 (right): 3D model of the human $GABA_A$ receptor (RCSB Protein Data Bank). Alpha1 subunit depicted in purple, Beta2 subunit depicted in turquoise. GABA binding site between the α1 and β2 subunits.

Figure 4 (left): Amino acid variation site (site 69 on the amino acid alignment) near the GABA binding site.

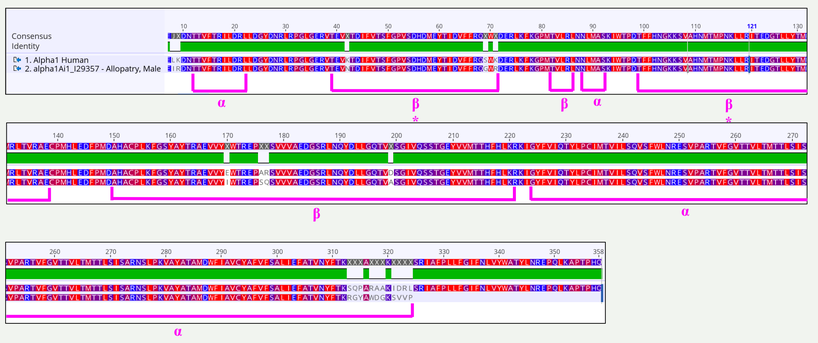

Figure 5 (left): Human $GABA_A$ receptor alpha1 subunit amino acid sequence aligned with an allopatric male's translated alpha1 subunit consensus sequence. Very little variation in amino acids is seen. Alignment regions are labeled by their locations on the GABA receptor: α denotes alpha helix, β denotes beta strand, star denotes sequences potentially near GABA-binding sites (GABRA1).

Conclusions & Future Directions

The variation in the alpha1 subunit *Pseudacris* nucleotide consensus sequences and lack of variation among the translated amino acid sequences demonstrate that this sequence is conserved over time. This was expected since the $GABA_A$ receptor is critical for a variety of brain functions, and any protein variation would have to alter those functions as well. Therefore, it is unlikely to discover protein variation in the $GABA_A$ receptor across individuals.

Although there was little variation in the alignment of the *Pseudacris* amino acid sequence and the human amino acid alpha1 subunit sequence, the few sites of variation include locations near the α1-β2 GABA binding site, which will have to be explored further. The lack of variation implies that most of the amino acids are conserved for specific function in the $GABA_A$ receptor.

Image 1 (left): *Pseudacris feriarum* (photo taken by Squier)

In future research, other subunits of the $GABA_A$ receptor must also be analyzed for amino acid variation across *Pseudacris* individuals. Variation must then be located on the $GABA_A$ receptor to determine if it affects function. Subsequently, the excitatory neurotransmitters AMPA and NMDA must also be analyzed for amino acid variation. Eventually, the project's main goal will be to discover if amino acid variation contributes to different mating call reception and selection, and therefore, speciation.

Acknowledgements

I would like to thank the UROP Program for this opportunity to discover and participate in research at FSU. Most importantly, I would like to thank Dr. Alan and Dr. Emily Lemmon for their mentorship, patience, and guidance on this continuing project and acceptance into their lab. Finally, a big Thank You to the other members of the Lemmon Lab for their kindness and support.