

## **Evolutionary Approach in the Characterization of Toll-like receptors in *Gryllus bimaculatus***

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Many insects rely on innate immunity to defend against pathogens and it was originally thought that Toll receptors played a key role; however, few Toll receptors have been shown to have a clear function in an insect's immune response. One example that we focused on for our research was *Drosophila melanogaster*, the common fruit fly. The purpose of looking into these Toll-like receptors lies in their ability to regulate cell death and cell survival which is known to assist in structural plasticity within the common fruit fly. This could indicate the potential role of Toll receptors in neuroplasticity.

Our research uses the common fruit fly proteins in order to determine which Tolls can be found in *Gryllus bimaculatus*, the two-spotted cricket. The cricket demonstrates unusual plasticity in which new pathways in the nervous system are formed as an effect of the environment, external stimuli, or injury which is defined by neuroplasticity. This mechanism by which this plasticity occurs is not understood in the cricket, so we turned to examples of plasticity in the common fruit fly, for which there is much more molecular information. Fruit flies have also shown plasticity in their olfactory system and have been viewed as a model for researching the plasticity of a sensory system. Synaptic plasticity describes how synapses are able to increase or decrease their potential activity as a result of the environment and any stimuli. Since fruit flies have demonstrated plasticity in their nervous system that is attributed to specific receptors, the purpose of our research is to find these receptors in crickets in order to uncover a potential mechanism for the plasticity in the cricket's nervous system.

We used Geneious to create a phylogenetic tree that illustrates the relationship among Tolls in a variety of insects, including crickets. Our transcriptome predicts all the mRNA transcripts that are expressed in any given tissue. In our case, the transcriptome we were observing was made from a control and deafferented prothoracic ganglia. We found that Tolls 1, 4, 6, 7, and 8 are conserved within *Gryllus bimaculatus* as predicted by our phylogenetic tree. Toll-2, -3, -4, and -5 did not follow the same patterns found in Toll-1, -4, -6, -7, and -8. We found that Toll-2 was closely related to Toll-7 and Tolls-3, -4 and -5 to be more closely related to Toll-1 (Figure1). A potential explanation for this could be that the signaling pathway for the first line of defense against pathogens may have been conserved so the Tolls that have clear functions in innate immunity are more conserved since they are essential to the organism's survival. The Tolls that do not have a clear role in defending against infection are less conserved which shows up in the tree that Geneious produced.

We also explored the role that Toll receptors played during development. Toll-like receptors have shown that they are expressed at different levels depending on the maturity of the fruit fly. Toll-3 and Toll-4 are expressed at lower levels during development while Toll-6, -7, and -8 are expressed at higher levels similar to Toll-1 and Toll-2 during development. Additionally, Toll-5 is expressed in adults and larvae exclusively. Since Toll receptors have a hand in developmental stages, this could point to a potential role in the structural plasticity found in the common fruit fly and possibly to the unusual neuroplasticity found in crickets. Further research would be beneficial to sequence the genome of the two-spotted cricket during development and whether patterns in these crickets mimic those in the common fruit fly.



