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

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Crickets have become model organisms for the study of regeneration and neuroplasticity— the ability to form new pathways in the nervous system as an effect of the environment, external stimuli or injury. The posterior cercal sensory system in *Gryllus bimaculatus* contains two thin protrusions, called cerci, that are covered in thin, wind-sensitive hairs. These hairs signal information regarding the location and direction of an attack, such as a lunging frog tongue, and whose function is critical for crickets to escape from predators. When a cercus is deafferented, the opposite cercus compensates and is able to transmit signals to both sides of the nervous system. On a cellular level, the neurons from the deafferented cercus will grow dendrites that cross the central nervous system's midline to form connections on the other side.

This summer, the Horch lab focused on protein families expressed in the prothoracic ganglion of *G. bimaculatus* that might play a role in their compensatory ability. My research was centered around Toll-like receptors which are proteins that are found across species ranging from humans to fruit flies. In humans, these proteins work alongside the immune system but are more closely linked with development and cell survival in insects. Using NCBI we found the protein sequences associated with Toll-like receptors in *D. melanogaster*; we chose this organism because of its evolutionary proximity to the cricket and its simple yet heavily studied genome. These sequences were then BLASTed against the cricket transcriptome using the software Geneious and phylogenetic trees were created. Furthermore, we added Toll-like receptor sequences from an array of other species to create an evolutionary overview of the protein family.

We found that Tolls 6, 7, and 8 (highlighted teal, blue, and green respectively) had distinct clades and grouping patterns within our tree, indicating that they have been conserved among most insects, including *G. bimaculatus.* To reaffirm our findings, we ran the sequences through NCBI to establish if the sequences were congruent with our phylogenetic tree. Only then did we rename the sequences to reflect that they were found in the cricket's genome. Clades containing Tolls 1 and 4 (highlighted purple and yellow) had a few outliers but we still determined that sequences in those groupings were accurate as they consisted of conserved domains characteristic of the Toll family. While our research reaffirmed the existence of Toll-like receptors in the adult cricket, it would be beneficial to sequence the genome of *G. bimaculatus* at the larval stage in order to get a better understanding of which Tolls are up or downregulated during development and whether patterns in *G. bimaculatus* mimic those in *D. melanogaster.*

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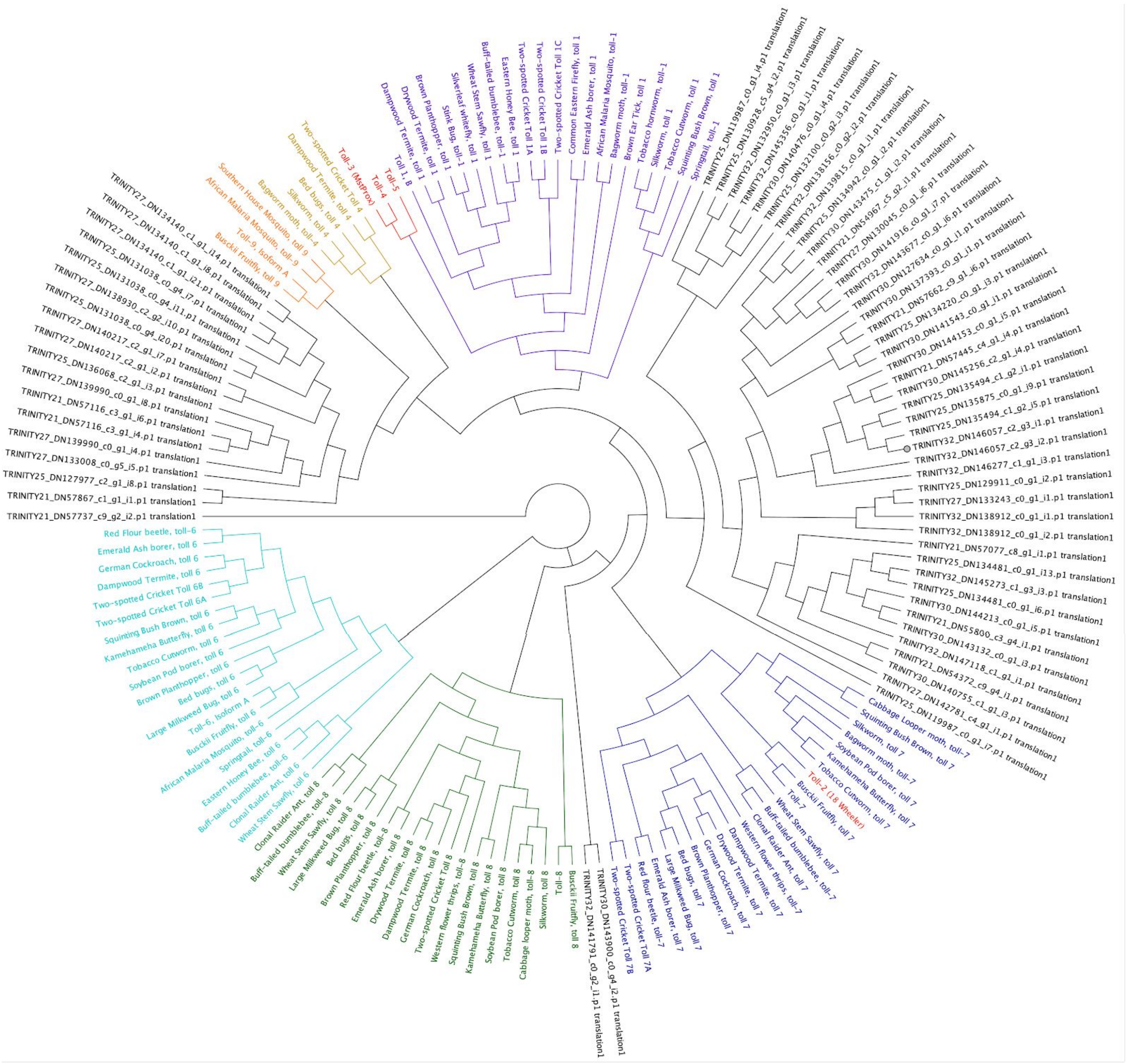
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Busckii Fruitfly, toll 9
Toll-9, Isoform A
African Malaria Mosquito, toll-9
Southern House Mosquito, toll 9
Bagworm moth, toll-4
Silkworm, toll 4
Bed bugs, toll 4
Dampwood Termite, toll 4
Two-spotted Cricket Toll 4
Toll-3 (MstProx)
Toll-4
Toll-5
Toll 1, B
Dampwood Termite, toll 1
Drywood Termite, toll 1
Brown Planthopper, toll 1
Stink Bug, toll-1
Silverleaf whitefly, toll 1
Wheat Stem Sawfly, toll 1
Buff-tailed bumblebee, toll-1
Eastern Honey Bee, toll 1
Two-spotted Cricket Toll 1A
Two-spotted Cricket Toll 1B
Two-spotted Cricket Toll 1C
Common Eastern Firefly, toll 1
Emerald Ash borer, toll 1
African Malaria Mosquito, toll-1
Bagworm moth, toll-1
Brown Ear Tick, toll 1
Tobacco hornworm, toll-1
Silkworm, toll 1
Tobacco Cutworm, toll 1
Squinting Bush Brown, toll 1
Springtail, toll-1
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Cabbage Looper moth, toll-7
Squinting Bush Brown, toll 7
Silkworm, toll 7
Bagworm moth, toll-7
Soybean Pod borer, toll 7
Kamehameha Butterfly, toll 7
Tobacco Cutworm, toll 7
Toll-2 (18 Wheeler)
Busckii Fruitfly, toll 7
Toll-7
Wheat Stem Sawfly, toll 7
Buff-tailed bumblebee, toll-7
Clonal Raider Ant, toll 7
Western flower thrips, toll-7
Dampwood Termite, toll 7
Drywood Termite, toll 7
German Cockroach, toll 7
Brown Planthopper, toll 7
Bed bugs, toll 7
Large Milkweed Bug, toll 7
Emerald Ash borer, toll-7
Red flour beetle, toll-7
Two-spotted Cricket Toll 7A
Two-spotted Cricket Toll 7B
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Busckii Fruitfly, toll 8
Toll-8
Silkworm, toll 8
Cabbage looper moth, toll-8
Tobacco Cutworm, toll 8
Soybean Pod borer, toll 8
Kamehameha Butterfly, toll 8
Squinting Bush Brown, toll 8
Western flower thrips, toll-8
Two-spotted Cricket Toll 8
German Cockroach, toll 8
Dampwood Termite, toll 8
Drywood Termite, toll-8
Emerald Ash borer, toll 8
Red Flour beetle, toll-8
Brown Planthopper, toll 8
Bed bugs, toll 8
Large Milkweed Bug, toll 8
Wheat Stem Sawfly, toll 8
Buff-tailed bumblebee, toll-8
Clonal Raider Ant, toll 8
Wheat Stem Sawfly, toll 6
Clonal Raider Ant, toll 6
Buff-tailed bumblebee, toll-6
Eastern Honey Bee, toll 6
Springtail, toll-6
African Malaria Mosquito, toll-6
Busckii Fruitfly, toll 6
Toll-6, Isoform A
Large Milkweed Bug, toll 6
Bed bugs, toll 6
Brown Planthopper, toll 6
Soybean Pod borer, toll 6
Tobacco Cutworm, toll 6
Kamehameha Butterfly, toll 6
Squinting Bush Brown, toll 6
Two-spotted Cricket Toll 6A
Two-spotted Cricket Toll 6B
Dampwood Termite, toll 6
German Cockroach, toll 6
Emerald Ash borer, toll 6
Red Flour beetle, toll-6