# Supplementary Note 6: Toll Receptors

**Authors: Teresa Shippy, Chris Cordola, Tracy Bell, David Hunter and Allison Izsak**

## Introduction

The Toll pathway is widely conserved in animals and plays a role in innate immunity in both vertebrates and insects. Toll receptors are transmembrane proteins characterized by extracellular leucine-rich repeats (LRRs) and cysteine-rich clusters, as well as a cytoplasmic Toll/Interleukin-1R (TIR) domain (see Imler and Zheng, 2004). Vertebrate Toll receptors, known as Toll-like receptors (TLRs) act as pattern recognition receptors and are directly activated by microbial components. In contrast, insect Toll receptors are indirectly activated by microbial components. Secreted pattern recognition receptors bind microbial molecules and then initiate a protease cascade that results in the cleavage of Spätzle. The cleaved form of Spätzle is able to bind to the Toll receptor, which then activates a downstream signal transduction pathway leading to the transcription of antimicrobial peptides (see Valanne et al. 2011)

The founding member of the Toll receptor family was the *Drosophila melanogaster* gene *Toll*, which was initially identified because of its role in embryonic development (Nüsslein-Volhard and Wieschaus, 1980). Eight more Toll receptor genes (*Toll-2* through *Toll*-9) have been found in the *D. melanogaster* genome. Comparison of the Toll receptors found in various insects suggests that there were only six ancestral Toll receptors: *Toll-1*, *Toll-6*, *Toll-2/7*, *Toll-8*, *Toll-9* and *Toll-10* (Evans et al. 2006; Benton et al. 2016). Extant insects have different numbers of Toll receptors due to lineage-specific duplications and losses (see Table 1). Most hemipterans whose genomes have been sequenced have one member of each ancestral class except *Toll*-9 (Gerardo et al. 2010, Bao et al. 2013, Wang et al. 2015, Benoit et al. 2016). The only hemipteran in which *Toll-9* has been found is the milkweed bug, *Oncopeltus fasciatus* (Benton et al. 2016). The only Toll receptor expansion reported in a hemipteran is in the pea aphid (*Acyrthosiphon pisum*), which has three *Toll-1* class genes (Gerardo et al. 2010).

## Methods

Toll receptor genes from other insects were used to query the predicted *D. citri* protein sets (Diacit_International_psyllid_consortium_proteins_v1 and Diacit_RefSeq_proteins_Release_100) at i5k@NAL. The loci encoding the matching proteins were identified and manually annotated in Web Apollo. BLAST searches to compare *D. citri* proteins to other insect proteins were performed at NCBI and Flybase. We also performed BLAST searches of the *D. citri* MCOT set to compare our gene models to *de novo* assembled transcripts when possible and to search evidence of genes not found in the current genome assembly. Multiple alignments of the predicted *D. citri* isoforms and their homologs were performed in Muscle

(http://www.ebi.ac.uk/Tools/msa/muscle/). We used MEGA7 to construct a phylogenetic tree via the neighbor-joining method. Sequences for multiple alignment and phylogenetic analysis were obtained from NCBI, FlyBase, ImmunoDB and the Bordenstein Lab (NSF DEB-1046149).

**Results and Discussion**

Using Toll receptor proteins from other insects to BLAST against *D. citri* predicted proteins, we identified four Toll receptors encoded by the *D. citri* genome. After manual annotation of these genes, we used the edited models to BLAST the MCOT database. When possible, we used *de novo* assemblies from MCOT as independent tests of our models. In one case (*Toll-1*), this method revealed repeated exons in the gene model due to improper contig assembly. We were able to improve the model by removing the repeated exons, although the model is still missing sequence that is present in the MCOT transcript but is not found in the assembled contig.

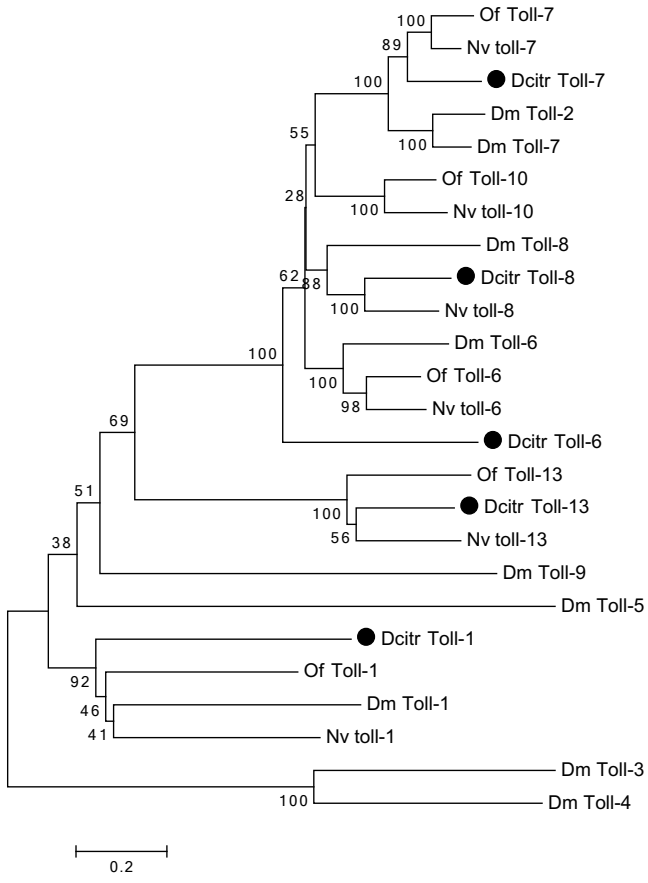


Figure 1. Neighbor-joining tree of Toll receptor proteins. Dm=*Drosophila melanogaster*, Of=*Oncopeltus fasciatus*, Nv=*Nilaparvata lugens*, Dcitr=*Diaphorina citri*

We were able to determine the apparent orthology of each of the Toll receptor genes by reciprocal BLAST searches (Table 2) and phylogenetic analysis (Fig. 1). Based on these

results, we named the genes *Toll-1*, *Toll-6*, *Toll-7* and *Toll-8*. No genes from the *Toll-9* or *Toll-10* classes were identified in the current assembly. We also searched the MCOT database for genes from these missing classes, but did not find any candidates.

In addition to the four typical Toll receptor genes, our BLAST searches also identified an ortholog of *Toll-13*, which has been found in other hemipteran species (Bao et al. 2013, Oncopeltus fasciatus Official Gene set v1.1. (Ag Data Commons http://dx.doi.org/10.15482/USDA.ADC/1173142). The protein encoded by *Toll-13* lacks the transmembrane region and the cytoplasmic Toll Interleukin-1 Receptor (TIR) domain, suggesting it might be secreted (Bao et al. 2013).

**References**


1. Bao, Y.-Y., Qu, L.-Y., Zhao, D., Chen, L.-B., Jin, H.-Y., Xu, L.-M., Cheng, J.-A., and Zhang, C.-X. (2013). The genome- and transcriptome-wide analysis of innate immunity in the brown planthopper, Nilaparvata lugens. Bmc Genomics *14*, 1–23.

2. Benoit, J., Adelman, Z., Reinhardt, K., Dolan, A., Poelchau, M., Jennings, E., Szuter, E., Hagan, R., Gujar, H., Shukla, J., et al. (2016). Unique features of a global human ectoparasite identified through sequencing of the bed bug genome. Nat Commun *7*, 10165.

3. Benton, M.A., Pechmann, M., Frey, N., Stappert, D., Conrads, K.H., Chen, Y.-T., Stamataki, E., Pavlopoulos, A., and Roth, S. (2016). Toll Genes Have an Ancestral Role in Axis Elongation. Curr Biol *26*, 1609–1615.

4. Evans, J., Aronstein, K., Chen, Y., Hetru, C., Imler, J.-L., Jiang, H., Kanost, M., Thompson, G., Zou, Z., and Hultmark, D. (2006). Immune pathways and defence mechanisms in honey bees Apis mellifera. Insect Mol Biol *15*, 645–656.

5. Gerardo, N., Altincicek, B., Anselme, C., Atamian, H., Barribeau, S., de Vos, M., Duncan, E., Evans, J., Gabaldón, T., Ghanim, M., et al. (2010). Immunity and other defenses in pea aphids, Acyrthosiphon pisum. Genome Biology *11*, R21.

6. Imler, J.-L., and Zheng, L. (2004). Biology of Toll receptors: lessons from insects and mammals. J Leukocyte Biol *75*, 18–26.

7. Nüsslein-Volhard, C., and Wieschaus, E. (1980). Mutations affecting segment number and polarity in Drosophila. Nature *287*, 795–801.

8. Valanne, S., Wang, J.-H., and Rämet, M. (2011). The Drosophila Toll Signaling Pathway. J Immunol *186*, 649–656.

9. (dataset) Vargas Jentzsch, Iris M.; Hughes, Daniel S. T.; Poelchau, Monica; Robertson, Hugh M.; Benoit, Joshua B.; Rosendale, Andrew J.; Armisén, David; Duncan, Elizabeth J.;

Vreede, Barbara M. I. ; Jacobs, Chris G. C.; Berger, Chloe; Burnett, Denielle L.; Chang, Chun-che; Chen, Yen-Ta; Chipman, Ariel D.; Andrew Cridge; Crumière, Antonin Jean Johan; Peter Dearden; Erezyilmaz, Deniz F.; Cassandra Extavour; Friedrich, Markus; Horn, Thorsten; Hsiao, Yi-min; Jones, Jeffery W.; Jones, Tamsin E.; Khila, Abderrahman; Leask, Megan; Lovegrove, Mackenzie; Lu, Hsiao-ling; Lu, Yong; Nair, Ajay; Palli, Subba R.; Pick, Leslie; Porter, Megan L.; Refki, Peter; Rivera Pomar, Rolando; Roth, Siegfried; Sachs, Lena; Santos, Emilia; Seibert, Jan; Sghaier, Essia; Shukla, Jayendra N.; Suzuki, Yuichiro; Tidswell, Olivia; Traverso, Lucila; van der Zee, Maurijn; Viala, Séverine; Richards, Stephen; Panfilio, Kristen A. (2015). Oncopeltus fasciatus Official Gene set v1.1. Ag Data Commons. http://dx.doi.org/10.15482/USDA.ADC/1173142

10. Wang, L., Tang, N., Gao, X., Guo, D., Chang, Z., Fu, Y., Akinyemi, I., and Wu, Q. (2016). Understanding the immune system architecture and transcriptome responses to southern rice black-streaked dwarf virus in Sogatella furcifera. Sci Reports *6*, 36254.