

Supplementary Note 7: Spaetzle proteins

Authors: Teresa Shippy

Introduction

Spaetzle proteins serve as ligands for Toll receptors. When a proper developmental or immune signal is received, Spaetzle is cleaved. This allows it to bind to the Toll receptor causing activation of downstream components of the Toll pathway (see Valanne et al 2011).

In *Drosophila*, there are six *spaetzle* genes, but the number of genes in other insects varies (see Viljakainen 2015 and Table 1). Phylogenetic analysis has shown that there are six major classes of *spaetzle* genes with one *Drosophila spaetzle* gene in each class (An et al. 2010, Sun et al. 2016).

Organism	Order	# of spätzle genes
<i>Tribolium castaneum</i>	Coleoptera	7
<i>Anopheles gambiae</i>	Diptera	6
<i>Drosophila melanogaster</i>	Diptera	6
<i>Acyrtosiphon pisum</i>	Hemiptera	6
<i>Nilaparvata lugens</i>	Hemiptera	8
<i>Apis mellifera</i>	Hymenoptera	2
<i>Nasonia vitripennis</i>	Hymenoptera	9
<i>Linepithsma humile</i>	Hymenoptera	5
<i>Bombyx mori</i>	Lepidoptera	3

Table 1. Gene counts of *spätzle* orthologs in representative insects.

Methods

At least one ortholog from each of the six classes of *spätzle* genes was used to query the predicted *D. citri* protein sets (Diacit_International_psyllid_consortium_proteins_v1 and Diacit_RefSeq_proteins_Release_100) at i5k@NAL. Putative *D. citri* orthologs were identified and manually annotated in Web Apollo. The predicted proteins were BLASTed against Insecta with NCBI BLAST to verify orthology. We also performed BLAST searches of the *D. citri* MCOT set at citrusgreening.org to compare the predicted proteins to proteins encoded by *de novo*-assembled transcripts. We used MEGA7 to construct a phylogenetic tree via the neighbor-joining method. Sequences for phylogenetic analysis were obtained from NCBI, FlyBase, ImmunoDB and the Bordenstein Lab (NSF DEB-1046149).

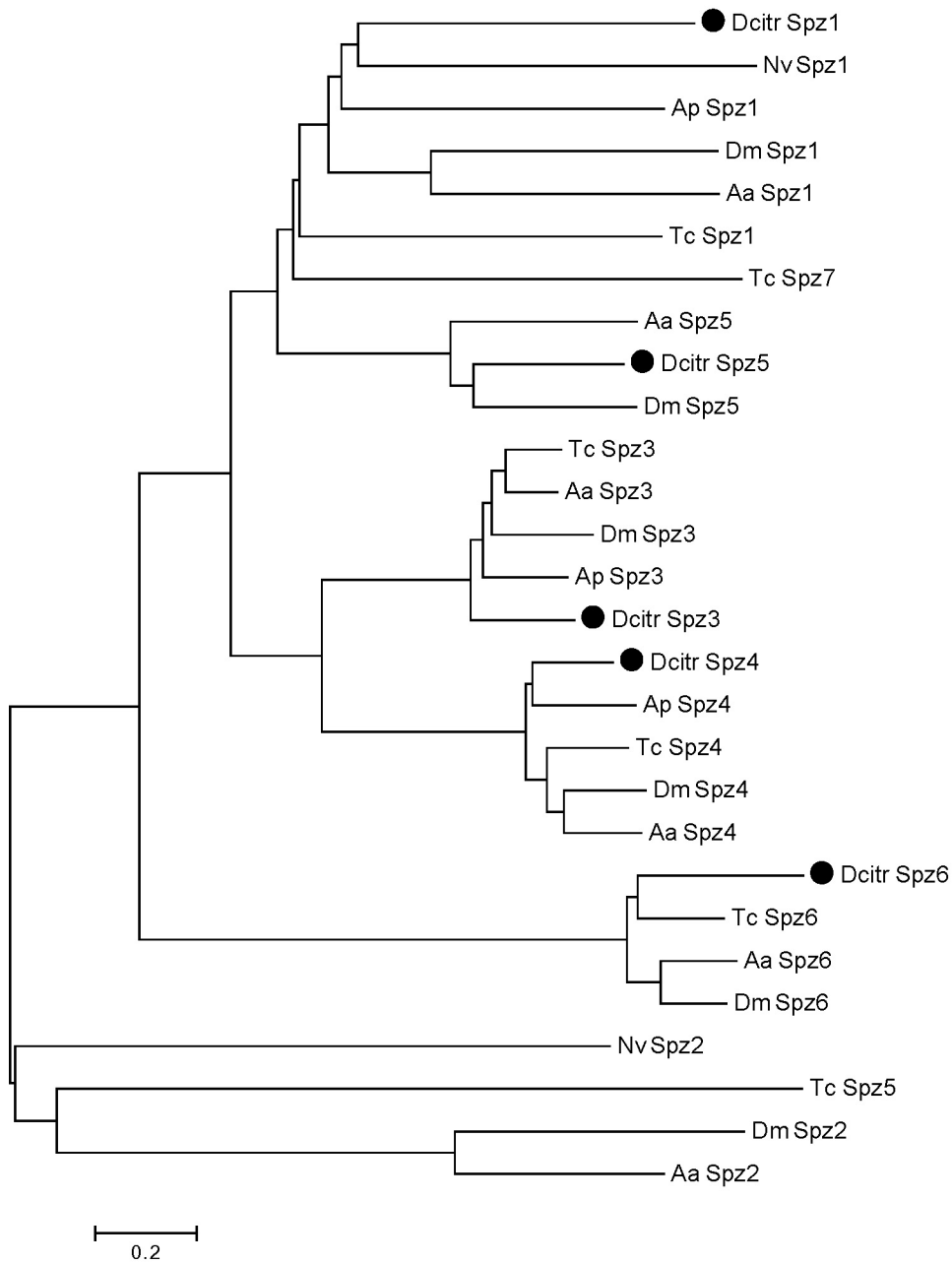


Figure 1. Phylogenetic tree of Spätzle orthologs from *Diaphorina citri* (Dc), *Nasonia vitripennis* (Nv), *Acyrthosiphon pisum* (Ap), *Drosophila melanogaster* (Dm), *Aedes aegypti* (Aa) and *Tribolium castaneum* (Tc). *D. citri* proteins are marked with a black dot.