

## Supplementary Note 4: PGRP and $\beta$ GRP

**Authors: Chris Cordola**

### Introduction

PGRPs are receptors that recognize and bind to peptidoglycan molecules which can be found in the cell walls of bacteria (Sun *et al.*, 2014). They have been conserved from insects to mammals as they are important in the detection of pathogens. The PGRP gene family is split into different size groups, two being PGRP long (30-90kDA) and PGRP small (20kDA) (Hoffmann, 2003). PGRP-S genes make up about half of the PGRP gene family present in the genome of *Drosophila melanogaster*, 7 out of 13 genes (Hoffmann, 2003). PGRP-S is an extracellular protein that bind to pathogen associated molecular patterns (PAMPs) which will cause the production of an immune response directing immune cells to degrade a pathogen or leading to the transcription of antimicrobial peptides via signaling pathways like Toll or IMD (Hoffmann, 2003). So far there has been one small PGRP found in the citrus psyllid's genome, but with the PGRPs being a first line of defense in the insect immune response, targeting them could result in a lack of cellular function that could dampen the *Diaphorina citri*'s immune system and response.

### Materials and Methods

The PGRP sequences from the following insects were used to search the genome of *Diaphorina citri*: *Drosophila melanogaster*, *Anopheles gambiae*, *Aedes aegypti*, *Culex pipiens* and *Cimex lectularius*. These sequences were used in the BLAT search tool in Web Apollo, BLAST from I5K, and the MCOT database of the citrus psyllid to find gene model predictions. The resulting hit was annotated using transcript and RNA sequences tracks as support.

Blastp was used to perform a pairwise alignment with other insects. The top hits from the previously mentioned insects plus the pea aphid were recorded along with accession, query, identity, and bit score information. The ExPasy Compute pI/MW tool was utilized to calculate the theoretical molecular weight of the annotated PGRP sequence. PGRP gene numbers were collected from Table 2 of Viljakainen, 2015.

The *D. citri* PGRP sequence and those from the following insects: *Culex quinquefasciatus* (XP\_001849091.1, XP\_001848058.1), *Halyomorpha halys* (XP\_014294583.1), *Anopheles gambiae* (XP\_001688526.1, XP\_310547.4), *Drosophila melanogaster* (NP\_573078.1, NP\_572727.1), *Aedes Aegypti* (XP\_001660103.2, XP\_001661789.2), and *Cimex lectularius* (XP\_014247856.1) were used to construct a multiple sequences alignment in MEGA7 with default MUSCLE settings. Subsequently, a neighbor-joining tree was made from the alignment with p-distance, 1000 bootstrap replicates, and complete deletion.

### Results and Discussion

In the search for the PGRPs in the citrus psyllid's genome one model was found after searches were conducted using BLAT, I5K BLAST, and MCOT search. The sequence collected from ACP contained one PGRP domain. The compute pl/MW tool was used to calculate the molecular weight of the PGRPs protein and result was about 23.85 kDa consistent with previous knowledge on PGRPs.

It was a surprise that there was only one gene found in version 1 of the psyllid genome (Table 1). On the other hand, it has been shown that the PGRPs are missing in the pea aphid, which is a closely related hemipteran to ACP (Table 1; Gerardo *et al.*, 2010). A Blastp pairwise alignment showed the sequence top matches were small PGRPs having two of the highest bit scores (Table 2). The neighbor-joining tree distance shows that the psyllid PGRP is closer to the small PGRPs (Figure 1). In the future this gene could be targeted as a means of possibly disrupting the insect's immunity.

Table 1: PGRP family member gene number in *D. citri*, *D. melanogaster*, *A. gambiae*, *A. pisum*, *P. humuculus*, *B. mori*, and *T. castaneum* (Gerardo *et al.*, 2010; Viljakainen 2015).

<b>PGRP gene count in <i>D. citri</i> and other insects</b>	
	PGRP
<i>Drosophila melanogaster</i>	13
<i>Anopheles gambiae</i>	11
<i>Acyrtosiphon pisum</i>	0
<i>Bombyx mori</i>	12
<i>Pediculus humanus</i>	1
<i>Tribolium castaneum</i>	7

Table 2: Top Blastp hit found when alignment the PGRP from *D. citri* with *D. melanogaster*, *A. aegypti*, *C. quinquefasciatus*, *A. gambiae*, and *C. lectularius*. Information includes: match description, accession, query, identity, and bit score.

<b>Blastp results for PGRP-S in <i>D. citri</i></b>					
<b>Organism</b>	<b>Match</b>	<b>Accession</b>	<b>Query</b>	<b>ID</b>	<b>Bit score</b>
<i>Drosophila melanogaster</i>	peptidoglycan recognition protein SB1	CAD89128.1	47%	38%	222
<i>Aedes aegypti</i>	peptidoglycan recognition protein (Long)	XP_001654275.1	48%	40%	217
<i>Culex quinquefasciatus</i>	peptidoglycan recognition protein sb2	XP_001849091.1	55%	38%	217
<i>Anopheles gambiae</i>	peptidoglycan recognition protein (long)	XP_558600.3	70%	27%	175
<i>Cimex lectularius</i>	PREDICTED: peptidoglycan-recognition protein SB2-like isoform X4	XP_014247859.1	47%	30%	136

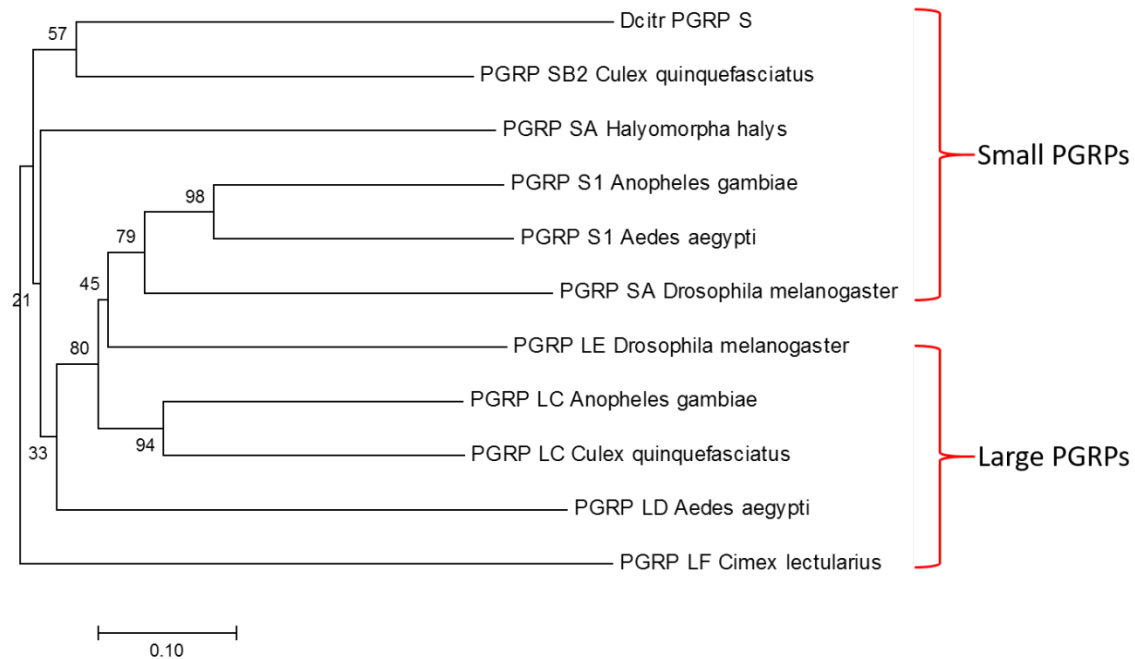


Figure 1: Neighbor-Joining tree constructed using small and large PGRP sequences from *D. citri* and other insects. The branches shown that the *D. citri* PGRP is closer to the other PGRPs, especially *C. quinquefasciatus*. Bootstrap values based on 1000 replicates are shown at the nodes.

## References

1. Dukhanina E, Lukyanova T, Romanova E, Guerriero V, Gnuchev N, Georgiev G, Yashin D, Sashchenko L. 2015. A new role for PGRP-S (Tag7) in immune defense: lymphocyte migration is induced by a chemoattractant complex of Tag7 with Mts1. *Cell Cycle* 14(22):3635-3643.
2. Dziarski R, Gupta D. 2006. The peptidoglycan recognition proteins (PGRPs). *Genome biology* 7(8):1.
3. Gerardo NM, Altincicek B, Anselme C, Atamian H, Barribeau SM, De Vos M, Duncan EJ, Evans JD, Gabaldón T, Ghanim M. 2010. Immunity and other defenses in pea aphids, *Acyrtosiphon pisum*. *Genome biology* 11(2):1.
4. Hoffmann JA. 2003. The immune response of *Drosophila*. *Nature* 426(6962):33-38.
5. Paredes JC, Welchman DP, Poidevin M, Lemaitre B. 2011. Negative regulation by amidase PGRPs shapes the *Drosophila* antibacterial response and protects the fly from innocuous infection. *Immunity* 35(5):770-779.
6. Sharma P, Dube D, Yadav SP, Sinha M, Kaur P, Sharma S, Singh TP. 2014. Structural and functional studies of mammalian peptidoglycan recognition protein, PGRP-S. *Journal of the Indian Institute of Science* 94(1):109-118.
7. Sun Q, Xu X-X, Freed S, Huang W-J, Zheng Z, Wang S, Ren S-X, Jin F-L. 2014. Molecular characterization of a short peptidoglycan recognition protein (PGRP-S) from Asian corn borer (*Ostrinia furnacalis*) and its role in triggering proPO activity. *World Journal of Microbiology and Biotechnology* 30(1):263-270.

8. Tydell CC, Yuan J, Tran P, Selsted ME. 2006. Bovine peptidoglycan recognition protein-S: antimicrobial activity, localization, secretion, and binding properties. *The Journal of Immunology* 176(2):1154-1162.
9. Viljakainen L. 2015. Evolutionary genetics of insect innate immunity. *Briefings in functional genomics:elv002*.