Gene Reports

Supplementary Note 1: C-type Lectins

Introduction

C-type Lectins (CTLs) are carbohydrate binding receptors which connect to mostly a wide range of carbohydrate structures (Cambi and Figdor 2003) and proteins as well (Dodd and Drickamer 2001). This gives them the ability to recognize pathogen associated molecular patterns (PAMPs) detecting different pathogens and possibly cancerous cells (Cambi Figdor 2003). They can be involved in transmission of signals after binding to a ligand. Some of them are also known as cell adhesion molecules (CAMs), which enable the transport of immune cells. These are involved in cells traveling to the site of infection through tissue proliferation during the immune response. The C-type Lectins can be either bound to the membrane by a transmembrane region or free-roaming (Weis et al. 1998). The silencing of these genes could lead to the death of an organism, since it will have difficulty preparing an immune response since a C-type Lectin might not be able to detect an organism through carbohydrates usually found on their cell surface, and the absence of CAMs will prevent immune cells from breaking down pathogens as they infect the organism.

Materials and Methods

The C-type Lectin sequences for the following insects were collected and used to search the genome of *Diaphorina citri*: *A. pisum, D. melanogastor, O. fasciatus, A. gambiae, A. aegypti, C. quinquefasciaitus* and *C. lectularius*. These sequences were used in the BLAT search tool in Web Apollo and BLAST from I5K to find gene model predictions. A total of 10 models were found and annotated, correcting anything that was not supported by evidence tracks like RNA sequence data, genomics, and proteomics tracks. Pairwise alignments using BLASTp were performed using the sequences of the completed models to find orthologues in other insects for additional support. Table 1 contains a list of the top results found in closely related insects as well as their ID, Query coverage, and bit score. The listed insects include: *Acyrthosiphon pisum, Cimex lectularius, Anopheles gambiae, Aedes aegypti,* and *Culex quinquefasciatus*.

The sequences from the annotated models were collected in FASTA format. Sequences of the same genes from other insects were found in NCBI and collected as FASTA files. Using a total of 38 sequences a multiple sequence alignment was made with default MUSCLE settings within MEGA 7. The phylogenetic tree was made with sequences from the following organisms: *Apis mellifera, Apis Dorsata, Bactrocera cucurbitae, Ceratitis capitate, Culex quinquefasciatus, Cimex lectularius, Acyrthosiphon pisum, Bombus impatiens, Nicrophorus vespilloides, Stomoxys calcitrans, Athalia rosae, Plutella xylostella, Homo sapiens, Pediculus humanus corporis,* and *Bombyx mori*. The names of most of the genes were simplified to abbreviations, and the species names to the first letter of the genus name and the whole species name. A neighbor joining tree was then

made, based on the previous alignment, with 1000 replicates for bootstrap, Poisson model, and pairwise deletion also within MEGA 7.

Results and Discussion

Ten C-type lectin gene models were located and annotated after using orthologs from closely related insects including *A. pisum, Drosophila melanogastor, Anopheles gambiae, Aedes aegypti, Culex quinquefasciaitus* and *Cimex lectulius*. Most of the models had a C-type Lectin domain that BLAST was able to detect. A multiple alignment was constructed which illustrated sections of conserved sequence. The phylogenetic tree shows that I have indeed found and annotated CTLs, like oxidized low- density lipoprotein receptor, due to their location on the tree in relation to orthologs (Figure 1). Even though the CTLs are conserved from metazoans to humans, there are little to no similarities between CTLs of vertebrates and invertebrates (Dodd and Drickamer 2001). SVEP had not paired with the orthologs found in *H.* sapiens. The lack of accurate pairing possibly indicates that there is a need for more sequences of more closely related organisms to illustrate relations, which was not available. 10 CTL sequences were found and annotated in the *Diaphorina citri* genome while 34 have been found in *Drosophila melanogaster,* 25 in *Anopheles gambiae,* 10 in *Apis mellifera,* and 16 in *Tribolium castaneum* (Tanaka et al. 2008*).* A comparison to the numbers of CTLs found in those insects show that possibly with better sequence and expression data there might be more CTLs found in the citrus psyllid's genome. Since these sequences have been supported as probable CTLs as more sequence data is available they could be knocked out with RNAi to determine if it could reduce the citrus psyllid population and prevent the spread of citrus greening.

Table 1: A comparison table between *D.citri* and *A. pisum, D. melanogastor, A. gambiae, A. aegypti, C. quinquefasciaitus* and *C. lectulius* derived from the BLAST results. Lists amino acid length of *D.citri* genes, their domains, query coverage, identity, and bit score.

Diaphorina citri gene comparison			
Dcitr-	**AA**		**Domains**
oxidized low density lipoprotein receptor 1	140	[1]smart0003	CLECT (CTL or CRD)
oxidized low density lipoprotein receptor 1	134	[1]cd00037	CLECT (CTL or CRD)
oxidized low density lipoprotein receptor 1	93	N/A	N/A
Agglucetin subunit alpha-1	156	[1]smart0003	CLECT (CTL or CRD)
C-type Lectin 8	283	[1]cd00037	CLECT (CTL or CRD)
C-type Lectin 3	188	[2]cd00037, [4]cd00041, [2]pfam00008, [1]cd00054	CLECT (CTL or CRD), CUB, EGF, EGF-CA
C-type Lectin 5	2355	[1]cd00020, [1]cd03590, [5]cd00033	ARM, CLECT DC-SIGN, CCP
E selectin	697	[1]pfam00754, [1]cd03590, [4]smart00032, [3]pfam00084	F5/8type C domain (discoidin; DS), CLECT DC-SIGN, CCP, Sushi
Selectin-like osteoblast-derived protein	794	[1]cd03591, [1]smart00034	CLECT (CTL or CRD), CLECT collectin like
Perlucin	128	[1]cd00037	CLECT (CTL or CRD)
Dcitr-	**AA**		**Domains**
oxidized low density lipoprotein receptor 1	140	[1]smart0003	CLECT (CTL or CRD)
oxidized low density lipoprotein receptor 1	134	[1]cd00037	CLECT (CTL or CRD)
oxidized low density lipoprotein receptor 1	93	N/A	N/A
Agglucetin subunit alpha-1	156	[1]smart0003	CLECT (CTL or CRD)
C-type Lectin 8	283	[1]cd00037	CLECT (CTL or CRD)
C-type Lectin 3	188	[2]cd00037, [4]cd00041, [2]pfam00008, [1]cd00054	CLECT (CTL or CRD), CUB, EGF, EGF-CA
C-type Lectin 5	2355	[1]cd00020, [1]cd03590, [5]cd00033	ARM, CLECT DC-SIGN, CCP
E selectin	697	[1]pfam00754, [1]cd03590, [4]smart00032, [3]pfam00084	F5/8type C domain (discoidin; DS), CLECT DC-SIGN, CCP, Sushi
Selectin-like osteoblast-derived protein	794	[1]cd03591, [1]smart00034	CLECT (CTL or CRD), CLECT collectin like
Perlucin	128	[1]cd00037	CLECT (CTL or CRD)

A. pisum				
Name	Accession	query	ID	Bit Score
PREDICTED: oxidized low-density lipoprotein receptor 1	XP_003245001.1	53%	56%	254
PREDICTED: oxidized low-density lipoprotein receptor 1	XP_003245001.1	75%	56%	342
PREDICTED: oxidized low-density lipoprotein receptor 1	XP_003245001.1	94%	52%	244
NA	NA	NA	NA	NA
PREDICTED: mucin-5AC	XP_001945032.2	91%	66%	663
PREDICTED: uncharacterized protein LOC100159662	XP_001950366.1	98%	77%	811
PREDICTED: uncharacterized protein LOC100167178 isoform X4	XP_016657887.1	61%	43%	1255
PREDICTED: sushi, von Willebrand factor type A, EGF and pentraxi	XP_001948504.2	66%	30%	458
PREDICTED: sushi, von Willebrand factor type A, EGF and pentraxi	XP_001948504.2	91%	27%	464
PREDICTED: perlucin isoform X2	XP_016663197.1	80%	60%	430

A. aegypti				
Name	Accession	query	ID	Bit Score
CTL9	XP_001663957.1	54%	44%	182
CTL9	XP_001663957.1	73%	36%	206
CTL9	XP_001663957.1	93%	46%	180
AAEL006825-PA	XP_001658055.1	66%	45%	252
CTL8	XP_001661274.2	78%	67%	631
CTLGA3	XP_001655089.1	98%	78%	833
CTL5	XP_001661639.2	13%	39%	467
C-Type Lectin (CTL) - selectin like	XP_001653527.2	69%	59%	1507
C-Type Lectin (CTL) - selectin like	XP_001653527.2	95%	43%	1571
C-Type Lectin (CTL) - mannose binding	XP_011493611.1	80%	63%	436

C.lectularius				
Name	Accession	query	ID	Bit Score
PREDICTED: oxidized low-density lipoprotein receptor 1-like	XP_014254818.1	54%	50%	223
PREDICTED: oxidized low-density lipoprotein receptor 1-like	XP_014254818.1	70%	63%	333
PREDICTED: oxidized low-density lipoprotein receptor 1-like	XP_014254818.1	96%	50%	211
NA	NA	NA	NA	NA
PREDICTED: uncharacterized protein LOC106673215 isoform	XP_014260729.1	91%	71%	679
PREDICTED: uncharacterized protein LOC106666810	XP_014249760.1	98%	81%	865
PREDICTED: uncharacterized protein LOC106663479 isoform	XP_014243827.1	84%	36%	1811
PREDICTED: CUB and sushi domain-containing protein 3	XP_014245415.1	85%	58%	1759
PREDICTED: CUB and sushi domain-containing protein 3	XP_014245415.1	95%	42%	1626
PREDICTED: perlucin isoform X2	XP_014243366.1	81%	58%	397

A. gambiae				
Name	Accession	query	ID	Bit Score
CTL9	XP_312307.1	53%	43%	170
CTL9	XP_312307.1	73%	33%	204
CTL9	XP_312307.1	59%	62%	191
CTL7	XP_309125.4	89%	45%	366
CTL8	XP_313387.4	91%	64%	618
CTLGA3	XP_319371.2	98%	77%	822
CTL5	XP_310650.4	70%	38%	2059
C-Type Lectin (CTL) - selectin like	XP_309111.3	78%	59%	1529
C-Type Lectin (CTL) - selectin like	XP_309111.3	96%	42%	1562
C-Type Lectin (CTL) - mannose binding	XP_315346.4	80%	63%	420

D.melanogaster				
Name	**Accession**	**query**	**ID**	**Bit Score**
C-type Lectin	NP_001138057.1	53%	43%	174
C-type Lectin	NP_001138057.1	66%	39%	235
C-type Lectin	NP_650444.1	64%	61%	187
C-type Lectin	NP_572269.1	89%	45%	355
narrow, isoform B	NP_001188963.1	90%	64%	613
C-type lectin 27kD, isoform A	NP_608858.3	98%	74%	798
cubilin ortholog	NP_727348.2	28%	32%	213
furrowed, isoform A	NP_511136.2	80%	56%	1494
furrowed, isoform A	NP_511136.2	95%	41%	1521
triforce, isoform A	NP_728586.1	81%	68%	445
C. quinquefasciatus				
Name	**Accession**	**query**	**ID**	**Bit Score**
conserved hypothetical protein	XP_001847975.1	57%	41%	169
conserved hypothetical protein	XP_001847975.1	73%	37%	210
NA	NA	NA	NA	NA
conserved hypothetical protein	XP_001868565.1	68%	45%	261
conserved hypothetical protein	XP_001866032.1	78%	66%	626
conserved hypothetical protein	XP_001847297.1	98%	79%	844
conserved hypothetical protein	XP_001862639.1	16%	43%	760
furrowed	XP_001869209.1	71%	28%	434
furrowed	XP_001869209.1	78%	32%	343
galactose-specific C-type lectin	XP_001842113.1	58%	45%	161
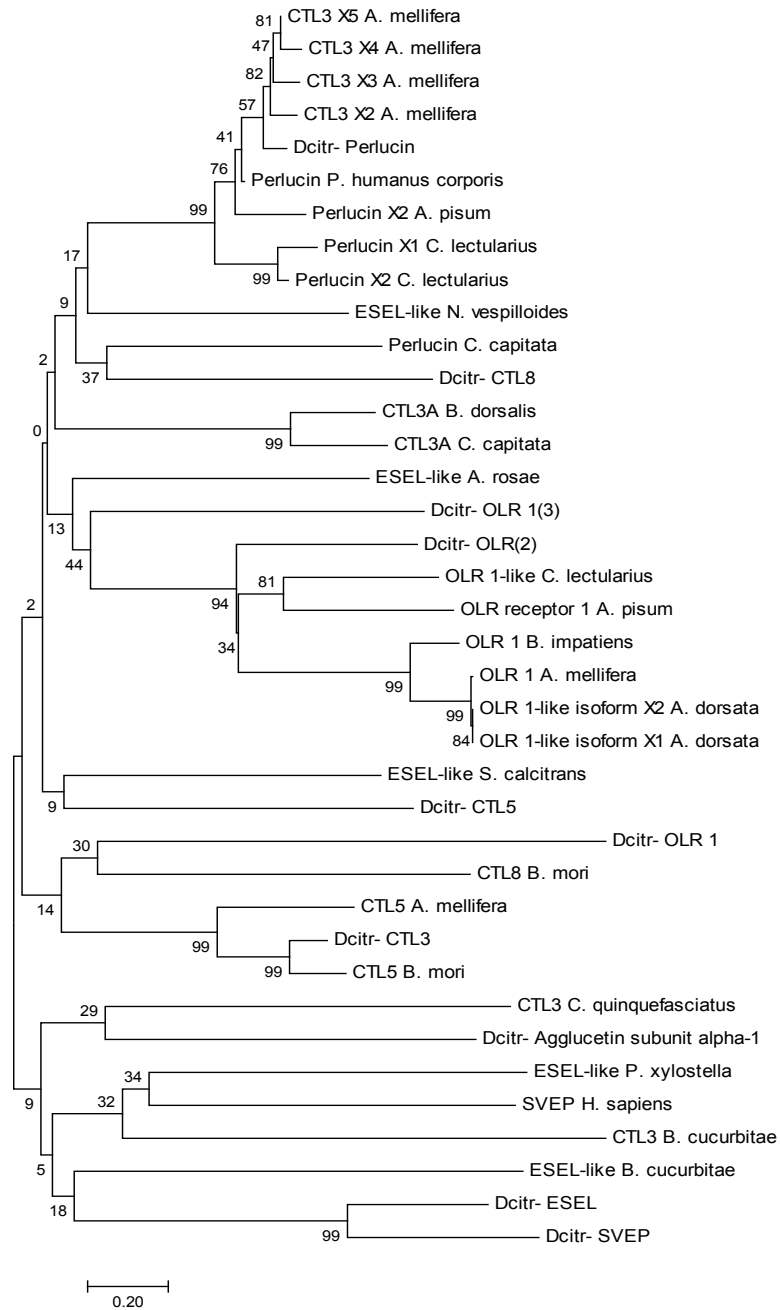
Figure1: Phylogenetic tree consisting of *Diaphorina citri* C-type Lectins and the same genes from other insects. The C-type Lectins found in *D.citri* are the following: oxidized low density lipoprotein receptor (OLR), C-type Lectin 3 (CTL3), C-type Lectin 5 (CTL5), E selectin (ESEL), Perlucin, Agglucetin subunit alpha, and selectin like osteoblast derived protein (SVEP). The species names of the additional organisms are shown next to the gene name. An ortholog from another insect could not be found for Agglucetin. Only a human ortholog could be found for SVEP. High bootstrap values show that some genes like OLR are closely related to those in other insects. OLR is also known as CTL8A.

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Supplementary Note 2: Galactoside-Binding Lectins

Introduction

Galactoside-binding lectins (galectins) bind β-galactose with their structurally similar carbohydrate-recognition domains (CRDs) (Cummings and Liu 2009). CRDs can function alone or in clusters creating a β-sandwich structure without Ca^{2+} binding sites (Leffler et al., 2004; Mitchell et al., 2001; Wang et al., 2012).

In the invertebrate *Clavelina picta*, it has been shown that galectin may be an effector molecule in the innate immune system (Vasta et al., 1999), whereas it can be similar in insects. *Anopheles* mosquito galectins were upregulated in the gut and salivary glands after infection of both malaria and bacteria (Dimopoulos et al., 1996, 1998, 1988). It was suggested that galectins may function as a pattern recognition receptor (PRR) by binding saccharides (β-galactose) on the pathogen surface to trigger the innate immune response or agglutinate bacteria in the midgut following blood-feeding (Dimopoulos et al., 1998).

Methods

Insecta galectins orthologs were collected from Immunodb and were used to blat against the *Diaphorina citri*, Asian citrus psyllid (ACP), genome. Identification and annotation of galectins was performed in WebApollo where RNA sequence data (adult, nymph, and egg) supported the manually curated gene models. BLASTp was used to confirm accuracy of the annotated galectins and determine the number of CRDs each galectin contains.

Galectin orthologs were collected from a limited NCBI search to include different insect orders. The conserved domains database in NCBI was used to identify and retrieve the CRD amino acid sequences of each collected galectin orthologs. ACP CRDs were globally pairwise aligned with *Acyrthosiphon pisum, Cimex lectularius, Diuraphis noxia,* and *Drosophila melanogaster* using NCBI Needleman-Wunsch tool. Multiple sequence alignments of ACP and orthologous insect galectins CRDs was performed in MEGA6 using ClustalW (Tamura et al 2003). The phylogenetic analysis of the CRDs included, constructing a midpoint rooted neighbor-joining tree in MEGA6 using p-distance to show evolutionary distance and 1,000 bootstrap replicates.

Results and Discussion

A total of three galectins and one partial galectin were identified and manually curated within the ACP genome (Table 1). *Acyrthosiphon pisum,* ACP closest relative, only has two galectin paralogs (Gerardo et al., 2010) (Table 3), which are isoforms (NCBI accession: XP_008186241 and XP_001943769). The naming of the ACP galectins are

based on NCBI Gnomon. Galectin-5 and 32 kDa beta-galactoside-binding lectin were identified in the same scaffold (WebApollo accession: gi|645507215|ref|NW_007377695.1|). Blastp showed that galectin-4, 32 kDa beta-galactoside-binding lectin, galectin-5, and galectin-4 partial has two CRDs, two CRDs, one CRD, and one CRD, respectively. ACP galectins containing two CRDs, like that of mammalian galectins, may mediate cell-cell communication or trigger signal cascades, as also suggested in *Drosophila melanogaster* (Pace et al., 2002; Liu, 2000; Hadari et al., 2000; Pace et al., 2000; Baum et al., 1995).

In the pairwise alignment analysis (Table 2), the ACP CRDs have an identity of 17% or greater compared to other related organisms. *D. citri* gal-4 CRD1, 32 kDa gal CRD2, and gal-5 CRD1 were analyzed with related insects CRDs from the orders Hymenoptera, and Coleoptera (Figure 1). The tree shows that the ACP CDRs have evolved from each other but still grouped with other hemipterans, further supporting the manually curated models. Coleoptera and Hymenoptera CRDs have also grouped with themselves independently.

Table 1: All the galectins found in the *D. citri* genome. Table shows all galectins with names based on NCBI Gnomon with BLASTp matches, and corresponding protein length, number of CRDs, and domains.

D. citri galectins	BLASTP match (organism, bit score)	Protein Length	Number of CRDs	Domains
Galectin 4	Galectin 4 (*Zootermopsis nevadensis*, 101)	286	2	Dimerization interface, sugar binding pocket, dimerization swap strand, Putative alternate dimerization interface, Gal-bind_lectin (CRD), Gal-bind_lectin (CRD), two Gal-bind_lectin, two GLECT, two GLECT superfamily
Galectin 4, partial	Galectin 9 (*Habropoda laboriosa*, 52.4)	142	1	Putative alternate dimerization interface, sugar binding pocket, Gal-bind_lectin (CRD), GLECT, Gal-bind_lectin, GLECT superfamily
Galectin 5	Galectin 8 (*Zootermopsis nevadensis*, 116)	218	1	Dimerization swap strand, sugar binding pocket, dimerization interface, putative alternate dimerization interface, Gal-bind_lectin (CRD), Gal-bind_lectin, GLECT, GLECT superfamily
32 kDa beta-galactoside-binding lectin	Galectin 8 (*Zootermopsis nevadensis*, 184)	494	2	Two sugar binding pocket, dimerization interface, dimerization swap strand, two putative alternate dimerization interface, two Gal-bind_lectin

					(CRD), two Gal-bind_lectin, two GLECT, two GLECT superfamily

Table 2: Global pairwise alignment of *D. citri* CRDs with related organisms displaying percent identity and (Needleman-Wunsch score).

	Galectin-4		32 kDa galectin		Galectin-5
Diaphorina citri	CRD1	CRD2	CRD1	CRD2	CRD1
Acyrthosiphon pisum (XP_008186241.1)	33% (146)	22% (-27)	28% (104)	20% (7)	28% (104)
Cimex lectularius (XP_014259365.1)	17% (12)	19% (-32)	31% (147)	18% (-29)	31% (147)
Diuraphis noxia (XP_015371421.1)	32% (139)	22% (-28)	27% (91)	19% (2)	27% (91)
Drosophila melanogaster (NP_001245813.1)	23% (52)	23% (-24)	31% (155)	20% (25)	31% (155)

Table 3: Number of galectins in *D. citri* and related organisms.

Organism	# of galectins
Diaphorina citri	3
Acyrthosiphon pisum	1
Cimex lectularius	1
D. melanogaster	1
Aedes aegypti	12
Anopheles gambiae	15
Culex quinquefasciatus	5

*Numbers based on Ensembl and NCBI, does not include isoforms/partial.
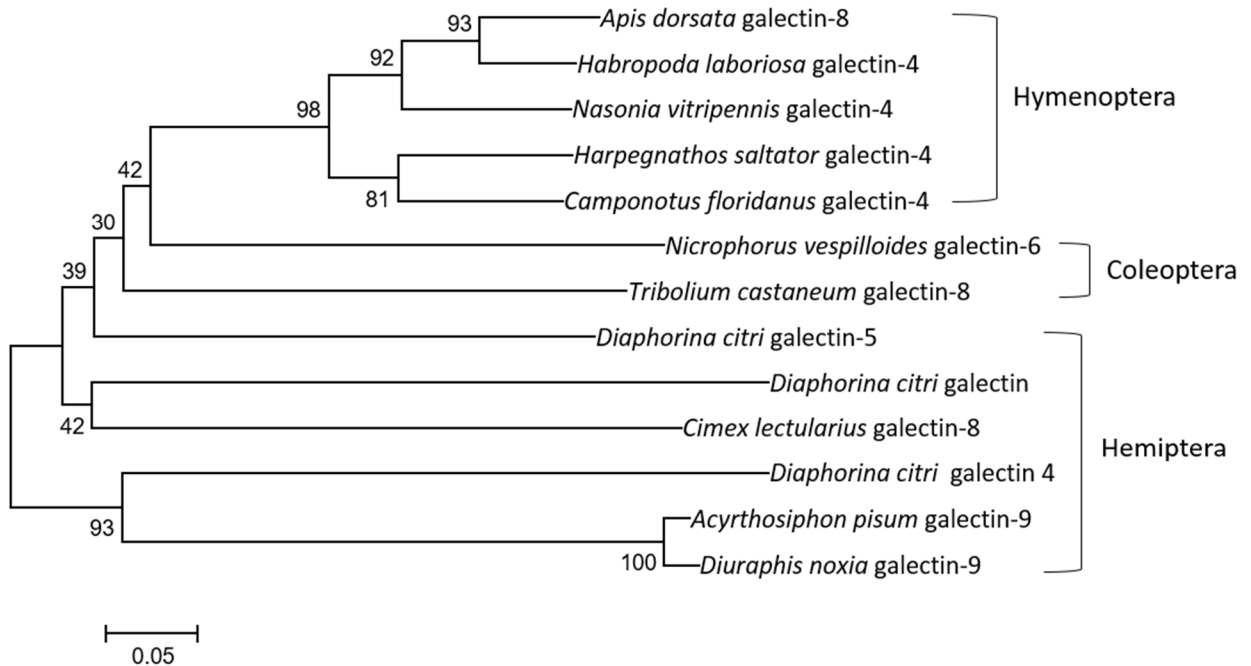
11

Figure 1: Neighbor-joining tree of 13 galectin CRDs was constructed in MEGA6 using 1000 bootstrap replications. The p-distance method was used to show evolutionary distance. A total of 60 amino acid positions were analyzed for the tree. NCBI accessions: *A. dorsata* [XP_006607868.1], *H. laboriosa* [XP_017794332.1], *N. vitripennis* [XP_008211704.1], *H. saltator* [EFN82261.1], *C. floridanus* [EFN73449.1], *N. vespilloides* [XP_017773770.1], *T. castaneum* [XP_015834566.1], *C. lectularius* [XP_014259365.1], *A. pisum* [XP_008186241.1], *D. noxia* [XP_015371421.1].

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Supplementary Note 3: Fibrinogen related proteins

Introduction

Fibrinogen related proteins (FREPS) include a highly conserved fibrinogen like domain (FBG domain) found universally in vertebrates and invertebrates (Wang et al, 2005). In invertebrates the FBG domains are predicted to function in innate immunity by recognizing carbohydrates and their derivatives on the surface of microorganisms. The FBG domain includes approximately 200 amino acids with high sequence similarity to the C terminus of fibrinogen, however some FREPs include a truncated domain (Middah and Wang, 2008). A number of N-terminal domains can be joined to the highly conserved FBG domain allowing for variability and antibodies against mammalian fibrinogen can be used to find proteins with FBG domains in invertebrates (Hanington and Zhang, 2010). FREP genes include angiopoietin, tenascin, scabrous, aslectin, microfibril associated proteins (MAP), ficolin, and tachylectin; all of which were searched for in the *D. citri* genome.

Methods

Fibrinogen related protein genes were collected from NCBI, i5k, Ensembl and FlyBase. BLAT searches were then performed at the i5k workspace to identify homologs in the *D. citri* genome. Potential hits within the *D. citri* genome were explored further using the WebApollo genome browser. NCBI Gnomon annotation gene models were manually annotated within WebApollo using BLAST and RNA-Seq evidence tracks. Completed gene models were analyzed by BlastP to confirm accuracy and completeness of the annotation.
Several models were found when searching the genome for the fibrinogen C-terminal domain. Sequences from completed annotated genes were used to perform BlastP searches against related insects and model organisms. Potential models found in this manner were used in BLASTp searches to verify. This includes gene models named angiopoietin, tenascin, and three fibrillin models.

Pairwise amino acid sequence comparisons were performed between *D citri*, *Acyrthosiphon pisum*, *Drosophila melanogaster*, *C. lectularius*, *Anopheles gambiae*, *A. aegypti*, and *C. quinquefasciatus*. Collected orthologs and annotated *D. citri* FREP sequences were used to generate a multiple sequence alignment using ClustalW in MEGA6. Sequences were chosen to identify close relationships to proteins that include the FBG domain. The *D. citri* proteins were named per the NCBI BLAST results. A neighbor joining phylogenetic tree was produced from the multiple sequence alignment to analyze the evolutionary relationships between the *D. citri* FREPS and well characterized sequences. (Figure 1) The unrooted Neighbor-Joining phylogenetic tree was constructed with the MEGA6 program using the bootstrap method (with 1000 replications) as a test of phylogeny and p-distance method as the substitution model.

The analysis includes 38 amino acid sequences with 53 positions in the data set of FREP genes proposed by NCBI.

Results and Discussion

Three FREPS were found within the *D. citri* genome. Several potential gene models had insufficient RNA sequence data and/or domains to facilitate an approved gene model. *Anopheles gambiae* has been shown to have 53 FREPS and *Drosophila melanogaster* to have 20 FREPS (Wang et al, 2005). (Table 1) It is likely the *D. citri* will have a closer amount to *D. melanogaster*, as it has been shown the FREPs have expanded in *A. gambiae* due to recognizing parasites from blood feeding.

One gene model is attributed to the ImmunoDB FREP ortholog search, scabrous protein, which includes the fibrinogen C-terminal domain. Scabrous protein has been shown in Drosophila melanogaster to be involved in the regulation of neurogenesis and (along with Gp150) as an endosomal protein that regulates Notch activity (Li et al, 2003). A search for the protein tenascin revealed one gene model although there are several members in the tenascin family. Tenascins are extracellular matrix proteins, which are associated with organogenetic processes, with neuronal and axon development, and mediate cell adhesion (Wang et al, 2005). A search for the protein angiopoietin revealed one gene model. Angiopoietin is involved with embryonic and postnatal angiogenesis. Other FREPS that were not found in the genome include ficolin, tachylectins and aslectin, which are likely involved in parasite detection within the innate immune system (Wang et al, 2005). Ficolin and tachylectin are involved with N-acetylglucosamine (GlcNAc) binding activity (Middah and Wang, 2008). Aslectin can also bind bacteria and GlcNAc.

The multiple sequence alignment and phylogenetic tree position the scabrous model in a separate clade alongside the other hemipteran scabrous proteins. The angiopoietin and tenascin models were also in the proper clades. (Figure 1)

Table 1: The numbers of named FREP gene copies in *Diaphorina citri*, *Acyrthosiphon pisum*, *Drosophila melanogaster*, *Cimex lectularius*, *Anopheles gambiae*, *Aedes aegypti*, and *Culex quinquefasciatus*. An extensive search was performed through NCBI search, ENSEMBL, and www.supfam.org to obtain final figures for numbers of FREP genes in other hemipterans.

Fibrinogen Related Proteins	Scabrous	MAP	Ficolin	Angiopoietin	Tenascin	Other
D. citri [a]	1	0	0	1	1	0
A. pisum [a]	1	1	0	1	1	0
C. lectularius [a]	1	1	0	1	0	N/A

D. melanogaster [a] [c]	1	1	0	0	2	16
A. gambiae [b] [c]	1	0	2	1	0	55
A. aegypti [b] [c]	1	1	0	0	0	33
C. quinquefasciatus [a] [c]	1	1	3	3	2	83

[a] NCBI search, [b] ENSEMBL, [c] www.supfam.org

Table 2: FREP sequence table. The protein sequences used for the phylogenetic analysis with their corresponding BLAST match results for specific species, with the bit scores followed by the coverage %, ID%, and accession numbers.

| *D. citri* Gene Named	*D. citri* AA Length	*A. pisum* BLAST Match Bit Score	*BLAST Query Percent*	*BLAST ID Percent*	*A. pisum* Acc #	*D. melanogaster* BLAST Match Bit Score	*BLAST Query Percent*	*BLAST ID Percent*	*D. melanogaster* Acc #
Scabrous	897	434	86	83	XP_001951011.3	419	87	78	NP_001188463.1
Angiopoietin	567	337	99	32	XP_001944698.3	337	95	33	AAA28880.1
Tenascin	977	313	72	31	XP_008178803.2	57.8	9	33	NP_609691.6
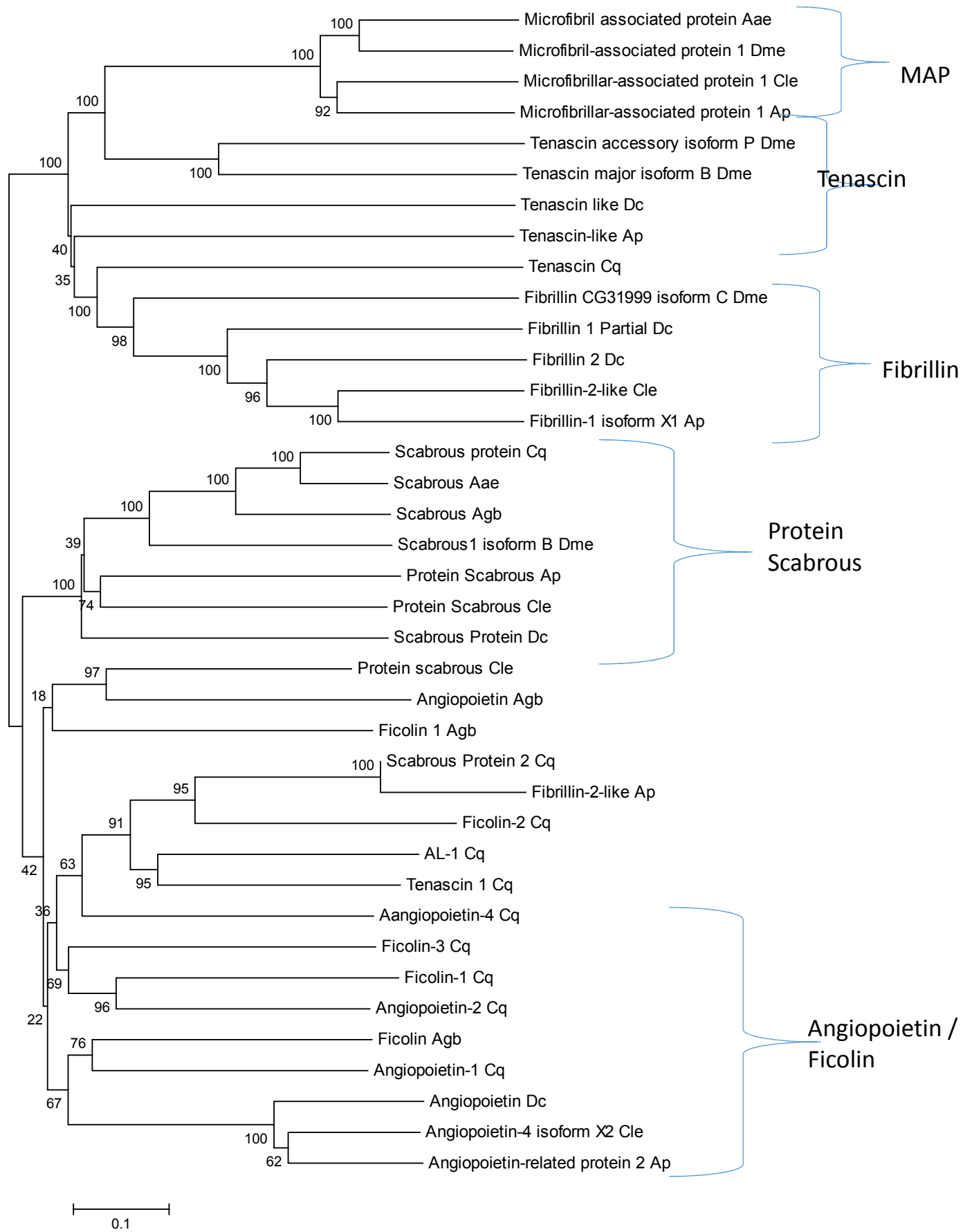
Figure 1: Phylogenetic analysis of Hemipteran FREP family protein sequences

The unrooted Neighbor-Joining phylogenetic tree was constructed with the MEGA6 program using the bootstrap method (with 1000 replications) as a test of phylogeny and p-distance method as the substitution model. The analysis includes 38 amino acid sequences with 53 positions in the data set of FREP genes proposed by NCBI. All bootstrap values are shown near the nodes. The *D. citri* proteins are numbered in the order found. The *A. pisum* proteins were named according to their EST numbers. The *D. Melanoaster, A gambiae,* and proteins were renamed from their accession number name. Abbreviations: *Diaphorina citri* Dc; *Acyrthosiphon pisum* Ap; *Anopheles gambiae* Agb; *Drosophila melanogaster* Dme; *Cimex lectularius* Cle, *Aedes aegypti* Aae, and *Culex quinquefasciatus* Cq.

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Supplementary Note 4: PGRP and βGRP

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 pl/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. castanium* (Gerardo et al., 2010; Viljakainen 2015).

PGRP gene count in *D. citri* and other insects	
	PGRP
Drosophila melanogaster	13
Anopheles gambiae	11
Acyrthosiphon pisum	0
Bombyx mori	12
Pediculus humanus	1
Tribolium castaneum	7

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

| Blastp results for PGRP-S in *D. citri*					
Organism	Match	Accession	Query	ID	Bit score
Drosophila melanogaster	peptidoglycan recognition protein SB1	CAD89128.1	47%	38%	222
Aedes aegypti	peptidoglycan recognition protein (Long)	XP_001654275.1	48%	40%	217
Culex quinquefasciatus	peptidoglycan recognition protein sb2	XP_001849091.1	55%	38%	217
Anopheles gambiae	peptidoglycan recognition protein (long)	XP_558600.3	70%	27%	175
Cimex lectularius	PREDICTED: peptidoglycan-recognition protein SB2-like isoform X4	XP_014247859.1	47%	30%	136
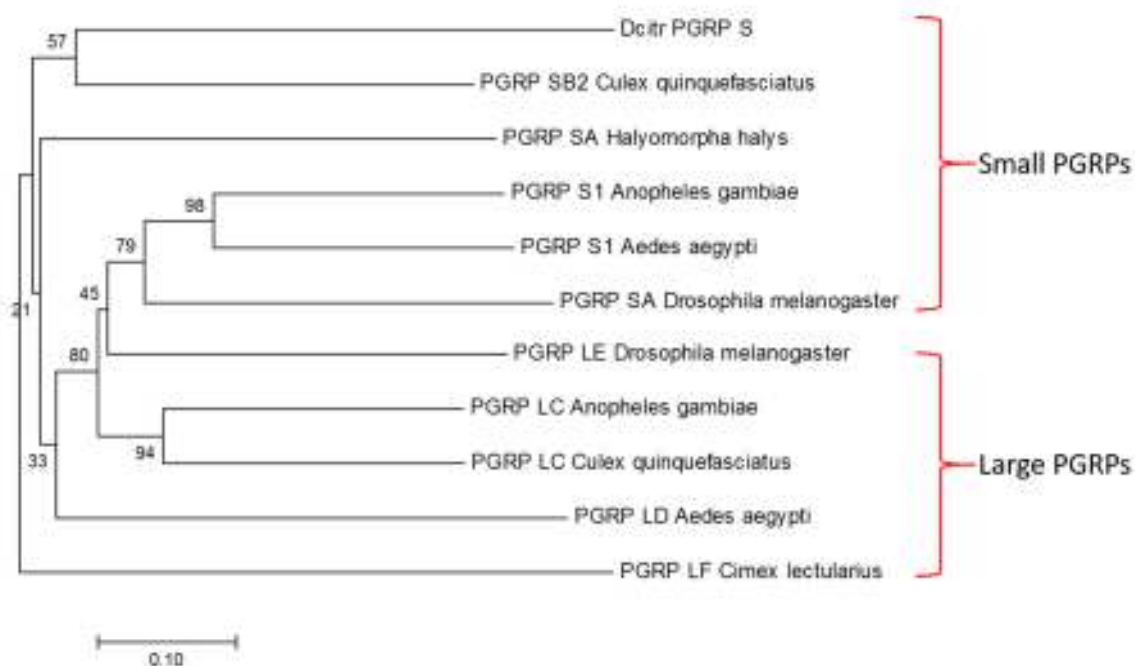
Figure 1: Neighbor-Joining tree constructed using small and large PGRP sequences from *D. citri* and other insects. The branches shown that the D. citri PGRP is closer to the other PGRPs, especially *C. quinquefasiatus*. Bootstrap values based on 1000 replicates are shown at the nodes.

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Supplementary Note 5: Thioester containing Proteins

Introduction

Thioester containing Proteins (TEPs) are members of an ancient protein family that includes vertebrate C complement and alpha-2-macroglobulin proteins (2). Insect TEPs seem to play a similar role to their vertebrate homologs, binding to invaders such as parasites or microbes, marking them for degradation (1). These proteins may be upregulated by the JAK/STAT pathway during innate immune response (1). In some TEPs, the interaction between invader and TEP occurs via a conserved thioester motif (GCGEQ), specifically TEP 1-4, but other TEPs lack this domain (2). TEPs also include a long C-terminal cysteine signature (2). Previous studies indicate that insect TEPs fall into three clades, with one clade being specific to mosquitos (3). Potential TEPs in *Diaphorina citri* were identified and investigated in comparison to sequences from other insects, including mosquito species, *Drosophila* species, and the pea aphid.

Methods

Investigation into potential TEPs in *Diaphorina citri* was initiated by first collecting known TEP sequences from other insects, including *Anopheles gambiae, Aedes aegypti, Culex quinquefasciatus, Drosophila melanogaster, Tribolium castaneum* and *Acrythosiphon pisum* (4) (7). Using the gathered sequences, individual searches were conducted for each of the 46 orthologs provided using the protein blat and blast functions in i5k and NCBI, respectively. Two predicted proteins were implicated as those most closely related to the known TEPs of related organisms. The corresponding sequences were then extracted from NCBI, and searched within the online annotation program, WebApollo. In WebApollo, the NCBI predicted sequence, the RNA seq data, and the Maker predicted genes were used as reference for manual annotation. Using the NCBI predicted sequences as a base, exons were edited based on the evidence provided by the RNAseq data. After ensuring that the NCBI model corresponded as precisely as possible to the RNAseq data, a multiple sequence alignment was created through MUSCLE to determine whether the constructed *D. citri* model was similar to the known sequences from the previously listed organisms. MEGA 7 was used to perform a local alignment on all of these sequences, from which a proposed phylogenetic relationship was created, using a neighbor-joining phylogeny, as illustrated in Figure 1.

Results and Discussion

Based on the current gene assembly, only two TEPs were able to be identified within the psyllid, *Diaphorina citri.* This number of TEPs is comparable to that of *Acrythosiphon*

pisum and *Nasonia vitripennis,* indicating that these are mostly likely the only two TEP genes in the psyllid's genome, although isoforms may be present.

Organism	Number of TEPs
Drosophila melanogaster	6
Anopheles gambiae	13
Tribolium castaneum	4
Apis mellifera	4
Nasonia vitripennis	3
Acyrthosiphon pisum	2
Diaphorina citri	2

Table 1 : TEP in *D. citri* and related organisms (5)(6)

The first gene TEP3 (Thioester_containing_protein 3) is 28,982 base pairs in length. It includes 24 exons, which are typically short and evenly spread between long introns. It was found to contain the distinct GCGEQ motif towards the C terminal.The second gene TEP6 (Thioester_containing_protein 6) is 30,776 base pairs in length. Within this gene there are 19 exons in three distinct clusters. The GCGEQ motif was not found within this protein, giving more evidence to its identity as an ortholog of TEP6. RNAseq data suggests that this model is one of many possible isoforms. Due to the length and the number of exons, determining the number of isoforms present was not possible at this time, and requires further investigation.

		A. pisum	*D. melanogaster*	*T. castaneum*
Thioester containing protein 3	Bit Score (name)	1400 (CD109 antigen)	704 (macroglobulin complement-related)	981 (CD 109 antigen)
	Query (Identity)	95% (65%)	95% (38%)	96% (46%)
	E value	0	0	0
Thioester containing protein 6	Bit Score (name)	1000 (CD109 antigen)	805 (macroglobulin complement-related)	834 (CD109 antigen)
	Query (Identity)	96% (58%)	93% (67%)	94% (69%)
	E value	0	0	0

Table 2: Blast matches of ACP TEP proteins to sequenced arthropods
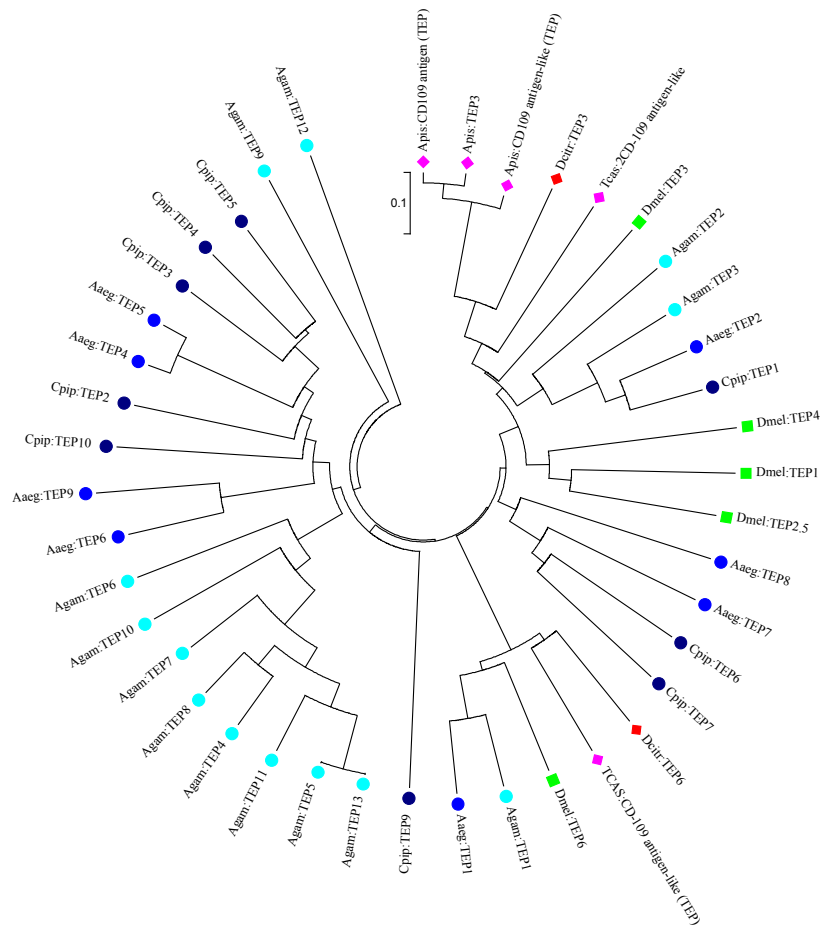
Figure 1: Proposed phylogenetic relationship between Diaphorina citri (Dcitr), Acrythosiphon pisum (Apis), Tribolium castaneum (Tcas), Anopheles gambiae (Agam), Aedes aegypti (Aaeg), Culex quinquefasciatus (Cpip) and Drosophila melanogaster (Dmel)

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Supplementary Note 6: Toll Receptors

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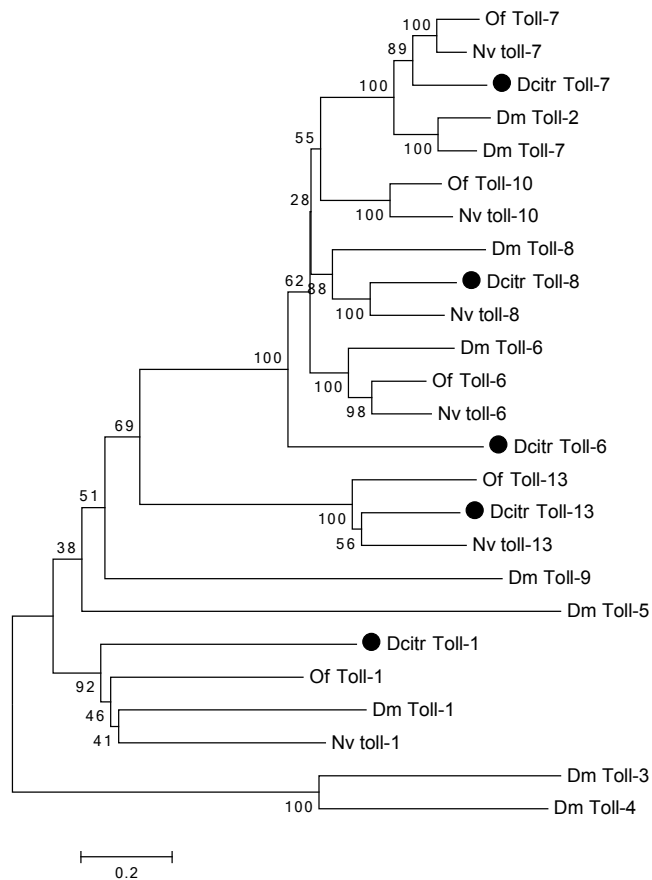
Figure 1. Neighbor-joining tree of Toll receptor proteins. Dm=*Drosophila melanogaster*, Of=*Oncopeltus fasciatus*, Nv=*Nilaparvata lugens*, Dcitr=*Diaphorina citri*

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).

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Supplementary Note 7: Spaetzle proteins

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
Tribolium castaneum	Coleoptera	7
Anopheles gambiae	Diptera	6
Drosophila melanogaster	Diptera	6
Acyrthosiphon pisum	Hemiptera	6
Nilaparvata lugens	Hemiptera	8
Apis mellifera	Hymenoptera	2
Nasonia vitripennis	Hymenoptera	9
Linepithsma humile	Hymenoptera	5
Bombyx mori	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).
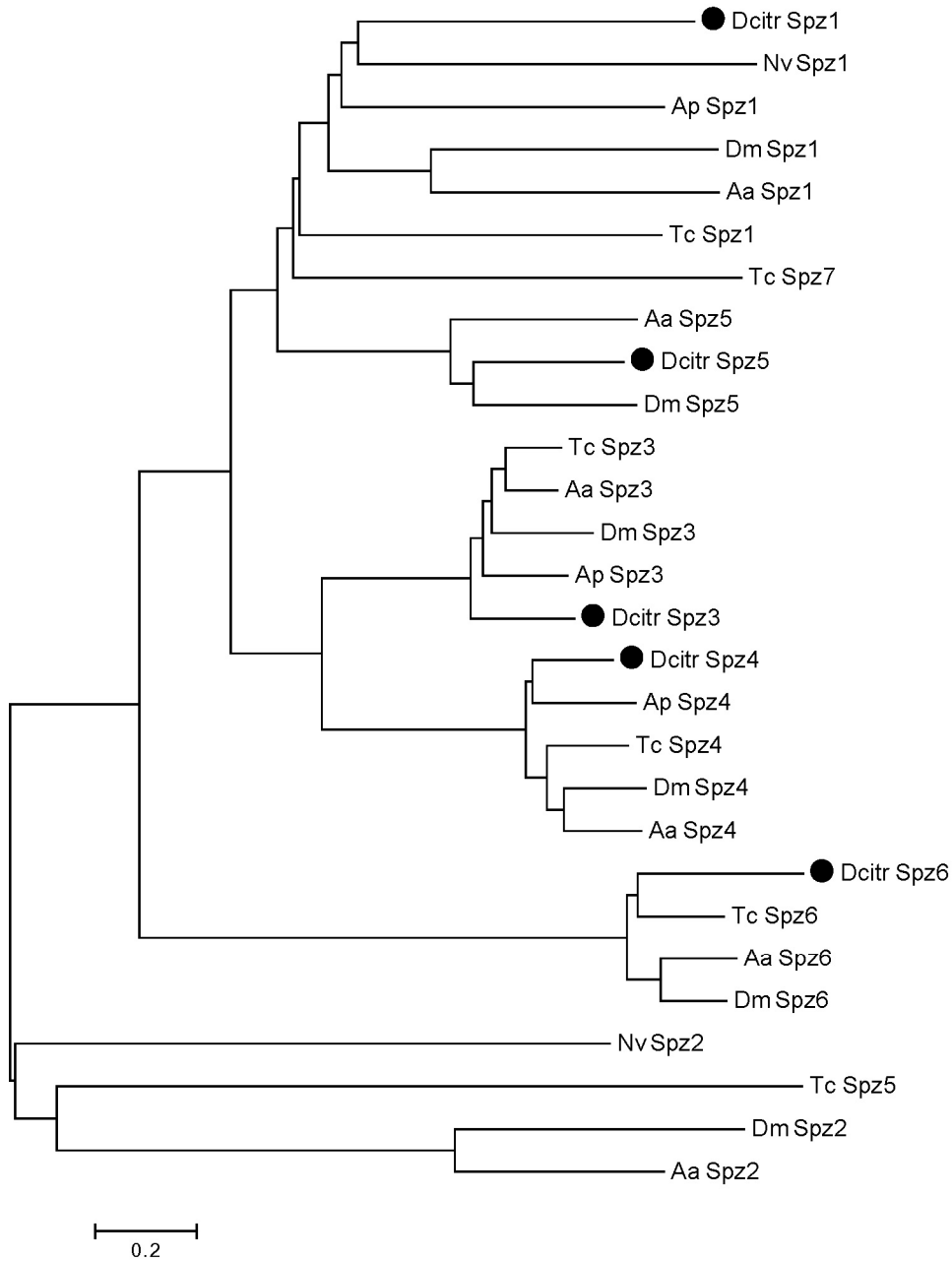
 Figure 1. Phylogenetic tree of Spaetzle orthologs from *Diaphorina citri* (Dc), *Nasonia vitripennis* (Nv), *Acyrthosiphon pisum* (Ap), *Drosophila melanogaster* (Dm), *Aedes aegypti* (Aa) and *Tribolium castaneum* (Tc). *D. citri* proteins are marked with a black dot.

Predicted Protein	Top BLASTp hit	Bitscore	E value	Percent Identity	Top D.melanogaster BLASTp hit	Bitscore	E value	Percent Identity
Spaetzle1 (MCOT15644.0.CT)	PREDICTED: LOW QUALITY PROTEIN: uncharacterized protein LOC100871556 [Apis florea]	110	9e-27	40%	spatzle, isoform I	82.8	2e-18	31%
Spaetzle3 (MCOT16194.0.CT)	hypothetical protein g.16433 [Cuerna arida]	395	8e-134	65%	spatzle 3	373	9e-124	54
Spaetzle4 (MCOT04678.2.CT)	spaetzle 4 precursor [Acyrthosiphon pisum]	335	2e-111	51%	spatzle 4	214 (316 total)	8e-64	70%
Spaetzle5 (MCOT21079.2.CO)	hypothetical protein g.5364, partial [Clastoptera arizonana]	267	8e-87	48%	spatzle 5	142 (217 total)	3e-39	62%
Spaetzle6 (MCOT04452.0.CT)	PREDICTED: uncharacterized protein LOC109036501 [Bemisia tabaci]	512	4e-179	60%	spatzle 6	392	1e-133	51%

Table 2. BLAST results for *D. citri* Spaetzle proteins.

Results and Discussion

The six Drosophila Spaetzle proteins and, when possible, their pea aphid orthologs were used to BLAST *Diaphorina citri* (*D.* citri) predicted protein sets. These searches identified potential orthologs for Spaetzle 1, 3, 4, 5, and 6. The *D. citri* proteins were then used to BLAST the MCOT protein set. For each Spaetzle class, at least one protein encoded by a de novo-assembled transcript was identified. These provided an assembly-independent alternative to the gene models.

A single Spaetzle1 ortholog was found in *D. citri*. The most complete sequence was found as MCOT15644.0.CT. This sequence mapped to scaffold gi|645505476|ref|NW_007378110.1, but problems with the assembly made it impossible to create a single accurate transcript. Some of the exons in the middle of the transcript were either missing or out of order. Therefore, two partial transcripts were created: spaetzle1 partial 5' and spaetzle1 partial 3'.

No Spaetzle2 ortholog was identified in either the predicted proteins or in MCOT.

We identified one Spaetzle3 ortholog in *D. citri*. The most complete protein sequence comes from MCOT16194.0.CT. Because of incomplete genome assembly, the sequences encoding this protein are found on three different scaffolds. Therefore, there are three partial transcripts associated with *spaetzle3*. spaetzle3 partial 5' is located on gi|645502920|ref|NW_007378693.1, spaetzle3 partial middle is on gi|645507426|ref|NW_007377648.1 and spaetzle3 partial 3' is on gi|645506656|ref|NW_007377820.1.

A single ortholog of Spaetzle4 was identified in *D. citri*. The MCOT04678.2.CT protein has a slightly longer C-terminus than the protein predicted by the best gene model. The exon encoding this additional C-terminal sequence is located in the wrong position in the assembly, so it was not included in the annotated transcript spaetzle4 partial.

One ortholog of Spaetzle5 was found in *D. citri*. Two MCOT proteins (MCOT21079.2.CO and MCOT21079.1.CO) matched the predicted protein from the Spaetzle5 gene model. These proteins differed in one stretch of amino acids, apparently as a result of the presence or absence of an alternative exon. We created two alternative models, one containing the alternative exon and one without it. The MCOT proteins also had additional N-terminal sequence compared to the gene model. This N-terminal sequence could not be located in the gi|645504497|ref|NW_007378346.1 scaffold. Therefore, we believe our annotated transcripts to be partial and named them spaetzle5 partial-RA and spaetzle5 partial-RB.

A single ortholog of Spaetzle6 was identified in *D. citri*. MCOT04452.0.CT appears to represent the most complete sequence available. The exons encoding the MCOT04452.0.CT protein map to gi|645504930|ref|NW_007378241.1, but the order is not consistent with the MCOT protein.

This appears to be a result of improper assembly. Only the exons from the 3' portion of the gene could be included in the gene model, so we named the transcript spaetzle6 partial.

The MCOT Spaetzle proteins from *D. citri* were used to BLAST against insect proteins and against *Drosophila melanogaster* proteins. The results confirmed the orthology of the *D. citri* proteins to the expected *Drosophila* proteins. (Table 2). A phylogenetic tree was also constructed using the *D. citri* MCOT Spaetzle proteins, as well as Spaetzle proteins from several other insects (Figure 1). In all cases, the *D. citri* Spaetzle proteins clustered with the expected class.

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Supplementary Note 8: Tube proteins

Introduction

Tube is a component of the Toll pathway, which is important for development and innate immunity in insects. In *Drosophila*, Tube acts as the central component in a complex with MyD88 and Pelle, each of which binds to a separate site in Tube's death domain (Sun et al. 2002). This interaction, which occurs when Toll is activated, allows Pelle to phosphorylate the inhibitory protein Cactus, targeting it for degradation (Daigneault et al 2013). Degradation of Cactus allows the Nf-kappa B transcription factors Dorsal and/or Dif to enter the nucleus and activate target genes (Wu and Anderson, 1998)

The presence of a single ortholog of *tube* appears to be the general rule in insect genomes (see Viljakainen 2015 and Table 1). However, many of these orthologs differ from *Drosophila* Tube in that they have a protein kinase domain in addition to the conserved death domain (Towb et al. 2009). This discovery allowed Towb et al. (2009) to determine that Tube and its binding partner Pelle are actually paralogous genes, which arose from a very ancient duplication. They also concluded that Tube is the ortholog of vertebrate IRAK4, while Pelle is orthologous to vertebrate IRAK1.

Organism	Order	# of tube genes
Tribolium castaneum	Coleoptera	1
Anopheles gambiae	Diptera	1
Drosophila melanogaster	Diptera	1
Acyrthosiphon pisum	Hemiptera	1
Nilaparvata lugens	Hemiptera	1
Apis mellifera	Hymenoptera	1
Nasonia vitripennis	Hymenoptera	1
Linepithsma humile	Hymenoptera	1
Bombyx mori	Lepidoptera	1

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

Methods

Tube orthologs 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. A locus encoding a putative Tube ortholog was identified and manually annotated in Web Apollo. The predicted protein was BLASTed against Insecta with NCBI BLAST to verify its identity. We also performed a BLAST search of the *D. citri* MCOT set to compare our gene model to *de novo* assembled transcripts. Multiple alignments 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

We used the *Tribolium castaneum* Tube ortholog (Towb et al. 2009) to perform a BLAST search of the *Diaphorina citri* (*D. citri*) predicted proteins. Several almost identical predicted proteins encoded by loci on several different scaffolds were identified. Most of these scaffolds are rather short and they appear to be artifacts of incomplete assembly rather than separate loci. We chose the locus on the longest scaffold (gi|645501093|ref|NW_007379137.1) for annotation. The NCBI model (XM_008481952.1) was a good match with the RNA-seq data, so it was renamed *tube*.

The Tube predicted protein was then used to BLAST the *D. citri* MCOT protein set. Two proteins encoded by de novo-assembled transcripts were nearly exact matches. MCOT03326.3.CO has four amino acid differences scattered throughout the protein. MCOT03326.1.CT appears to be truncated at the N-terminus and also has hree of the four amino acid differences seen in MCOT03326.3.CO. Finding only minor differences between the MCOT proteins and the gene model protein increases our confidence in the accuracy of the gene model.

We next used Tube to BLAST against other insect proteins. The top hit is annotated as a putative plant-type serine-threonine protein kinase from the body louse *Pediculus humanus corporis* (Table 2). Moving down the list were several interleukin-1 receptor-associated kinase 4-like (IRAK-4-like) proteins from other Hemipterans. Since Tube is orthologous to vertebrate IRAK4, it appears that these are Tube orthologs. However, the list also includes several good matches that are annotated as Pelle orthologs.

Predicted Protein	Top Blastp hit	Bitscore	E value	Percent Identity
Tube	serine-threonine protein kinase, plant-type, putative [Pediculus humanus corporis]	295	3e-93	36%

Table 2. BLAST analysis of the predicted Tube protein.

D. citri has a separate *pelle* ortholog (see *pelle* gene report), but we wanted to be sure that *tube* was really *tube* and not another *pelle* gene. In most insects, Tube and Pelle both have a death domain and a protein kinase domain. However, there are a few diagnostic residues that distinguish the two groups. Tube orthologs have previously been shown to share several conserved death domain residues that are required for interaction with MyD99 and Pelle (Towb et al. 2009). Multiple alignment with *Anopheles gambiae* Tube indicates that the three residues required for MyD99 interaction are perfectly conserved in Tube, but the residues involved in

Pelle interaction are only weakly conserved. Most strikingly, a GP-A motif in the Pelle interaction site that is conserved in previously described Tube orthologs (Towb et al. 2009), is not found in Tube. The significance of these differences for interaction of Tube and Pelle in *D. citri* is not clear. Another diagnostic residue is found in the protein kinase domain. Tube orthologs have an arginine (R) preceding an aspartate (D) in sequence subdomain VI, putting them in the RD class of protein kinases (Towb et al. 2009). Pelle orthologs have a glycine (G) instead. Tube has an R in that position, as would be expected for a Tube ortholog. Taken together, these observations suggest that the Tube is, in fact, a Tube ortholog.

We constructed a phylogenetic tree to compare Tube and Pelle to Tube and Pelle orthologs from several other insects. As expected, Dcitri_Tube clustered with other Tube orthologs, while Dcitri_Pelle grouped with other Pelle orthologs (Fig. 1)

Figure 1. A phylogenetic tree constructed with Pelle and Tube orthologs from several insects.

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Supplementary Note 9: Pelle proteins

Introduction

Pelle is a protein kinase that is a component of the Toll pathway. In *Drosophila*, it is responsible for the phosphorylation of the inhibitory protein Cactus (Daigneault et al 2013), targeting it for degradation so that the transcription factor Dorsal and/or Dif can translocate to the nucleus and activate target genes (Wu and Anderson, 1998). Pelle activity requires the formation of a heterotrimeric complex with MyD88 and Tube (Sun et al. 2002)

Tube and Pelle are paralogs, arising from an ancient duplication before the separation of the vertebrate and invertebrate lineages. They are orthologs of vertebrate IRAK4 and IRAK1, respectively (Towb et al. 2009). In Drosophila, Pelle and Tube are easily distinguished because Tube has no protein kinase domain. However, in many other insects both proteins have a death domain and a protein kinase domain (Towb et al. 2009. *pelle* is a single-copy gene in those insects that have been sequenced (see Viljakainen 2015 and Table 1).

Organism	Order	# of tube genes
Tribolium castaneum	Coleoptera	1
Anopheles gambiae	Diptera	1
Drosophila melanogaster	Diptera	1
Acyrthosiphon pisum	Hemiptera	1
Nilaparvata lugens	Hemiptera	1
Apis mellifera	Hymenoptera	1
Nasonia vitripennis	Hymenoptera	1
Linepithsma humile	Hymenoptera	1
Bombyx mori	Lepidoptera	1

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

Methods

Pelle orthologs 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 best hits was BLASTed against Insecta with NCBI BLAST to check their orthology. The locus encoding Pelle was identified and manually annotated in Web Apollo. We also performed a BLAST search of the *D. citri* MCOT set at citrusgreening.org. We used MEGA7 to construct a phylogenetic tree via the neighbor-joining method. Sequences for BLAST searches and phylogenetic analysis were obtained from NCBI, FlyBase, ImmunoDB and the Bordenstein Lab (NSF DEB-1046149).

Results

We used *Acyrthosiphon pisum* Pelle to BLAST against the *Diaphorina citri* (*D. citri*) predicted proteins at i5K. This identified a locus on gi|645505624|ref|NW_007378073.1 that was the clear best match. The next best hit (which had a much lower bitscore) was the Tube ortholog (see *tube* gene report), suggesting that there is only one *pelle* gene in the current *D. citri* assembly.

When the NCBI-predicted Pelle protein was used to BLAST against other insect proteins, the best matches were Pelle orthologs from other hemipterans (Table 2). These proteins show identity to the predicted Pelle protein in all regions except the C-terminus of the predicted protein. However, RNA-seq data indicates that the region encoding the C-terminus appears in the majority of the transcripts. Therefore, we accepted the NCBI annotation as the gene model for *pelle*. We then used Pelle to BLAST the MCOT predicted proteins.   However, the only match was to another assembly-predicted protein, so we were unable to find independent verification of the gene model.

Predicted Protein	Top Blastp hit	Bitscore	E value	Percent Identity
Serine threonine-protein kinase pelle	PREDICTED: serine/threonine-protein kinase pelle [Acyrthosiphon pisum]	435	6e-142	48%

Table 2. BLAST analysis of the predicted Pelle protein

We constructed a phylogenetic tree containing Pelle and Tube orthologs from several insects. Pelle clearly clustered with other Pelle orthologs (Fig. 1), supporting our conclusion that the locus encodes a Pelle ortholog.

Figure 1. A phylogenetic tree constructed with Pelle and Tube orthologs from several insects.

References

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2. Sun, H., Bristow, B., Qu, G., and Wasserman, S. (2002). A heterotrimeric death domain complex in Toll signaling. Proc Natl Acad Sci *99*, 12871–12876.

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Supplementary Note 10: MyD88 proteins

Introduction

MyD88 is a component of the Toll pathway in both vertbrates and invertebrates. *Drosophila* MyD88 has a Toll/Interleukin 1-R (TIR) domain that binds to the Toll TIR (Horng and Medzhitov, 2001; Tauszig-Delamasure et al 2002) and a death domain that interacts with Tube (Sun et al 2002). The formation of a trimeric complex containing MyD88, Tube and Pelle (Sun et al. 2002) allows Pelle to phosphorylate Cactus (Daigneault et al 2013). Once Cactus is phosphorylated, it is degraded, releasing its inhibition of the transcription factors Dorsal and/or Dif and allowing them to move to the nucleus to activate their target genes (Wu and Anderson, 1998).

MyD88 is found in a single copy in almost all insect genomes that have been examined (see Viljakainen 2015 and Table 1). Exceptions include the brown planthopper *Nilaparvata lugens*, which has two *MyD88* genes (Bao et al. 2015), and the diamondback moth *Plutella xylostella* where *MyD88* has not been found (Xia et al. 2015).

Organism	Order	# of MyD88 genes
Tribolium castaneum	Coleoptera	1
Anopheles gambiae	Diptera	1
Drosophila melanogaster	Diptera	1
Acyrthosiphon pisum	Hemiptera	1
Nilaparvata lugens	Hemiptera	2
Apis mellifera	Hymenoptera	1
Nasonia vitripennis	Hymenoptera	1
Linepithsma humile	Hymenoptera	1
Bombyx mori	Lepidoptera	1
Plutella xylostella	Lepidoptera	0?

Table 1. Gene counts of *MyD88* orthologs in representative insects. The question mark indicates that the absence of *MyD88* has not been proven.

Methods

Oncopeltus fasciatus MyD88 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. A locus encoding a putative MyD88 ortholog was identified and manually annotated in Web Apollo. The predicted protein was BLASTed against Insecta with NCBI BLAST to verify its identity. We also performed a BLAST search of the *D. citri* MCOT set at citrusgreening.org. 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).

Results

We used *Oncopeltus fasciatus* MyD88 to BLAST against *Diaphorina citri* (*D. citri*) predicted proteins at i5k. There were only two predicted proteins that showed significant identity to *Oncopeltus* MyD88, and both mapped to a single locus on gi|645507901|ref|NW_007377556.1. The two predicted proteins were encoded by NCBI gene model (XM_008489802.1) and maker gene model (maker-s119-augustus-gene-0.138-mRNA-1). The two models differed primarily at the 5' end, with the maker model having additional 5' exons. BLAST searches with the two predicted proteins showed that the maker model was a fusion of two genes. Comparison of the NCBI model to RNA-seq data suggested that it was missing one exon near the 3' end, so we manually merged that exon from the maker model with the NCBI model. The new model was named *MyD88*. We performed a BLAST with MyD88 against MCOT to try to obtain independent verification for our model, but the only match was to another predicted from the genome assembly.

When MyD88 is used as a query against other insect proteins, the top hit is bed bug MyD88 (Table 2). The domain analysis from NCBI BLAST indicates that, as expected, MyD88 has both a death domain and a TIR domain.

Predicted Protein	Top BLASTp hit	Bitscore	E value	Percent Identity
MyD88	PREDICTED: myeloid differentiation primary response protein MyD88 [Cimex lectularius]	117	2e-27	31%

Table 2. BLAST analysis of the predicted MyD88 protein.

We constructed a phylogenetic tree using MyD88 proteins from a variety of insects. Surprisingly, MyD88 appears to be a outgroup to all of the other proteins rather than clustering with the hemipteran MyD88 orthologs (Fig. 1). To understand this result, we compared BLAST alignments using either MyD88 or its closest match, bed bug (*Cimex lectularius*) MyD88, as the query sequence. The results suggest that MyD88 has diverged more rapidly than other MyD88 orthologs. While bed bug MyD88 is the best hit for MyD88, the converse is not true. MyD88 does not even appear on the list of top bed bug MyD88 matches. The stretches of identity shared between MyD88 and bed bug MyD88 are noticeably shorter than those shared by bed bug MyD88 with other insect orthologs. The significance of the rapid divergence of MyD88 is unclear, but it could have implications for Toll signaling in *D. citri*.
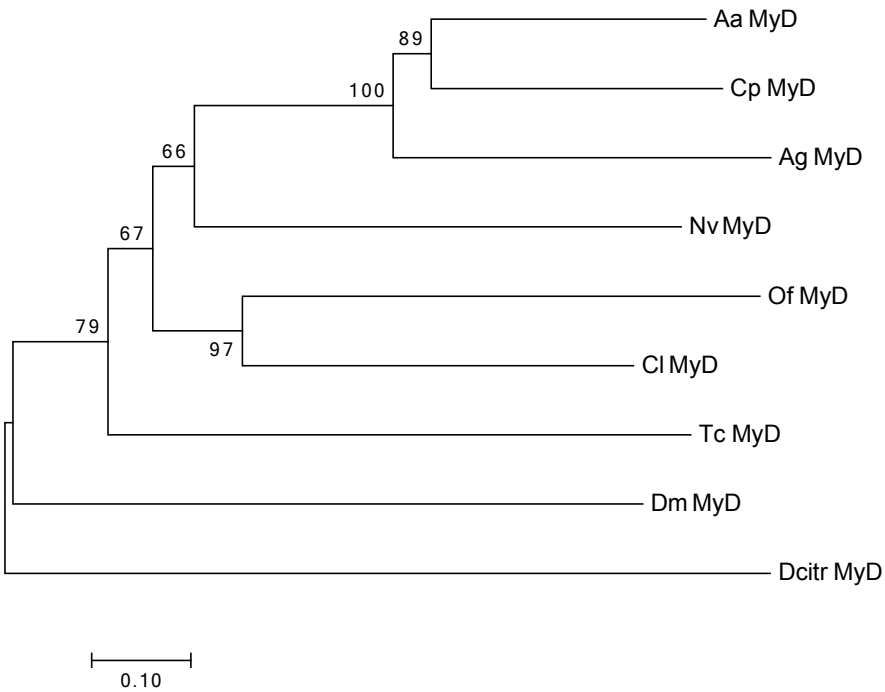
Figure 1. A phylogenetic tree constructed with MyD88 orthologs from *Aedes aegypti* (Aa), *Culex pipiens* (Cp), *Anopheles gambiae* (Ag), *Nasonia vitripennis* (Nv), *Oncopeltus fasciatus* (Of), *Cimex lectularius* (Cl), *Tribolium castaneum* (Tc), *Drosophila melanogaster* (Dm), and *Diaphorina citri* (Dcitr).

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Supplementary Note 11: Traf6 proteins

Introduction

TRAF6 is an adaptor protein that acts in several signaling pathways in the immune system of vertebrates (see Walsh et al. 2015 for review). This multifunctionality stems from its ability to interact with many different proteins. TRAF6 also acts as a non-conventional E3 ubiquitin ligase. In the Toll pathway, TRAF6 interacts with the IRAK complex to activate the TAK1 complex by ubiquitination (reviewed by Valanne et al. 2011).

There have been mixed reports as to whether the Drosophila ortholog of TRAF6, also known as dTRAF2, functions in Drosophila innate immunity. Shen et al. (2001) showed that TRAF6 can interact with Pelle *in vitro* and that the two proteins act synergistically to activate Dorsal when transfected into Schneider cells. Moreover, a null mutant of TRAF6 appears to have a deficient immune system, showing reduced transcription of the antimicrobial peptide genes *diptericin and drosomycin* in response to infection (Cha et al. 2003). In contrast, Sun et al. (2002) reported that RNAi against TRAF6 in cultured cells with constitutively active Toll receptors had no effect on the expression of a *drosomycin* reporter construct, suggesting that TRAF6 is not required for Toll signaling.

As in vertebrates, *Drosophila* TRAF6 has been shown to function in other pathways besides Toll. There have been reports of TRAF6 acting in JNK (Tang et al. 2013) and Notch (Mishra et al. 2014) signaling.

Most insects seem to have a single TRAF6 ortholog, although duplications have occasionally been described (Table 1).

Organism	Order	# of TRAF6 genes
Tribolium castaneum	Coleoptera	1
Drosophila melanogaster	Diptera	1
Plutella xylostella	Lepidoptera	2
Bombyx mori	Lepidoptera	1
Cimex lectularius	Hemiptera	1

Table 1. Gene counts of *TRAF6* orthologs in several insects.

Methods

Drosophila TRAF6 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. A locus encoding a putative TRAF6 ortholog was identified and manually annotated in Web Apollo. The predicted protein was BLASTed against Insecta with NCBI BLAST to verify its identity. We also performed a BLAST search of the

D. citri MCOT set at citrusgreening.org. Multiple alignments were performed using MUSCLE (http://www.ebi.ac.uk/Tools/msa/muscle/). 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).

Results

We used *Drosophila melanogaster* TRAF6 to BLAST against *Diaphorina citri* (*D. citri*) predicted protein sets. Matching proteins were encoded on two separate scaffolds, gi|645498610|ref|NW_007379755.1and gi|645508310|ref|NW_007377463.1. Multiple alignments showed that the proteins were virtually identical (data not shown), making it unlikely that they were actually separate loci. Both scaffolds had duplications of portions of the TRAF6-encoding sequence, probably due to assembly problems. The longest predicted protein on the longest scaffold (XP_008472878.1 on gi|645508310|ref|NW_007377463.1) was used to BLAST the MCOT data set. A protein encoded by a *de novo*-assembled transcript, MCOT13375.2.CT, differed by only one amino acid, suggesting that our gene model is accurate. We also BLASTed the predicted protein from our gene model against insect proteins at NCBI. The top matches were TRAF6 proteins (Table 2.) We therefore named our gene model TRAF6.

Predicted Protein	Top BLASTp hit	Bitscore	E value	Percent Identity
TRAF6	TNF receptor-associated factor 6 [Papilio xuthus]	157	3e-40	27%

Table 2. BLAST analysis of the predicted TRAF6 protein.

We constructed a phylogenetic tree using predicted TRAF6 proteins from *D. citri* and other insects (Figure 1). Traf6 groups with TRAF6 from the bed bug, *Cimex lectularius.*
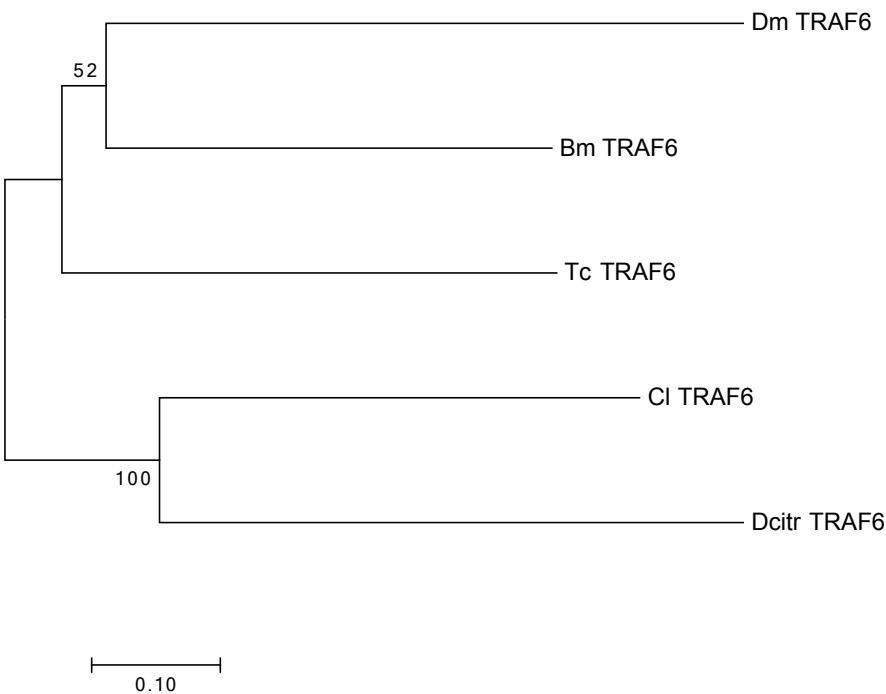
Figure 1. A phylogenetic tree constructed with TRAF6 orthologs from *Bombyx mori* (Bm), *Cimex lectularius* (Cl), *Tribolium castaneum* (Tc), *Drosophila melanogaster* (Dm), and *Diaphorina citri* (Dcitr).

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Supplementary Note 12: Cactus proteins

Introduction

Cactus is an I kappa B factor that acts in the Toll pathway in insects (Geisler et al, 1992). In *Drosophila*, Cactus binds the NF-kappa B transcription factors Dorsal and Dif, keeping them in the cytoplasm. When the Toll pathway is activated by the presence of an infectious agent (usually Gram-positive bacteria or fungi), Cactus is phosphorylated and then degraded. This releases Dorsal or Dif to translocate to the nucleus to activate target genes such as antimicrobial peptides (see Valanne et al. 2011)

Most insects whose genomes have been sequenced have a single ortholog of Cactus (see Viljakainen 2015 and Table 1), although the honeybee *Apis mellifera* has three (Evans et al 2006) and the Argentine ant *Linepithsma humile* has two (Smith et al 2011).

Organism	Order	# of cactus genes
Tribolium castaneum	Coleoptera	1
Anopheles gambiae	Diptera	1
Drosophila melanogaster	Diptera	1
Acyrthosiphon pisum	Hemiptera	1
Nilaparvata lugens	Hemiptera	1
Apis mellifera	Hymenoptera	3
Nasonia vitripennis	Hymenoptera	1
Linepithsma humile	Hymenoptera	2
Bombyx mori	Lepidoptera	1

Table 1. Gene counts of *cactus* orthologs in various insects

Predicted Protein	Top BLASTp hit	Bitscore	E value	Percent Identity
Cactus (MCOT19824.0.CT)	PREDICTED: NF-kappa-B inhibitor cactus [Bemisia tabaci]	212	6e-63	39%

Table 2. BLAST analysis of the predicted Cactus protein.

Methods

Acyrthosiphon pisum Cactus 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. The best hit was BLASTed against Insecta with NCBI BLAST to verify its identity. The locus encoding this protein was identified and

manually annotated in Web Apollo. We also performed a BLAST search of the *D. citri* MCOT set to compare our gene model to *de novo* assembled transcripts. Multiple alignments 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

Using *Acyrthosiphon pisum* Cactus as a BLAST query, we identified a single *D. citri cactus* gene. An existing NCBI gene model was manually edited to include additional exons encoding motifs shared with other Cactus orthologs. However, comparing the predicted protein to orthologous proteins reveals that these motifs do not occur in the expected order, suggesting a possible problem with the genome assembly in this region. We then used the predicted Cactus protein to BLAST the MCOT predicted protein set. We identified a single MCOT protein (MCOT19824.0.CT), which perfectly matched several stretches of the predicted Cactus protein. Again, these regions were not in the same order. The region corresponding to the C-terminal portion of the MCOT protein was located in the middle of the assembly-predicted protein. Since the MCOT protein is a *de novo*-assembled transcript from Trinity and the order of its sequence matches that found in Cactus genes from other insects, we conclude that the genome is incorrectly assembled in this region causing the *cactus* exons to be in the wrong order. Therefore, we used the MCOT Cactus protein for the remainder of our analysis.

Using the MCOT Dcitri_Cactus protein to BLAST against insect proteins retrieves Cactus orthologs from many insects. The top hit is to the Cactus ortholog of another hemipteran, *Bemisia tabaci*, the silverleaf whitefly (Table 2). In a phylogenetic tree, MCOT Dcitri_Cactus also clusters with another hemipteran Cactus ortholog (Fig. 1)
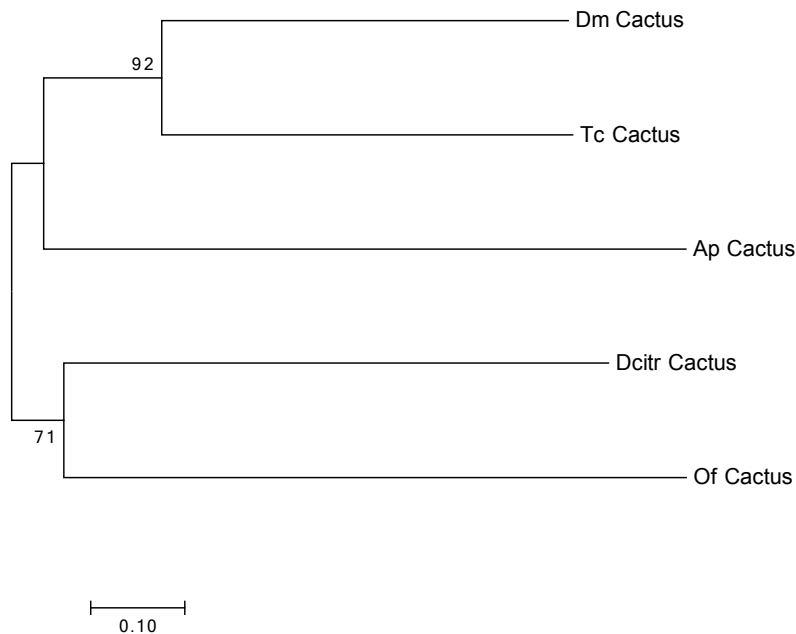
Figure 1. A neighbor-joining tree of Cactus proteins from *Drosophila melanogaster* (Dm), *Tribolium castaneum* (Tc), *Acyrthosiphon pisum* (Ap), *Diaphorina citri* (Dcitr) and *Oncopeltus fasciatus* (Of).

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Supplementary Note 13: Dorsal proteins

Introduction

Rel/NF☐B transcription factors are widely conserved in the animal kingdom. These proteins are defined by the presence of a Rel Homology Domain (RHD), which includes a DNA binding domain and a protein dimerization domain. In insects, Rel proteins play important roles in both development and immunity. *Drosophila melanogaster*, where these genes have been best studied, has three Rel genes: *dorsal* (*dl*), *Dorsal-related immunity factor* (*Dif*) and *Relish* (Ganesan et al., 2011). Dorsal and DIF both respond to Toll signaling (Belvin and Anderson, 1996; Meng et al., 1999), while Relish responds to IMD signaling (Hedengren et al., 1999; Khush et al., 2001). Relish also differs in that it contains ankyrin repeats (Dushay et al., 1996).

Methods

Rel 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 locus encoding the matching protein was identified and manually annotated in Web Apollo using RNA-Seq data to deduce the structure of transcripts. Multiple alignments of the predicted *D. citri* isoforms and their homologs were performed in Muscle (http://www.ebi.ac.uk/Tools/msa/muscle/). A phylogenetic tree was constructed using the neighbor-joining method in MEGA7. All sequences for multiple alignment and phylogenetic analysis were obtained from NCBI, FlyBase, ImmunoDB and the Bordenstein Lab (NSF DEB-1046149).

Results and Discussion
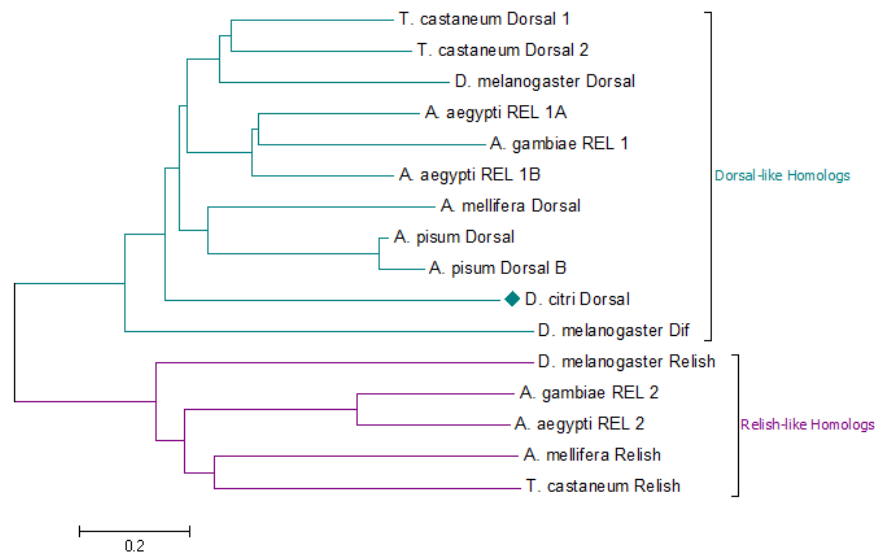
Figure 1: Phylogenetic tree depicting clustering of insect Rel proteins

In *D. citri*, BLASTp searches with known Rel proteins identified only one locus encoding a predicted Rel family member. The predicted RHD is much more similar to Dorsal than to either DIF or Relish. Moreover, the locus does not seem to encode the ankyrin repeats that are found in Relish orthologs. Consistent with these observations, phylogenetic analysis shows that the *D. citri* protein clusters with Dorsal proteins from other insects. Note that *dorsal* and *Dif* seem to be the result of a duplication in the *Drosophila* lineage (Meng et al., 1999; Christophides et al., 2004). *Dif* has diverged more rapidly (hence its position as an outgroup), so homologs in other insects have generally been named *dorsal* (Chen et al., 2000; Ursic-Bedoya et al., 2009; Gerardo et al., 2010). Following this convention, we will refer to this gene as *D. citri dorsal* (*dorsal*).

		D. citri Dorsal isoform A	*D. citri* Dorsal isoform B
D. melanogaster Dorsal isoform A	Bit score	254	244
	QC (Identity)	58% (45%)	45%(44%)
	E value	1.00E-76	3.00E-71
D. melanogaster Dorsal isoform B	Bit score	249	258
	QC (Identity)	58% (44%)	73% (45%)
	E value	1.00E-72	4.00E-74
D. melanogaster Dif	Bit score	184	180
	QC (Identity)	59% (38%)	42% (40%)
	E value	2.00E-50	5.00E-48
D. melanogaster Relish	Bit score	127	126
	QC (Identity)	59% (33%)	42% (34%)
	E value	1.00E-30	1.00E-29
A. gambiae REL 1	Bit score	171	171
	QC (Identity)	37% (46%)	29% (46%)
	E value	9.00E-51	1.00E-49
A. gambiae REL 2	Bit score	55.5	54.7
	QC (Identity)	14% (38%)	11% (38%)
	E value	1.00E-08	1.00E-08
A. aegypti REL 1A	Bit score	287	281
	QC (Identity)	57% (50%)	43% (50%)
	E value	3.00E-90	1.00E-86
A. aegypti REL 1B	Bit score	282	262
	QC (Identity)	55% (51%)	45% (48%)
	E value	4.00E-86	5.00E-80
A. aegypti REL 2	Bit score	102	105
	QC (Identity)	59% (31%)	49% (31%)
	E value	5.00E-23	2.00E-23
A. mellifera Dorsal Isoform A	Bit score	290	274
	QC (Identity)	97% (36%)	44% (49%)
	E value	7.00E-91	3.00E-83
A. mellifera Dorsal Isoform B	Bit score	271	287
	QC (Identity)	56% (49%)	74% (50%)
	E value	7.00E-82	3.00E-86
A. mellifera Relish	Bit score	48.1	45.1
	QC (Identity)	12% (42%)	7% (45%)
	E value	1.00E-05	1.00E-04
T. castaneum Dorsal 1	Bit score	270	256
	QC (Identity)	98% (37%)	45% (46%)
	E value	1.00E-83	7.00E-77
T. castaneum Dorsal 2	Bit score	194	191
	QC (Identity)	56% (39%)	44% (39%)
	E value	6.00E-57	1.00E-54
T. castaneum Relish	Bit score	99.8	99.8
	QC (Identity)	54% (30%)	42% (30%)
	E value	8.00E-22	2.00E-21
A. pisum Dorsal	Bit score	279	274
	QC (Identity)	56% (51%)	46% (49%)
	E value	3.00E-88	1.00E-84
A. pisum Dorsal B	Bit score	282	275
	QC (Identity)	97% (39%)	50% (47%)
	E value	2.00E-88	8.00E-84

Table 1: Bit score, query coverage, identity and e-value results from blast analysis of predicted dorsal_RA and dorsal_RB proteins

In several insect species, *dorsal* genes produce alternative transcripts that generate different protein isoforms (Gross et al., 1999; Shin et al., 2005; Zou et al., 2007). Therefore, it was not surprising that RNA-Seq data suggests that *dorsal* produces at least two alternative transcripts (*dorsal_RA* and *dorsal_RB*) that are very similar in structure to those seen in other insects.

Species	# Dorsal-like genes	# Relish-like genes
Drosophila melanogaster	2	1
Anopheles gambiae	1	1
Aedes aegypti	2	1
Apis mellifera	1	1
Tribolium castaeum	2	1
Acyrthosiphon pisum	2	0
Diaphorina citri	1	0

Table 2: Rel/NFkB transcription factors in *D. citri* and related organisms

No ortholog of *Relish* was found in the *D. citri* genome. While it is possible that we were unable to find *Relish* due to gaps in the *D. citri* genome, the fact that *Relish* is missing in the pea aphid genome (Gerardo et al., 2010) suggests that it could be absent in *D. citri* as well. It is interesting to note that both the pea aphid and the Asian citrus psyllid, two organisms known for their ability to host a variety of bacterial symbionts, appear to be lacking multiple members of the IMD pathway (including *Relish*) (Gerardo et al., 2010). The IMD pathway is known to be involved in the detection and elimination of Gram negative bacteria (Hedengren et al., 1999). Several Gram negative bacteria have been identified as *D. citri* symbionts, including the bacteria *Wolbachia*, *Candidatus* Carsonella, *Candidatus* Profftella armature, an as yet unidentified enteric bacteria closely related to *Klebsiella variicola* and *Salmonella enterica*, and *Candidatus* Liberibacter asiaticus, which causes citrus greening disease (Jagoueix et al., 1994; Nakabachi et al., 2006; Hilgenboecker et al., 2008; Saha et al., 2012; Sloan and Moran, 2012; Nakabachi et al., 2013). Given this information it is tempting to speculate that a reduction in IMD pathway genes may in fact aid in *D. citri's* ability to acquire and harbor these Gram negative symbionts.

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Supplementary Note 14: Imd Pathway CASPAR (FAF1), IKKB, TAK1, TAB proteins

Introduction

Fas-associated factor-1 (FAF1) is a component of the death-inducing signing complex (DISC) and is able to enhance Fas-induced apoptosis when overexpressed. FAF1 is characterized by containing two ubiquitin-homologous domains in its N-terminus and a UX domain (Ryu et al., 2001, 2003). FAF1 is a homolog of *Drosophila* caspar (Chu et al., 1995; Ryu et al., 2003; Park et al., 2004). It has been shown that caspar suppresses the immune response by inhibiting nuclear localization of Relish induced by bacterial infection (Kim et al., 2006). Disruption of the IKK complex can occur when FAF1 binds to I-kappaB kinase-beta (IKKB) (Park et al., 2007). IKKB is the catalytic subunit of the IKK complex (~400 kDa) in IMD signaling (Silverman and Takashi, 2005; Silverman and Maniatis, 2001). In *Drosophila*, the IKK complex is responsible for phosphorylating Relish, which triggers cleavage of Relish mediated by DREDD. TAK1 (Transforming growth factor beta-activated kinase 1) activates the IKK complex and is required for expression of anti-microbial peptide genes through Relish activation (Silverman and Takashi, 2005).

Methods

Dipteran caspar, IKKB, and TAK1 orthologs were collected from Immunodb and were used to blat against the *Diaphorina citri*, Asian citrus psyllid (ACP), genome. Identification of the genes were performed in WebApollo where RNA sequence data (adult, nymph, and egg) supported the manually curated gene models. BLASTp was used to confirm accuracy of the annotated the gene models.

FAS-associated factor 1, IKKB, and TAK1 orthologs were collected through an NCBI search for the phylogenetic analysis. Multiple sequence alignments (MSA) of ACP and orthologous insect FAF1 protein sequences was performed in MEGA6 using ClustalW (Tamura et al 2003). MSA was repeated for IKKB and TAK1. The phylogenetic analysis of FAF1, IKKB, and TAK1 included, constructing two midpoint rooted neighbor-joining trees in MEGA6 using p-distance to show evolutionary distance and 1,000 bootstrap replicates.

Results

In the ACP genome, one FAF1 was identified along with two partials (Table 1). The two partials were located on small contigs, ~4,000 bp and below. Between hemipterans and dipterans, only one TAK1 and IKKB genes were found, including in the ACP (Table 1 & 2). TAB was found in the ACP, however, the N-terminus and C-terminus of the gene are on separate contigs. TAK1 is activated by overexpression of TAK1 interacting proteins, TAB (Shibuya et al. 1996). *D. citri* is missing many components of the IMD pathway, including DREDD, FADD, IMD, and IKKG

(kenny). The pea aphid also have many IMD members missing (Gerardo et al., 2010). In *Drosophila*, this pathway is crucial to elicit an immune response when infected with Gram-negative bacteria (Boutros et al., 2002; Lemaitre et al., 1995).

Table 1: IMD pathway members found in the *D. citri* genome. Table shows members with BLASTp matches, corresponding protein length, and domains.

IMD members	BLASTP match (organism, bit score)	Protein Length	Domains
Fas-associated factor-1	FAF1 (Zootermopsis nevadensis, 171)	862	UAS family, FAF1 subfamily, Faf1 UBX domain, UAS domain, UBX domain, UBA-like domain, thioredoxin (TRX)-like superfamily, hydrophobic patch, charged pocket
FAF1, partial	FAF1 (Locusta migratoria manilensis, 98.6)	198	UBA like domain, UBA like domain
FAF1, partial	FAF1 (Zootermopsis nevadensis, 117)	152	UAS family, FAF1 subfamily, thioredoxin (TRX)-like superfamily
TAK1	TAK1 (Zootermopsis nevadensis, 367)	257	STKc_TAK1, KIND, PKc like superfamily, STYKc, Pkinase_Tyr, SPS1, PTZ00267, TOMM_kin_cyc
IKKB	IKKB (Halyomorpha halys, 508)	765	DCX (6), XLIS mutations, SBH mutations, hydrophobic patch, charged pocket, active site, ATP binding site (2), polypeptide substrate binding site, UBQ superfamily (2), PKc like superfamily (2), STKc_DCKL, pk1, STYKc1, Pkinase, SPS1, Pkinase_Tyr, TOMM_kin_cyc (2),

Table 2: Number of IMD pathway members in *D. citri* and related organisms.

Organism	FAF1/caspar	TAK1	IKKB
Diaphorina citri	1	1	1
Acyrthosiphon pisum	1	1	1
Cimex lectularius	2	1	1
D. melanogaster	1	1	1
Aedes aegypti	2	1	1
Anopheles gambiae	2	1	1
Culex quinquefasciatus	1	1	1
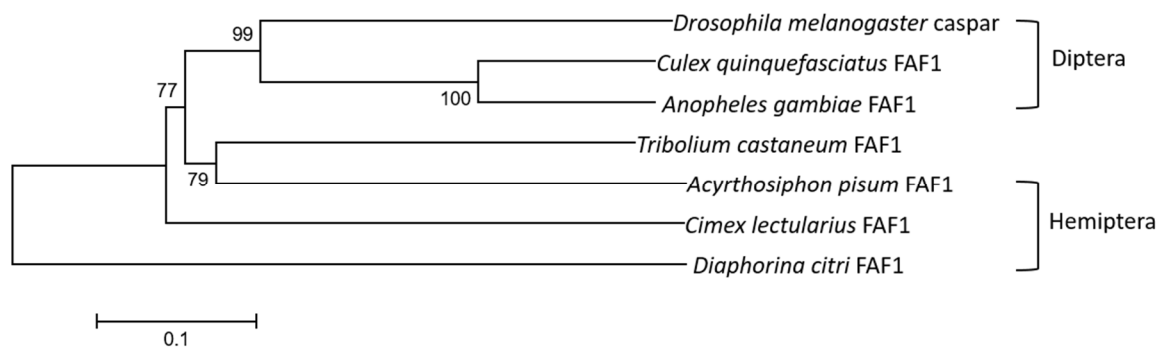
Figure 1: Neighbor-joining tree of 7 FAF1 was constructed in MEGA6 using 1000 bootstrap replications. The p-distance method was used to show evolutionary distance. A total of 546 amino acid positions were analyzed for the tree. NCBI accessions: *A. pisum* [XP_008188590.1], *T. castaneum* [XP_008195902.1], *A. gambiae* [XP_316513.4], *C. quinquefasciatus* [EDS36965.1], *C. lectularius* [XP_014254010.1], *D. melanogaster* [NP_611080.1].
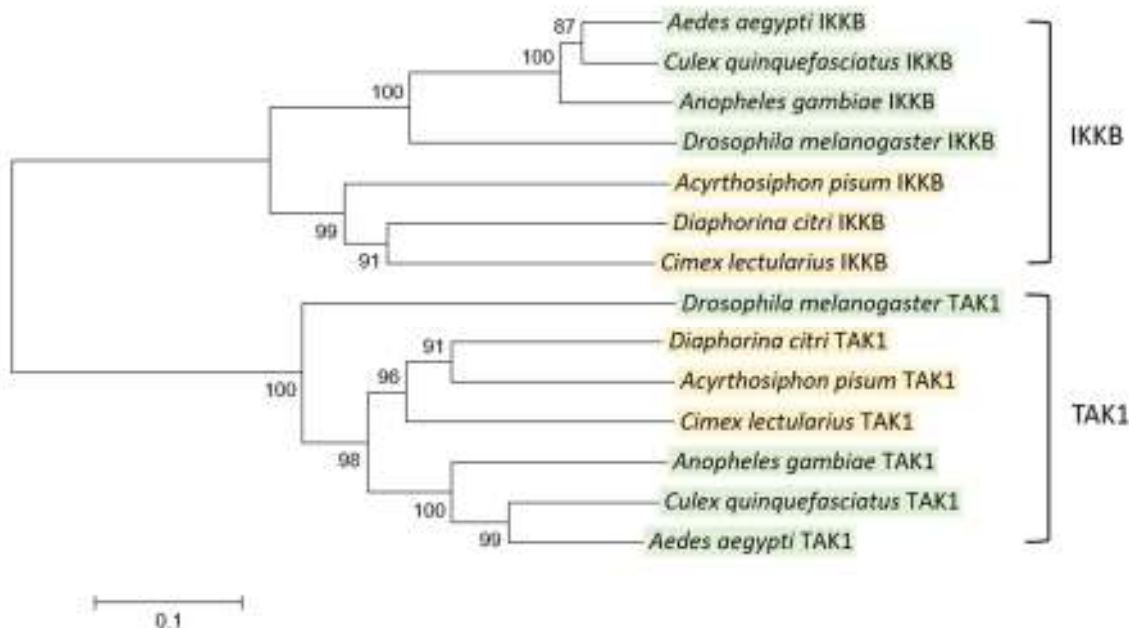
Figure 2: Neighbor-joining tree of 14 FAF1 was constructed in MEGA6 using 1000 bootstrap replications. The p-distance method was used to show evolutionary distance. A total of 237 amino acid positions were analyzed for the tree. Organisms highlighted in yellow and green belong to orders Hemipteran and Diptera, respectively.

The phylogenetic analysis (Figure 1) shows the clustering of ACP FAF1 with other hemipterans and the relationship between. *T. castaneum* (Order: Coleoptera) and Dipterans. Since, TAK1 and IKKB are both kinases, a neighbor-joining tree (Figure 2) was constructed showing the evolution of these proteins. Table 1 shows that TAK1 and IKKB share some domains.

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Supplementary Note 15: JAK/STAT pathway

Introduction

The Janus kinase/signal transducer of activators of transcription (JAK/STAT) pathway is a somewhat-simple, signaling pathway that provides direct communication between the membrane and nucleus (O'shea *et al.*, 2015). A variety of molecules could signal this pathway, two being cytokines and growth factors (O'shea *et al.*, 2015). Janus kinase and signal transducer of activators of transcription are the mammalian names given for these genes. They have been called hopscotch and marelle or STAT92E in insects, specifically *Drosophila melanogaster* (Hou *et al., 1996).* Domeless is the third component of this pathway, and it is similar to the cytokine receptor in mammals (Brown *et al.*, 2001). They still carry out, generally, the same purposes like development and immunity. In the genome of *Diaphorina citri* a search for orthologs of these genes resulted in finding one of each, establishing the presence of the pathway in the Asian citrus psyllid.

Materials and Methods

To find JAK/HOP, STAT, and DOME in the psyllid's genome orthologs from five organisms were obtained: *Acyrthosiphon pisum* (pea aphid), *Aedes aegypti, Anopheles gambiae, Drosophila melanogaster* (fruit fly), and *Culex pipiens*. The sequences were used in the BLAT tool in Web Apollo, the BLAST tool at I5K, and the MCOT tool in the psyllid database. GNOMON predicted models were manually annotated using supporting evidence tracks. The sequences of the models were used to perform comparisons in Blastp at NCBI.

The *D. citri* sequences were used with sequences from other insects collected from NCBI to make a multiple sequence alignment using default MUSCLE settings in MEGA7. A Neighbor-Joining tree was then made using a 1000 bootstrap test, complete deletion, and Poisson model.

Table 1: Blastp top results for DOME, HOP, and STAT genes in *D. citri.* Includes Name, accession, query, identity, and bit score.

Top Blastp Hit for JAK/STAT pathway members in *D. citri*					
Gene	Match	Accession	Query	ID	Bit score
DOME	Cytokine receptor [Zootermopsis nevadensis]	KDR09708.1	82%	37%	1120
HOP	PREDICTED: LOW QUALITY PROTEIN: tyrosine-protein kinase hopscotch-like [Atta cephalotes]	XP_012054793.1	83%	33%	922
STAT	PREDICTED: signal transducer and activator of transcription 5B isoform X3 [Megachile rotundata]	XP_012146903.1	97%	46%	1053

Table 2: Gene numbers of the JAK/STAT pathway members present in the listed insects, including *D. citri* (Viljakainen, 2015).

JAK/STAT member count in *D. citri* vs. other insects			
	DOME	HOP	STAT
Drosophila melanogaster	1	1	1
Anopheles gambiae	1	1	2
Acyrthosiphon pisum	4	1	2
Bombyx mori	1	1	1
Pediculus humanus	1	1	1
Tribolium castaneum	1	1	1
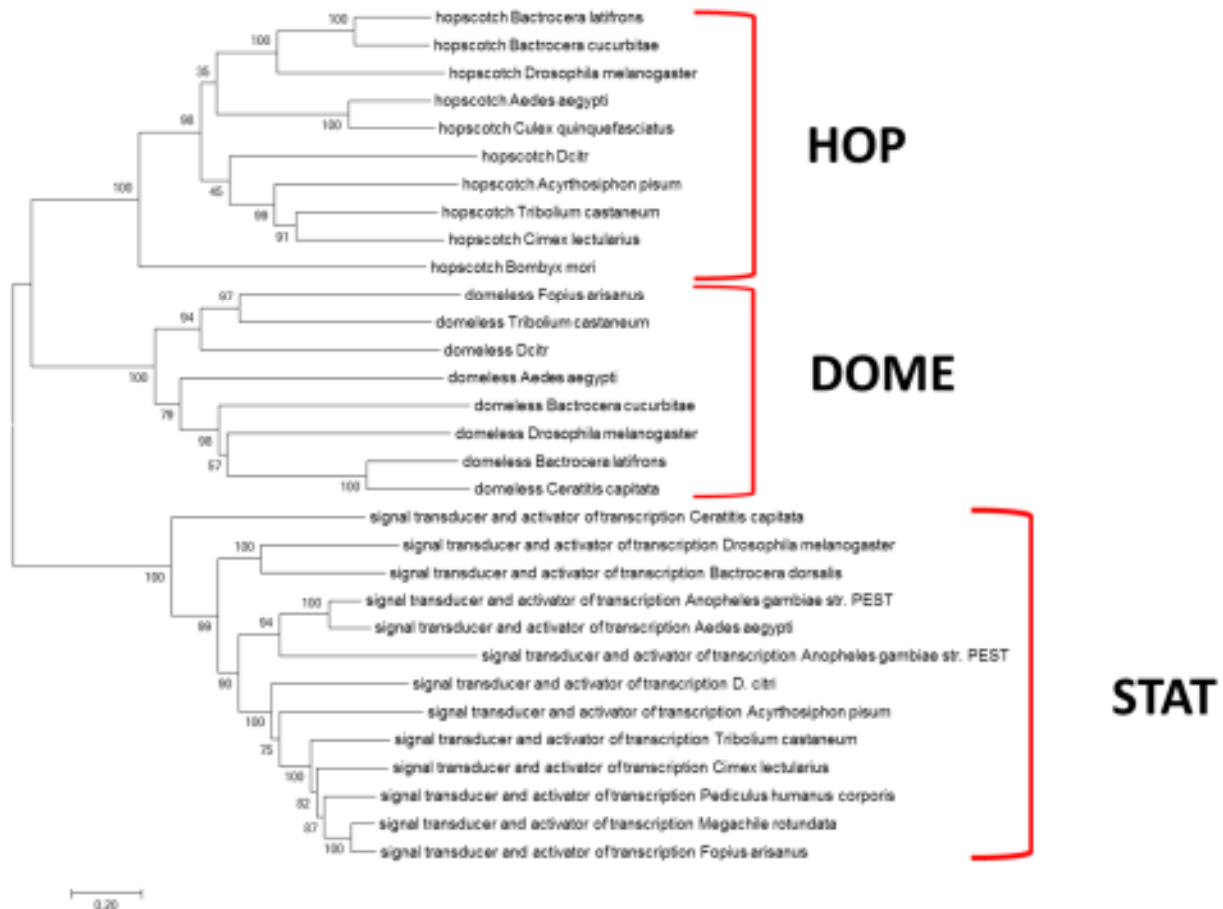
Figure1: Neighbor- Joining tree for JAK/STAT genes in *D. citri* using 1000 bootstrap replicates, Poisson model, and complete deletion. *D. citri* consistently appears close to fellow hemipteran *A. pisum* and beetle *T. castaneum*

Results and Discussion

Annotation revealed one ortholog for each component of the JAK/STAT signaling pathway. Blastp gives insect matches with high query and bit score values (Table 2). The gene numbers found in the psyllid is consistent with what has been seen in other insects (Table 2). The

phylogenetic tree shows clades formed for each gene, along the psyllid gene being close to pea aphid and *T. castaneum*. The JAK/STAT pathway members have been successfully found and annotated. The next step would be to use them as targets in treatments.

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Supplementary Note 16: Lysozymes

Introduction

Lysozymes (LYS) hydrolyze the β-(1,4)-glycosidic linkage between *N*-acetylmuramic acid and *N*-acetylglucosamine of bacterial peptidoglycan. Many insects will produce lysozymes, particularly c-type, and secrete them into the hemolymph, following bacterial infection. LYS c-type have been reported in many different insect orders, such as Diptera, Hemiptera, and Lepidoptera (Callewaert and Michiels, 2010). Gram-positive bacteria are detected by these LYS, while only a few insect LYS are antibacterial against Gram-negative bacteria (Abraham et al. 1995; Yu et al. 2002). LYS i-type has isopeptidase (destabilase) activity, it hydrolyzes fibrin molecules in coagulated blood (Callewaert and Michiels, 2010). Studies analyzing whether LYS i-type have muramidase and/or isopeptidase activity in insects are negligible (Paskewitz et al. 2008). Although, *Anopheles gambiae* have shown increased overexpression of i-type LYS in the midgut after blood-feeding, indicating a possible isopeptidase activity (Paskewitz et al. 2008).

Methods

Lysozyme orthologs were collected from Immunodb and were used to blat against the *Diaphorina citri*, Asian citrus psyllid (ACP), genome. Identification of LYS was performed in WebApollo where RNA sequence data (adult, nymph, and egg) supported the manually curated gene models. BLASTp was used to confirm accuracy of the annotated LYS. Further analysis of LYS were done through the MCOT database found on citrusgreening.org. Lysozyme orthologs were collected through an NCBI search for the phylogenetic analysis. Multiple sequence alignments of ACP and orthologous insect LYS protein sequences was performed in MEGA6 using ClustalW (Tamura et al 2003). The phylogenetic analysis of LYS included, constructing a midpoint rooted neighbor-joining tree in MEGA6 using p-distance to show evolutionary distance and 1,000 bootstrap replicates.

Table 1: All the LYS found in the *D. citri* genome. Table shows all LYS with names based on NCBI Gnomon with BLASTp matches, corresponding protein length, and domains.

D citri LYS	BLASTP match (organism, bit score)	Protein Length	Domains
LYS i-type 1	i-type LYS (Nilaparvata lugens, 137)	165	Destabilase, Destabilase superfamily
LYS i-type 2	i-type LYS 3 (Nilaparvata lugens, 100)	131	Destabilase, Destabilase superfamily
LYS i-type 3	LYS i-type (Acyrthosiphon pisum, 169)	159	Destabilase, Destabilase superfamily
LYS c-type 1	Lysozyme (Tribolium castaneum, 167)	349	Lys catalytic site, lys catalytic cleft, Ca^{2+} binding site (2), lysozyme like superfamily, LYZ1, Lys

LYS c-type 2	Lysozyme (Papilio polytes, 92.8)	181	Lys catalytic site, Ca^{2+} binding site, LYZ1, Lys, lysozyme like superfamily

Table 2: Number of LYS in *D. citri* and related organisms.

Organism	LYS c-type	LYS i-type
Diaphorina citri	2	3
Acyrthosiphon pisum	0	3
Cimex lectularius	8	2
D. melanogaster	12	9
Aedes aegypti	6	2
Anopheles gambiae	7	2
Culex quinquefasciatus	5	2
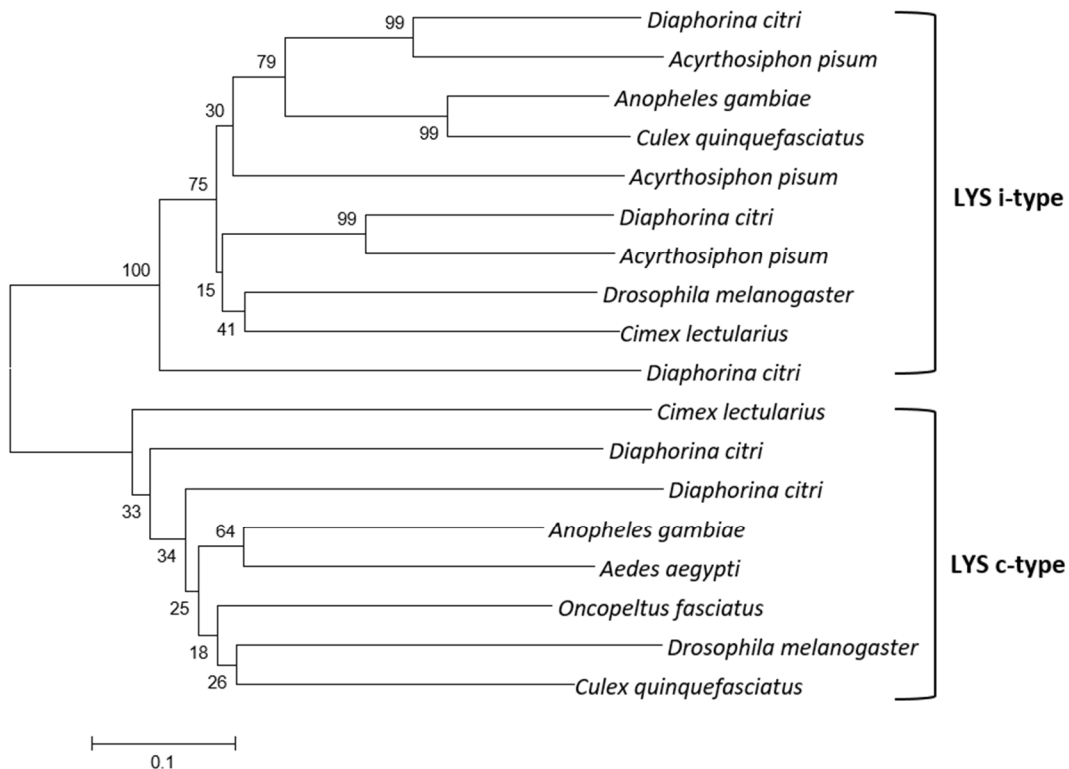
Figure 1: Neighbor-joining tree of 18 LYS was constructed in MEGA6 using 1000 bootstrap replications. The p-distance method was used to show evolutionary distance. A total of 75 amino acid positions were analyzed for the tree.

Results and Discussion

Within the ACP genome, three LYS i-type and two c-type have been identified. LYS c-type was not found initially with a blat search. The MCOT database predicted two c-type genes and was then found on Webapollo. All LYS i-type found contains the destabilase domain, as shown in Table 1. The function of this destabilase activity is not clear in many insects (Callewaert and Michiels, 2010), therefore it can be inferred no known function in the ACP. *Acyrthosiphon pisum* (aphid)*,* ACP closest relative, lacks the c-type lysozyme but contains three LYS i-type (Table 2) (Gerardo et al., 2010). Bacterioltyic activity assay were performed on immune-challenged aphids suggesting that aphid hemolymph has little to no lysozyme activity (Altincicek et al., 2008).

The neighbor-joining tree of the LYS reveal the divergence of i-type and c-type (Figure 1). It is shown that *D. citri* LYS are evolutionary closer to other hemipterans than dipterans.

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Supplementary Note 17: Superoxide dismutases

Introduction

Superoxide dismutases (SODs) combat reactive oxygen species (ROS) by converting negatively charged oxygen molecules to hydrogen peroxide that is then destroyed by catalase, as an enzymatic defense in aerobic metabolism (Parker et al., 2004). ROS are harmful to cellular components as evidence implicates ROS as a major cause of aging (Perez-Campo et al., 1998; Finkel & Holbrook, 2000; Arking et al., 2002; Barja, 2002; Sohal et al., 2002; Hasty et al., 2003; Hekimi & Guarente, 2003).

In animals, there are three distinct SODs: CuZn SOD (SOD1), CuZn SOD (SOD3), and Mn SOD (SOD2) (Bordo et al., 1994; Colinet et al., 2011). SOD1 is located primarily in the cytoplasm and found in small amounts in the mitochondrial inter-membrane space (Okado and Fridovich, 2001). SOD3 is similar to SOD1 in structure but contains an N-terminal signal peptide and a C-terminal region involved in extracellular binding (Stenlund et al., 2002). SOD2 is completely different from SOD1 in protein sequence and structure. SOD2 is located in the inner matrix of the mitochondria (Weisiger and Fridovich, 1973).

Methods

Insecta SOD orthologs were collected from Immunodb and were used to Blat against the *Diaphorina citri*, Asian citrus psyllid (ACP), genome. Identification of the SODs was performed in WebApollo where RNA sequence data (adult, nymph, and egg) supported the manually curated gene models. BLASTp was used to confirm accuracy of the annotated SODs.

Superoxide dismutase orthologs were collected through an NCBI search for the phylogenetic analysis. Multiple sequence alignments of ACP and orthologous insect SODs protein sequences was performed in MEGA6 using ClustalW (Tamura et al 2003). The phylogenetic analysis of SODs included, constructing a midpoint rooted neighbor-joining tree in MEGA6 using p-distance to show evolutionary distance and 1,000 bootstrap replicates.

Table 1: All the SODs found in the *D. citri* genome. Table shows all SODs with names based on NCBI Gnomon with BLASTp matches, corresponding protein length, and domains.

D. citri SODs	BLASTP match (organism, bit score)	Protein Length	Domains
SOD [Mn-Fe]	Mn superoxide dismutase (*Bemisia tabaci*, 296)	224	Sod_Fe_N, Sod_Fe_N superfamily, SodA, PLN02471, Sod_Fe_C, Sod_Fe_C superfamily
SOD [Cu-Zn]	Cu/Zn superoxide dismutase	155	Active site, Cu^{2+} binding site, Zn^{2+} binding site, E-class dimer interface, P-class dimer interface, Cu-Zn_Superoxide_Dismutase, Sod_Cu,

	(*Coptotermes formosanus*, 239)		SodC, Cu-Zn_Superoxide_Dismutase superfamily, PLN02386, PLN02957
SOD [Cu-Zn]	superoxide dismutase [Cu-Zn] (*Acyrthosiphon pisum,* 178)	214	Active site, Zn^{2+} binding site, P-class dimer interface, Cu-Zn_Superoxide_Dismutase, Sod_Cu, SodC, Cu-Zn_Superoxide_Dismutase superfamily, PLN02957
SOD [Cu-Zn], partial	Superoxide dismutase [Cu-Zn] (*Acyrthosiphon pisum*, 87.8)	69	Cu-Zn_Superoxide_Dismutase, Sod_Cu, SodC, Cu-Zn_Superoxide_Dismutase superfamily, PLN02386, PLN02957

Table 2: Number of SODs in *D. citri* and related organisms.

Organism	# of SODs
Diaphorina citri	3
Acyrthosiphon pisum	3
Cimex lectularius	5
D. melanogaster	8
Aedes aegypti	7
Anopheles gambiae	6
Culex quinquefasciatus	11

*Numbers based on Ensembl and NCBI, does not include isoforms/partial.
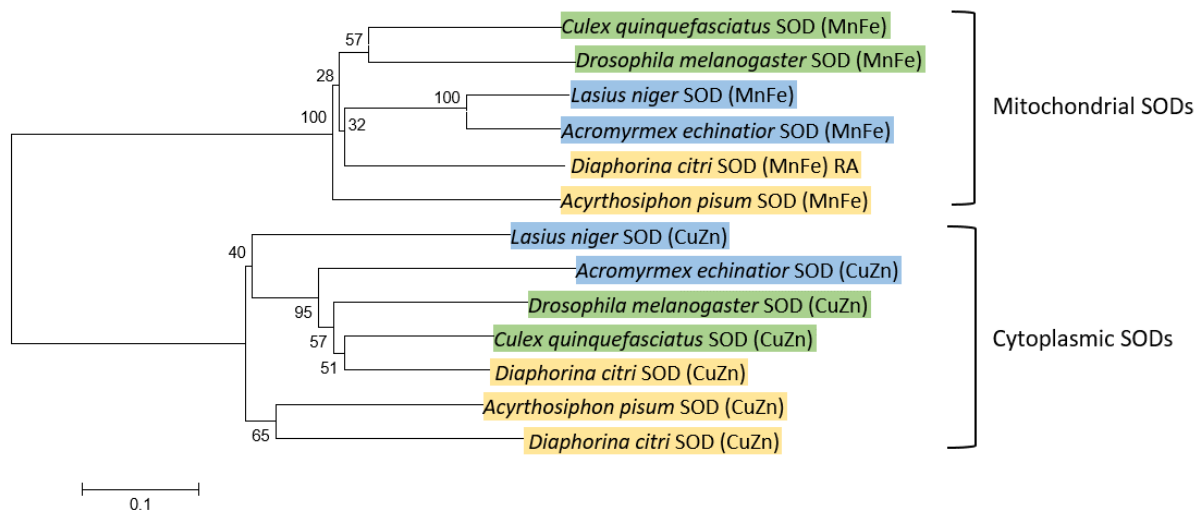
Figure 1: Neighbor-joining tree of 13 CuZn and MnFe SODs was constructed in MEGA6 using 1000 bootstrap replications. The p-distance method was used to show evolutionary distance. A total of 132 amino acid positions were analyzed for the tree. Organisms highlighted in green, blue, and yellow,

belong to orders: Diptera, Hymenoptera, and Hemiptera, respectively. NCBI accessions: *D. melanogaster* [NP_001286503.1 & NP_001261700.1], *C. quinquefasciatus* [EDS43182.1 & EDS44530.1], *A. echinatior* [EGI68366.1 & EGI70961.1], *L. niger* [KMQ93269.1 & KMQ95190.1], *A. pisum* [BAH70786.1 & NP_001156153.1].

Results and Discussion

It was identified that *D. citri* has a total of four SODs (Table 1). Three of them represent SOD CuZn, and one SOD2. It was shown in *Drosophila melanogaster* that both Cu/Zn SOD and Mn SOD were found to increase life span without effecting metabolism, when induced (Sune et al. 2002). However, transgenic fruit flies with an extra copy of Mn SOD did not exhibit the same effect (Mockett *et al.* 1999). These results suggest that overexpression/silencing of SOD can increase/decrease life span in other related organisms, including the ACP.

Insect mitochondrial and cytoplasmic SODs are clearly distinct from each other as shown in the phylogenetic tree (Figure 1). These SODs share the same function, but there are many reasons to argue against a common ancestor. SOD1 and SOD2 have completely different crystal structures, differ in metal cofactors, and distinctive catalytic mechanism (Zelko et al., 2002).

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Supplementary Note 18: CLIP

Introduction

Exclusive to the hemolymph of arthropods, Clip-domain serine proteases are "paper clip"-like non-digestive proteases that are composed of one or more N-terminal clip domains (Jang IH, Nam HJ, and Lee WJ 2008). Clip-domains are followed by a linker sequence and a carboxyl-terminal s1A family serine protease domain (Jiang H and Kanost MR 2000). CLIP protease genes have evolved as four clades (CLIPA, CLIPB, CLIPC, and CLIPD) that are present as multigene families in insect genomes and function as hemolymphs in innate immune responses (Jiang H and Kanost MR 2015). In immune pathways that have been characterized, CLIP proteases are involved via a proteinase cascade method (Whaley and Lemercier, 1993). CLIPC is activated by stimulation of an initiating modular serine protease by microbial surface molecules. CLIPC then activates CLIPB which, in turn, cleaves and activates effector molecules proSpätzle or prophenoloxidase. As CLIPA proteins lack proteolytic activity (pseudoprotease), some CLIPA proteins can function as regulators of the other CLIP proteases, forming immune complexes with high molecular weight. The functions of CLIPD and most other CLIP proteases are currently unknown.

Methods

The orthologs of CLIPs were collected from NCBI and their scaffolds were screened for predicted gene models using BLAT from Web Apollo and i5k BLAST, where necessary. Gene models were manually annotated and verified using RNAseq data. Further analysis was also performed using NCBI's BLASTP program.

Once the genes models had been annotated, pairwise alignments between amino acid sequences *Diaphorina citri* and *Acyrthosiphon pisum* were performed using the ClustalW program in MEGA6 software (Tamura, Stecher, Peterson, Filipski, and Kumar 2013).

Table 1. Current CLIP domain proteases found in *D.citri* genome (CLIPD annotation is incomplete). Table shows names based on NCBI Gnomon (*) with Blastp matchs, location and accession number, and protein length

D. citri CLIP Serine Protease	LOCATION [Accession #]	Protein Length	Domains
CLIPA7-trypsin 1 like protease	gi|645506705|ref| NW_007377811.1 [XP_008470938.1]	368 bp	
CLIPA10 -Kallikrein-15-like	gi|645505574|ref| NW_007378085.1 [XP_008473780.1]	143 bp	**Tryp_SpC**

CLIPA6-serine proteinase stubble	gi|645499856|ref| NW_007379449.1 [XP_017303128.1]	667 bp	
ClIPA15-serine proteinase stubble	gi|645507848|ref| NW_007377568.1 [XP_008487216.1]	690 bp	
CLIPB1	gi|645502691|ref| NW_007378736.1 [XP_017302078.1]	514 bp	
CLIPB2	gi|645506135|ref| NW_007377957.1 [XP_008472575.1]	310 bp	
CLIPB3 (Partial)	[XP_017302079.1]	-	
CLIPB4 (Partial)	-	-	
CLIPB5	gi|645508403|ref| NW_007377443.1 [XP_017303373.1]	254 bp	Tryp_SpC
CLIPB6	gi|645507848|ref| NW_007377568.1 [XP_008488270.1]	485 bp	
CLIPB7	gi|645506135|ref| NW_007377957.1 [XP_017299783.1]	496 bp	
CLIPC11-serine protease snake-like	gi|645507171|ref| NW_007377706.1 [XP_008469523.1]	384 bp	
CLIPC3 -Serine protease snake-like	gi|645504563|ref| NW_007378328.1 [XP_008475910.1]	184 bp	
CLIPD- serine protease (Partial)	-	-	

Table 2. Represents Query Coverage (Identity) and E-value of the annotated gene models pairwise aligned to orthologues in other species. Pairwise alignment was performed using NCBI blast.

Diaphorina citri	CLIPB1 QC (ID) E-value	CLIPB2 QC (ID) E-value	CLIPB5 QC (ID) E-value	CLIPB6 QC (ID) E-value	CLIPB7 QC (ID) E-value
Acyrthosiphon pisum	81%(36%) 5e46	98%(57%) 2e-124	100%(45%) 6e-78	90%(56%) 0.0	91%(61%) 2e-67
Halyomorpha halys	86%(37%) 1e-60	87%(60%) 7e-131	99%(47%) 4e-69	90%(53%) 5e-163	95%(57%) 2e-80
Cimex lectularius	82%(37%) 3e-46	85%(64%) 6e-139	99%(43%) 2e-70	89%(57%) 1e-180	84%(73%) 8e-80
Anopheles gambiae	82%(41%) 2e-61	85%(66%) 6e-140	100%(38%) 1e-58	89%(48%) 3e-117	77%(75%) 2e-69
Aedes aegypti	81%(40%) 7e-55	80%(68%) 9e-137	100%(41%) 1e-65	69%(49%) 3e-122	77%(73%) 7e-66

	CLIPA6 QC (ID) E-value	CLIPA7 QC (ID) E-value	CLIPA10 QC (ID) E-value	CLIPA15 QC (ID) E-value	CLIPC3 QC (ID) E-value	CLIPC11 QC (ID) E-value
Acyrthosiphon pisum	34% (37%) 1e-43	65% (46%) 1e-66	36% (60%) 2e-19e	96% (58%) 0.0	86% (47%) 2e-39	95% (44%) 1e-60
Halyomorpha halys	45% (68%) 6e-140	66% (57%) 4e-82	37% (68%) 3e-24	36% (37%) 0.0	80% (43%) 2e-35	90% (37%) 1e-47
Cimex lectularius	32% (33%) 9e-32	66% (61%) 1e-93	35% (59%) 1e-18	35% (39%) 0.0	80% (40%) 4e-33	100%(39%) 8e-53
Anopheles gambiae	31% (40%) 4e-50	59% (59%) 2e-99	79% (78%) 1e-26	86% (64%) 5e-169	97% (42%) 2e-39	90% (34%) 9e-54
Aedes aegypti	95% (47%) 9e-166	66% (58%) 1e-94	37% (79%) 2e-27	93%(59%) 0.0	80% (43%) 1e-35	96% (40%) 1e-54

Table 3. This table shows the number of CLIP domain proteases within *D.citri, A. pisum, H. halys, C. Lectularius, A. gambiae* and *A. aegypti*.

	Number of CLIP Genes
Diaphorina citri	11 found
Acyrthosiphon pisum	11
Halyomorpha halys	18
Cimex lectularius	7
Anopheles gambiae	41
Aedes aegypti	79

Figure 1. Neighbor joining tree of 11 annotated CLIP domain proteases was constructed using standard parameters.
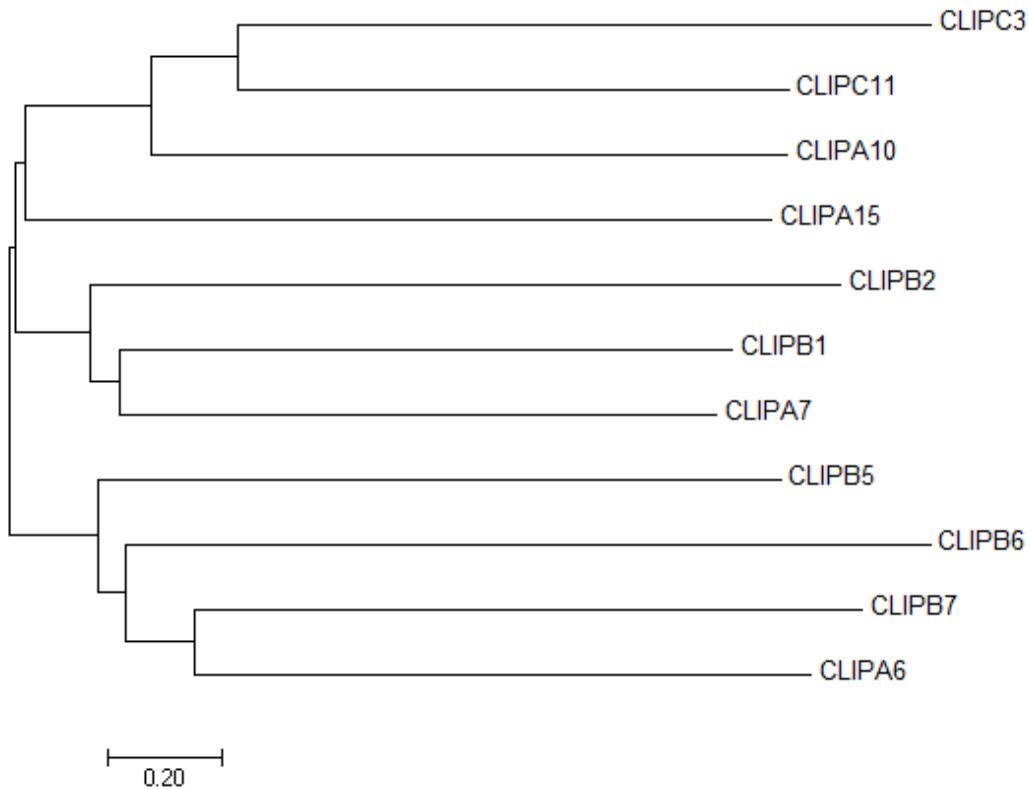
Figure 2. Neighbor joining tree constructed using standard parameters. Eleven annotated CLIP domain proteases phylogenetic relationships were compared within various insect species. 4 Tribolium (Tc), 4 Drosophila (Dm), 3 Manduca (Ms), 7 Anopeles (Ag), Aedes (Aa), and 5 Acrythosiphon (Ap).
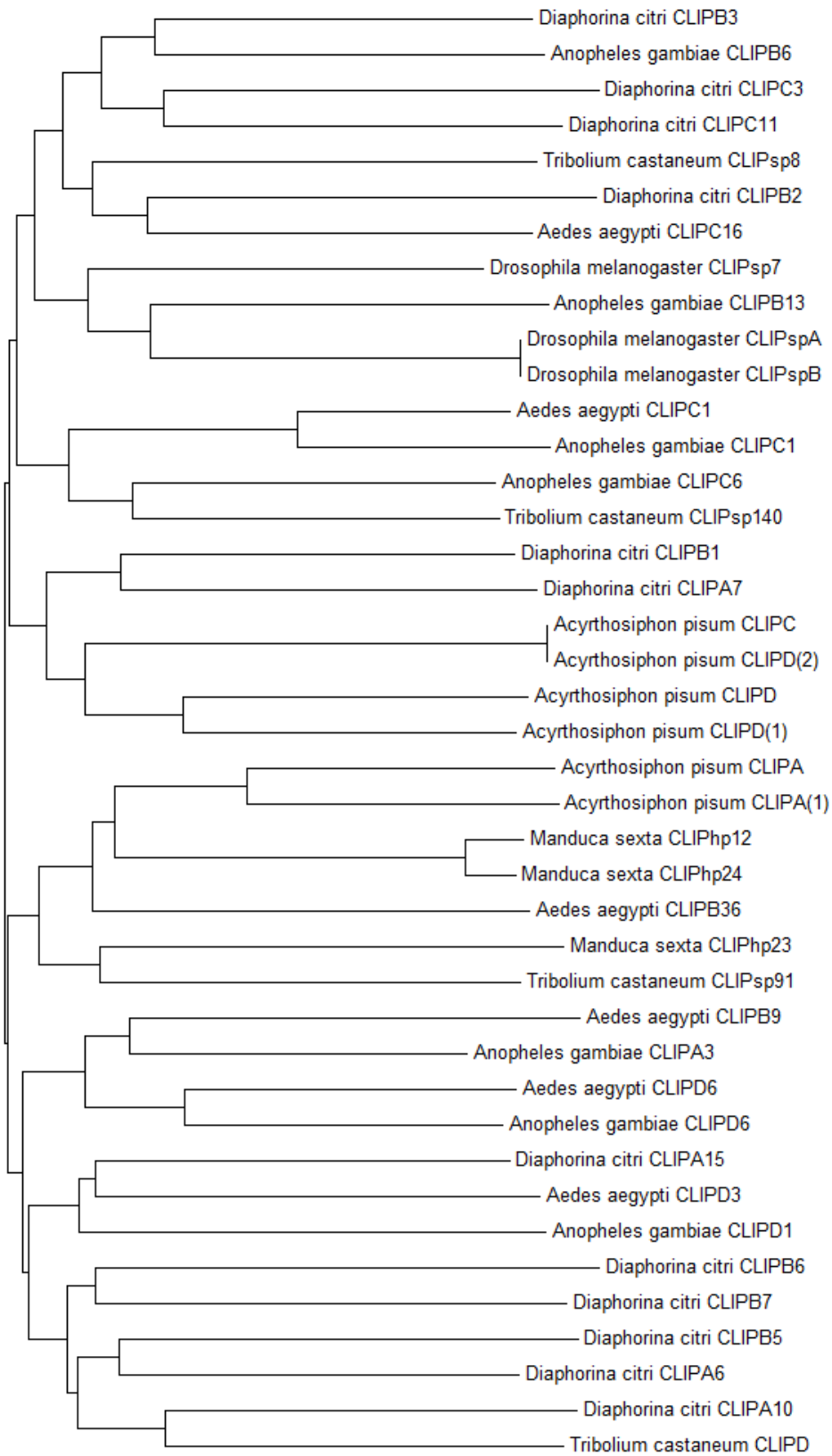
Table 3. MCOT blasts of annotated CLIP sequences. Sequences were blasted against the *Diaphorina citri* MCOT protein database using blastp.

	SubjectId	id%	Aln	evalue	Score
CLIPA7	MCOT03907.0.CO	99.54	217/218	e-155	448
CLIPA10	MCOT02226.0.CT	100	88/88	3.00E-51	177
CLIPA6	MCOT05567.0.CT	96.06	634/660	0	1149
CLIPA15	MCOT01310.0.CT	72.57	471/649	0	810
CLIPC11	MCOT00897.2.CO	89.67	243/271	e-170	484
CLIPC3	MCOT12281.1.CO	94.02	173/184	e-126	360
CLIPB1	MCOT23305.0.CC	100	334/334	0	676
CLIPB2	MCOT16598.0.CC	100	310/310	0	643
CLIPB5	MCOT05156.0.CT	92.13	234/254	e-167	471
CLIPB6	MCOT19384.1.CT	78.67	413/525	0	799
CLIPB7	MCOT16016.2.CC	99.53	211/212	e-153	443

Table 3 Cont.

	Description
CLIPA7	| Serine protease H2 | Similar to D6WBT0 | ***- | PANTHER PTHR24256 | PANTHER PTHR24256:SF203 | Pfam PF00089 Length = 440
CLIPA10	| Serine proteinase stubble | Similar to A0A034V6F0 | *-*- | PANTHER PTHR24258 | PANTHER PTHR24258:SF96 | Pfam PF00089
CLIPA6	| Serine proteinase stubble | Similar to A0A034V6F0 | *-*- | PANTHER PTHR24258 | PANTHER PTHR24258:SF96 | Pfam PF00089 Length = 1010
CLIPA15	| Serine protease 42 | Similar to W8C5K3 | *-*- | PANTHER PTHR24258 | PANTHER PTHR24258:SF105 | Pfam PF00089 Length = 762

CLIPC11	| Trypsin-like serine protease | Similar to Q9XY63 | ***- | PANTHER PTHR24256 | Pfam PF00089 Length = 391
CLIPC3	| Trypsin-like serine protease | Similar to Q9XY63 | ***- | PANTHER PTHR24256 | Pfam PF00089 Length = 308
CLIPB1	| Serine protease | Similar to Q6RX66 | ***- | PANTHER PTHR24256 | Pfam PF00089 Length = 360
CLIPB2	| Serine protease | Similar to W5JJ65 | ***- | PANTHER PTHR24256 | PANTHER PTHR24256:SF218 | Pfam PF00089 Length = 310
CLIPB5	| Serine protease | Similar to Q8MP08 | ***- | PANTHER PTHR24256 | Pfam PF00089 | Pfam PF12032 Length = 379
CLIPB6	| Trypsin-like serine protease | Similar to A8QL65 | ***- | PANTHER PTHR24258 | PANTHER PTHR24258:SF99 | Pfam PF00089 Length = 550
CLIPB7	| Trypsin-like serine protease | Similar to A8QL65 | ***- | PANTHER PTHR24256 | PANTHER PTHR24256:SF158 | Pfam PF00089 Length = 335

Results and Discussion

Eleven CLIPs (excluding partial models), from four subfamilies, within the *D. citri* genome were manually annotated using Web Apollo software (Table 1). A neighbor-joining tree was constructed using standard parameters (Fig 1). Phylogenetic relationships of the annotated CLIP domain proteases were compared with various insect species [Tribolium (Tc) XP_015839950.1, XP_008193916.1, EFA07452.2, EFA11600.2, Drosophila (Dm) 2XXL_A, NP_001097020.1, Manduca (Ms) AAZ91695.1, AAZ91694.1, AAZ91697.1, Anopeles (Ag) EAA03300.4, XP_307757.4, CAO83683.1, XP_552698.3, XP_001687772.2, XP_312523.4, XP_312099.2, Aedes (Aa) XP_001657092.1, XP_011492919.1, XP_001655815.2, XP_001662819.1, XP_001662898.2, XP_001654732.1, and Acrythosiphon (Ap) XP_001943192.2, XP_016663806.1, NP_001155379.1, BAH71307.1, XP_008178923.2].

A pairwise alignment (Table 2) identified several *A. pisum* CLIPA orthologs (CLIPA6, CLIPA7, CLIPA10, CLIPA15) with 37–60% identity, and 34–65% query coverage for CLIPA6, CLIPA7, and CLIPA10, while a query coverage of 96% was displayed in CLIPA15. Higher identities and query coverages (qc) for CLIPA6, CLIPA7, and CLIPA10 were found in *Aedes aegypti* (47% ident, 95% qc), *Cimex lectularius* (61% ident, 66% qc), and *Anopheles gambiae* (78% ident, 79% qc) respectively. Orthologs of CLIPC (3 and 11) and CLIPB (1, 2, 5, 6, and 7) in *A. pisum* were also found in *D. citri* with 36–61% identity and 81–100% query coverage.

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Supplementary Note 19: Autophagy

Introduction

Autophagy is the regulated breakdown of unnecessary or dysfunctional components of the cell. This process is critical and is highly conserved among all animals and is critical to the regulation of cell degradation and recycling of cellular components. The main pathway is marcoautophagy, where specific cytoplasmic components are isolated from the remaining cell in a double-membraned vesical called the autophagosome[1-3].

Materials and Methods

Drosophila melanogaster autophagy-related genes (Twenty total) were used to identify putative autophagy genes within the ACP genome and MCOT datasets. Blastx (e value = 10^{-10} limit) was used to compared the *Drosophila* autophagy-related genes to ACP predicted and MCOT protein sets.   The protein sequence for each corrected model was then subjected to a blastp at NCBI to ensure the correct autophagy gene had been identified.

Table 1. A comparison table between *D.citri* and *D. melanogastor* autophagy genes derived from the BLAST results.

Drosophila autophagy-related gene	E-value	Bit score	Predicted gene	Bit score	E value	MCOT gene
Atg1	7.37E-113	356.68	XP_008477352	357.45	2.99E-111	MCOT02628.0.CT_Serine/threonine-protein_kinase_N
Atg10	2.45E-14	67.4	XP_008486870	65.86	3.14E-13	MCOT03774.0.CT_Ubiquitin-like-conjugating_enzyme_ATG10
Atg101	2.62E-83	251.14	XP_008472596	251.52	4.40E-83	MCOT22746.3.CT_Autophagy-related_protein
Atg12	NA	NA	NA	105.92	1.15E-29	MCOT15209.2.CC_Ubiquitin-like_protein_ATG12
Atg13	6.37E-48	166.39	XP_008468708	223.02	5.00E-66	MCOT04981.0.CO_Autophagy-related_protein_13
Atg14	NA	NA	NA	162.16	1.67E-43	MCOT10433.0.OO_Beclin_1-associated_autophagy-related_key_regulator
Atg16	4.27E-66	211.46	XP_008479241	371.32	1.27E-124	MCOT22842.0.CO_Autophagy-related_protein_16-1
Atg17	3.99E-39	153.3	XP_008469662	342.43	1.36E-98	MCOT13132.0.CO_RB1-inducible_coiled-coil_protein_1
Atg18a	3.15E-64	209.92	XP_008476026	432.56	1.49E-148	MCOT11696.0.MO_WD_repeat_domain_phosphoinositide-interacting_protein_2
Atg18b	5.06E-33	127.49	XP_008476026	328.56	1.60E-106	MCOT19872.0.CO_WD_repeat_domain_phosphoinositide-interacting_protein_2
Atg2	1.07E-34	144.44	XP_008482845	297.36	4.81E-82	MCOT19312.0.CT_Autophagy-related_protein_2-like_protein_B
Atg3	5.48E-55	179.49	XP_008480509	380.95	5.48E-131	MCOT18670.0.CT_Autophagy-related_protein_3
Atg4a	2.26E-64	206.84	XP_008483713	378.64	9.81E-128	MCOT20756.0.CT_Cysteine_protease_ATG4B
Atg4b	1.79E-24	101.68	XP_008483713	183.34	9.46E-51	MCOT20756.0.CT_Cysteine_protease_ATG4B
Atg5	2.68E-78	240.35	XP_008475187	293.12	2.62E-98	MCOT13950.0.CT_Autophagy_protein_5
Atg6	1.52E-143	422.16	XP_008474622	465.69	5.56E-161	MCOT09494.0.MO_Beclin-1-like_protein
Atg7	1.21E-66	232.65	XP_008477335	525.01	3.14E-177	MCOT01675.2.CT_Ubiquitin-like_modifier-activating_enzyme_ATG7
Atg8a	7.77E-79	231.49	XP_008471089	213	7.12E-71	MCOT11945.0.MM_Adenosine_monophosphate-protein_transferase_FICD,_partial
Atg8b	3.82E-67	201.44	XP_008471089	183.34	3.53E-59	MCOT11945.0.MM_Adenosine_monophosphate-protein_transferase_FICD,_partial
Atg9	3.10E-157	476.48	XP_008486164	482.26	9.99E-158	MCOT13355.0.CT_Autophagy-related_protein_9A

Table 2. BLAST-based analyses of *D. citri* autophagy-associated genes comapred to those from the fruit fly (*Drosophila melanogaster*), bed bug (*Cimex lectularius*), and pea aphid (*Acyrthosiphon pisum*).

MCOT Identification	Autophagy gene name	*Drosophila melanogaster*				*Cimex lectularius*				*Acyrthosiphon pisum*			
		E-value	Greatest positive %	Name	Greatest bit score	E-value	Greatest positive %	Name	Greatest bit score	E-value	Greatest positive %	Name	Greatest bit score
MCOT01675	Atg7	4.2E-178	69.81132075	FBpp0085890	527.709	3.31E-78	78.0701754	CLEC000439-RA	259.61	0	64.893617	ACYPI008937-PA	585.489
MCOT02628	Atg1	5.2E-122	81.15942029	FBpp0289788	385.956	0	69.470405	CLEC009361-RA	592.808	7.1E-173	65.0297619	ACYPI000902-PA	511.916
MCOT03774	Atg10	6.43E-13	51.2605042	FBpp0309509	65.855	3.95E-13	44.3548387	CLEC003625-RA	65.4698	9.59E-23	67.5	ACYPI53898-PA	93.2041
MCOT04981	Atg13	5.44E-67	51.50115473	FBpp0081333	225.713	7E-69	76.300578	CLEC000332-RA	219.55	2.66E-63	58.0110497	ACYPI41944-PA	214.157
MCOT09494	Atg6	2.1E-148	73.98568019	FBpp0083975	433.721	3.6E-167	79.5294118	CLEC006228-RA	481.1	3E-165	77.8042959	ACYPI004000-PA	476.093
MCOT10433	Atg14	2.42E-51	55.76923077	FBpp0073859	184.496	8.2E-99	63.8826185	CLEC011142-RA	308.145	1.73E-67	57.5342466	ACYPI005194-PA	226.868
MCOT11696	Atg18a	2.9E-149	79.60339943	FBpp0076416	434.876	7.4E-144	82.9581994	CLEC000472-RA	418.698	8E-170	76.1445783	ACYPI006216-PA	488.419
MCOT11945	Atg8a	1.12E-70	74.35897436	FBpp0071427	213.001	2.34E-70	74.025974	CLEC002365-RA	211.46	7.37E-65	72.5490196	ACYPI004701-PA	197.593
MCOT13132	Atg17	7.5E-108	55.55555556	FBpp0311128	369.007	2.6E-102	64.2857143	CLEC013328-RA	332.028	5E-125	50.3286385	ACYPI38993-PA	410.223
MCOT13355	Atg9	2.3E-167	68	FBpp0293153	507.294	9.7E-135	69.3304536	CLEC011740-RA	423.32	1.2E-159	75	ACYPI000660-PA	482.641
MCOT13950	Atg5	2.82E-90	72.3880597	FBpp0071023	272.707	2.93E-68	71.8918919	CLEC007777-RA	212.231	3.98E-97	73.4082397	ACYPI005861-PA	289.656
MCOT15209	Atg12	1.97E-29	62.96296296	FBpp0289288	105.531	5.31E-24	60.5504587	CLEC000229-RA	90.8929	3.4E-39	72.5663717	ACYPI000775-PA	130.183
MCOT18670	Atg3	1.7E-136	77.97619048	FBpp0074822	395.201	3.9E-141	79.0625	CLEC005511-RA	405.216	2.2E-132	72.3342939	ACYPI072921-PA	384.415
MCOT19312	Atg2	1.63E-73	73.54085603	FBpp0305632	270.011	0	59.8319328	CLEC010004-RA	843.573	1.8E-161	81.3688213	ACYPI009929-PA	531.176
MCOT19872	Atg18b	3.8E-149	80.74712644	FBpp0302864	434.876	1.3E-143	82.9581994	CLEC000472-RA	418.313	2.5E-167	84.7507331	ACYPI006216-PA	482.256
MCOT20756	Atg4b	1.6E-127	69.68838527	FBpp0304824	378.637	1.3E-174	70.9976798	CLEC005624-RA	498.434	1.1E-179	84.2975207	ACYPI001437-PD	510.76
MCOT20756	Atg4a	1.6E-127	69.68838527	FBpp0304824	378.637	1.3E-174	70.9976798	CLEC005624-RA	498.434	1.1E-179	84.2975207	ACYPI001437-PD	510.76
MCOT22746	Atg101	4.1E-83	67.76859504	FBpp0074406	251.906	3.76E-97	71.0743802	CLEC007299-RA	286.96	4.18E-81	70.8333333	ACYPI005306-PA	246.128
MCOT22842	Atg16	1E-121	72.66666667	FBpp0084895	370.163	5.32E-75	77.486911	CLEC009445-RA	240.35	3.7E-170	79.6568627	ACYPI065154-PA	491.886

Results and Discussion

Based upon the blast results, we were able to identify 17/20 autophagy-related genes from the ACP genome and MCOT gene sets (Table 1; Table 2). There is only a single autophagy-related 8 gene in ACP gene sets compared to two for the *Drosophila* gene set, but this is common for non-dipteran insects[2]. Thus, as expected, psyllids have the required repertoire of autophagy-related genes to undergo marcoautophagy. This suggests that utilized of the gene sets from the genome and MCOT can identify conserved genes for ACP by comparison to other insects such as *Drosophila*.

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Supplementary Note 20: Dicer

Introduction

RNase III enzymes are molecules which exhibit specificity for dsRNA. Three subclasses of RNase III enzymes have been identified to date; the *Escherichia coli* RNase III class (class I), the Drosha class (class II) and the Dicer-like class (class III). Typically, enzymes in the Dicer-like class contain an N-terminal DEXH-box RNA helicase domain, a domain of unknown function known as DUF283, a PAZ (PIWI-Argonaute-Zwille) domain, two RNase III domains and a dsRNA binding domain. The presence of these domains allow Dicer (Dcr) to function as a ribonuclease, cleaving long dsRNA molecules into small effector molecules, in both the miRNA and RNAi pathways (reviewed in (Carmell and Hannon, 2004). In vertebrates and some invertebrates, such as *Caenorhabditis elegans*, one protein (Dcr-1) is involved in both pathways (Bernstein et al., 2001; Ketting et al., 2001; Knight and Bass, 2001). In other organisms, such as *Drosophila melanogaster*, different Dcr proteins function in each pathway. In *D. melanogaster* Dm-Dcr-1 functions in the miRNA pathway while Dm-Dcr-2 functions in the RNAi pathway (Lee et al., 2004).

Methods

Insect Dicer protein sequences were obtained from NCBI, ImmunoDB and the i5k workspace. BLASTp analysis was used to query the predicted *Diaphorina citri* protein sets (Diacit_International_psyllid_consortium_proteins_v1 and Diacit_RefSeq_proteins_Release_100) at i5k@NAL. Loci encoding putative homologs were identified and manually annotated in Web Apollo using RNA-Seq data as evidence for transcript structure. EMBL EBI's online InterPro program was used for domain identification. MEGA7 software was used to produce multiple sequence alignments of insect Dicer and RNase III enzyme homologs (ClustalW). Neighbor-joining phylogenetic trees were constructed in MEGA7 using multiple sequence alignments of predicted protein sequences.

Table 1: Number of Dicer homologs in insect species.

Species	Dicer 1	Dicer 2
D. melanogaster	1	1
A. gambiae	1	1
A. aegypti	1	1
C. quinquefasciatus	1	1
T. castaneum	1	1
C. lectularius	1	1
D. citri	1 or 2	1 or 2

Table 2: Bit score, query coverage, identity and e-value results from BLAST analysis of predicted Dicer1, Dicer2RA, Dicer2RB, Dicer like partial and Endonuclease Dicer to insect Dcr homologs. The scores given are the scores from the named ortholog in each insect species. In the cases of Dicer like partial and Endonuclease Dicer whether the hit was to Dicer1 or Dicer2 has been denoted in the Bit Score column.

	Top Blastp Hit		*D. melanogaster*	*A. gambiae*	*A. aegypti*	*C. quinquefasciatus*	*T. castaneum*	*C. lectularius*
D. citri Dicer 1	Dcr 1 *C. costatus*	**Bit score**	639	688	724	668	639	1145
		QC (Identity)	92% (59%)	90% (35%)	90% (36%)	89% (61%)	79% (65%)	94% (49%)
		E value	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
D. citri Dicer 2RA	Dicer 2 *N. lugens*	**Bit score**	94	91.3	99	92.8	97.4	113
		QC (Identity)	15% (41%)	16% (39%)	63% (41%)	68% (42%)	42% (41%)	40% (41%)
		E value	1.00E-19	5.00E-19	2.00E-21	2.00E-19	1.00E-20	8.00E-26
D. citri Dicer 2RB	Dicer 2 *N. lugens*	**Bit score**	94.7	91	99	92.8	97.4	113
		QC (Identity)	15% (41%)	16% (39%)	63% (41%)	68% (42%)	42% (41%)	40% (41%)
		E value	1.00E-19	5.00E-19	2.00E-21	2.00E-19	1.00E-20	8.00E-26
D. citri Dicer like partial	Dicer like *N. vespilloides*	**Bit score**	166 (Dcr1)	180 (Dcr1)	187 (Dcr2)	191 (Dcr2)	208 (Dcr2)	191 (Dcr1)
		QC (Identity)	45% (39%)	52% (37%)	59% (41%)	48% (42%)	69% (43%)	61% (48%)
		E value	2.00E-42	1.00E-47	5.00E-50	4.00E-51	2.00E-56	1.00E-60
D. citri Endonuclease Dicer	Endoribonuclease Dicer *C. floridanus*	**Bit score**	82 (Dcr1)	84 (Dcr1)	97.1 (Dcr2)	87.8 (Dcr2)	80.9 (Dcr2)	83.2 (Dcr2)
		QC (Identity)	61% (34%)	61% (34%)	64% (39%)	63% (34%)	55% (39%)	66% (29%)
		E value	1.00E-17	8.00E-19	2.00E-23	4.00E-20	2.00E-17	2.00E-18

Results and Discussion

In *D. citri*, initial BLASTp searches with known insect Dcr proteins identified four loci encoding predicted Dcr proteins (Table 1). InterPro analysis of the first Dcr identified (Dicer1) revealed that the predicted protein contained a DEXH box RNA helicase domain, RNase III domains and a PAZ domain validating its identification as a Dcr protein. However, incomplete assembly of the *D. citri* genome has made analysis of the final three *Dcr* loci more difficult. Dicer2, as currently annotated, contains both a helicase and PAZ domain but seems to be lacking the RNase III domain. Whether this represents a true domain loss or is an artifact of inaccurate assembly or incomplete annotation is unknown. RNA-Seq data suggests that at least two isoforms of this gene are produced, they have been designated *Dicer2A* and *Dicer2B*. The third *Dcr* loci identified (*endoribonuclease Dicer*) is a fragment of DNA on a very short contig (~3,000bp) suggesting that this portion of the genome may not be assembled correctly. Analysis of this fragment revealed that this sequence only encodes a PAZ domain. As PAZ domains are only found in Dcr and Argonaute (AGO) proteins (Carmell and Hannon, 2004) it is possible that this fragment (*endoribonuclease Dicer*) may in fact belong to one of the previously identified Dcr or AGO proteins but has been incorrectly assembled resulting in a partial gene fragment (although multiple sequence alignments of *D. citri* AGO and Dcr proteins does not support this idea). Alternatively, it could represent a fragment of a duplicated *Dicer* gene as yet unidentified. The final *Dcr* loci identified (*Dicer like partial*) is at the end of a contig and is thus incomplete.

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Domain analysis of this fragment indicates a helicase domain and RNase III domain are present in the available sequence. Reciprocal blast analysis of the four identified loci all indicate that these proteins share homology with other insect Dcr proteins but determining true orthology for Dicer like partial and Endonuclease Dicer is not possible at this time (Table 2).

Figure 1: Phylogenetic tree of inset Dicer proteins. Dicer 1 is represented in blue Dicer 2 is represented in purple. *D. citri* proteins have been identified with a taxon marker.
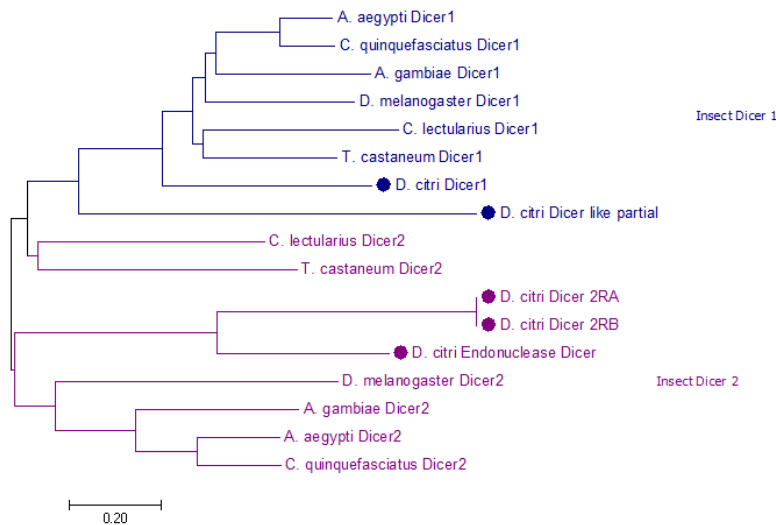
Due to incomplete gene models we are unable to make a definitive determination of *Dcr* gene number in *D. citri* but there are likely three and perhaps four *Dcr* genes in *D. citri* (Table 1). Incomplete sequences may influence phylogenetic tree analysis but based on the data we have Dicer1 is orthologous to other insect Dcr1 proteins (Figures 1 and 2). The isoforms Dicer 2RA and Dicer 2RB, as currently annotated, cluster with other Dicer2 proteins (as does the Endoribonuclease Dicer fragment) (Figure 1). Finally, Dicer like partial shows similarity with both Dcr-1 (Figure 1) and Dcr-2 (Figure 2). More complete gene models or complete sequences from experimental data is necessary to determine the exact relationship between *D. citri* Dcr proteins and other insect Dcrs.

Figure 2: Phylogenetic tree depicting clustering of insect Class II (Drosha) and Class III (Dicer) RNase III enzymes. Blue represents likely Dicer1 homologs. Purple represents likely Dicer2 homologs. Teal represents Drosha homologs. *D. citri* proteins have been marked with a taxon marker.

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Supplementary Note 21: Drosha

Introduction

Drosha belongs to a class (class II) of RNase III enzymes. Drosha proteins characteristically contain two tandem RNase III domains and a C-terminal dsRNA binding domain. One of Drosha's primary roles in the cell is in the processing of microRNAs (miRNAs). Drosha's catalytic activity allows it to cleave primary miRNA (pri-miRNA) molecules, which have been transcribed by the cell, into precursor miRNA (pre-miRNA) molecules which are transported from the nucleus via Exportin-5 and then are further processed by Dicer (a class III RNase III enzyme) into their mature form (reviewed in (Ha and Kim, 2014; Connerty et al., 2015).
Drosha is conserved in animals, and most insects with a sequenced genome (and annotated RNAi and miRNA genes) have been shown to have 1 Drosha homolog (Campbell et al., 2008; Tomoyasu et al., 2008; Jaubert-Possamai et al., 2010; Xu et al., 2013).

Methods

Insect Drosha protein sequences were obtained from NCBI, ImmunoDB and the i5k workspace. BLASTp analysis was used to query the predicted *Diaphorina citri* protein sets (Diacit_International_psyllid_consortium_proteins_v1 and Diacit_RefSeq_proteins_Release_100) at i5k@NAL. Loci encoding putative homologs were identified and manually annotated in Web Apollo using RNA-Seq data as evidence for transcript structure. EMBL EBI's online InterPro program was used for domain identification. MEGA7 software was used to produce multiple sequence alignments of insect Drosha and RNase III homologs (ClustalW). Neighbor-joining phylogenetic trees were constructed in MEGA7 using multiple sequence alignments of predicted protein sequences.

Table 1: Number of Drosha homologs in insect species

Species	Drosha
D. melanogaster	1
A. gambiae	1
A. aegypti	1
C. quinquefasciatus	1
T. castaneum	1
C. lectularius	1
D. citri	2 or 3

Table 2: Bit score, query coverage, identity and e-value results from BLAST analysis of predicted Drosha1, Drosha2 partial and Drosha3 to insect Drosha homologs.

		D. melanogaster	*A. gambiae*	*A. aegypti*	*C. quinquefasciatus*	*T. castaneum*	*C. lectularius*
D. citri Drosha 1	**Bit score**	567	588	587	42	522	592
	QC (Identity)	95% (72%)	96% (73%)	96% (73%)	83.2% (16%)	94% (67%)	955 (73%)

	E value	0.00E+00	0.00E+00	0.00E+00	4.00E-04	2.00E-176	0.00E+00
D. citri Drosha 2 partial	**Bit score**	917	967	974	731	1060	1044
	QC (Identity)	78% (57%)	77% (58%)	81% (57%)	64% (56%)	77% (64%)	81% (62%)
	E value	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
D. citri Drosha 3	**Bit score**	347	366	370	379	443	443
	QC (Identity)	61% (45%)	60% (45%)	67% (45%)	51% (48%)	59% (54%)	59% (54%)
	E value	2.00E-100	3.00E-107	7.00E-109	2.00E-116	1.00E-136	1.00E-136

Figure 1: Phylogenetic tree of insect Drosha homologs. Insect species are depicted with differing colors. *D. citri* proteins are marked with a taxon marker.
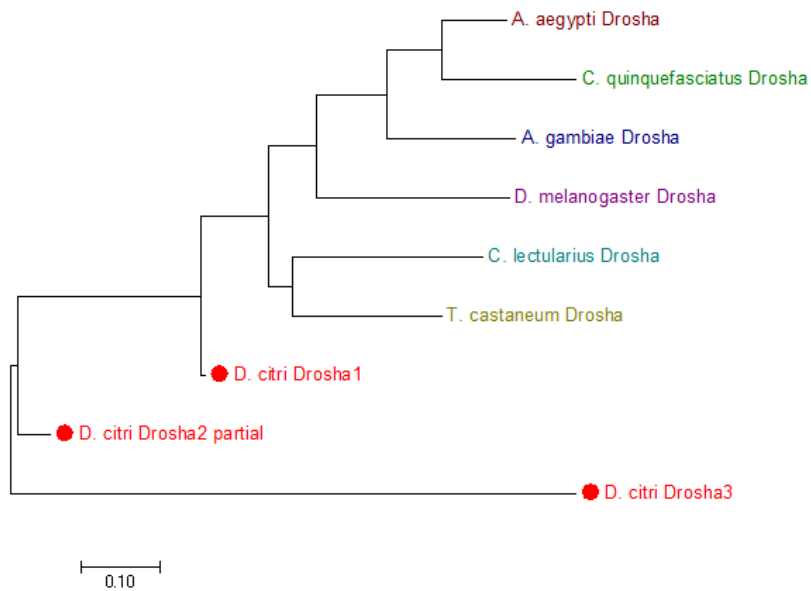

Figure 2: Phylogenetic tree depicting clustering of insect Class II (Drosha) and Class III (Dicer) RNase III enzymes. Blue represents likely Dicer1 homologs. Purple represents likely Dicer2 homologs. Teal represents Drosha homologs. *D. citri* proteins have been marked by a taxon marker.

A. aegypti Dicer1
C. quinquefasciatus Dicer1
A. gambiae Dicer1
D. melanogaster Dicer1 — RNase Class III Dicer 1
T. castaneum Dicer1
C. lectularius Dicer1
● D. citri Dicer1

C. lectularius Dicer2
T. castaneum Dicer2
● D. citri Dicer like partial
D. melanogaster Dicer2 — RNase Class III Dicer 2
A. gambiae Dicer2
A. aegypti Dicer2
C. quinquefasciatus Dicer2
● D. citri endonuclease dicer
● D. citri Dicer 2RA
● D. citri Dicer 2RB

● D. citri Drosha3
A. aegypti Drosha
C. quinquefasciatus Drosha
A. gambiae Drosha
D. melanogaster Drosha — RNase Class II Drosha
C. lectularius Drosha
T. castaneum Drosha
● D. citri Drosha1
● D. citri Drosha2 partial

0.20

Results and Discussion

In *D. citri*, initial BLASTp searches with known insect Drosha proteins identified three loci encoding predicted Drosha proteins (Table 1). While all three genes identified show homology to Drosha, as shown by reciprocal BLAST analysis (Table 2), their position in the current *D. citri* genome assembly has made their annotation and analysis difficult. The first gene identified, currently named *D. citri drosha2 partial* (*drosha2 partial*), is located at the end of a contig, causing an artificial truncation of the gene. The second gene, currently named *D. citri drosha1* (*drosha1*) is near the end of another contig and was found within an intron of another annotated gene, *D. citri transmembrane protein 179*, making analysis of RNA-Seq data difficult. The third gene, currently named *D. citri drosha 3* (*drosha3*) is also near the end of a contig and, of the three, shares the least similarity to other known Drosha proteins.

EMBL EBI's InterPro analysis of Drosha1 revealed that despite its small size this predicted protein is the most complete of all the *D. citri* Drosha proteins as it contains two RNase III domains and the dsRNA binding domain. InterPro analysis of Drosha2 partial identified one full and one truncated RNase III domain. This result is no doubt the result of *drosha2 partial's* position at the end of the contig, resulting in the loss of the C-terminal end of the protein sequence, as opposed to a true loss of the second RNase III domain and dsRNA binding domain. InterPro analysis of Drosha3 only identified a dsRNA binding domain in this sequence. This

result could indicate that this third *drosha* gene has become highly diverged over time or this may not be a complete gene and may represent the 3' end of *drosha2 partial*.

Phylogenic analysis of insect Drosha proteins (Figure 1), and of RNase III class II and class III proteins (Figure 2) indicate that the three loci identified are homologous to other insect Drosha proteins. However, conclusions about the exact number of *D. citri* homologs and the relationship between Drosha proteins are unable to be drawn due to incomplete sequence information and genome fragmentation.

At least two and possibility three *drosha* genes have been identified in *D. citri*. Two *pasha* genes (the partner of *drosha*) have also been identified. This increase in miRNA processing components may increase the ability of *D. citri* cells to produce miRNAs but it is important to note the Drosha and Pasha have been shown to have other cellular functions within the cell (Han et al., 2009; Karginov et al., 2010; Macias et al., 2012). Therefore, their duplication and subsequent maintenance in the *D. citri* genome may be unrelated to their function in the miRNA pathway.

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Supplementary Note 22: Pasha protein

Introduction

Pasha is the dsRNA binding protein (dRBP) partner of Drosha (Denli et al., 2004). dRBPs have a motif allowing them to interact with dsRNA and have been shown to perform a variety of cellular functions within the cell (Saunders and Barber, 2003). Pasha is known to be specifically involved in the microRNA (miRNA) pathway through interactions with its RNase III protein partner Drosha (Denli et al., 2004). When miRNA precursors are transcribed from non-coding genes within a cell these primary transcripts form imperfect dsRNA hairpins which are bound by Pasha and processed by Drosha before leaving the nucleus where they are further processed by Dicer into mature miRNAs (reviewed in (Ouellet et al., 2006; Lucas and Raikhel, 2013).

Methods

Pasha homolog sequences from *Drosophila melanogaster*, *Anopheles gambiae*, *Aedes aegypti*, *Culex quinquefasciatus*, *Tribolium castaneum* and *Cimex lectularius* were used to query the predicted *Diaphorina citri* protein sets (Diacit_International_psyllid_consortium_proteins_v1 and Diacit_RefSeq_proteins_Release_100) at i5k@NAL via BLASTp analysis. Putative homologs were identified, renamed, and manually annotated in Web Apollo using RNA-Seq data as evidence for transcript structure. MEGA 7 software was used to create multiple sequence alignments (ClustalW) and build neighbor-joining phylogenic trees from full length Pasha homologs.

Table 1: Number of Pasha homologs in insect species

Species	Pasha
D. melanogaster	1
A. gambiae	1
A. aegypti	1
C. quinquefasciatus	1
T. castaneum	1
C. lectularius	1
D. citri	2

Table 2: Bit score, query coverage, identity and e-value results from BLAST analysis of predicted Pasha 1 and Pasha 2 to insect Pasha homologs.

		D. melanogaster	*A. gambiae*	*A. aegypti*	*C. quinquefasciatus*	*T. castaneum*	*C. lectularius*
D. citri Pasha 1	**Bit score**	434	327	398	410	423	409
	QC (Identity)	79% (44%)	78% (46%)	81% (41%)	83% (41%)	77% (46%)	80% (45%)
	E value	9.00E-146	3.00E-103	2.00E-132	3.00E-135	1.00E-141	3.00E-136

D. citri Pasha 2	Bit score	340	312	303	301	326	301
	QC (Identity)	86% (37%)	79% (34%)	84% (34%)	84% (33%)	81% (38%)	85% (33%)
	E value	6.00E-110	3.00E-98	5.00E-96	4.00E-94	2.00E-104	6.00E-95

Figure 1: Phylogenetic tree depicting clustering of insect Pasha homologs. Blue represents insects in the order Diptera. Teal represents insects in the order Coleoptera. Purple represents insects in the order Hemiptera. *D. citri* proteins are designated with a taxon marker.
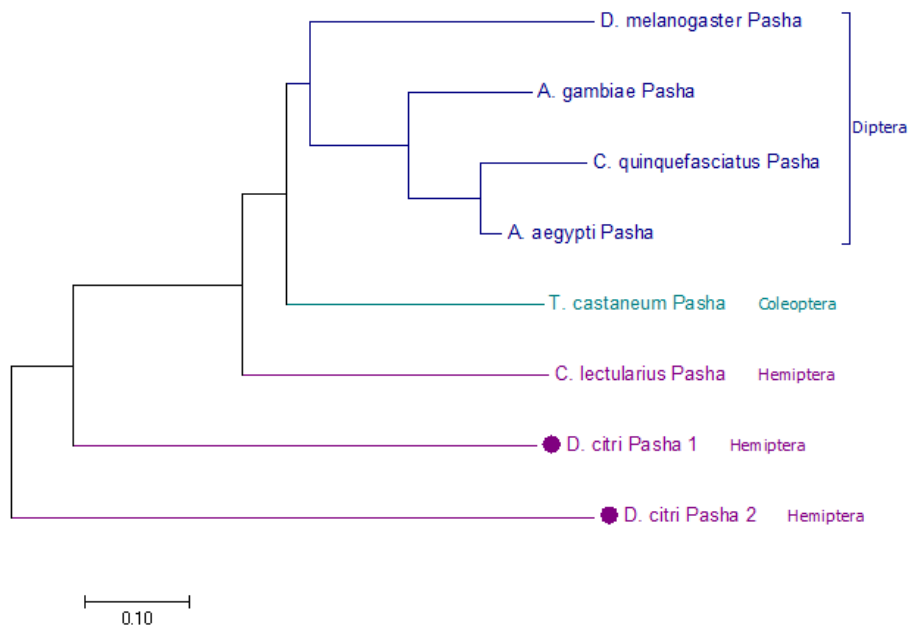
Results and Discussion

BLASTp analysis of the *D. citri* genome revealed two putative Pasha homologs (Table 1). We have named these genes *D. citri pasha 1* (*pasha 1*) and *D. citri pasha 2* (*pasha 2*). Both of these identified proteins have dsRNA binding domains and show homology to fly, mosquito, beetle and bedbug Pasha homologs (Table 2).

Phylogenetic analysis illustrates appropriate clustering of insect Pasha homologs, with the Diptera forming one clade and the Hemiptera proteins located more basally (Figure 1). While a single Pasha has been identified in the *D. melanogaster*, *A. gambiae*, *A. aegpti*, *C. quinquefasciatus*, *T. castaneum* and *C. lectularius* genomes, two have been found in the *D. citri* genome. This could be the result of gene loss of one Pasha homolog in studied model insects or, more likely, could be the result of gene duplication and rapid divergence of Pasha paralogs in *D. citri*. Interestingly, multiple Drosha proteins have also been identified in *D. citri*.

Figure 2: Phylogenetic tree depicting clustering of insect dRBPs. Loquacious homologs are shown in blue. R2D2 homologs are shown in teal. Pasha homologs are shown in purple. *D. citri* proteins are designated with a taxon marker.
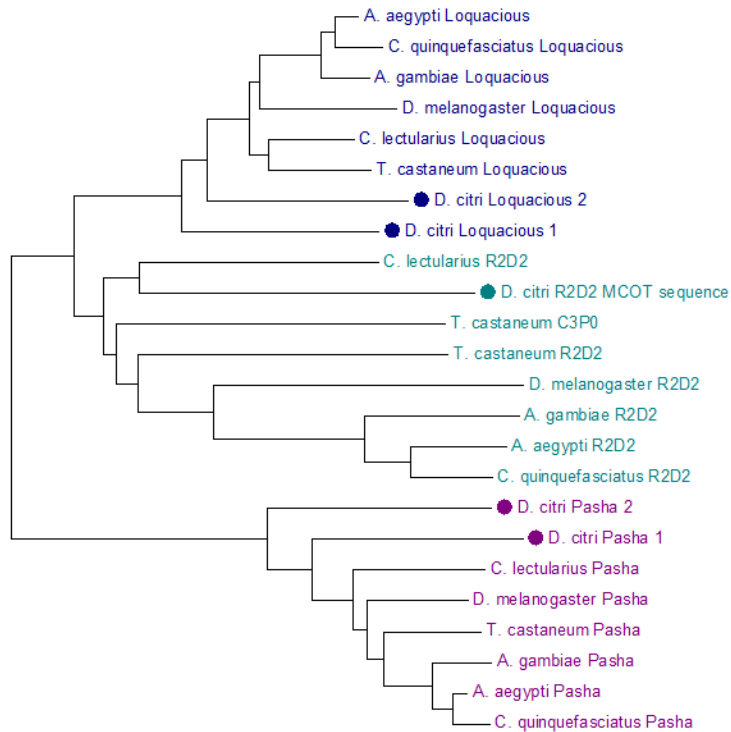
It is important to note that, to our knowledge, extensive functional analysis has not been performed on RNAi and miRNA core machinery genes in insects outside of *Drosophila*. However, if we assume that as in *Drosophila* Pasha 1 and Pasha 2 act as partners to Drosha one must speculate whether a specific *D. citri* Pasha protein partners with a specific Drosha protein, or whether these proteins participate in more of a free-for-all approach with available Pasha proteins partnering with any available Drosha protein. Further phylogenetic analysis of all known dRBPs involved in RNA silencing demonstrates Pasha 1 and Pasha 2 are more similar to other insect Pasha's than other dRBPs (Loquacious and R2D2) (Figure 2) confirming the results of our reciprocal BLAST analysis (Table 2). It has been reported that the pea aphid *Acyrthosiphon pisum* has seen a duplication of genes involved in the miRNA pathway with 4 *pasha* genes, 2 *loquacious* genes, 2 *Dicer-1* genes, and 2 *Argonaute 1* genes. In contrast, the number of genes involved in the RNAi pathway in *A. pisum* is similar to the number reported in other insects with sequenced genomes (Jaubert-Possamai et al., 2010). This first genome assembly in *D. citri* seems to show a similar trend with duplicated *drosha*, *pasha*, *loquacious* and possibly *Dicer-1* genes. Determining the function of these additional miRNA machinery genes, which have diverged but been maintained over evolutionary time, would be an enticing endeavor.

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Supplementary Note 23: Loquacious and R2D2 proteins

Introduction

Loquacious (Loqs) and R2D2 are dsRNA binding proteins (dRBP) which play an essential role in the miRNA and RNAi pathways. These dsRBPs partner with Dicer during cleavage of dsRNA into small effector molecules (miRNAs and siRNAs) and, in the case of R2D2, aid in loading these small RNA molecules into the RNA-induced silencing complex (RISC) (Liu et al., 2003; Leuschner et al., 2005; Saito et al., 2005; Liu et al., 2006; Liu et al., 2007; Okamura et al., 2011). In *Drosophila melanogaster* these two proteins have subfunctionalized so that Loqs interacts specifically with Dicer1 in the miRNA pathway and R2D2 interacts specifically with Dicer2 in the RNAi pathway (reviewed in (Paroo et al., 2007).

Thus far there are limited functional studies of the core RNAi and miRNA machinery genes outside of the leading insect model *D. melanogaster*. However, the few studies which have been performed suggest that perhaps this subfunctionalization of dsRBPs may be a relatively recent event in the dipteran lineage. In *Aedes aegypti* an isoform of Loqs has recently been found to interact with siRNA components and influences the quantity of siRNAs present in mosquito cells (Haac et al., 2015). In *Tribolium castaneum* knockdown of Loqs via RNAi did not result in the loss of miRNA phenotype seen in Ago1 knocked down individuals, suggesting the dsRBPs may act redundantly in small RNA pathways in *T. castaneum* (Tomoyasu et al., 2008). Furthermore, more extensive studies in *D. melanogaster* indicate that in some situations Loqs can also associate with Dicer2 in the siRNA pathway (Czech et al., 2008).

Methods

Loqs and R2D2 homolog sequences based on peer-reviewed literature were collected from NCBI and were used to query the predicted *Diaphorina citri* protein sets (Diacit_International_psyllid_ consortium_proteins_v1 and Diacit_RefSeq_proteins_Release_100) at i5k@NAL with the BLASTp tool. Plausible homologs were run through the Web Apollo genome browser in order to identify *D. citri* putative homologs. These homologs were manually annotated in Web Apollo using RNA-Seq data as evidence for transcript structure. Resulting protein sequences were run through a BLAST search on NCBI to confirm homology and domain conservation was confirmed through the domain database on NCBI and by EMBL EBI's online InterPro program. MEGA 7 software was used to create multiple sequence alignments (ClustalW) and build neighbor-joining phylogenic trees from full length homologs.

Table 1: Number of Loquacious and R2D2 homologs in insect species. * Indicates evidence for this homolog was found in MCOT

Species	Loquacious	R2D2
D. melanogaster	1	1

A. gambiae	1	1
A. aegypti	1	1
C. quinquefasciatus	1	1
T. castaneum	1	2
C. lectularius	1	1
D. citri	2	1*

Table 2: Bit score, query coverage, identity and e-value results from BLAST analysis of predicted Loq1 and Loqs2 to insect Loqs Homologs

	Top Blastp Hit		D. melanogaster	A. gambiae	A. aegypti	C. quinquefasciatus	T. castaneum
D. citri Loqs1	M. destructor Loqs	Bit score	223	208	208	202	233
		QC (Identity)	80% (40%)	84% (41%)	85% (41%)	92% (38%)	84% (43%)
		E value	1.00E-68	1.00E-64	1.00E-64	9.00E-62	1.00E-73
D. citri Loqs2	M. destructor Loqs	Bit score	213	202	222	207	224
		QC (Identity)	98% (40%)	75% (46%)	75% (49%)	97% (44%)	96% (43%)
		E value	1.00E-65	2.00E-63	8.00E-71	6.00E-65	2.00E-71

Results and Discussion

Most insects used in our BLASTp analysis have been shown to have one *loqs* ortholog and one *r2d2* ortholog. The one exception to this is *T. castaneum* which has been shown to have a second, highly diverged, *r2d2* paralog named *C3P0* (Tomoyasu et al., 2008) (Table 1). BLASTp analysis of the *D. citri* genome identified two loci similar to both Loqs and R2D2. Reciprocal BLAST analysis of these two putative homologs identified them both as Loqs orthologs (Table 2).

Figure 1: Phylogenetic tree depicting clustering of insect dRBPs. Loquacious homologs are shown in blue. R2D2 homologs are shown in teal. Pasha homologs are shown in purple. *D. citri* proteins are designated with a taxon marker.

A. aegypti Loquacious
C. quinquefasciatus Loquacious
A. gambiae Loquacious
D. melanogaster Loquacious
C. lectularius Loquacious
T. castaneum Loquacious
D. citri Loquacious 2
D. citri Loquacious 1
C. lectularius R2D2
D. citri R2D2 MCOT sequence
T. castaneum C3P0
T. castaneum R2D2
D. melanogaster R2D2
A. gambiae R2D2
A. aegypti R2D2
C. quinquefasciatus R2D2
D. citri Pasha 2
D. citri Pasha 1
C. lectularius Pasha
D. melanogaster Pasha
T. castaneum Pasha
A. gambiae Pasha
A. aegypti Pasha
C. quinquefasciatus Pasha
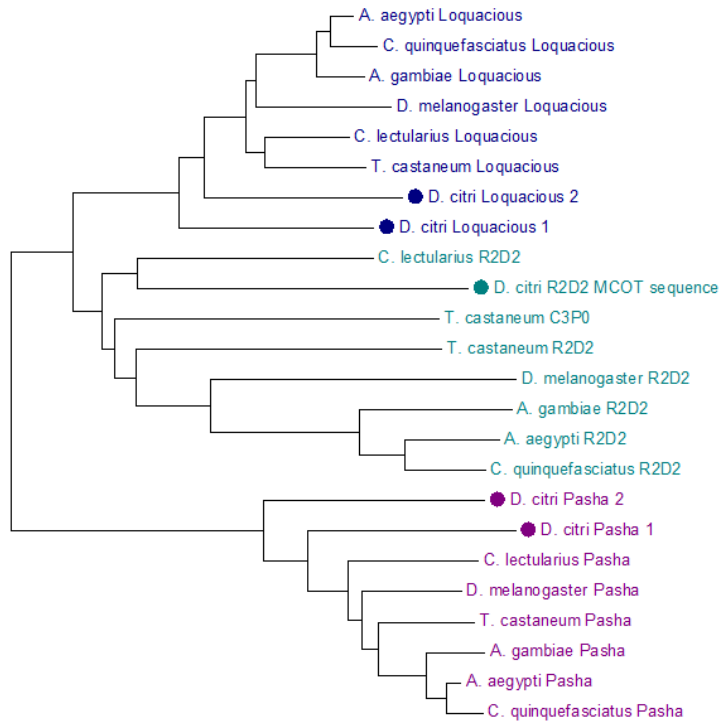
Furthermore, phylogenetic analysis of all three dsRBPs involved in RNA silencing mechanisms show that these *D. citri* genes are found in the insect Loqs clade (Figure 1). Therefore, we have tentatively named these two genes *D. citri loqs1* (*loqs1*) and *D. citri loqs2* (*loqs2*). The presence of multiple *loqs* paralogs is not unprecedented as two *loqs* paralogs have also been identified in the pea aphid *Acyrthosiphon pisum* (Jaubert-Possamai et al., 2010). Initially we are unable to identify a true R2D2 ortholog in the *D. citri* genome, therefore, we used insect R2D2 orthologs to search the MCOT transcriptome. This analysis identified a gene showing homology to insect R2D2 proteins and while fragments of this gene could be found in the *D. citri* genome, fragmentation and possible misassembly of the genome at this location made annotation impossible. Future genome assemblies will need to be examined to determine if a true *r2d2* ortholog exists in the *D. citri* genome. If *D. citri* is in fact lacking an *r2d2* ortholog this is not unprecedented as the recent publication of the grasshopper *Shirakiacris shirakii* transcriptome suggests that it may lack an *r2d2* ortholog (Qiu et al., 2016) and, as suggested in the introduction, dsRBPs may be capable of functioning outside of their canonical pathway. If this is the case in *D. citri*, the loss of R2D2 would not necessarily be catastrophic, as one of the two Loqs proteins could potentially act in the RNAi pathway. Loqs and R2D2 are very similar proteins and domain analysis of insect Loqs and R2D2 orthologs indicate that Loqs typically contains three dsRNA binding domains, at least one interferon-inducible dsRNA-dependent protein kinase activator A domain and a Staufen domain while R2D2 contains only two dsRNA binding domains. Domain analysis of the Loqs orthlogs found in the *D. citri* genome and the R2D2 ortholog found in the MCOT transcriptome showed similar trends with Loqs2 containing three dsRNA binding domains, 2 interferon-inducible dsRNA-dependent protein kinase activator A domains and a Staufen domain, Loqs1 containing two dsRNA binding domains and an

interferon-inducible dsRNA-dependent protein kinase activator A domain and the potential *D. citri* R2D2 sequence containing two dsRNA binding domains. Future experimental and functional analysis is required to provide further insights into the evolutionary origin and history of the *loqs/r2d2* homologs in *D. citri* and other species.

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Supplementary Note 24: Argonaute and PIWI proteins

Introduction

Argonaute (AGO) proteins play an essential role in RNA-silencing mechanisms and have been implicated in the RNA interference (RNAi), micro RNA (miRNA), and transcriptional gene silencing (rasiRNA/piRNA) pathways (reviewed in (Parker and Barford, 2006; Meister, 2013). They function to present small RNA guide molecules to their complementary targets through the RNA-induced silencing complex (RISC) or the RNA-induced initiation of transcription gene silencing (RITS) complex (Hammond et al., 2001; Verdel et al., 2004). Additionally, AGO proteins provide the 'Slicer' catalytic activity that is required for mRNA cleavage in some RNA silencing pathways (Liu et al., 2004; Meister et al., 2004). AGO proteins are varied with respect to sequence and can be divided into subfamilies including the AGO and PIWI subfamilies. Despite their variability AGO proteins contain four conserved domains, an amino terminal (N) domain, a PAZ (PIWI-Argonaute-Zwille) domain, a MID domain, and a C-terminal PIWI domain (reviewed in (Parker and Barford, 2006; Meister, 2013). Structural studies of these domains have revealed that the PAZ domain functions in binding the 3' end of small RNA guides (Lingel et al., 2003; Song et al., 2003; Yan et al., 2003; Ma et al., 2004), the N domain is responsible for small RNA loading (Kwak and Tomari, 2012), the MID domain is responsible for anchoring the 5' end of the guide RNA (Jinek and Doudna, 2009), and the PIWI domain provides the 'Slicer' enzymatic activity of the protein (Liu et al., 2004; Rivas et al., 2005; Parker and Barford, 2006; Meister, 2013).

In many model organisms, including *Arabidopsis thaliana*, *Caenorhabditis elegans* and *Drosophila melanogaster*, AGO proteins have subfunctionalized with specific AGO proteins operating in specific RNA-silencing pathways (Cikaluk et al., 1999; Lynn et al., 1999; Tabara et al., 1999; Grishok et al., 2001). In *D. melanogaster*, the insect model for which the RNAi core machinery genes have been best studied, there are five AGO family members. Dm-AGO1 is involved in the miRNA pathway, Dm-AGO2 is involved in the RNAi pathway (Hammond et al., 2000; Okamura et al., 2004) while Dm-AGO3, Dm-PIWI and Dm-Aub (all members of the PIWI subfamily) are involved in transcriptional silencing (Kennerdell et al., 2002; Pal-Bhadra et al., 2002; Aravin et al., 2004) (reviewed in (Lippman and Martienssen, 2004; Meister and Tuschl, 2004; Parker and Barford, 2006; Meister, 2013). Functional studies in *Tribolium castaneum* indicate that similar subfunctionalization exists in beetles as well with Tc-Ago1 functioning in the miRNA pathway and Tc-Ago 2a and Tc-Ago 2b functioning in the RNAi pathway (Tomoyasu et al., 2008).

Methods

AGO homolog sequences from a variety of insects were obtained from NCBI, ImmunoDB and the i5k workspace. These sequences were then used to query the predicted *Diaphorina citri* protein sets (Diacit_International_psyllid_consortium_proteins_v1and Diacit_RefSeq_

proteins_Release_100) at i5k@NAL. Loci encoding putative homologs were identified and manually annotated in Web Apollo using RNA-Seq data as evidence for transcript structure. EMBL EBI's online InterPro program was used for domain identification. Multiple sequence alignments of insect Ago homologs were performed using the ClustalW program (MEGA7 software). A neighbor-joining phylogenetic tree was constructed using full-length protein sequences in MEGA7.

Table 1: Argonaute family genes in *D. citri* and related organisms. Note: Many mosquito genes are given the PIWI nomenclature even though they are Aub-like.

Species	AGO1	AGO2	AGO3	PIWI/Aub
D. melanogaster	1	1	1	2
A. gambiae	1	1	1	2
A. aegypti	2	1	1	7
C. quinquefasciatus	1	2	1	6
T. castaneum	1	2	1	1
C. lectularius	2	2	1	4
D. citri	1	1	1	1

Table 2: Bit score, query coverage, identity and e-value results from BLAST analysis of predicted AGO1, AGO2, AGO3, Aub RA and Aub RB proteins to their presumptive orthologs.

| Species		*D. citri* AGO1	*D. citri* AGO2	*D. citri* AGO3	*D. citri* Aub RA	*D. citri* Aub RB
D. melanogaster	Bit score	1245	214	629	376	368
	QC (Identity)	91% (90%)	98% (47%)	87% (41%)	82% (35%)	82% (34%)
	E value	0	2.00E-59	0	6.00E-121	4.00E-118
A. gambiae	Bit score	1248	238	696	349	363
	QC (Identity)	91% (93%)	94% (45%)	92% (43%)	94% (36%)	93% (35%)
	E value	0	4.00E-68	0	5.00E-110	5.00E-115
A. aegypti	Bit score	1258	274	728	408	396
	QC (Identity)	99% (86%)	100% (50%)	96% (43%)	93% (37%)	93% (36%)
	E value	0	2.00E-80	0	4.00E-132	5.00E-128
C. quinquefasciatus	Bit score	1257	261	533	401	386
	QC (Identity)	96% (87%)	99% (51%)	76% (40%)	93% (37%)	93% (36%)
	E value	0	6.00E-76	4.00E-177	4.00E-130	1.00E-124
T. castaneum	Bit score	320	289	657	381	362
	QC (Identity)	18% (96%)	99% (56%)	94% (41%)	92% (38%)	92% (36%)
	E value	7.00E-105	2.00E-86	0	2.00E-121	2.00E-114
C. lectularius	Bit score	301	300	664	456	450
	QC (Identity)	85% (33%)	99% (59%)	100% (39%)	91% (42)	91% (41%)
	E value	1.00E-89	2.00E-89	0	3.00E-154	6.00E-149

Results and Discussion

In *D. citri*, BLASTp searches with known insect AGO proteins identified four loci encoding predicted AGO proteins (Table 1). Following the naming convention from previously studied insects these genes have been named *D. citri Argonaute 1* (*AGO1*), *D. citri Argonaute 2* (*AGO2*), *D. citri Argonaute 3* (*AGO3*) and *D. citri aubergine* (*aub*). RNA-Seq data suggests that *aub* may have at least two isoforms and they have been designated *aubRA* and *aubRB* (Table 2). This result is not surprising as isoforms of the PIWI/Aub class of Ago genes have been identified in other insects.

Domain analysis using EMBL-EBI's InterPro indicate AGO1 contains the N, Mid, PAZ and PIWI domains characteristic of AGO proteins. In contrast, AGO2, AGO3, and Aub were only found to contain the PAZ and PIWI domains. Phylogenetic analysis shows that *D. citri* AGO1, AGO2, AGO3 and Aub cluster with representative genes from other insect taxa (Figure 1).

It is interesting to note that *D. citri* appears to have a minimal number of AGO proteins as has also been observed in other insects such as *Bombyx mori (Wang et al., 2013)*, *Nilaparvata lugens (Xu et al., 2013)*, *T. castaneum (Tomoyasu et al., 2008),* and *D. melanogaster*. It does not appear to have undergone expansions in the PIWI class of AGO proteins, as seen in several species of mosquitoes (Campbell et al., 2008) nor does it have AGO proteins showing homology to the expanded class of secondary AGO proteins seen in *C. elegans* (Yigit et al., 2006).

Figure 1: Phylogenetic tree depicting clustering of insect Argonaute family proteins. Purple clades represent the canonical AGO subclass of proteins typically involved in post transcriptional silencing (RNAi and miRNA pathways). Blue clades represent the PIWI subclass of proteins typically involved in transcriptional silencing (rasiRNA/piRNA pathways). *D. citri* proteins are marked with a taxon marker.
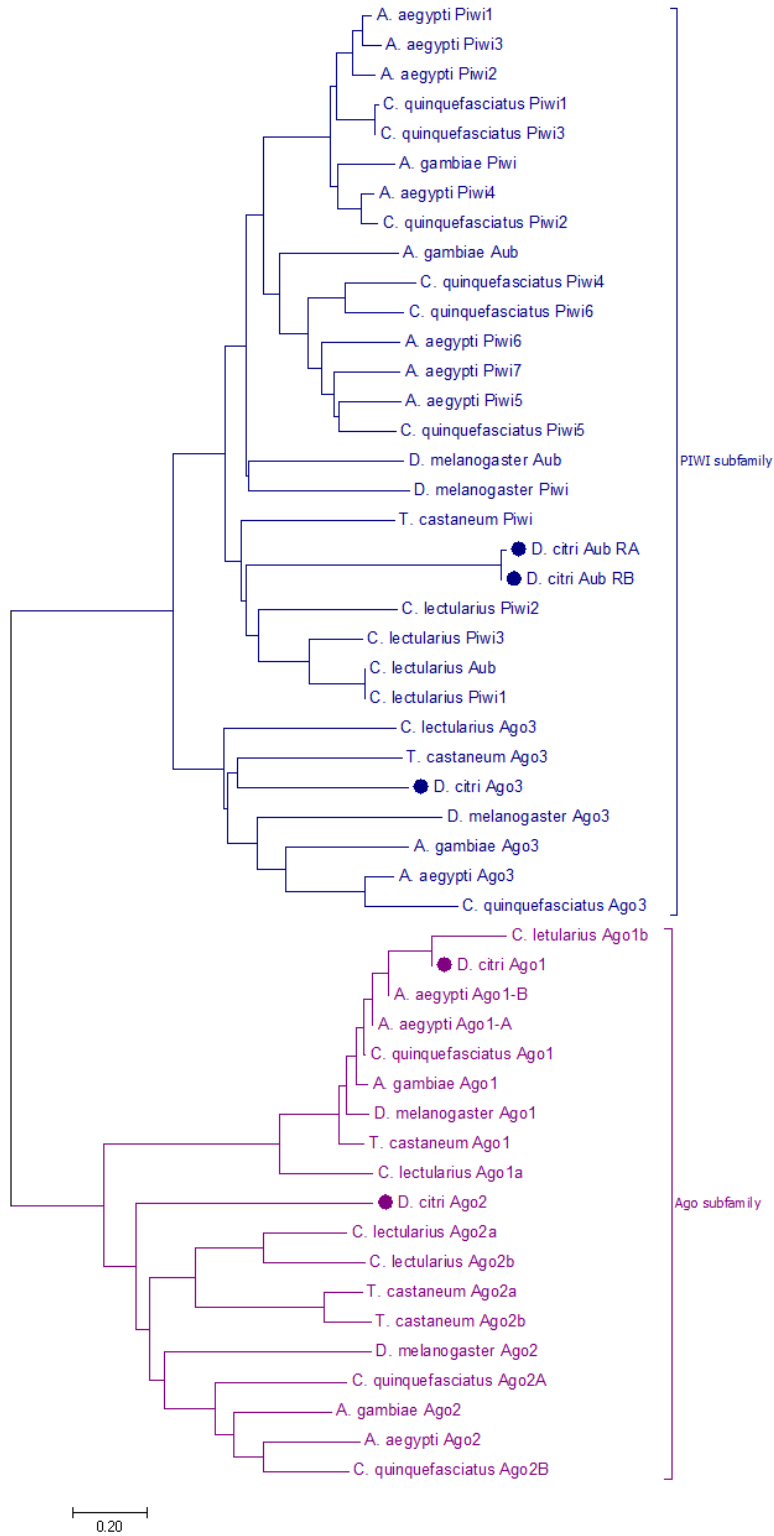
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Supplementary Note 25: Tudor Staphylococcal Nuclease protein

Introduction

Tudor Staphylococcal Nuclease protein (Tudor SN or TSN) has been found to be a component of the holo-RISC complex in *Drosophila melanogaster*, *Caenorhabditis elegans* and mammals. TSN in *D. melanogaster* contains five Staphylococcal/Micrococcal nuclease domains and a Tudor domain (Caudy et al., 2003).

Methods

TSN protein sequences were used to query the predicted *Diaphorina citri* protein sets (Diacit_International_psyllid_consortium_proteins_v1and Diacit_RefSeq_proteins_Release _100) at i5k@NAL via the BLASTp tool. Loci encoding putative homologs were identified and manually annotated in Web Apollo using RNA-Seq data as evidence for transcript structure. EMBL EBI's online InterPro program was used for domain identification. NCBI databases were used for reciprocal blast analysis of predicted proteins. Multiple sequence alignments of insect homologs were performed using the ClustalW program (MEGA7 software). A neighbor-joining phylogenetic tree was constructed using full-length protein sequences in MEGA7.

Table 1: Number of TSN homologs in insect species

	TSN
D. melanogaster	1
A. gambiae	1
A. aegypti	1
C. quinquefasciatus	1
D. citri	2

Table 2: Bit score, query coverage, identity and e-value results from BLAST analysis of predicted TSN1 and TSN2 to TSN insect orthologs.

		D. melanogaster	*A. gambiae*	*A. aegypti*	*C. quinquefasciatus*
D. citri TSN1	**Bit score**	402	409	429	429
	QC (Identity)	96% (45%)	95% (45%)	98% (46%)	96% (46%)
	E value	2.00E-130	5.00E-136	1.00E-141	2.00E-141
D. citri TSN2	**Bit score**	256	246	266	268
	QC (Identity)	96% (38%)	95% (39%)	95% (41%)	95% (40%)
	E value	9.00E-77	1.00E-73	8.00E-81	2.00E-81

Results and Conclusions

Initial genome searches identified four loci with significant BLAST hits to TSN insect homologs. However, reciprocal BLAST analysis suggests that only two of these genes are likely orthologous

to fly and mosquito TSN (Table 2). We have named these genes *D. citri TSN1* (*TSN1*) and *D. citri TSN2* (*TSN2*) (Table 1).

Domain analysis reveals that *D. citri* TSN1 contains 3 staphylococcal nuclease domains and one tudor domain. *D. citri* TSN 2 contains 3 staphylococcal nuclease domains and does not contain a tudor domain. However, *D. citri* TSN2 is located at the end of a contig and the predicted protein is likely missing C-terminal sequence information. Both *D. citri* TSN proteins have fewer staphylococcal nuclease domains than is seen in *D. melanogaster* but this is not surprising as the number of staphylococcal domains in TSN homologs varies across species (4 in *Bombyx mori* and humans, 5 in mosquitoes) (Li et al., 2008; Zhu et al., 2013). Phylogenetic analysis depicting the relationship between insect TSN homologs is shown below.

Figure 1: Phylogenetic tree of insect TSN homologs. Each species is depicted with a different color. *D. citri* is marked with a taxon marker.
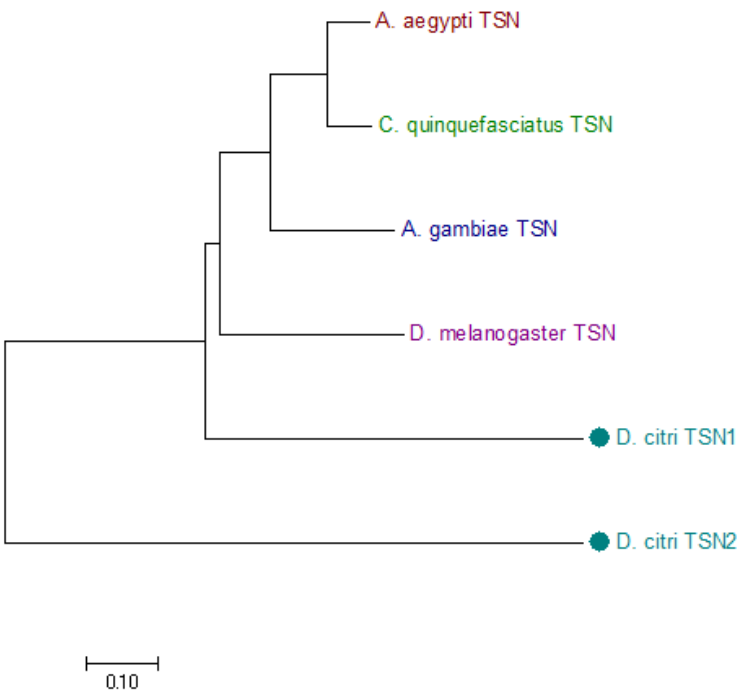
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Supplementary Note 26: Vasa intronic gene

Introduction

When the RNA-induced silencing complex (RISC) was first identified in *Drosophila melanogaster* S2 cells, two RNA binding proteins, Fragile X Mental Retardation (FMR) and Vasa Intronic Gene (VIG) were found to be components of the complex (Caudy et al., 2002). VIG has since been shown to play an important role in RNAi in *Caenorhabditis elegans* as well, as inactivation of Ce VIG-1 resulted in decreased RNAi activity (Caudy et al., 2003), however the function of VIG in the RISC is still poorly understood.

Methods

VIG sequences were used to query the predicted *Diaphorina citri* protein sets (Diacit_International_psyllid_consortium_proteins_v1and Diacit_RefSeq_proteins_Release _100) at i5k@NAL via the BLASTp tool. Loci encoding putative homologs were identified and manually annotated in Web Apollo using RNA-Seq data as evidence for transcript structure. NCBI databases were used for reciprocal blast analysis of the predicted protein. Multiple sequence alignments of insect homologs were performed using the ClustalW program (MEGA7 software). A neighbor-joining phylogenetic tree was constructed using full-length protein sequences in MEGA7.

Table 1: Number of VIG homologs seen in related insect species

	VIG
D. melanogaster	2
A. gambiae	1
A. aegypti	1
D. citri	1

Table 2: Bit score, query coverage, identity and e-value results from BLAST analysis of predicted VIG to insect VIG homologs.

		D. melanogaster	*A. gambiae*	*A. aegypti*
D. citri VIG	**Bit score**	116	114	104
	QC (Identity)	70% (43%)	70% (39%)	74% (40%)
	E value	1.00E-29	2.00E-29	5.00E-26

Results and Conclusions

BLASTp analysis of the *D. citri* genome revealed one putative VIG ortholog (Table 1). We have named this gene *D. citri VIG* (*VIG*). Blast analysis indicates that *D. melanogaster* has two VIG genes, although to our knowledge VIG2 has not been implicated in RNAi. The mosquito species *Anopheles gambiae* and *Aedes aegypti* each have one VIG ortholog, although only the *A. aegypti* gene appears to be annotated (Table 1). Reciprocal BLAST analysis with the predicted *D.*

citri VIG protein sequence confirms that *D. citri* VIG is orthologous to fly and mosquito VIG (Table 2).
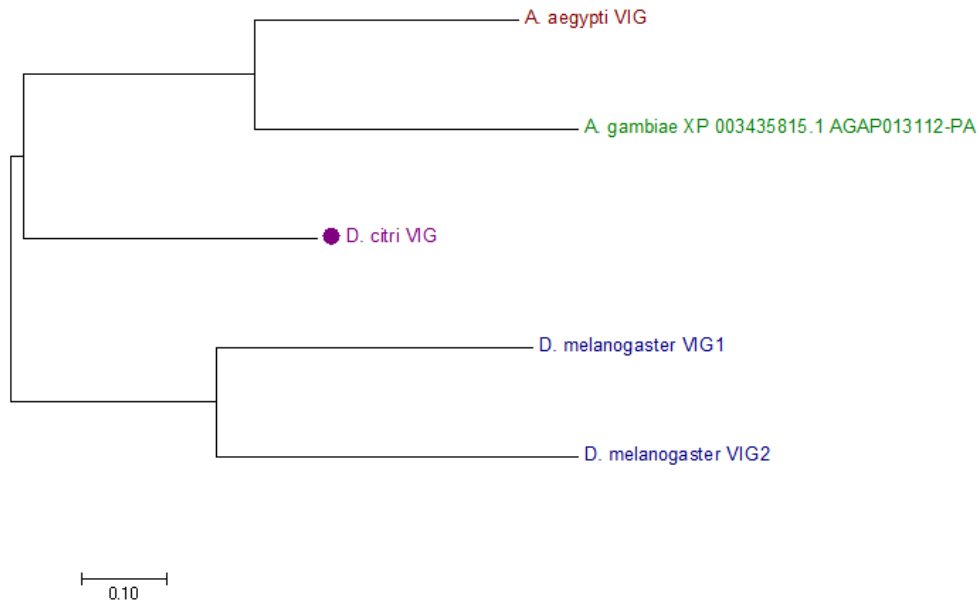
Phylogenetic analysis of fly and mosquito VIG homologs indicate that the two *D. melanogaster* genes are likely paralogs, with *D. citri VIG* showing more similarity to mosquito *VIG* genes (Figure 1).

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Supplementary Note 27: Armitage

Introduction

Armitage (Armi) is a homolog of the *Arabidopsis thaliana* gene SDE3. SDE3 has been identified as an ATP dependent RNA helicase involved in RNAi in plants (Dalmay et al., 2001; Willmann, 2001) and has been shown, in a variety of studies, to be required for RNAi in the fruit fly *Drosophila melanogaster*. Armi is necessary for silencing select mRNAs involved in oogenesis (Cook et al., 2004) and *armi* mutants have been shown to be defective in the production of RISC (Tomari et al., 2004). Furthermore, Armi has been shown to be one of many factors essential for primary piwiRNA (piRNA) biogenesis (Malone et al., 2009; Haase et al., 2010; Olivieri et al., 2010).

Methods

Armi protein sequences were used to query the predicted *Diaphorina citri* protein sets (Diacit_International_psyllid_consortium_proteins_v1and Diacit_RefSeq_proteins_Release _100) at i5k@NAL via the BLASTp tool. Loci encoding putative homologs were identified and manually annotated in Web Apollo using RNA-Seq data as evidence for transcript structure. NCBI databases were used for reciprocal blast analysis of the predicted protein. Multiple sequence alignments of insect homologs were performed using the ClustalW program (MEGA7 software). A neighbor-joining phylogenetic tree was constructed using full-length protein sequences in MEGA7.

Table 1: Number of Armitage homologs seen in related insect species

| Species	Armitage
D. melanogaster	1
A. gambiae	1
A. aegypti	2
C. quinquefasciatus	2
D. citri	1

Table 2: Bit score, query coverage, identity and e-value results from BLAST analysis of predicted Armitage to insect Armitage homologs.

		D. melanogaster	*A. gambiae*	*A. aegypti*	*C. quinquefasciatus*
D. citri Armitage	**Bit score**	289	247	266	158
	QC (Identity)	51% (41%)	52% (41%)	54% (38%)	43% (35%)
	E value	3.00E-81	1.00E-67	2.00E-74	2.00E-39

Results and Conclusions

BLASTp analysis of the *D. citri* genome revealed one putative Armi ortholog (Table 1).

We have named this gene *D. citri armitage* (*armitage*). Reciprocal BLAST analysis with the predicted *D. citri* Armi protein sequence to fly and mosquito genome databases confirm that *D. citri* Armi is orthologous to fly and mosquito Armi (Table 2). Phylogenetic analysis of insect Armi homologs show clustering of *Aedes aegypti* and *Culex quinquefasciatus* paralogs and *D. citri's* Armi is positioned as the outgroup (Figure 1).

Figure 1: Phylogenetic tree of related insect Armitage proteins. Individual insect species are depicted with different colors. *D. citri* is marked with a taxon marker.
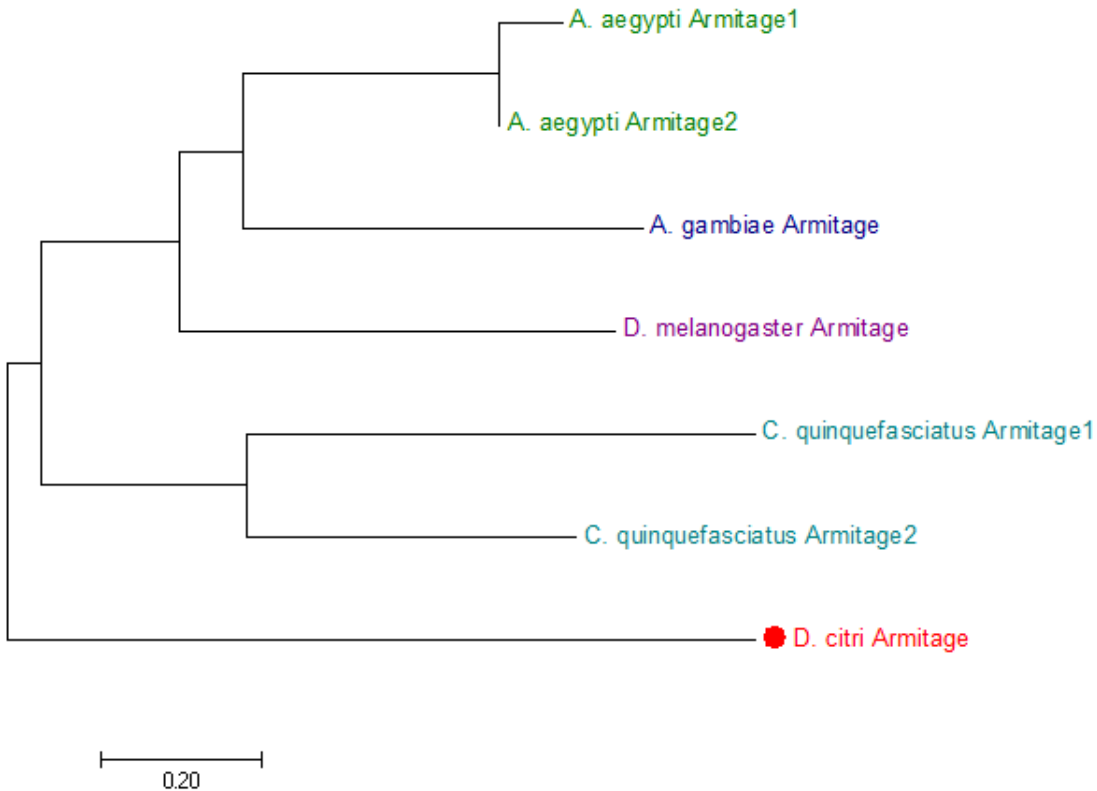
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Supplementary Note 28: Fragile X Mental Retardation Protein

Introduction

Fragile X syndrome is a major cause of autism and mental retardation in humans. The mutation responsible for this syndrome is an expansion of a CGG trinucleotide repeat which suppresses expression of the *fragile X mental retardation 1* (FMR1) gene found on the X chromosome in humans. This loss of protein expression causes a loss of neuronal connectivity resulting in the syndrome's symptoms (reviewed in (Lin, 2015)). In insects FMR1 was identified as playing a role in the RNAi response when it was identified as part of the RNA-induced silencing complex (RISC) in *Drosophila melanogaster* S2 cells (Caudy et al., 2002; Ishizuka et al., 2002). This data indicating FMR1's role in RISC has led to further investigations identifying FMR1's role in gene expression regulation through the miRNA pathway (reviewed in (Lin, 2015).

Methods

FMR1 sequences were used to query the predicted *Diaphorina citri* protein sets (Diacit_International_psyllid_consortium_proteins_v1and Diacit_RefSeq_proteins_Release _100) at i5k@NAL via the BLASTp tool. Loci encoding putative homologs were identified and manually annotated in Web Apollo using RNA-Seq data as evidence for transcript structure. EMBL EBI's online InterPro program was used for domain identification. NCBI databases were used for reciprocal blast analysis of the predicted protein. Multiple sequence alignments of insect homologs were performed using the ClustalW program (MEGA7 software). A neighbor-joining phylogenetic tree was constructed using full-length protein sequences in MEGA7.

Table 1: Number of FMR1 homologs in insect species

	FMR1
D. melanogaster	1
A. gambiae	0
A. aegypti	1
C. quinquefasciatus	1
D. citri	1

Table 2: Bit score, query coverage, identity and e-value results from BLAST analysis of predicted FMR1 to FMR1 insect orthologs.

		D. melanogaster	*A. gambiae*	*A. aegypti*	*C. quinquefasciatus*
D. citri FMR1	**Bit score**	202	No significant hits	245	234
	QC (Identity)	54% (44%)		50% (50%)	54% (49%)
	E value	3.00E-57		2.00E-73	2.00E-69

Results and Conclusions

BLASTp analysis indicates that *D. citri* has one *fragile x mental retardation* ortholog (Table 1 and 2). Following conventional nomenclature, we have named this gene *D. citri FMR1* (*FMR1*). RNA-Seq data suggests that alternative splicing does occur at this loci and multiple isoforms for this gene probably exist. This is not a surprising result as at least 9 isoforms of *FMR1* have been identified in *Drosophila*. As currently annotated the *D. citri* FMR1 protein has two Agenet-like domains and a K homology domain but does not contain the Fragile X related 1 protein C terminal core as seen in flies and mosquitoes.

Whether this domain is missing due to inaccurate annotation or true loss is unknown. Phylogenetic analysis shows expected relationships between fly, mosquito and psyllid FMR1 proteins (Figure 1).

Figure 1: Phylogenetic tree of insect FMR1 homologs. Each species is depicted with a different color. *D. citri* is marked with a taxon marker.
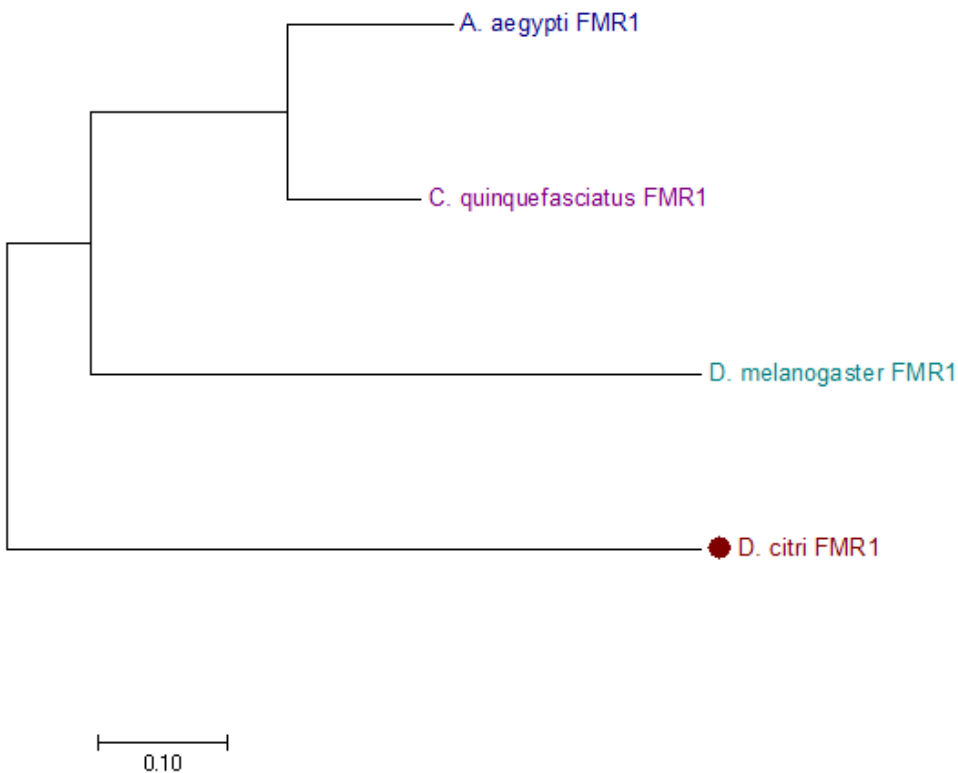
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Supplementary Note 29: Spindle_E (homeless)

Introduction

Spindle-E (homeless) mutations were first characterized by the role the protein product played in fertility in *Drosophila melanogaster* (Stapleton et al., 2001; Aravin et al., 2004). Soon after this work was preformed *spindle-E* was identified as having a role in RNA silencing pathways when *spindle-E* mutants were shown to prevent RNAi activation during egg maturation and prevent translation control during oogenesis (Kennerdell et al., 2002). Later, *spindle-E* mutants were shown to be defective in heterochromatic silencing indicating *spindle-E* has a role in transcriptional silencing in germline cells (Pal-Bhadra et al., 2004).

Methods

Spindle-E protein sequences were used to query the predicted *Diaphorina citri* protein sets (Diacit_International_psyllid_consortium_proteins_v1and Diacit_RefSeq_proteins_Release _100) at i5k@NAL via the BLASTp tool. Loci encoding putative homologs were identified and manually annotated in Web Apollo using RNA-Seq data as evidence for transcript structure. NCBI databases were used for reciprocal blast analysis of predicted proteins. Multiple sequence alignments of insect homologs were performed using the ClustalW program (MEGA7 software). A neighbor-joining phylogenetic tree was constructed using full-length protein sequences in MEGA7.

Table 1: Number of Spindle-E homologs in insect species

Species	Spindle_E
D. melanogaster	1
A. gambiae	1
A. aegypti	1
C. quinquefasciatus	1
D. citri	2

Table 2: Bit score, query coverage, identity and e-value results from BLAST analysis of predicted Spindle-E1 and Spindle-E2 to insect Spindle-E homologs.

		D. melanogaster	*A. gambiae*	*A. aegypti*	C. quinquefasciatus
D. citri Spindle-E1	**Bit score**	242	243	250	244
	QC (Identity)	54% (52%)	89% (49%)	60% (52%)	80% (50%)
	E value	7.00E-68	5.00E-68	2.00E-70	2.00E-68
D. citri Spindle-E2	**Bit score**	175	158	161	171
	QC (Identity)	26% (36%)	72% (33%)	74% (33%)	78% (33%)
	E value	2.00E-47	4.00E-39	3.00E-40	3.00E-45

Results and Conclusions

BLASTp analysis of the *D. citri* genome has identified at least two Spindle-E orthologs (Table 1). These two genes have been named *D. citri spindle-E1* (*spindle-E1*) and *D. citri spindle-E2* (*spindle-E2*). Reciprocal BLAST analysis confirms these putative orthologs (Table 2) and phylogenetic analysis shows appropriate clustering of insect Spindle-E proteins (Figure 1). In the case of *spindle-E2* RNA-Seq data suggests alternative splicing may occur producing a variety of different isoforms.

Putative isoforms have been named *spindle-E2 RA*, *spindle-E2 RB*, *spindle-E2 RC* and *spindle-E2 RD*. *spindle-E2 RA* was used as the representative isoform for BLAST and phylogenetic analysis.

Figure 1: Phylogenetic tree of related insect Spindle-E proteins. Individual insect species are depicted with different colors. *D. citri* is marked with a taxon marker.
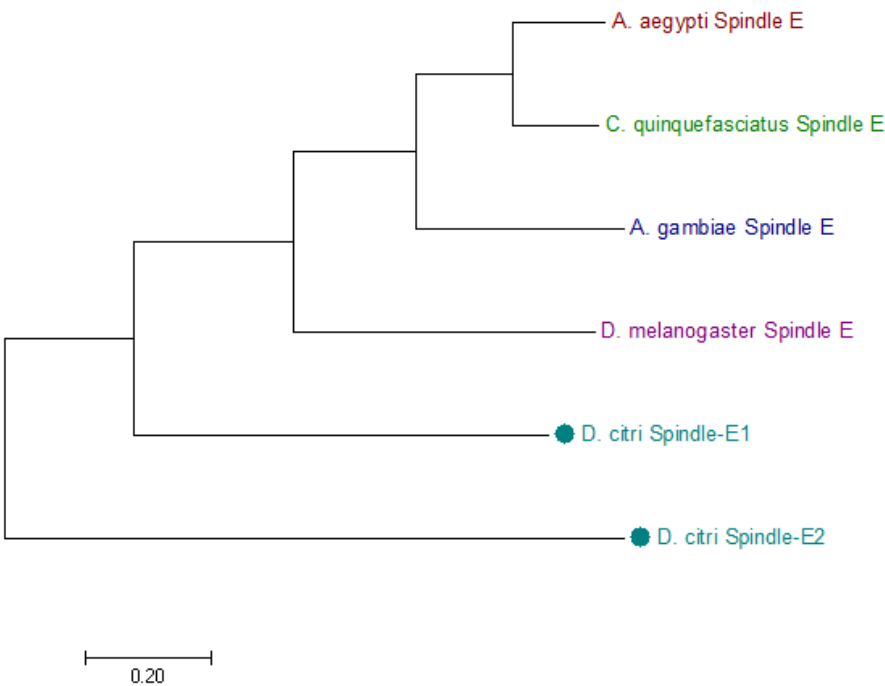
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Supplementary Note 30: Rm62 protein

Introduction

Rm62 (also known as Lip) is the ortholog of human p68. It is an RNA helicase which has been shown to be involved in the RNA interference (RNAi) pathway (Huang and Liu, 2002; Ishizuka et al., 2002), antiviral defense (Zambon et al., 2006) and in retrotransponson maintenance in *Drosophila melanogaster* (Lei and Corces, 2006). While only Rm62 has been linked to RNAi in *Drosophila* six other RNA helicase genes have also been identified (NM165103, NM141510, NM1401752, NM132792, NM139805, NM132196) (Campbell et al., 2008). Furthermore, in the mosquito species *Anopheles gambiae*, *Aedes aegypti* and *Culex quinquefasciatus* six, nine and ten Rm62-like genes have been respectively identified and expression has been confirmed for many of these genes (Campbell et al., 2008).

Methods

Rm62 sequences were used to query the predicted *Diaphorina citri* protein sets (Diacit_International_psyllid_consortium_proteins_v1and Diacit_RefSeq_proteins_Release_100) at i5k@NAL via the BLASTp tool. Loci encoding putative homologs were identified and manually annotated in Web Apollo using RNA-Seq data as evidence for transcript structure. NCBI databases were used for reciprocal blast analysis of the predicted protein. Multiple sequence alignments of insect homologs were performed using the ClustalW program (MEGA7 software). A neighbor-joining phylogenetic tree was constructed using full-length protein sequences in MEGA7.

Table 1: Number of Rm62 homologs in insect species. Note: While there is only 1 Rm62 in *Drosophila* there are 6 more Rm62 like proteins. Therefore, the mosquito homologs listed here are not necessarily orthologous to Rm62 but could be orthologs of other *Drosophila* RNA helicases.

	Rm62
D. melanogaster	1
A. gambiae	6
A. aegypti	9
C. quinquefasciatus	10
D. citri	1

Table 2: Bit score, query coverage, identity and e-value results from BLAST analysis of predicted Rm62 to Rm62 insect homologs. For the mosquito species the top BLAST hit has been identified in the Bit Score column.

		D. melanogaster	*A. gambiae*	*A. aegypti*	*C. quinquefasciatus*
D. citri Rm62	**Bit score**	516	506 (Rm62A)	505 (Rm62C)	506 (Rm62H)
	QC (Identity)	78% (58%)	81% (56%)	92% (57%)	83% (56%)
	E value	8.00E-180	6.00E-174	1.00E-175	2.00E-174

Results and Conclusions

The RNA helicase family in Diptera is clearly a large, complex family. Because Rm62 is currently the only RNA helicase in this family known to be involved in RNAi we limited our search of the *D. citri* genome to orthologs of this particular gene. We found one putative ortholog to Rm62 which we have named *D. citri Rm62* (*Rm62*) and included in our analysis (Table 1). It is important to note, however, that other RNA helicase family members could also exist in the *D. citri* genome which have not been annotated. Reciprocal BLASTp analysis of Rm62 indicates that this gene is orthologous to *Drosophila's* Rm62. However, the top BLASTp hits to mosquito databases suggest that this gene may be more closely related to *A. gambiae's* Rm62A, *A. aegypti's* Rm62C and *C. quinquefasciatus'* Rm62H than to other mosquito *Rm62* genes (Table 2).

Figure 1: Phylogenetic tree of insect Rm62 homologs. Each species is depicted with a different color. *D. citri* is marked with a circular taxon marker. Proteins that received the top BLAST score on a reciprocal BLAST analysis are marked with a diamond.
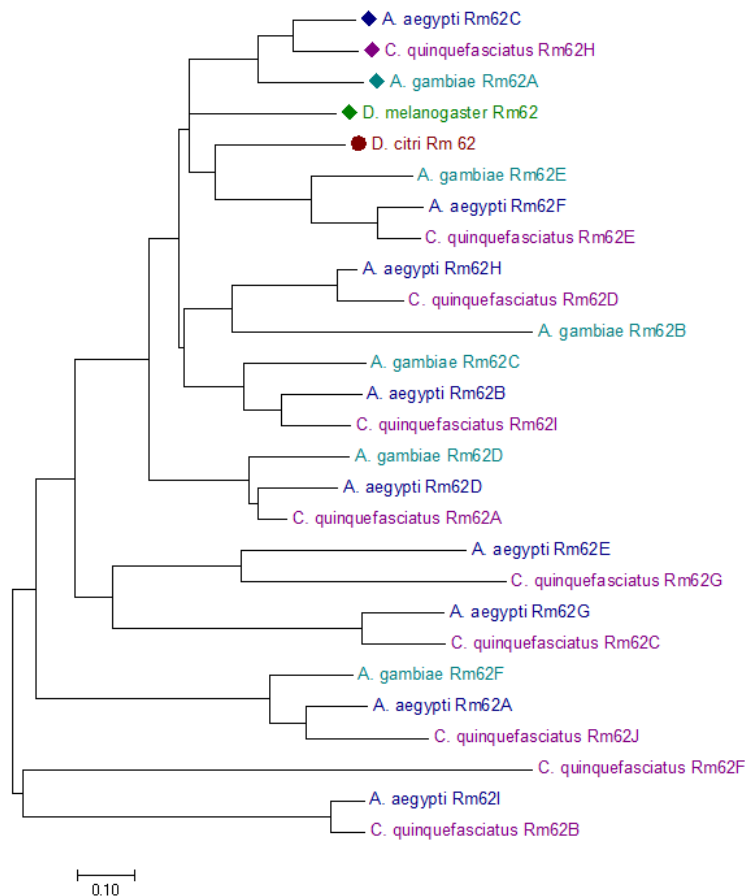
Exact relationships between these RNA helicase family members is difficult to determine as bootstrap scores at some nodes are quite low. However, Rm62, *Drosophila* Rm62 and mosquito

top BLASTp hits do fall into the same clade (top reciprocal BLAST hits are depicted by the colored diamonds) (Figure 1).

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Supplementary Note 31: Ras-related nuclear protein

Introduction

Ran (Ras-related nuclear protein) is a GTPase which regulates many cellular functions related to the cell cycle, including spindle assembly and nuclear membrane assembly (reviewed in (Chen et al., 2015). In addition to its role in the cell cycle Ran has been implicated in the nucleocytoplasmic transport of various molecules (Shibata et al., 2006). With respect to RNA silencing Ran plays an essential role in miRNA biogenesis as Exportin-5 functions to transport primary miRNA precursors from the nucleus to the cytoplasm in a Ran-dependent manner (Yi et al., 2003; Bohnsack et al., 2004; Lund et al., 2004; Wang et al., 2011)

Methods

Ran sequences were used to query the predicted *Diaphorina citri* protein sets (Diacit_International_psyllid_consortium_proteins_v1and Diacit_RefSeq_proteins_Release _100) at i5k@NAL via the BLASTp tool. Loci encoding putative homologs were identified and manually annotated in Web Apollo using RNA-Seq data as evidence for transcript structure. EMBL EBI's online InterPro program was used for domain identification. NCBI databases were used for reciprocal blast analysis of the predicted protein. Multiple sequence alignments of insect homologs were performed using the ClustalW program (MEGA7 software). A neighbor-joining phylogenetic tree was constructed using full-length protein sequences in MEGA7.

Table 1:   Number of Ran homologs in insect species

	Ran
D. melanogaster	1
A. gambiae	1
A. aegypti	1
C. quinquefasciatus	1
D. citri	1

Table 2: Bit score, query coverage, identity and e-value results from BLAST analysis of predicted Dcitr_Ran to Ran insect orthologs.

		D. melanogaster	*A. gambiae*	*A. aegypti*	*C. quinquefasciatus*
D. citri Ran	**Bit score**	418	424	423	423
	QC (Identity)	100% (91%)	99% (93%)	99% (93%)	99% (93%)
	E value	5.00E-151	7.00E-154	2.00E-153	2.00E-153

Results and Conclusions

BLASTp analysis confirms the *D. citri* genome contains one Ran GTPase orthologous to *Drosophila* Ran (Table 1). We have named this gene *D. citri ran* (*ran*). Reciprocal BLASTp analysis

to fly and mosquito databases show orthologous Ran proteins to be the only significant hit to Ran (Table 2) and domain analysis confirms *D. citri* Ran as a Ran GTPase belonging to the small GTPase superfamily. Phylogenetic analysis shows expected relationships between fly, mosquito and psyllid Ran proteins (Figure 1).

Figure 1: Phylogenetic tree of insect Ran homologs. Each species is depicted with a different color. *D. citri* is marked with a taxon marker.
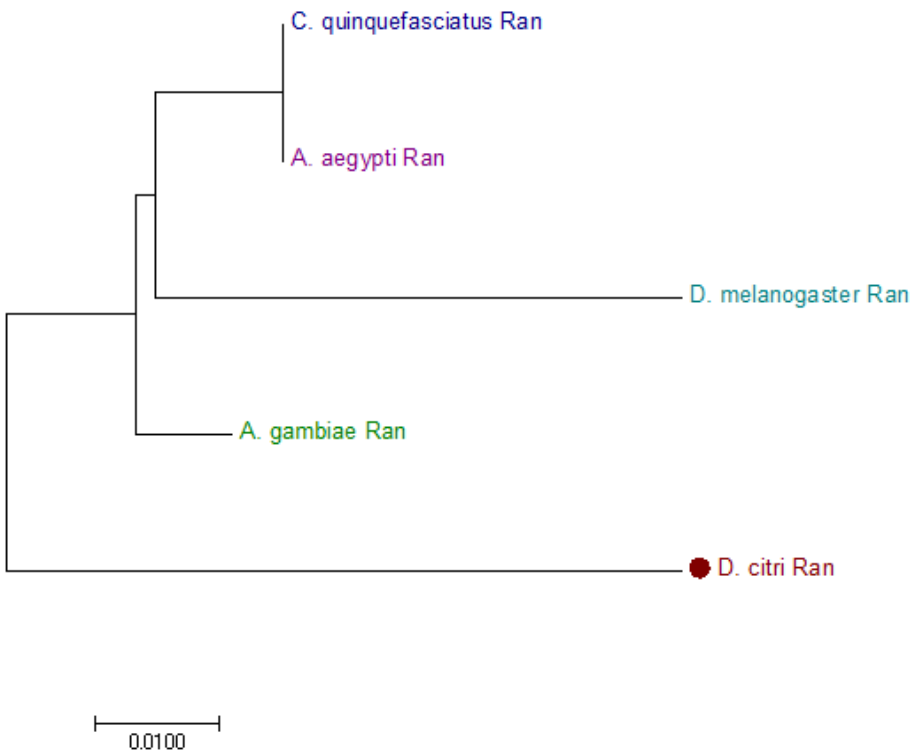
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Supplementary Note 32: Glycolysis pathway

Introduction

The glycolysis pathway for three carbon compounds is an essential pathway for eukaryotic life. We have attempted to annotate 15 genes involved in this pathway in the Asian citrus psyllid (*Diaphorina citri*), an agrarian pest species, by comparison to the genome of a model insect species: *Drosophila melanogaster*.

Methods

Gene Identification

A list of genes associated with glycolysis in *D. melanogaster* were gathered using the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (http://www.genome.jp/kegg/) (2, 3). The sequences for these genes were downloaded from FlyBase (1), and the putative homologs in *D. citri* were identified by BLASTing these sequences against the transcriptome of *D. citri* using CLC Genomics Workbench 7.0 (https://www.qiagenbioinformatics.com), using E-value to determine best matches; genes with non-unique matches were assigned unique matches based on next lowest E-value.

Gene Annotation

Gene annotations were performed using the JBrowse/Web Apollo *Diaphorina citri* on the i5k Workspace@NAL (http://i5k.nal.usda.gov/) (4). A search was performed for each gene sequence and gene models were created from various evidence tracks. The sequence of each newly created model was BLASTed at NCBI to check for congruence.

Table 1: List of genes involved in the core module of glycolysis involving three-carbon compounds (2, 3)

Gene	Name
Dmel_CG11249	CG11249 gene product from transcript CG11249-RA (EC:2.7.1.40) [KO:K00873]
Dmel_CG12055	Gapdh1; Glyceraldehyde 3 phosphate dehydrogenase 1 (EC:1.2.1.12) [KO:K00134]
Dmel_CG12229	CG12229 gene product from transcript CG12229-RA (EC:2.7.1.40) [KO:K00873]
Dmel_CG1721	Pglym78; Phosphoglyceromutase [KO:K01834]
Dmel_CG17645	Pglym87; CG17645 gene product from transcript CG17645-RA [KO:K01834]
Dmel_CG17654	Eno; Enolase (EC:4.2.1.11) [KO:K01689]
Dmel_CG2171	Tpi; Triose phosphate isomerase (EC:5.3.1.1) [KO:K01803]
Dmel_CG2964	CG2964 gene product from transcript CG2964-RA (EC:2.7.1.40) [KO:K00873]
Dmel_CG3127	Pgk; Phosphoglycerate kinase (EC:2.7.2.3) [KO:K00927]
Dmel_CG7069	CG7069 gene product from transcript CG7069-RA (EC:2.7.1.40) [KO:K00873]
Dmel_CG7070	PyK; Pyruvate kinase (EC:2.7.1.40) [KO:K00873]
Dmel_CG7362	CG7362 gene product from transcript CG7362-RB (EC:2.7.1.40) [KO:K00873]
Dmel_CG8893	Gapdh2; Glyceraldehyde 3 phosphate dehydrogenase 2 (EC:1.2.1.12) [KO:K00134]
Dmel_CG9010	CG9010 gene product from transcript CG9010-RA (EC:1.2.1.12) [KO:K00134]
Dmel_CG9961	CG9961 gene product from transcript CG9961-RB (EC:2.7.2.3) [KO:K00927]

Table 2: Results from CLC Genomics Workspace 7.0 BLAST of *D. melanogaster* genes against *D. citri* RNA.

Query	Lowest E-value	Accession (E-value)	Next Best Match	Resulting E-value
FBgn0250906	0.00	XM_008475651	-	-
FBgn0001092	0.00	XM_008481620	-	-
FBgn0000579	0.00	XM_008483244	-	-
FBgn0001091	1.01E-175	XM_008481620	XM_08481619	1.01E-175
FBgn0031451	3.76E-145	XM_008475651	XM_008475652	2.33E-143
FBgn0034173	2.76E-138	XM_008481620	XM_008474604	3.79E-138
FBgn0267385	1.63E-119	XM_008482433	-	-
FBgn0031462	7.46E-79	XM_008482433	XM_008476979	2.47E-69
FBgn0014869	1.67E-69	XM_008489682	-	-
FBgn0011270	5.23E-68	XM_008474451	-	-
FBgn0038952	6.55E-67	XM_008482433	XM_008482434	7.84E-49
FBgn0086355	3.66E-62	XM_008476234	-	-
FBgn0038258	1.20E-47	XM_008482433	XM_008483412	3.17E-4
FBgn0037115	1.23E-7	XM_008482433	XM_008479557	0.36
FBgn0036723	2.05	XM_008481206	-	-

Table 3: Results from BLAST at NCBI of gene model

Gene Name	mRNA	E-Value	Max score	Total score	Query cover	E value	Ident
phosphoglycerate kinase, transcript variant X1, mRNA	XM_008475651	0	2294	2804	94.00%	0	100.00%
Glyceraldehyde-3-phosphate dehydrogenase 2, transcript variant x2	XM_008481620	0	499	1885	29.00%	1.00E-136	100.00%
Enolase	XM_008483244	0	1561	2077	74.00%	0	100.00%
Glyceraldehyde-3-phosphate dehydrogenase 2, transcript variant x1	XM_008481619	1.01E-175	2422	2422	100.00%	0	100.00%
phosphoglycerate kinase, transcript variant X2, mRNA	XM_008475652	2.33E-143	2289	2436	95.00%	0	99.00%
Glyceraldehyde-3-phosphate dehydrogenase-like	XM_008474604	3.79E-138	2381	2381	100.00%	0	100.00%
Pyruvate kinase-like	XM_008482433	1.63E-119	2988	5121	32.00%	0	100.00%
Pyruvate kinase-like	XM_008476979	2.47E-069	3520	3520	100.00%	0	100.00%
Phosphoglycerate mutase 1-like	XM_008489682	1.67E-069	496	496	91.00%	1.00E-136	100.00%
Phosphoglycerate mutase 1-like	XM_008474451	5.23E-068	826	826	100.00%	0	100.00%
Pyruvate kinase-like	XM_008482434	7.84E-049	2113	2113	100.00%	0	100.00%
Triosephosphate isomerase-like	XM_008476234	3.66E-062	1690	1690	100.00%	0	100.00%

Results

Out of fifteen genes identified as potential matches for the glycolysis pathway in *D. citri* only five unique best matches based on E-value were found, with one predicted gene (XM_008482433) being the best match for five genes in the pathway. After reassignment, twelve unique predicted genes had an E-value below E-10, which was used as an arbitrary cutoff. In all cases for the remaining 12 models the subsequent check using NCBI BLAST confirmed the model, though low Max Score/Total Score ration was observed in one case involving transcript variants.

Discussion

Despite being an integral pathway for metabolism, there was amount of variation in the length of similar sequences, with some *D. citri* sequences only matching a stretch totaling less than 1/8th of the query gene. There could be a case made against the use of solely E-value as a determinant of best matches in this case, especially considering the number of non-unique matches. The number of transcript variants and related proteins in this pathway was also no doubt a contributing factor to difficulty in distinguishing its related components.

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Supplementary Note 33: Aquaporin

Introduction

Aquaporins are integral membrane proteins primarily responsible for transporting water and, depending on the protein subset, small solutes across biological membranes[1-3].   A part of the major intrinsic protein (MIP) family, this subset of proteins has a common motif of 6 membrane-spanning elements, which form a selective pore allowing water or other molecules to pass through the cell membrane. Thirteen of these proteins have been identified in mammalian samples, and greater or fewer numbers in lower species including, plants, amphibians, insects, and bacteria[1,4]. Insects typically have 6-8 aquaporins[2,5] with higher flies have 8-12 aquaporins[6]

Methods and Results

A quick GO term search of the FlyBase Drosophila melanogaster gene sets generated a list of eight genes with water transmembrane transporter activity proteins (flybase.org). Using the tblastx function in the CLC Genomics Workbench 9.0 software searching against a local Diaphorina citri RNA gene sets, eight aquaporin genes were identified that match those from D. melanogaster. The best matches of those genes, determined by E-value and bit scores (Table 1) were targeted for annotation using the WebApollo.

In cases where matches were uncertain or redundant, the next closest gene targets were annotated. Using the MAKER predicted gene set, the NCBI predicted gene set, and RNA-seq read mapping data, gene models were aligned and adjusted in the assembled *D. citri* genome. The final curated gene model was then confirmed using the NCBI BLAST search of the predicted protein.

For the most part, gene models were readily identified, and at least partially corrected with little issue. However, in the case of three closely related *D. melanogaster* proteins (see Table 1), psyllid AQP genes were difficult to pinpoint, were found to be closely related to more than one predicted gene, and/or difficult to correct.   These incorrect models prevented the generation of a phylogenetic tree for *D. citri* AQPs.   To determine correct AQP sequences the assembled MCOT was utilized to supplement the genome-based analyses. The combination of the MCOT and genome analyses revealed that there are eight unique aquaporin genes (Figure 1, Table 2) and provided the complete sequences for each of these genes. From this combinatory analysis, we were able to confirm that we have identified eight aquaporin genes that includes the typical water transporters (Drip, AQP2), AQP4/AQP5/Eglps (three sequences), AQP6 (unorthodox), and Bib. This number falls within the range of most insects (6-8)[2,5].

Figure 1. Comparison of predicted aquaporins from *D. melanogaster* and *D. citri*. Neighbor-joining tree was produced using MEGA6 using Dayhoff Model and pairwise matching; branch values indicate support following 1500 bootstraps; values below 50% are omitted.
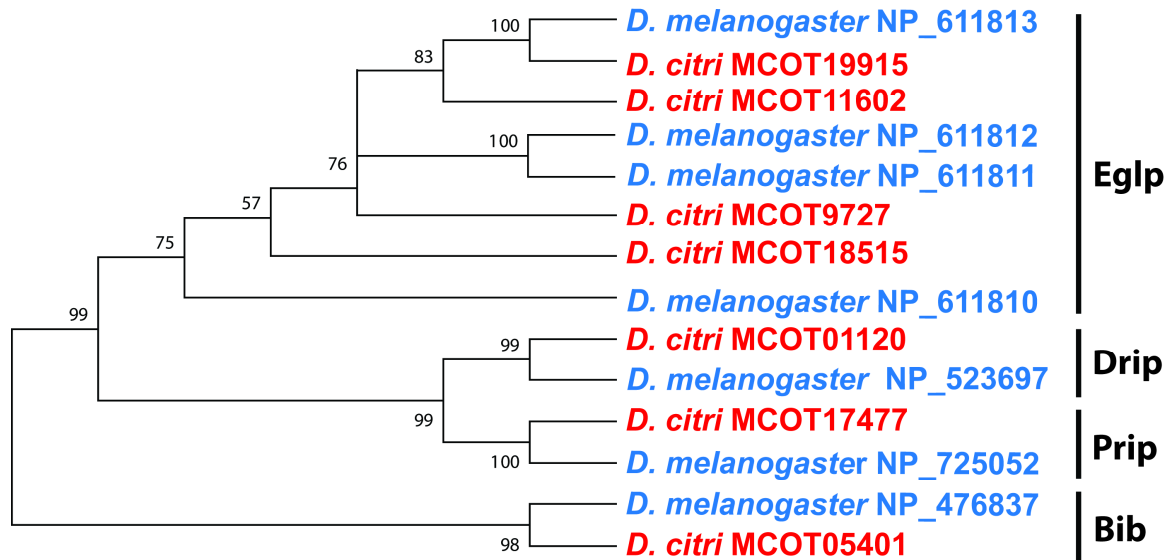
Table 1: *D. citri* annotated gene list. Homologous blast search from eight *D. melagonaster* aquaporin genes (flybase.org) against *D. citri* genome were mostly successful in identifying genes to annotate. In cases marked with (*), the next closest match from the BLAST search was annotated.

mRNA:	Symbol (Predicted):	Scaffold:	*D. melanogaster*
XM_008476342	bib (big brain)	NW_007378164.1	FBgn0000180
XM_008471675	Aquaporin AQPA.e_1	NW_007377733.1	FBgn0033635
XM_008486010	Aquaporin AQPAe.a_2 (drip)	NW_007381649.1	FBgn0015872
XM_008486171	Aquaporin 12-like	NW_007381869.1	FBgn0033807
XM_008489052	Aquaporin-4 like	NW_007459679.1	FBgn0034882
XM_008475207*	Aquaporin AQPAe.a-like*	NW_007378044.1*	FBgn0034885
XM_008489051*	Aquaporin-2-like*	NW_007459679.1*	FBgn0034884
XM_008483237*	Aquaporin-4 like*	NW_007379533.1*	FBgn0034883

Table 2. BLAST-based analyses of *D. citri* aquaporin genes compared to those from the fruit fly (*Drosophila melanogaster*), bed bug (*Cimex lectularius*), and pea aphid (*Acyrthosiphon pisum*).

	Drosophila melanogaster				Cimex lectularius				Acyrthosiphon pisum			
MCOT Identification	E-value	Greatest positive %	Name	Greatest bit score	E-value	Greatest positive %	Name	Greatest bit score	E-value	Greatest positive %	Name	Greatest bit score
MCOT00250	5.28E-60	66.48648649	FBpp0289514	192.586	1.96E-66	68.2795699	CLEC009415-RA	208.379	4.37311	53.968254	ACYPI40602-RA	29.261
MCOT01120	2.4E-87	70.20408163	FBpp0303807	263.848	3.62E-58	67.4033149	CLEC007371-RA	186.037	3.39E-79	66.3967611	ACYPI006387-RB	246.899
MCOT05401	2.61E-67	64.3454039	FBpp0079519	238.424	3.98E-57	68.3982684	CLEC002337-RA	202.986	4.31E-53	58.0838323	ACYPI010017-RA	199.519
MCOT09727	1.13E-49	59.74576271	FBpp0072014	169.088	3.91E-56	63.5983264	CLEC013397-RA	193.741	2.37E-35	51.2195122	ACYPI009194-RA	135.576
MCOT11602	1.87E-18	49.375	FBpp0087235	80.4925	1.66E-21	53.1034483	CLEC007371-RA	87.4261	1.96E-20	52.4137931	ACYPI006387-RB	87.0409
MCOT17477	7.4E-62	67.77777778	FBpp0308570	199.519	5.34E-53	61.6858238	CLEC007784-RA	175.637	2.22E-43	63.7254902	ACYPI006387-RB	154.066
MCOT18515	5.53E-50	58.62068966	FBpp0072012	169.088	1.12E-44	57.7092511	CLEC011798-RA	153.68	6.5E-46	58.6666667	ACYPI009194-RA	165.236
MCOT19915	1.29E-59	64.12556054	FBpp0072012	194.512	1.12E-50	60.7929515	CLEC011798-RA	169.859	6.45E-55	59.6774194	ACYPI009194-RA	190.66

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Supplementary Note 34: Cathepsins and Cysteine Proteases

Introduction

Cysteine cathepsins are usually found in lysozymes where they are optimally active due to the slightly acidic environment (Turk et al, 2012). Cathepsins are found in vertebrates and invertebrates; in insects, cathepsins function in digestion, growth, and embryological development. There are several forms of cathepsins, designated by letters that can be divided into functional groups. Cathepsin B has its own group, cathepsins L, O, H, K, and S are in another group, and cathepsins W and F potentially make a third group (Wang et al, 1998). Normal organismal functioning depends on the different cathepsins; for example, cathepsin F is involved with MHC class II antigen presentation in macrophages (Turk et al, 2001). Cathepsin B has been attributed to several biological processes including apoptosis during metamorphosis, in embryogenesis with mobilization and degradation of yolk proteins, and digestion in the midgut (Kutsukake et al, 2004).

Methods

Cathepsin genes were collected from NCBI, i5k, Ensembl and FlyBase. BLAT searches were then performed at the i5k workspace to identify homologs in the ACP genome. Potential hits within the ACP genome were explored further using the WebApollo genome browser. NCBI Gnomon annotation gene models were manually annotated within WebApollo using BLAST and RNA-Seq evidence tracks. Completed gene models were analyzed by BlastP to confirm accuracy and completeness of the annotation.

Sequences from completed annotated genes were used to perform BlastP searches against related insects and model organisms. Pairwise amino acid sequence comparisons were performed between ACP, *Acyrthosiphon pisum* and *Drosophila melanogaster.* Collected orthologs and annotated ACP Cathepsin B protein sequences were used to generate a multiple sequence alignment using ClustalW in MEGA6. Sequences were chosen to identify close relationships to *A. pisum*. The ACP proteins were numbered in the order found. A neighbor joining phylogenetic tree was produced from the multiple sequence alignment to analyze the evolutionary relationships between the ACP cathepsins and well characterized sequences. (Figure 1) The unrooted Neighbor-Joining phylogenetic tree was constructed with the MEGA6 program using the bootstrap method (with 1000 replications) as a test of phylogeny and Poisson correction as the substitution model. The analysis includes 53 amino acid sequences of cathepsin genes proposed by NCBI and Ensembl, 607 positions with the ambiguous positions removed for each sequence pair in the data set.

Results and Discussion

Within the Asian citrus psyllid genome, a total of 34 cathepsins and/or cysteine proteinase orthologs were found. (Table 1) Manual curation of NCBI Gnomon annotation gene models

allowed for evidence-based gene associations. The NCBI gene models included several partial genes that included peptidase domains within Apollo. Areas flanking the partial models were investigated, but did not reveal the absent sequences. Annotation of full length cathepsin B genes contained 5-6 exons, as found by Rispe et al in 2008. Several *A. pisum* cathepsin B orthologs were identified in the ACP with 32-65% amino acid identity, and 71-100% query coverage. (Table 2) Two *D. melanogaster* cathepsin B orthologs were identified in the ACP with 31% to 69% amino acid identity. Cathepsin H, L, K, O, and W also have orthologs in ACP. Two clades were identified through phylogenetic analysis within the cathepsin B orthologs, B-1 and B-2.

The evolutionary process of gene duplication can contribute to development of new biological functions or phenotypic traits, and is important to study within the cathepsin family. There was research that previously found many cathepsin B-like genes in the pea aphid (Table 1), and 11 were found in the psyllid genome (Rispe et al, 2008). Pairwise alignments and multiple sequence alignments were created to analyze the evolutionary relationships between the cathepsin B's, which showed a similar expansion from 2 B-like genes in most model organisms, to the 28 in the pea aphid (Figure 1). Cathepsin B and cathepsin F have undergone genetic expansion, including 11 cathepsin B and B like proteins, and 9 cathepsin F and F like proteins. This is similar to the pea aphid, whom was shown to have a genetic expansion of cathepsin B proteins likely due to their phloem feeding lifestyle. RNAi inhibition of psyllid cathepsin B is considered a potential approach to control psyllid vectored disease.

Table 1: Cathepsin gene copies. The numbers of named gene copies in *Diaphorina citri*, *Acyrthosiphon pisum*, *Drosophila melanogaster*, *Cimex lectularius*, *Anopheles gambiae*, *Aedes aegypti*, and *Culex quinquefasciatus*, and *Mus musculus*. The letters designate the type of cathepsin.

Cathepsin	B	F	H	L / L1	K	Other
D. citri	11	9	1	5	0	8
A. pisum	28	1	0	2	1	8
C. lectularius	8	0	0	9	0	6
D. melanogaster	2	1	0	1	1	0
A. gambiae	6	2	0	2	0	4
A. aegypti	7	0	0	1	0	4
C. quinquefasciatus	5	0	0	1	0	2
M. musculus	2	2	2	2	1	21

Table 2: Cathepsin B sequence table. The protein sequences used for the phylogenetic analysis with their corresponding BLAST match results for specific species, with the bit scores followed by the coverage %, ID%, and accession numbers. The *D. citri* proteins are numbered in the order found. The A. pisum proteins were named according to their EST numbers. The D. melanogaster proteins were renamed from their accession number name.

D. citri Gene Named	D. citri AA Length	A. pisum BLAST Match Name and Bit Score	BLAST Query Percent	BLAST ID Percent	A. pisum Acc #	D. melanogaster BLAST Match Name and Bit Score	BLAST Query Percent	BLAST ID Percent	D. melanogaster Acc #
B Prot	131	B348 77.4	90	32	NP001119608	B Iso A 53.5	29	49	NP572920
B Prot 1	253	B5880 133	84	34	NP001119619	B Iso A 105	82	31	NP572920
B Prot 1 Iso 2	344	B348 229	99	38	NP001119608	B Iso A 126	96	37	NP572920
B Prot 2	180	B348 240	97	65	NP001119608	B Iso A 256	98	69	NP572920
B-like iso X1 prot 3	399	B348 355	91	55	NP001119608	B Iso A 340	91	57	NP572920
B-like iso X2 prot 4	399	B348 356	91	55	NP001119608	B Iso A 345	91	57	NP572920
B-like cp 5	374	B91418 231	71	42	NP001119614	B2 3074 211	69	31	NP726176
B-like cp 6	362	B84 242	95	37	NP001119618	B Iso A 227	89	39	NP572920
B-like prot 7 part	143	B1852 123	100	40	NP001119610	B Iso A 116	99	40	NP572920
B-like prot 8 part	110	B3074 47.8	88	34	DAA06100	B Iso A 46.6	75	37	NP572920
B-like cp 4 prot 9 part	90	B10270 67	84	42	NP001128401	B Iso A 27.7	86	31	NP572920
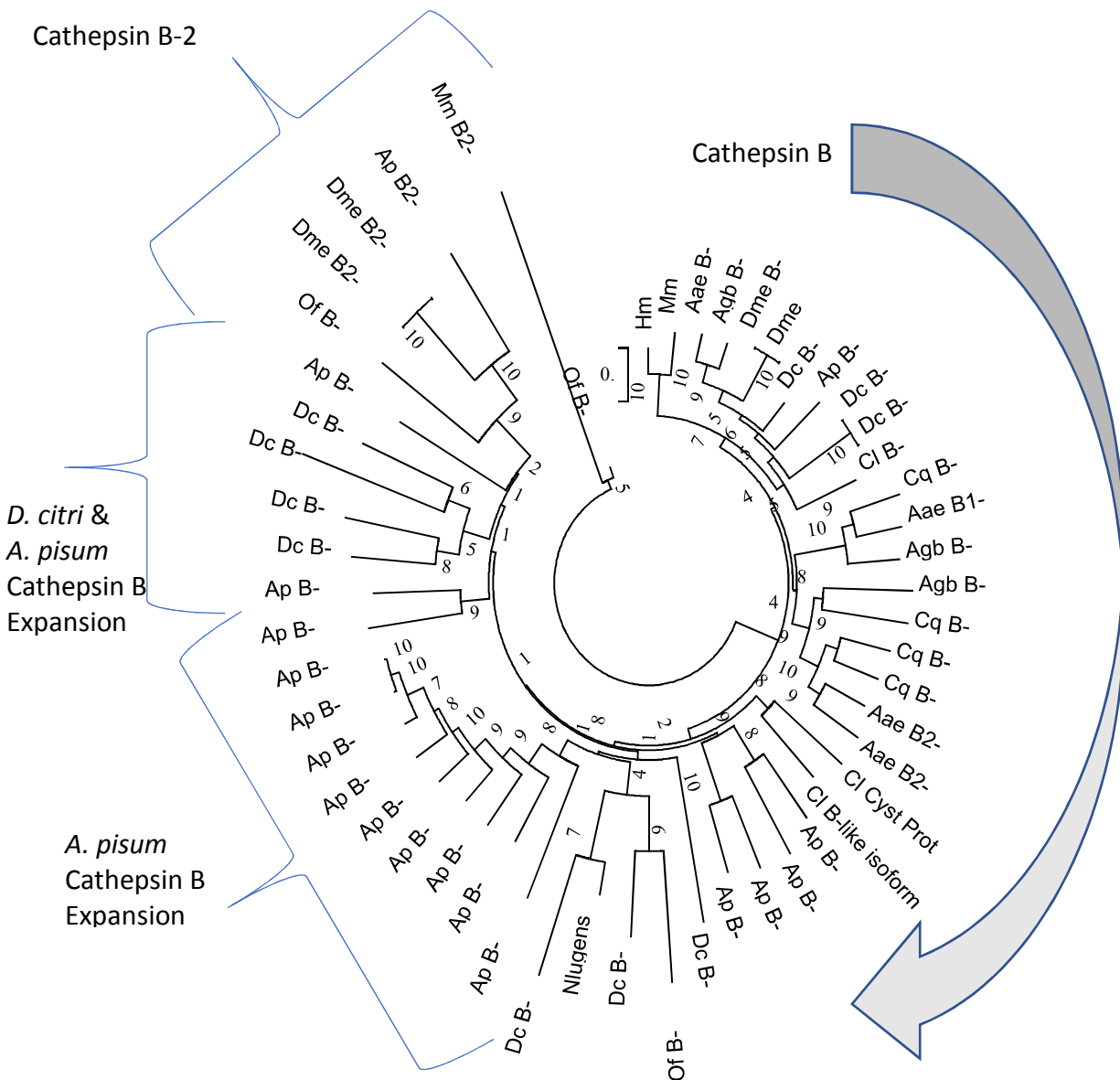
Figure 1: Phylogenetic analysis of *Diaphorina citri* cathepsin B family protein sequences
The unrooted Neighbor-Joining phylogenetic tree was constructed with the MEGA6 program using the bootstrap method (with 1000 replications) as a test of phylogeny and Poisson correction as the substitution model. The analysis includes 53 amino acid sequences with 607 positions in the data set of cathepsin genes proposed by NCBI. Bootstrap values are shown near the nodes. The *D. citri* proteins are numbered in the order found. The *A. pisum* proteins were named according to their EST numbers. *Cimex lectularius, Aedes aegypti, Culex quinquefasciatus* were renamed using the last four digits of their

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accession numbers, and *D. Melanoaster* proteins were renamed from their accession number name. Abbreviations: *Diaphorina citri* Dc; *Acyrthosiphon pisum* Ap; *Anopheles gambiae* Agb; *Drosophila melanogaster* Dme; *Cimex lectularius Cl; Aedes aegypti Aae; Culex quinquefasciatus* Cq; *Homo sapien* Hm; *Mus musculus* Mm; and *Nilaparvata lugens* Nlugens.

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Supplementary Note 35: Early developmental genes (Gap and Pair Rule genes)

Introduction

Early developmental genes are responsible for determining anterior/posterior (A/P) and the dorsal/ventral (D/V) axis of the embryo. The establishment of the axes enables the embryo to undergo segmentation. Segmentastion, or the subdivision of the developing embryo into serially homologous units, is one of the hallmarks of arthropods development. Arthropod segmentation is best understood in the fly *D. melanogaster*. *D. melanogaster* is different from most arthropods in that all segments are formed from the early blastoderm and segments are formed simultaneously (so called long-germ developmental mode). In most other arthropods only the anterior segments are formed in a similar way to *D. melanogaster* with the posteria sections added one at a time or in pairs of two from cell material derived from a posterior growth zone (so called short-germ developmental mode). Insects, show a range of short to long germ development however segmentation mechanisms are not universally conserved and only little is known about the genetic patterning of the anterior segments in *D. citri,* hence the need to identify early development genes in *D. citri*.

Methods

The choice of early developmental genes to annotate was informed on the basis of GO term annotations in *D. melanogaster*. Protein sequences for developmental genes for *D. melanogaster* were obtained from http://flybase.org/ [3]. Contig sequences were searched for homology to the selected protein sequences using tbastn. Gene models that aligned with the regions of highest homology identified by tbastn search were selected for further analysis. RNAseq mapped reads were compared with the gene models to determine the transcribed regions. The transcribed regions were used to determine protein sequences of the gene. Protein sequences were used in reciprocal blast (blastx NCBI) to confirm the homology of the orthologs. Gene models were edited to resolve conflicts between RNAseq, blastx and homology data. Many of these were partial models and some had frameshifts and/or internal stop codons. A list of most (but not all) of the sequences is provided in the Psyllid annotation workbook. *Diaphorina citri* gene models identified by MCOT generated from a comparison of maker and cufflinks models with de novo assemblies by Trinity and Oases were searched for homology to known *D. melanogaster* early development genes. MCOT Gene models that aligned with the regions of highest homology identified by tbastn search were selected for further analysis by reciprocal blast against model insect genomes.

Results

One of the main reasons for choosing to sequence the *Diaphorina citri* genome was to identify novel targets for biological control. For this reason, it was of special interest to analyse its

developmental gene complement. As developmental genes are a potential targets for gene silencing by RNA interference (RNAi), which is emerging as a promising tool to control *D. citri* through targeting nymphy development pathways [1]. In total, we annotated ~10 genes that are known, in other insects, to be involved in developmental processes. We identified a further ~15 gene models in MCOT data base that also mapped to proteins involved in early developmental processes in insects. These include both genes encoding transcription factors and members of signaling pathway. These genes are identified and named as distinct development genes in accordance with the nomenclature from *Drosophila melanogaster*. There are also numerous developmental gene fragments, incomplete and putative models/genes predicted from the assembly. The genes identified indicate that *D. citri* utilizes conical insect developmental pathway, including Hox genes, and can be expected to contain components require to establish a normal anterior/posterior axis pattern. Normally the number of early developmental genes does not vary in arthropod species due to the conserved nature of development in this order [2]. In confirmation of this the early patterning genes of *D. citri,* appear mainly conserved relative to what is known from other insects. No gene duplication in the early development genes was observed. However a number of gap and pair rule genes know from *Drosophila* were not found in *D. citri* however we conclude that the absence from the genome is evidence of the incomplete coverage of the sequencing effort, rather than a true absence.

Genes	Species	
	D. melanogaster	*D. citri*
crocodile	✓	✓
knirps	✓	✓
even-skipped	✓	✓
hairy	✓	✓
odd-paired	✓	✓
runt	✓	✓
runxA/lozenge	✓	✓
runxB	✓	✓
antennapedia	✓	✓
sex combs reduced	✓	✓

Table 1 Presence/absence of early patterning genes in the genomes of Hemiptera *Drosophila melanogaster* and *Diaphorina citri*

D. citri Gap Genes	LOCATION [Accession #]	Protein Length	Domains
crocodile	gi|645504644|ref|NW_007378308.1 [XP_008475713.1]	322 aa	FH Superfamily

knirps	gi|645504350|ref|NW_007378383.1 [XP_008476344.1]	344 aa	zinc finger C4
D. citri Pair Rule Genes	LOCATION [Accession #]	Protein Length	Domains
even-skipped	gi|645499728|ref|NW_007379481.1 [XP_008481312.1]	165 aa	
hairy	gi|645499404|ref|NW_007379550.1 [XP_008481515.1]	297 aa	HLH Superfamily Hairy-orange Superfamily
odd-paired	gi|645497036|ref|NW_007380134.1 [XP_008482718.1]	316 aa	zinc finger M2C2
runt	gi|645490800|ref|NW_007381688.1 [XP_008484261.1]	155 aa	runt superfamily
runxA/lozenge	gi|645501180|ref|NW_007379113.1 [XP_008480084.1]	258 aa	runt superfamily
runxB	gi|645501995|ref|NW_007378890.1 [XP_008479194.1]	130 aa	runt superfamily
D. citri Hox Genes	LOCATION [Accession #]	Protein Length	Domains
antennapedia	gi|645505921|ref|NW_007378005.1 [XP_008481317.1]	317 aa	Homeobox
sex combs reduced	gi|645504043|ref|NW_007378448.1.1 [XP_008476811.1]	313 aa	Homeobox

Table 2. Current Early Developmental Genes identified in the *D. citri* genome.

The table lists Gap Genes, Pair Rule Genes and Hox Genes models, model location and accession number, protein length, and protein domain identified in the model

Diaphorina citri	crocodile QC (ID) Bit Score	knirps QC (ID) Bit Score	even skipped QC (ID) E-value	hairy QC (ID) E-value	odd paired QC (ID) Bit Score
Acyrthosiphon pisum	64% (63%) 246	31% (87%) 207	35% (95%) 52	100% (45%) 192	37% (76%) 202
Halyomorpha halys	79% (55%) 233	43% (74%) 216	26% (51%) 47	62% (47%) 160	32% (44%) 98
Cimex lectularius	97% (50%) 248	36% (80%) 204	29% (52%) 51	61% (48%) 144	37% (81%) 206
Anopheles gambiae	67% (54%) 227	28% (94%) 202	20% (83%) 60	73% (50%) 195	37% (66%) 209
Aedes aegypti	94% (74%) 244	40% (73%) 199	20% (83%) 60	100% (45%) 232	93% (44%) 230
Drosophila melanogaster	69% (56%) 231	27% (86%) 196	20% (93%) 60	100% (40%) 170	40% (73%) 209

Diaphorina citri	runt QC (ID) Bit Score	RunxA QC (ID) E-value	RunxB QC (ID) E-value	antennapedia QC (ID) E-value	sex combs reduced QC (ID) E-value
Acyrthosiphon pisum	100% (66%) 211	62% (82%) 284	89% (84%) 206	89% (80%) 190	94% (81%) 68
Halyomorpha halys	100% (70%) 233	55% (89%) 274	78% (85%) 191	100% 67% 326	97% (46%) 89
Cimex lectularius	92%	58%	82%	100%	94%

	(78%) 245	(89%) 292	(83%) 198	(66%) 322	(51%) 104
Anopheles gambiae	99% (72%) 243	48% (80%) 233	81% (67%) 154	99% (47%) 219	57% (81%) 54
Aedes aegypti	89% (73%) 220	59% (87%) 283	90% (76%) 189	99% (48%) 218	57% (81%) 53
Drosophila melanogaster	94% (75%) 244	64% (80%) 293	82% (82%) 194	95% (61%) 218	25% (58%) 50

Table 3. Represents Query Coverage (Identity) and E-value of the annotated gene models pairwise aligned to orthologues in other species. Pairwise alignment was performed using NCBI blast.

D. citri Genes	MCOT Model Number
cap 'n' collar	MCOT01547.1.CT
caudal	MCOT08625.0.OO
collier/knot	MCOT22397.0.CO
crocodile	MCOT13724.0.CC
empty spiracles	MCOT04329.0.CO
hunchback	MCOT05092.1.CC
kruppel	MCOT03100.0.CO
orthodenticle	MCOT05861.1.CO
sloppy paired	MCOT19895.0.CT
tailless	MCOT23139.2.CC
even skipped	MCOT05103.0.CT
hairy	MCOT00149.0.CT

odd paired	MCOT12397.2.CT
runt	MCOT14329.0.CT

Table 4. Current Early Developmental Genes identified in the *D. citri* MCOT database. The table lists Gap Genes, and Pair Rule Genes model number

Gap Genes

Diaphorina citri	cap 'n' collar QC (ID) E-value	caudal QC (ID) E-value	collier QC (ID) E-value	crocodile QC (ID) E-value	empty spiracles QC (ID) Bit Score
Acyrthosiphon pisum	39% (63%) 234	43% (71%) 141	51% (77%) 471	61% (62%) 244	63% (79%) 238
Halyomorpha halys	66% (44%) 381	90% (49%) 154	82% (87%) 797	76% (55%) 233	46% (87%) 194
Cimex lectularius	66% (43%) 417	79% (58%) 162	82% (87%) 792	93% (49%) 248	60% (75%) 214
Anopheles gambiae	24% (72%) 230	54% (71%) 169	16% (78%) 36	51% (68%) 223	30% (79) 174
Aedes aegypti	35% (44%) 249	61% (66%) 168	82% (83%) 792	89% (46%) 239	47% (57%) 177
Drosophila melanogaster	23% (66%) 207	37% (76%) 134	82% (84%) 798	65% (57%) 230	46% (60%) 183

Diaphorina citri	hunchback QC (ID) E-value	kruppel QC (ID) E-value	orthodenticle QC (ID) E-value	sloppy paired QC (ID) Bit Score	tailless QC (ID) E-value

Acyrthosiphon pisum	15% (51%) 148	27% (67%) 164	22% (89%) 116	20% (76%) 147	95% (42%) 263
Halyomorpha halys	17% (58%) 187	50% (48%) 223	49% (65%) 172	77% (51%) 187	94% (51%) 338
Cimex lectularius	18% (53%) 184	51% (57%) 265	44% (72%) 171	82% (51%) 208	98% (51%) 360
Anopheles gambiae	16% (55%) 174	56% (51%) 265	32% (66%) 129	41% (69%) 174	82% (78%) 201
Aedes aegypti	15% (51%) 148	57% (59%) 279	23% (89%) 133	41% (69%) 174	95% (44%) 295
Drosophila melanogaster	16% (55%) 187	53% (56%) 271	30% (76%) 135	42% (73%) 210	95% (42%) 298

Pair Rule Genes

Diaphorina citri	even skipped QC (ID) Bit Score	hairy QC (ID) E-value	odd paired QC (ID) E-value	runt QC (ID) Bit Score
Acyrthosiphon pisum	38% (91%) 160	92% (48%) 223	44% (79%) 320	35% (76%) 208
Halyomorpha halys	24% (88%) 149	59% (53%) 202	42% (83%) 316	56% (64%) 254
Cimex lectularius	40% (90%) 154	57% (54%) 184	ND	52% (70%) 268
Anopheles gambiae	24% (90%) 152	68% (55%) 234	47% (70%) 332	54% (66%) 253
Aedes aegypti	25% (85%)	92% (46%)	61% (44%)	49% (67%)

	152	258	220	250
Drosophila melanogaster	24% (88%) 149	57% (56%) 196	46% (76%) 328	49% (69%) 253

Table 5. Represents Query Coverage (Identity) and E-value of the MCOT gene models pairwise aligned to orthologues in other species. Pairwise alignment was performed using NCBI blast.
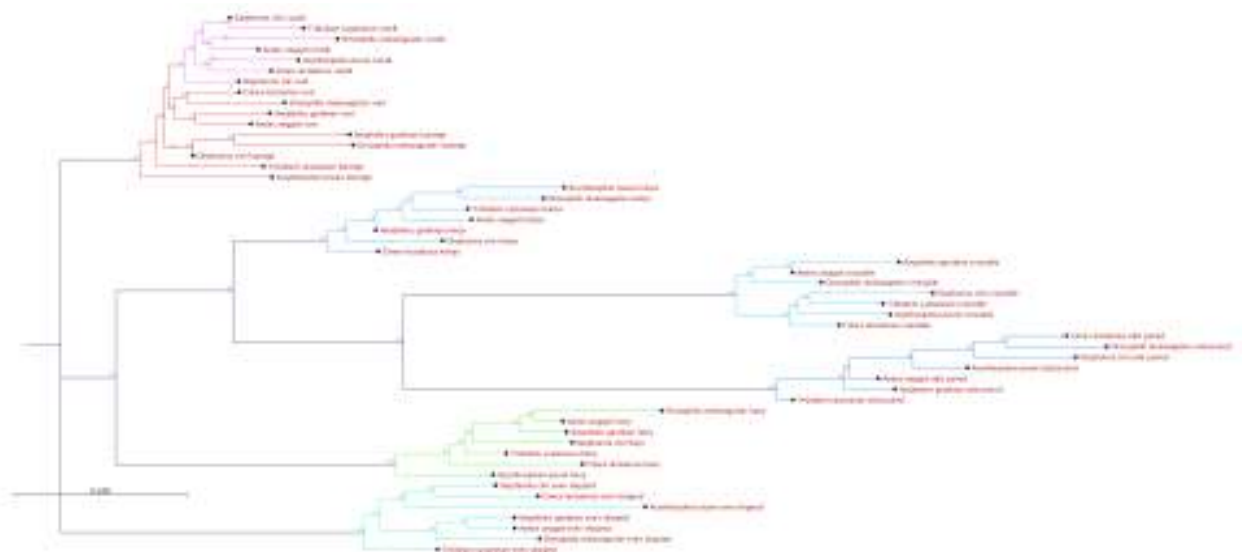
Figure1: Neighbor- Joining tree for early development genes genes in *D. citri* using 1000 bootstrap replicates, Poisson model, and complete deletion. *D. citri* consistently appears close to fellow hemipteran *A. pisum* and beetle *T. castaneum.*

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Supplementary Note 36: Cuticle proteins

Introduction

The cuticle of arthropods provides the structural and mechanical support and protection that has contributed to the success of these organisms in diverse habitats (Neville, 1975). Arthropod cuticles are primarily composed of chitin fibers embedded in a proteinaceous matrix (Neville, 1975) that consists of a variety of cuticle proteins (reviewed in Willis et al., 2005). The number and types of cuticle proteins present are diverse among the arthropods with individual species containing some subset of approximately a dozen families of cuticle protein (Ioannidou et al, 2014; Willis, 2010). Families are generally classified by the presence of conserved motifs, with the CPR family, characterized by the "R&R Consensus" domain (Rebers and Riddiford, 1988), being the largest. Certain families, including CPR, CPAP1 and CPAP3, are common among arthropods whereas families such as CPCFC and CPLCW are restricted to certain orders and/or even smaller taxonomic groups (Ioannidou et al, 2014; Willis, 2010). However, the understanding of the diversity of cuticle proteins is currently evolving as more arthropod genomes are sequenced and expression patterns of cuticle protein genes are examined.

Methods

The two assembly versions for *Diaphorina citri* (diaci1.1 generated by the Baylor College of Medicine Human Genome Sequencing Center (BCM-HGSC**)**, and the NCBI GenBank- and RefSeq-processed version; NCBI-diaci1.1) were blasted (BLASTp; McGinnis and Madden, 2004) using sequence motifs that are characteristic of several families of cuticle proteins (Willis, 2002). Predicted cuticle proteins were further analyzed with CutProtFam-Pred, a cuticular protein family prediction tool described in Ioannidou et al. (2014), to assign genes to specific gene families of cuticle proteins. To find the closest putative homolog to cuticle protein genes from *D. citri*, genes were searched against the RefSeq protein sets downloaded from the National Center for Biotechnology Information (NCBI), or the official gene set for *Acyrthosiphon pisum* (Refseq), *Cimex lectularius* (maker v. 5.3, BCM-HGSC; Benoit et al., 2016), Gerris buenoi (maker v. 5.3, BCM-HGSC), *Oncopeltus fasciatus* (maker v. 5.3, BCM-HGSC), *Rhodnius prolixus* (CDC v. 3.1, downloaded from VectorBase), *Drosophila melanogaster* (Refseq), *Bombyx mori* (Refseq), *Tribolium castaneum* (Refseq), *Apis mellifera* (Refseq), and *Pediculus humanus corporis* (Refseq). The protein sequence with the lowest e-value was considered the closest putative homolog. Due to the relatively few number of cuticle protein genes found in the assembled genome, MCOT v1.0 transcriptome was also analyzed for putative cuticle proteins using the same methods described above.

Table 1. Number of genes identified as putative cuticle proteins per family in the genome of *Diaphorina citri*.							
	CPR[a]						
RR-1	**RR-2**	**Uncl**	**CPAP1**	**CPAP3**	**CPF**	**TWDL**	**Total**
9	26	3	11	8	3	3	63

[a]Sequences that scored above the assigned cutoffs for the RR-1 and RR-2 models were classified as the corresponding type, whereas sequences with scores below the assigned cutoffs but above 0 were characterized as "unclassified" (Uncl). For more information, see Ioannidou et al. (2014).

Table 1b. Number of genes identified as putative cuticle proteins per family in the genome of *Diaphorina citri*.

	CPR[a]						
RR-1	**RR-2**	**Uncl**	**CPAP1**	**CPAP3**	**CPF**	**TWDL**	**Total**
11	33	20	16	7	2	3	92

[a]Sequences that scored above the assigned cutoffs for the RR-1 and RR-2 models were classified as the corresponding type, whereas sequences with scores below the assigned cutoffs but above 0 were characterized as "unclassified" (Uncl). For more information, see Ioannidou et al. (2014).

Table 2. Number of genes identified as putative cuticle proteins per species in the genomes of several insect orders.										
Order	**Species**	**CPR**	**CPAP1**	**CPAP3**	**CPCFC**	**CPF**	**CPLCA**	**CPLCG**	**TWDL**	**Total**
Hemiptera	*Acyrthosiphon pisum* [b]	117	11	10	1	3	0	0	3	145
	Cimex lectularius [c]	121	15	6	0	5	0	0	3	149
	Diaphorina citri [a]	38	11	8	0	3	0	0	3	63
	Gerris buenoi [a]	126	10	6	0	3	0	0	10	155
	Oncopeltus fasciatus [a]	133	11	7	0	7	0	0	3	173
	Rhodnius prolixus [a]	91	10	6	1	2	0	0	1	111
Diptera	*Drosophila melanogaster* [b]	137	29	10	1	5	13	4	29	228
Lepidoptera	*Bombyx mori* [b]	144	13	6	1	1	2	0	4	171
Coleoptera	*Tribolium castaneum* [b]	110	13	7	2	5	1	1	3	142
Hymenoptera	*Apis mellifera* [b]	38	15	7	0	4	0	0	2	66
Phthiraptera	*Pediculus humanus* [b]	41	12	6	1	1	0	0	2	63

[a] Cuticle protein numbers determined by analyzing gene sets with CutProtFam-Pred (Ioannidou et al., 2014).
[b] Cuticle protein numbers determined from Ioannidou et al. (2014).
[c] Cuticle protein numbers determined from Benoit et al. (2015).

Results

Sixty-three genes encoding for putative cuticle proteins were identified in *D. citri* by searching the genome with sequence motifs characteristic of different cuticle protein families as established by Willis (2002). CutProtFam-Pred (Ioannidou et *al.*, 2014) was employed to assign these genes to one of five families (CPR, CPAP1, CPAP3, CPF, and TWDL; Table 1). The total number of cuticle protein genes identified in *D. citri* (63) is much less than in other hemipterans (111-173; Ioannidou et al., 2014), but is within the range of that observed in other insects (Table 2). It is unclear if this reduced number accurately reflects the number of genes in *D. citri*, or is a reflection of a suboptimal genome assembly. As with other insects, the number of CPR genes (38) constituted the largest group of cuticle protein genes in the *D. citri* genome; however, this number is less than in other hemipterans and accounted for the reduced number of total cuticle protein genes. The number of genes in the protein families CPAP1, CPAP3, CPF, and TWDL was similar to the number in other insects (Ioannidou et al., 2014; Table 2). Within MCOT transcriptome 92 genes were identified as putative cuticle proteins. The greater number of cuticle protein transcripts was partially due to multiple isoforms of single genes; however, some were unique from the identified genes, suggesting the assembled genome may be lacking several expected genes.

References

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Supplementary Note 37: Secretome

Introduction

Saliva is secreted by insects and this product make contact with the plant tissue during the feeding process (Moreno et al., 2012). Secreted proteins (including potentially effectors) from insect plant pest thought to be involved in pathogenicity are encoded in their genomes and can be identified by sequence analyses based on secretion signals (Rodriguez and Bos, 2013;Jaouannet et al., 2014). To date effectors from insect pest pathogens have been identified bioinformatically identified from transcript data obtained from insect tissue containing saliva and from saliva extracts (Bos et al., 2010).

Materials and Methods

The presence of signal peptide secretion signals is a universal feature of effectors plant, insect and mammalian parasitic microbes and can be used as an initial filtering step (Shang et al., 2016). We screened all predicted proteins for the presence of signal peptides using SignalP version 2.0 (Nielsen et al., 1997) and for the absence of transmembrane domains with TMHMM version 2.0 (Krogh et al., 2001). Secreted proteins with a SignalP HMM score > 0.9, NN cleavage site within 10 and 40 amino acids (aa), and no transmembrane domains found at >40 amino acids away from the starting amino acid methionine, were selected. From the secretome list, we performed a search for proteins with 2% or higher % of cysteines and no more than 250 amino acids in length. We used the group of small cysteine rich proteins and described the presence of nuclear localization signals (NLSs) using NLStradamus (Nguyen Ba et al., 2009) and Chloroplast motifs using ChloroP v1.0 (Emanuelsson et al., 1999).

Table 1: List of predicted cysteine rich proteins

Gene_ID	Description
XP_008482042.1	15 kDa selenoprotein [Diaphorina citri] --HMM score = 0.963 --Signal peptide length = 24 PredHel = 0 Topology = o len = 176
XP_017298172.1	ABC transporter A family member 9-like [Diaphorina citri] --HMM score = 0.920 --Signal peptide length = 15 PredHel = 1 Topology = i2-24o len = 140
XP_008482159.1	acidic mammalian chitinase-like [Diaphorina citri] --HMM score = 1.000 --Signal peptide length = 24 PredHel = 0 Topology = o len = 84
XP_017299188.1	alpha-galactosidase A-like [Diaphorina citri] --HMM score = 1.000 --Signal peptide length = 22 PredHel = 0 Topology = o len = 146
XP_017299189.1	alpha-galactosidase A-like [Diaphorina citri] --HMM score = 1.000 --Signal peptide length = 22 PredHel = 0 Topology = o len = 146
XP_008478669.1	apolipoprotein D-like [Diaphorina citri] --HMM score = 0.971 --Signal peptide length = 32 PredHel = 0 Topology = o len = 189
XP_008484002.1	astakine-like, partial [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 20 PredHel = 0 Topology = o len = 72
XP_008472105.1	autophagy-related protein 2 homolog A-like [Diaphorina citri] --HMM score = 1.000 --Signal peptide length = 19 PredHel = 0 Topology = o len = 147

XP_008477626.1	basic salivary proline-rich protein 3-like [Diaphorina citri] --HMM score = 0.986 --Signal peptide length = 27 PredHel = 1 Topology = i7-29o len = 123
XP_008475283.1	cathepsin B-like [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 16 PredHel = 0 Topology = o len = 128
XP_008480568.1	chondroitin proteoglycan 2-like [Diaphorina citri] --HMM score = 1.000 --Signal peptide length = 20 PredHel = 0 Topology = o len = 247
XP_008487969.1	chorion class high-cysteine HCB protein 13-like [Diaphorina citri] --HMM score = 1.000 --Signal peptide length = 20 PredHel = 0 Topology = o len = 74
XP_008485271.1	clustered mitochondria protein homolog, partial [Diaphorina citri] --HMM score = 0.940 --Signal peptide length = 24 PredHel = 0 Topology = o len = 79
XP_008472726.1	connective tissue growth factor-like [Diaphorina citri] --HMM score = 0.993 --Signal peptide length = 22 PredHel = 0 Topology = o len = 159
XP_008473317.1	ctenidin-1-like isoform X1 [Diaphorina citri] --HMM score = 0.991 --Signal peptide length = 21 PredHel = 0 Topology = o len = 203
XP_017303134.1	cystatin-C-like isoform X1 [Diaphorina citri] --HMM score = 1.000 --Signal peptide length = 20 PredHel = 0 Topology = o len = 150
XP_008481260.1	cystatin-C-like isoform X2 [Diaphorina citri] --HMM score = 1.000 --Signal peptide length = 20 PredHel = 0 Topology = o len = 150
XP_008481261.1	cystatin-C-like isoform X3 [Diaphorina citri] --HMM score = 1.000 --Signal peptide length = 20 PredHel = 0 Topology = o len = 144
XP_008487197.1	cystatin-C-like, partial [Diaphorina citri] --HMM score = 1.000 --Signal peptide length = 20 PredHel = 0 Topology = o len = 140
XP_008479809.1	eclosion hormone-like [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 25 PredHel = 0 Topology = o len = 65
XP_008484466.1	eclosion hormone-like [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 25 PredHel = 0 Topology = o len = 80
XP_017300318.1	ejaculatory bulb-specific protein 3-like [Diaphorina citri] --HMM score = 0.992 --Signal peptide length = 21 PredHel = 0 Topology = o len = 156
XP_008478140.1	ejaculatory bulb-specific protein 3-like [Diaphorina citri] --HMM score = 0.993 --Signal peptide length = 22 PredHel = 0 Topology = o len = 137
XP_008473947.1	ejaculatory bulb-specific protein 3-like [Diaphorina citri] --HMM score = 0.998 --Signal peptide length = 23 PredHel = 0 Topology = o len = 135
XP_008478451.1	ejaculatory bulb-specific protein 3-like [Diaphorina citri] --HMM score = 0.998 --Signal peptide length = 23 PredHel = 1 Topology = o4-21i len = 75
XP_008478860.1	ejaculatory bulb-specific protein 3-like [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 19 PredHel = 0 Topology = o len = 124
XP_008482474.1	ejaculatory bulb-specific protein 3-like [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 19 PredHel = 0 Topology = o len = 88
XP_008478138.1	ejaculatory bulb-specific protein 3-like [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 20 PredHel = 0 Topology = o len = 128
XP_017303149.1	ejaculatory bulb-specific protein 3-like [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 20 PredHel = 0 Topology = o len = 136
XP_017303150.1	ejaculatory bulb-specific protein 3-like [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 20 PredHel = 0 Topology = o len = 136
XP_008478141.1	ejaculatory bulb-specific protein 3-like [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 20 PredHel = 0 Topology = o len = 67

XP_008471452.1	ejaculatory bulb-specific protein 3-like [Diaphorina citri] --HMM score = 1.000 -- Signal peptide length = 18 PredHel = 0 Topology = o len = 124
XP_008471453.1	ejaculatory bulb-specific protein 3-like [Diaphorina citri] --HMM score = 1.000 -- Signal peptide length = 18 PredHel = 0 Topology = o len = 124
XP_008481200.1	ejaculatory bulb-specific protein 3-like [Diaphorina citri] --HMM score = 1.000 -- Signal peptide length = 18 PredHel = 0 Topology = o len = 67
XP_008486851.1	ejaculatory bulb-specific protein 3-like, partial [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 19 PredHel = 1 Topology = i5-24o len = 59
XP_017301641.1	epidermal growth factor-like protein 7 [Diaphorina citri] --HMM score = 0.949 -- Signal peptide length = 24 PredHel = 0 Topology = o len = 103
XP_017304908.1	esterase 6-like, partial [Diaphorina citri] --HMM score = 1.000 --Signal peptide length = 20 PredHel = 1 Topology = o4-26i len = 63
XP_008474275.1	extensin-1-like [Diaphorina citri] --HMM score = 0.995 --Signal peptide length = 21 PredHel = 0 Topology = o len = 144
XP_017304793.1	Fanconi anemia group I protein homolog, partial [Diaphorina citri] --HMM score = 0.947 --Signal peptide length = 21 PredHel = 0 Topology = o len = 180
XP_008468088.1	folate receptor beta-like [Diaphorina citri] --HMM score = 0.958 --Signal peptide length = 17 PredHel = 0 Topology = o len = 211
XP_008468232.1	frizzled-2-like [Diaphorina citri] --HMM score = 0.997 --Signal peptide length = 23 PredHel = 1 Topology = i7-29o len = 117
XP_008469244.1	gamma-interferon-inducible lysosomal thiol reductase-like [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 23 PredHel = 0 Topology = o len = 212
XP_008470659.1	general odorant-binding protein 83a-like [Diaphorina citri] --HMM score = 0.989 -- Signal peptide length = 23 PredHel = 0 Topology = o len = 140
XP_017302279.1	geranylgeranyl transferase type-2 subunit beta-like, partial [Diaphorina citri] --HMM score = 0.988 --Signal peptide length = 21 PredHel = 0 Topology = o len = 163
XP_008469206.1	GILT-like protein C02D5.2 [Diaphorina citri] --HMM score = 0.996 --Signal peptide length = 23 PredHel = 0 Topology = o len = 208
XP_008469207.1	GILT-like protein C02D5.2 [Diaphorina citri] --HMM score = 0.996 --Signal peptide length = 23 PredHel = 0 Topology = o len = 208
XP_008469208.1	GILT-like protein C02D5.2 [Diaphorina citri] --HMM score = 0.996 --Signal peptide length = 23 PredHel = 0 Topology = o len = 208
XP_017297739.1	glutaredoxin-C4-like [Diaphorina citri] --HMM score = 0.967 --Signal peptide length = 24 PredHel = 1 Topology = o4-26i len = 117
XP_017297749.1	glutaredoxin-like isoform X1 [Diaphorina citri] --HMM score = 0.992 --Signal peptide length = 20 PredHel = 0 Topology = o len = 122
XP_008487182.1	glycine-rich cell wall structural protein 1.0-like isoform X1 [Diaphorina citri] --HMM score = 0.991 --Signal peptide length = 21 PredHel = 0 Topology = o len = 199
XP_008487183.1	glycine-rich cell wall structural protein 1.0-like isoform X2 [Diaphorina citri] --HMM score = 0.991 --Signal peptide length = 21 PredHel = 0 Topology = o len = 196
XP_008473318.1	glycine-rich cell wall structural protein 1.0-like isoform X2 [Diaphorina citri] --HMM score = 0.991 --Signal peptide length = 21 PredHel = 0 Topology = o len = 200
XP_008488257.1	hypertrehalosaemic prohormone-like [Diaphorina citri] --HMM score = 0.983 -- Signal peptide length = 24 PredHel = 1 Topology = i7-29o len = 74
XP_017299390.1	insulin gene enhancer protein isl-2a-like [Diaphorina citri] --HMM score = 0.971 -- Signal peptide length = 18 PredHel = 0 Topology = o len = 146

XP_008468127.1	insulin-2-like [Diaphorina citri] --HMM score = 0.980 --Signal peptide length = 26 PredHel = 0 Topology = o len = 136
XP_008473632.1	jacalin-related lectin 34-like isoform X2 [Diaphorina citri] --HMM score = 1.000 --Signal peptide length = 18 PredHel = 0 Topology = o len = 228
XP_008470248.1	lachesin-like [Diaphorina citri] --HMM score = 1.000 --Signal peptide length = 24 PredHel = 0 Topology = o len = 147
XP_008478723.1	latex clearing protein-like, partial [Diaphorina citri] --HMM score = 0.934 --Signal peptide length = 31 PredHel = 0 Topology = o len = 225
XP_017303321.1	leucine-rich repeat and immunoglobulin-like domain-containing nogo receptor-interacting protein 1-B [Diaphorina citri] --HMM score = 0.998 --Signal peptide length = 21 PredHel = 0 Topology = o len = 124
XP_017299004.1	leucine-rich repeat-containing protein 26-like, partial [Diaphorina citri] --HMM score = 0.991 --Signal peptide length = 25 PredHel = 0 Topology = o len = 133
XP_008468126.1	LIRP-like [Diaphorina citri] --HMM score = 0.980 --Signal peptide length = 26 PredHel = 0 Topology = o len = 136
XP_017297981.1	LIRP-like [Diaphorina citri] --HMM score = 0.980 --Signal peptide length = 26 PredHel = 0 Topology = o len = 136
XP_017302049.1	lysozyme-like isoform X1 [Diaphorina citri] --HMM score = 0.944 --Signal peptide length = 22 PredHel = 1 Topology = i7-25o len = 165
XP_008478360.1	lysozyme-like isoform X2 [Diaphorina citri] --HMM score = 0.944 --Signal peptide length = 22 PredHel = 1 Topology = i7-25o len = 165
XP_008485854.1	malectin-A-like, partial [Diaphorina citri] --HMM score = 0.966 --Signal peptide length = 26 PredHel = 1 Topology = i9-31o len = 70
XP_008474209.1	mesencephalic astrocyte-derived neurotrophic factor homolog [Diaphorina citri] --HMM score = 0.982 --Signal peptide length = 24 PredHel = 0 Topology = o len = 176
XP_017301153.1	neprilysin-2-like [Diaphorina citri] --HMM score = 0.997 --Signal peptide length = 15 PredHel = 0 Topology = o len = 100
XP_017304350.1	neuroendocrine protein 7B2 isoform X1 [Diaphorina citri] --HMM score = 0.996 --Signal peptide length = 17 PredHel = 0 Topology = o len = 238
XP_008484960.1	neuroendocrine protein 7B2 isoform X2 [Diaphorina citri] --HMM score = 0.996 --Signal peptide length = 17 PredHel = 0 Topology = o len = 238
XP_017304351.1	neuroendocrine protein 7B2 isoform X3 [Diaphorina citri] --HMM score = 0.996 --Signal peptide length = 17 PredHel = 0 Topology = o len = 238
XP_017302603.1	neuroligin-4, Y-linked-like [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 22 PredHel = 0 Topology = o len = 150
XP_008468890.1	neuropeptide SIFamide-like [Diaphorina citri] --HMM score = 0.991 --Signal peptide length = 23 PredHel = 1 Topology = i5-23o len = 75
XP_008468892.1	neuropeptide SIFamide-like [Diaphorina citri] --HMM score = 0.991 --Signal peptide length = 23 PredHel = 1 Topology = i5-23o len = 75
XP_008487361.1	nucleoside diphosphate kinase-like, partial [Diaphorina citri] --HMM score = 0.982 --Signal peptide length = 23 PredHel = 0 Topology = o len = 75
XP_008471862.1	peroxiredoxin-4-like [Diaphorina citri] --HMM score = 0.992 --Signal peptide length = 22 PredHel = 0 Topology = o len = 208
XP_008470698.1	peroxiredoxin-5, mitochondrial-like [Diaphorina citri] --HMM score = 0.955 --Signal peptide length = 19 PredHel = 0 Topology = o len = 174
XP_017298995.1	phospholipase A2-like [Diaphorina citri] --HMM score = 0.994 --Signal peptide length = 23 PredHel = 1 Topology = o4-21i len = 171

XP_017300972.1	pleiotrophin-A-like isoform X1 [Diaphorina citri] --HMM score = 1.000 --Signal peptide length = 19 PredHel = 0 Topology = o len = 154
XP_017300973.1	pleiotrophin-A-like isoform X2 [Diaphorina citri] --HMM score = 1.000 --Signal peptide length = 19 PredHel = 0 Topology = o len = 149
XP_008477640.1	probable G-protein coupled receptor Mth-like 5, partial [Diaphorina citri] --HMM score = 0.993 --Signal peptide length = 30 PredHel = 0 Topology = o len = 158
XP_008486968.1	probable protein disulfide-isomerase A6 isoform X1 [Diaphorina citri] --HMM score = 0.998 --Signal peptide length = 18 PredHel = 0 Topology = o len = 83
XP_008486974.1	probable protein disulfide-isomerase A6 isoform X2 [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 18 PredHel = 0 Topology = o len = 83
NP_001306216.1	probable xyloglucan-specific endo-beta-1,4-glucanase A precursor [Diaphorina citri] --HMM score = 1.000 --Signal peptide length = 22 PredHel = 0 Topology = o len = 230
XP_008474579.1	prohormone-3 [Diaphorina citri] --HMM score = 0.966 --Signal peptide length = 21 PredHel = 0 Topology = o len = 179
XP_008469102.1	protein quiver-like [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 38 PredHel = 0 Topology = i len = 64
XP_017299179.1	protein slit-like [Diaphorina citri] --HMM score = 0.980 --Signal peptide length = 32 PredHel = 1 Topology = o15-37i len = 147
XP_008481158.1	protein twisted gastrulation-like [Diaphorina citri] --HMM score = 0.948 --Signal peptide length = 30 PredHel = 0 Topology = o len = 114
XP_008470818.1	protein-L-isoaspartate O-methyltransferase 1-like [Diaphorina citri] --HMM score = 0.996 --Signal peptide length = 23 PredHel = 0 Topology = o len = 199
XP_008473927.1	putative odorant-binding protein A10 [Diaphorina citri] --HMM score = 0.993 --Signal peptide length = 18 PredHel = 0 Topology = o len = 121
XP_008473937.1	putative odorant-binding protein A10 [Diaphorina citri] --HMM score = 0.995 --Signal peptide length = 22 PredHel = 0 Topology = o len = 127
XP_008473631.1	putative per-hexamer repeat protein 5 isoform X1 [Diaphorina citri] --HMM score = 1.000 --Signal peptide length = 18 PredHel = 0 Topology = o len = 239
XP_008481743.1	quinone oxidoreductase-like [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 22 PredHel = 0 Topology = o len = 191
XP_017302036.1	RNA polymerase II transcriptional coactivator-like isoform X1 [Diaphorina citri] --HMM score = 0.994 --Signal peptide length = 21 PredHel = 0 Topology = o len = 139
XP_017302037.1	RNA polymerase II transcriptional coactivator-like isoform X1 [Diaphorina citri] --HMM score = 0.994 --Signal peptide length = 21 PredHel = 0 Topology = o len = 139
XP_017302038.1	RNA polymerase II transcriptional coactivator-like isoform X1 [Diaphorina citri] --HMM score = 0.994 --Signal peptide length = 21 PredHel = 0 Topology = o len = 139
XP_017302039.1	RNA polymerase II transcriptional coactivator-like isoform X1 [Diaphorina citri] --HMM score = 0.994 --Signal peptide length = 21 PredHel = 0 Topology = o len = 139
XP_017302041.1	RNA polymerase II transcriptional coactivator-like isoform X1 [Diaphorina citri] --HMM score = 0.994 --Signal peptide length = 21 PredHel = 0 Topology = o len = 139
XP_008478347.1	RNA polymerase II transcriptional coactivator-like isoform X2 [Diaphorina citri] --HMM score = 0.994 --Signal peptide length = 21 PredHel = 0 Topology = o len = 119
XP_008477322.1	serine protease VLSP-1-like, partial [Diaphorina citri] --HMM score = 0.998 --Signal peptide length = 21 PredHel = 0 Topology = o len = 125
XP_017301918.1	SPARC-like protein 1 [Diaphorina citri] --HMM score = 1.000 --Signal peptide length = 22 PredHel = 0 Topology = o len = 190

XP_008482273.1	thioredoxin domain-containing protein 12-like [Diaphorina citri] --HMM score = 0.987 --Signal peptide length = 19 PredHel = 0 Topology = o len = 158
XP_008482491.1	thioredoxin domain-containing protein 12-like [Diaphorina citri] --HMM score = 0.998 --Signal peptide length = 20 PredHel = 0 Topology = o len = 148
XP_008485322.1	thioredoxin-2-like isoform X1 [Diaphorina citri] --HMM score = 0.942 --Signal peptide length = 22 PredHel = 0 Topology = o len = 115
XP_017300397.1	transmembrane 9 superfamily member 2-like [Diaphorina citri] --HMM score = 0.993 --Signal peptide length = 18 PredHel = 0 Topology = o len = 64
XP_008487451.1	tribbles homolog 3-like, partial [Diaphorina citri] --HMM score = 0.912 --Signal peptide length = 24 PredHel = 0 Topology = o len = 144
XP_008475553.1	turripeptide Lol9.1-like isoform X1 [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 23 PredHel = 1 Topology = o4-21i len = 89
XP_008475554.1	turripeptide Lol9.1-like isoform X2 [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 23 PredHel = 1 Topology = o4-21i len = 89
XP_008481695.1	tyrosine-protein kinase transmembrane receptor Ror-like [Diaphorina citri] --HMM score = 0.943 --Signal peptide length = 29 PredHel = 1 Topology = o4-26i len = 207
NP_001284620.1	uncharacterized LOC103519184 precursor [Diaphorina citri] --HMM score = 1.000 --Signal peptide length = 17 PredHel = 0 Topology = o len = 148
XP_008486709.1	uncharacterized monothiol glutaredoxin ycf64-like, partial [Diaphorina citri] --HMM score = 0.917 --Signal peptide length = 25 PredHel = 1 Topology = o10-29i len = 66
XP_008467481.1	uncharacterized protein LOC103504956 [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 21 PredHel = 0 Topology = o len = 120
XP_008467997.1	uncharacterized protein LOC103505443 [Diaphorina citri] --HMM score = 0.929 --Signal peptide length = 19 PredHel = 0 Topology = o len = 223
XP_008468059.1	uncharacterized protein LOC103505499 isoform X2 [Diaphorina citri] --HMM score = 0.994 --Signal peptide length = 22 PredHel = 0 Topology = o len = 83
XP_008468200.1	uncharacterized protein LOC103505626 isoform X1 [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 23 PredHel = 1 Topology = o4-26i len = 112
XP_008468201.1	uncharacterized protein LOC103505626 isoform X2 [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 23 PredHel = 1 Topology = o4-26i len = 78
XP_008468499.1	uncharacterized protein LOC103505904 [Diaphorina citri] --HMM score = 0.973 --Signal peptide length = 17 PredHel = 0 Topology = o len = 188
XP_008468771.1	uncharacterized protein LOC103506162 [Diaphorina citri] --HMM score = 0.998 --Signal peptide length = 22 PredHel = 0 Topology = o len = 169
XP_017298314.1	uncharacterized protein LOC103506345 [Diaphorina citri] --HMM score = 0.975 --Signal peptide length = 28 PredHel = 1 Topology = o4-26i len = 118
XP_008469242.1	uncharacterized protein LOC103506625 [Diaphorina citri] --HMM score = 0.967 --Signal peptide length = 20 PredHel = 0 Topology = o len = 129
XP_008469286.1	uncharacterized protein LOC103506671 [Diaphorina citri] --HMM score = 0.998 --Signal peptide length = 24 PredHel = 0 Topology = o len = 126
XP_008469287.1	uncharacterized protein LOC103506671 [Diaphorina citri] --HMM score = 0.998 --Signal peptide length = 24 PredHel = 0 Topology = o len = 126
XP_008469288.1	uncharacterized protein LOC103506672 [Diaphorina citri] --HMM score = 0.998 --Signal peptide length = 24 PredHel = 0 Topology = o len = 126
XP_008469597.1	uncharacterized protein LOC103506945 [Diaphorina citri] --HMM score = 0.925 --Signal peptide length = 25 PredHel = 0 Topology = o len = 145

XP_008469915.1	uncharacterized protein LOC103507240 [Diaphorina citri] --HMM score = 0.998 --Signal peptide length = 16 PredHel = 0 Topology = o len = 152
XP_008470076.1	uncharacterized protein LOC103507388 [Diaphorina citri] --HMM score = 0.995 --Signal peptide length = 25 PredHel = 1 Topology = i5-27o len = 132
XP_008470083.1	uncharacterized protein LOC103507391 [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 20 PredHel = 0 Topology = o len = 140
XP_008470176.1	uncharacterized protein LOC103507484 [Diaphorina citri] --HMM score = 1.000 --Signal peptide length = 20 PredHel = 0 Topology = o len = 138
XP_008470863.1	uncharacterized protein LOC103508106 [Diaphorina citri] --HMM score = 1.000 --Signal peptide length = 17 PredHel = 0 Topology = o len = 170
XP_008470924.1	uncharacterized protein LOC103508171 [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 22 PredHel = 0 Topology = o len = 199
XP_008470940.1	uncharacterized protein LOC103508185 [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 19 PredHel = 0 Topology = o len = 242
XP_008471218.1	uncharacterized protein LOC103508449 [Diaphorina citri] --HMM score = 0.988 --Signal peptide length = 31 PredHel = 1 Topology = i7-26o len = 134
XP_017299347.1	uncharacterized protein LOC103508661 isoform X1 [Diaphorina citri] --HMM score = 0.989 --Signal peptide length = 20 PredHel = 0 Topology = o len = 140
XP_008471455.1	uncharacterized protein LOC103508661 isoform X2 [Diaphorina citri] --HMM score = 0.989 --Signal peptide length = 20 PredHel = 0 Topology = o len = 130
XP_008471535.1	uncharacterized protein LOC103508742 [Diaphorina citri] --HMM score = 1.000 --Signal peptide length = 21 PredHel = 0 Topology = o len = 106
XP_008471554.1	uncharacterized protein LOC103508759 [Diaphorina citri] --HMM score = 0.925 --Signal peptide length = 25 PredHel = 0 Topology = o len = 102
XP_008471577.1	uncharacterized protein LOC103508782 isoform X4 [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 21 PredHel = 0 Topology = o len = 245
XP_017299457.1	uncharacterized protein LOC103508938 [Diaphorina citri] --HMM score = 0.972 --Signal peptide length = 30 PredHel = 1 Topology = o10-32i len = 133
XP_008471753.1	uncharacterized protein LOC103508946 [Diaphorina citri] --HMM score = 0.982 --Signal peptide length = 29 PredHel = 1 Topology = o10-32i len = 140
XP_017299481.1	uncharacterized protein LOC103508984 isoform X1 [Diaphorina citri] --HMM score = 0.986 --Signal peptide length = 19 PredHel = 0 Topology = o len = 153
XP_008471797.1	uncharacterized protein LOC103508984 isoform X2 [Diaphorina citri] --HMM score = 0.986 --Signal peptide length = 19 PredHel = 0 Topology = o len = 153
XP_017299482.1	uncharacterized protein LOC103508984 isoform X3 [Diaphorina citri] --HMM score = 0.986 --Signal peptide length = 19 PredHel = 0 Topology = o len = 152
XP_017299682.1	uncharacterized protein LOC103509026 [Diaphorina citri] --HMM score = 0.996 --Signal peptide length = 24 PredHel = 1 Topology = i7-29o len = 137
XP_008471850.1	uncharacterized protein LOC103509036 [Diaphorina citri] --HMM score = 0.963 --Signal peptide length = 19 PredHel = 0 Topology = o len = 132
XP_008471928.1	uncharacterized protein LOC103509115 [Diaphorina citri] --HMM score = 0.993 --Signal peptide length = 23 PredHel = 0 Topology = o len = 134
XP_008471929.1	uncharacterized protein LOC103509116 [Diaphorina citri] --HMM score = 0.969 --Signal peptide length = 24 PredHel = 1 Topology = i7-26o len = 134
XP_017299608.1	uncharacterized protein LOC103509257 [Diaphorina citri] --HMM score = 0.942 --Signal peptide length = 21 PredHel = 0 Topology = o len = 136

XP_008472279.1	uncharacterized protein LOC103509439 [Diaphorina citri] --HMM score = 0.998 --Signal peptide length = 19 PredHel = 0 Topology = o len = 85
XP_008472722.1	uncharacterized protein LOC103509867 [Diaphorina citri] --HMM score = 0.927 --Signal peptide length = 31 PredHel = 0 Topology = o len = 193
XP_008472811.1	uncharacterized protein LOC103509953 [Diaphorina citri] --HMM score = 0.996 --Signal peptide length = 25 PredHel = 0 Topology = i len = 102
XP_008473711.1	uncharacterized protein LOC103510798 [Diaphorina citri] --HMM score = 0.991 --Signal peptide length = 22 PredHel = 0 Topology = o len = 244
XP_008474025.1	uncharacterized protein LOC103511092 [Diaphorina citri] --HMM score = 0.961 --Signal peptide length = 30 PredHel = 0 Topology = o len = 102
XP_008474120.1	uncharacterized protein LOC103511179 [Diaphorina citri] --HMM score = 0.976 --Signal peptide length = 21 PredHel = 0 Topology = o len = 119
XP_017300687.1	uncharacterized protein LOC103511954 [Diaphorina citri] --HMM score = 0.912 --Signal peptide length = 23 PredHel = 1 Topology = o6-28i len = 139
XP_017300752.1	uncharacterized protein LOC103512119 isoform X2 [Diaphorina citri] --HMM score = 0.991 --Signal peptide length = 28 PredHel = 1 Topology = i9-31o len = 83
XP_008475221.1	uncharacterized protein LOC103512248 [Diaphorina citri] --HMM score = 0.997 --Signal peptide length = 19 PredHel = 0 Topology = o len = 106
XP_008475227.1	uncharacterized protein LOC103512253 [Diaphorina citri] --HMM score = 0.951 --Signal peptide length = 22 PredHel = 0 Topology = o len = 164
XP_008475409.1	uncharacterized protein LOC103512422 [Diaphorina citri] --HMM score = 0.998 --Signal peptide length = 17 PredHel = 0 Topology = o len = 197
XP_008475418.1	uncharacterized protein LOC103512433 [Diaphorina citri] --HMM score = 0.972 --Signal peptide length = 25 PredHel = 0 Topology = o len = 115
XP_008475639.1	uncharacterized protein LOC103512644 isoform X2 [Diaphorina citri] --HMM score = 0.967 --Signal peptide length = 21 PredHel = 0 Topology = o len = 137
XP_008475641.1	uncharacterized protein LOC103512645 isoform X2 [Diaphorina citri] --HMM score = 0.967 --Signal peptide length = 21 PredHel = 0 Topology = o len = 137
XP_017301155.1	uncharacterized protein LOC103513080 [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 22 PredHel = 0 Topology = o len = 227
XP_008476191.1	uncharacterized protein LOC103513160 [Diaphorina citri] --HMM score = 1.000 --Signal peptide length = 25 PredHel = 1 Topology = i5-27o len = 112
XP_008476330.1	uncharacterized protein LOC103513290 [Diaphorina citri] --HMM score = 0.998 --Signal peptide length = 19 PredHel = 0 Topology = o len = 143
XP_008476658.1	uncharacterized protein LOC103513591 [Diaphorina citri] --HMM score = 0.997 --Signal peptide length = 22 PredHel = 0 Topology = o len = 228
XP_008476773.1	uncharacterized protein LOC103513701 [Diaphorina citri] --HMM score = 0.936 --Signal peptide length = 19 PredHel = 0 Topology = o len = 194
XP_008477393.1	uncharacterized protein LOC103514302 [Diaphorina citri] --HMM score = 0.997 --Signal peptide length = 22 PredHel = 1 Topology = o4-23i len = 130
XP_008478010.2	uncharacterized protein LOC103514871 [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 22 PredHel = 0 Topology = o len = 173
XP_008478017.1	uncharacterized protein LOC103514875 [Diaphorina citri] --HMM score = 0.992 --Signal peptide length = 21 PredHel = 0 Topology = o len = 230
XP_017302085.1	uncharacterized protein LOC103515145 [Diaphorina citri] --HMM score = 1.000 --Signal peptide length = 19 PredHel = 0 Topology = o len = 168

XP_017302015.1	uncharacterized protein LOC103515150, partial [Diaphorina citri] --HMM score = 0.990 --Signal peptide length = 28 PredHel = 0 Topology = o len = 179
XP_008478400.1	uncharacterized protein LOC103515249 [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 20 PredHel = 0 Topology = o len = 147
XP_008478473.1	uncharacterized protein LOC103515309 isoform X1 [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 17 PredHel = 0 Topology = o len = 132
XP_008478472.1	uncharacterized protein LOC103515309 isoform X2 [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 17 PredHel = 0 Topology = o len = 132
XP_008478649.1	uncharacterized protein LOC103515486 [Diaphorina citri] --HMM score = 1.000 --Signal peptide length = 23 PredHel = 0 Topology = o len = 172
XP_008478682.1	uncharacterized protein LOC103515524, partial [Diaphorina citri] --HMM score = 0.976 --Signal peptide length = 18 PredHel = 0 Topology = o len = 113
XP_008478727.1	uncharacterized protein LOC103515567 [Diaphorina citri] --HMM score = 0.986 --Signal peptide length = 23 PredHel = 0 Topology = o len = 137
XP_008478809.2	uncharacterized protein LOC103515652, partial [Diaphorina citri] --HMM score = 0.977 --Signal peptide length = 22 PredHel = 0 Topology = o len = 178
XP_017302433.1	uncharacterized protein LOC103516168 isoform X1 [Diaphorina citri] --HMM score = 1.000 --Signal peptide length = 23 PredHel = 0 Topology = o len = 176
XP_017302434.1	uncharacterized protein LOC103516168 isoform X2 [Diaphorina citri] --HMM score = 1.000 --Signal peptide length = 23 PredHel = 0 Topology = o len = 176
XP_017302499.1	uncharacterized protein LOC103516340 [Diaphorina citri] --HMM score = 1.000 --Signal peptide length = 19 PredHel = 0 Topology = o len = 134
XP_008479535.1	uncharacterized protein LOC103516350 [Diaphorina citri] --HMM score = 0.998 --Signal peptide length = 22 PredHel = 0 Topology = o len = 159
XP_017302718.1	uncharacterized protein LOC103516847 [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 20 PredHel = 0 Topology = o len = 203
XP_008480359.1	uncharacterized protein LOC103517116 [Diaphorina citri] --HMM score = 0.998 --Signal peptide length = 20 PredHel = 0 Topology = o len = 216
XP_008480375.1	uncharacterized protein LOC103517129 [Diaphorina citri] --HMM score = 0.966 --Signal peptide length = 29 PredHel = 0 Topology = o len = 192
XP_008480572.1	uncharacterized protein LOC103517322 [Diaphorina citri] --HMM score = 0.979 --Signal peptide length = 19 PredHel = 0 Topology = o len = 102
XP_008481275.1	uncharacterized protein LOC103518002 [Diaphorina citri] --HMM score = 0.984 --Signal peptide length = 22 PredHel = 0 Topology = o len = 177
XP_008481410.1	uncharacterized protein LOC103518133 [Diaphorina citri] --HMM score = 1.000 --Signal peptide length = 25 PredHel = 1 Topology = i7-29o len = 168
XP_008481488.1	uncharacterized protein LOC103518205 [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 21 PredHel = 0 Topology = o len = 129
XP_008481512.1	uncharacterized protein LOC103518230 [Diaphorina citri] --HMM score = 0.962 --Signal peptide length = 15 PredHel = 0 Topology = o len = 105
XP_008481831.1	uncharacterized protein LOC103518532 [Diaphorina citri] --HMM score = 0.978 --Signal peptide length = 23 PredHel = 0 Topology = o len = 126
XP_008481832.1	uncharacterized protein LOC103518533 [Diaphorina citri] --HMM score = 0.981 --Signal peptide length = 23 PredHel = 1 Topology = o5-27i len = 126
XP_008482380.1	uncharacterized protein LOC103519077 [Diaphorina citri] --HMM score = 0.974 --Signal peptide length = 34 PredHel = 1 Topology = i12-34o len = 120

XP_008482381.1	uncharacterized protein LOC103519077 [Diaphorina citri] --HMM score = 0.974 --Signal peptide length = 34 PredHel = 1 Topology = i12-34o len = 120
XP_017303539.1	uncharacterized protein LOC103519077 [Diaphorina citri] --HMM score = 0.974 --Signal peptide length = 34 PredHel = 1 Topology = i12-34o len = 120
XP_008482489.1	uncharacterized protein LOC103519182 [Diaphorina citri] --HMM score = 1.000 --Signal peptide length = 17 PredHel = 0 Topology = o len = 149
XP_008482531.1	uncharacterized protein LOC103519226 [Diaphorina citri] --HMM score = 0.998 --Signal peptide length = 20 PredHel = 0 Topology = o len = 127
XP_008483107.1	uncharacterized protein LOC103519797 [Diaphorina citri] --HMM score = 0.968 --Signal peptide length = 25 PredHel = 0 Topology = o len = 152
XP_008483348.1	uncharacterized protein LOC103520032, partial [Diaphorina citri] --HMM score = 0.989 --Signal peptide length = 25 PredHel = 1 Topology = i5-27o len = 107
XP_008483947.1	uncharacterized protein LOC103520623 isoform X1 [Diaphorina citri] --HMM score = 0.963 --Signal peptide length = 21 PredHel = 0 Topology = o len = 156
XP_008483946.1	uncharacterized protein LOC103520623 isoform X2 [Diaphorina citri] --HMM score = 0.957 --Signal peptide length = 21 PredHel = 0 Topology = o len = 156
XP_008484013.1	uncharacterized protein LOC103520692 [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 25 PredHel = 0 Topology = o len = 100
XP_008484243.1	uncharacterized protein LOC103520923, partial [Diaphorina citri] --HMM score = 0.989 --Signal peptide length = 25 PredHel = 1 Topology = i7-29o len = 86
XP_008484322.1	uncharacterized protein LOC103520998, partial [Diaphorina citri] --HMM score = 0.996 --Signal peptide length = 22 PredHel = 0 Topology = o len = 191
XP_008484331.1	uncharacterized protein LOC103521004 [Diaphorina citri] --HMM score = 0.998 --Signal peptide length = 21 PredHel = 0 Topology = o len = 125
XP_008484388.1	uncharacterized protein LOC103521057, partial [Diaphorina citri] --HMM score = 0.995 --Signal peptide length = 17 PredHel = 0 Topology = o len = 143
XP_008485033.1	uncharacterized protein LOC103521704 [Diaphorina citri] --HMM score = 0.981 --Signal peptide length = 23 PredHel = 0 Topology = o len = 234
XP_008485252.1	uncharacterized protein LOC103521922 [Diaphorina citri] --HMM score = 0.997 --Signal peptide length = 24 PredHel = 0 Topology = o len = 123
XP_008485511.1	uncharacterized protein LOC103522187 isoform X1 [Diaphorina citri] --HMM score = 0.984 --Signal peptide length = 24 PredHel = 0 Topology = o len = 134
XP_008485512.1	uncharacterized protein LOC103522187 isoform X2 [Diaphorina citri] --HMM score = 0.984 --Signal peptide length = 24 PredHel = 0 Topology = o len = 134
XP_017304464.1	uncharacterized protein LOC103522187 isoform X3 [Diaphorina citri] --HMM score = 0.984 --Signal peptide length = 24 PredHel = 0 Topology = o len = 134
XP_008485549.1	uncharacterized protein LOC103522225, partial [Diaphorina citri] --HMM score = 0.997 --Signal peptide length = 22 PredHel = 0 Topology = o len = 140
XP_008485803.1	uncharacterized protein LOC103522478, partial [Diaphorina citri] --HMM score = 0.927 --Signal peptide length = 32 PredHel = 0 Topology = o len = 113
XP_008485893.1	uncharacterized protein LOC103522571 [Diaphorina citri] --HMM score = 0.988 --Signal peptide length = 28 PredHel = 0 Topology = o len = 156
XP_008486403.1	uncharacterized protein LOC103523112 [Diaphorina citri] --HMM score = 0.996 --Signal peptide length = 22 PredHel = 0 Topology = o len = 128
XP_008486405.1	uncharacterized protein LOC103523114 [Diaphorina citri] --HMM score = 0.992 --Signal peptide length = 21 PredHel = 0 Topology = o len = 167

XP_017304775.1	uncharacterized protein LOC103523676, partial [Diaphorina citri] --HMM score = 0.979 --Signal peptide length = 23 PredHel = 0 Topology = o len = 231
XP_008487473.1	uncharacterized protein LOC103524243 [Diaphorina citri] --HMM score = 0.921 --Signal peptide length = 20 PredHel = 0 Topology = o len = 108
XP_008487527.1	uncharacterized protein LOC103524291 [Diaphorina citri] --HMM score = 0.997 --Signal peptide length = 22 PredHel = 0 Topology = o len = 193
XP_017305073.1	uncharacterized protein LOC103524376 [Diaphorina citri] --HMM score = 0.916 --Signal peptide length = 16 PredHel = 0 Topology = o len = 110
XP_008487632.1	uncharacterized protein LOC103524398 [Diaphorina citri] --HMM score = 0.997 --Signal peptide length = 25 PredHel = 1 Topology = o4-26i len = 131
XP_008487825.1	uncharacterized protein LOC103524576 [Diaphorina citri] --HMM score = 0.997 --Signal peptide length = 23 PredHel = 0 Topology = o len = 145
XP_008488071.1	uncharacterized protein LOC103524816 [Diaphorina citri] --HMM score = 0.972 --Signal peptide length = 20 PredHel = 0 Topology = o len = 173
XP_008488275.1	uncharacterized protein LOC103525009 [Diaphorina citri] --HMM score = 1.000 --Signal peptide length = 19 PredHel = 0 Topology = o len = 204
XP_017298578.1	uncharacterized protein LOC108251836 [Diaphorina citri] --HMM score = 0.992 --Signal peptide length = 22 PredHel = 0 Topology = o len = 210
XP_017298676.1	uncharacterized protein LOC108252130 [Diaphorina citri] --HMM score = 0.996 --Signal peptide length = 18 PredHel = 0 Topology = o len = 144
XP_017298791.1	uncharacterized protein LOC108252162 [Diaphorina citri] --HMM score = 0.982 --Signal peptide length = 28 PredHel = 0 Topology = o len = 146
XP_017298792.1	uncharacterized protein LOC108252163 [Diaphorina citri] --HMM score = 0.982 --Signal peptide length = 28 PredHel = 0 Topology = o len = 146
XP_017298994.1	uncharacterized protein LOC108252218 [Diaphorina citri] --HMM score = 0.996 --Signal peptide length = 23 PredHel = 1 Topology = o4-21i len = 135
XP_017299295.1	uncharacterized protein LOC108252313 [Diaphorina citri] --HMM score = 1.000 --Signal peptide length = 20 PredHel = 0 Topology = o len = 114
XP_017300042.1	uncharacterized protein LOC108252542 [Diaphorina citri] --HMM score = 0.995 --Signal peptide length = 24 PredHel = 1 Topology = i7-29o len = 64
XP_017301248.1	uncharacterized protein LOC108252888 [Diaphorina citri] --HMM score = 0.997 --Signal peptide length = 23 PredHel = 0 Topology = o len = 122
XP_017302258.1	uncharacterized protein LOC108253225 isoform X1 [Diaphorina citri] --HMM score = 0.998 --Signal peptide length = 21 PredHel = 0 Topology = o len = 106
XP_017302259.1	uncharacterized protein LOC108253225 isoform X2 [Diaphorina citri] --HMM score = 0.998 --Signal peptide length = 21 PredHel = 0 Topology = o len = 95
XP_017302492.1	uncharacterized protein LOC108253299 [Diaphorina citri] --HMM score = 0.983 --Signal peptide length = 24 PredHel = 0 Topology = o len = 143
XP_017302533.1	uncharacterized protein LOC108253312 [Diaphorina citri] --HMM score = 0.987 --Signal peptide length = 28 PredHel = 0 Topology = o len = 101
XP_017302851.1	uncharacterized protein LOC108253431 [Diaphorina citri] --HMM score = 0.994 --Signal peptide length = 24 PredHel = 1 Topology = o10-32i len = 113
XP_017302907.1	uncharacterized protein LOC108253446 [Diaphorina citri] --HMM score = 0.963 --Signal peptide length = 21 PredHel = 0 Topology = o len = 193
XP_017303819.1	uncharacterized protein LOC108253753 isoform X1 [Diaphorina citri] --HMM score = 0.949 --Signal peptide length = 24 PredHel = 1 Topology = i7-29o len = 124

XP_017303820.1	uncharacterized protein LOC108253753 isoform X2 [Diaphorina citri] --HMM score = 0.949 --Signal peptide length = 24 PredHel = 1 Topology = i7-29o len = 124
XP_017304064.1	uncharacterized protein LOC108253867 [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 22 PredHel = 0 Topology = o len = 127
XP_008483080.1	UPF0729 protein AAEL015238 [Diaphorina citri] --HMM score = 0.938 --Signal peptide length = 21 PredHel = 0 Topology = o len = 92
XP_008488184.1	vasotab [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 21 PredHel = 1 Topology = o4-26i len = 101
XP_017303584.1	viresin-like [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 19 PredHel = 1 Topology = i5-24o len = 67
XP_008482275.1	waprin-Thr1 [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 20 PredHel = 0 Topology = o len = 107
XP_017303632.1	waprin-Thr1 [Diaphorina citri] --HMM score = 0.999 --Signal peptide length = 20 PredHel = 0 Topology = o len = 107
XP_008473202.1	yrdC domain-containing protein, mitochondrial [Diaphorina citri] --HMM score = 0.958 --Signal peptide length = 20 PredHel = 0 Topology = o len = 245

Results

Our goal was to identified from genomic data a predicted set of secreted proteins in the Asian citrus psyllid *Diaphorina citri*. We used all predicted gene encoding proteins from version 1.1 and run a search of proteins with N-terminal signal peptide. The predicted *Diaphorina citri* secretome contains a total of 1,247 proteins that is equivalent to ~6% of the proteome. 246 out of 1,247 secreted proteins are small cysteine rich (SCR) proteins that included a SCR protein annotated as "basic salivary proline-rich protein 3-like (XP_008480568.1)". We also found four cystatin-like domain and one Kazal-like domain inhibitors of cysteine and serine plant proteases, respectively.

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Supplementary Note 38: Heat shock proteins

Introduction

Heat shock proteins (hsps) are molecular chaperones that facilitates protein folding and stabilization, act as proteases, and aiding the degradation of damaged proteins (Gerardo et al 2010; Pockley 2003). In various organisms, hsps are produced in response to stress and may serve as signaling proteins during immune responses (Henderson 2006; Pockley 2003).

Hsps are grouped into several families, including small hsps, hsp60, hsp70, hsp90, and hsp100 (Pockley, 2003). These hsp families involve many different functions. Heat shock protein 90 (hsp90) was shown to be upregulated in *Galleria mellonella* after a mild heat shock and microbial infection, suggesting that Hsp90 may be involved in both insect immunity and heat shock pathways (Wojda and Jakubowicz 2007). However, compared to the symbiotic relationship between *Acyrthosiphon pisum* and *Serratia symbiotica*, *A. pisum* hsp gene expression induces little change (Burke and Moran, 2011), showing hsps may not affect insect immunity in this example.

Methods

Hemipteran hsps orthologs were collected from NCBI and i5k Blast. The orthologs were used to blat against the *Diaphorina citri*, Asian citrus psyllid (ACP), genome. Identification and annotation of hsps was performed in WebApollo where RNA sequence data (adult, nymph, and egg) supported the manually curated gene models. BLASTp was used to confirm accuracy of the annotated hsps and ExPASy was used to identify molecular weight.

Hsps orthologs were collected through i5k Blast and a limited NCBI search to include different insect orders. Local pairwise amino acid sequence alignments were done in BLASTp between *D. citri*, *Acyrthosiphon pisum, Oncopeltus fasciatus, Halyomorpha halys, Cimex lectularius, Gerris buenoi, Homalodisca vitripennis,* and *Pachypsylla venusta.* Multiple sequence alignments of ACP and orthologous insect hsps sequences was performed in MEGA6 using ClustalW (Tamura et al 2003). The phylogenetic analysis of the hsps included, constructing a midpoint rooted neighbor-joining tree in MEGA6 using p-distance to show evolutionary distance and 1,000 bootstrap replicates.

Table 1: All the hsps found in *D. citri* genome. Table shows all hsp, names based on NCBI Gnomon ([A]) with BLASTp matches and domain organization, functional description from Uniprot ([B]) for each gene, and corresponding molecular weight and protein length.

D. citri Hsps [A]	BLASTP match (organism, bit score)	Function[B]	MW (kDa)	Protein Length	Domains
10 kDa Hsp, mitochondrial (2)	11.1 kDa Hsp (*Oxya chinensis*, 114)	Chaperone that binds to hsp60 to enhance protein folding	12	111 residues	CPN10, GroES, oligomerization

					interface, mobile loop, roof hairpin
Hsp 23	Heat shock protein (*Aedes aegypt*, 67)	No known function	17	113 residues	Metazoan_ACD, HSP20, IbpA
Hsp beta-1 (2 isoforms)	Hsp beta-1 (*Musca domestica*, 253)	Involved in stress resistance and actin organization	21	RA= 187 residues RB= 151 residues	metazoan ACD, IbpA, hsp20, ACD hsps p23 superfamily, putative dimer interface
Alpha-crystallin A chain	Alpha-crystallin A chain (*Halyomorpha halys*, 204)	has chaperone-like activity	21	189 residues	metazoan ACD, IbpA, hsp20, ACD hsps p23 superfamily
Hsp 60 kDa, mitochondrial	60 kDa Hsp, mitochondrial (*Lasius niger*, 720)	Folds/transports newly imported proteins into the mitochondria	45	421 residues	GroEL, chaperonin like superfamily, thermosome_arch
Hsp 68 (partial)	-		-	-	-
Hsp 70 A1 (4)	Hsp 70 (*Bemisia tabaci*, 1118)	Stabilizes existing proteins against aggregation and mediates the folding of newly translated proteins	69	626,629, 629, 629 residues	NBD of HSPA1, NBD of sugar kinase hsp 70 actin superfamily, hsp70, DnaK, SBD interface, BAG/hsp70 and EF/hsp70 interaction site
Hsp 70 kDa protein 14 (partial)	Hsp 70 kDa protein 14 (*A. pisum*, 84.7)		23	213 residues	NBD of sugar kinase hsp 70 actin superfamily
Hsp 75 kDa, mitochondrial (partial)	Hsp 75 kDa, mitochondrial (*Athalia rosae*, 577)	Chaperone with ATP activity	46	402 residues	HSP90
Hsp 83	Hsp 90 (*Laodelphax striatella*, 1226)	Molecular chaperone of specific proteins of the cell cycle and signal transduction	83	723 residues	Hsp90 superfamily, HATPase_c, ATP binding site, Mg2+ binding site, G-X-P motif
Hsp 81-3	Hsp 83 (*H. saltator*, 444)		74	642 residues	Hsp90 superfamily, HATPase_c, ATP binding site, Mg2+ binding site, G-X-P motif
Hsp 83	Hsp90 (Nilaparvata lugens, 903)		64	553 residues	Hsp90 and superfamily

Hsp 90 (partial)	-	-	-	-	-
97 kDa Hsp	97 kDa Hsp (*Amyelois transitella*, 733)	No known function	85	763 residues	Hsp70, DnaK, NBD sugar kinase hsp 70 superfamily, SBD interface, hsp70 interaction site
Hsp 105 kDa (partial)	-	Chaperone with ATP activity	-	-	-

Table 2: Pairwise Alignment of *D. citri* hsps with related organisms in Hemiptera. Sequence % identity, (sequence % similiarity), and [bit score]. No sequence found for the organism represented by (-), and values for alpha-crystallin represent query coverage of the domain only.

Diaphorina citri	Hsp 10	Hsp 23	Hsp β / α-crystallin	Hsp 60	Hsp 70	Hsp 83
Acyrthosiphon pisum	45% (76%) [96.3]	-	- / 72% (82%) [261]	77% (88%) [633]	84% (91%) [1107]	82% (91%) [1156]
Oncopeltus fasciatus	58% (81%) [112]	31% (58%) [50.4]	- / 63% (73%) [143]	87% (92%) [712]	79% (88%) [1085]	84% (93%) [1185]
Halyomorpha halys	58% (80%) [112]	34% (57%) [46.6]	- / 87% (94%) [193]	88% (93%) [719]	84% (92%) [1118]	84% (92%) [1174]
Cimex lectularius	58% (79%) [111]	34% (63%) [52]	- / 68% (79%) [240]	87% (94%) [712]	84% (92%) [1114]	84% (92%) [1161]
Gerris buenoi	55% (80%) [113]	30% (47%) [48.9]	- / 60% (74%) [136]	85% (93%) [701]	82% (91%) [1084]	79% (87%) [952]
Homalodisca vitripennis	40% (61%) [93.6]	33% (58%) [49.7]	- / 94% (97%) [123]	74% (80%) [679]	85% (93%) [1130]	84% (92%) [1174]
Pachypsylla venusta	-	30% (61%) [42]	- / 40% (70%) [65.9]	79% (83%) [644]	89% (94%) [1065]	89% (96%) [1247]

Table 3: Number of heat shock proteins in *D. citri* and related organisms.

Organism	# of hsps
Diaphorina citri	17
Acyrthosiphon pisum	15
Cimex lectularius	15
D. melanogaster	24
Aedes aegypti	23
Anopheles gambiae	27
Culex quinquefasciatus	22

Results and Discussion

A total of 17 hsps, representing five hsp families, were identified and manually curated within the ACP genome (Table 1). Only 15 hsps were found in the closest relative of *D. citri*, *A. pisum* (Gerardo et al 2010) (Table 3). The naming convention is based on NCBI Gnomon. Misassembly within the ACP genome prevented annotation of a couple hsps, including hsp 68 and hsp 105 kDa. All hsp 70 genes were found in a cluster together in a particular scaffold (WebApollo accession: gi|645503904|ref|NW_007378480.1|), the gene structure were similar between them containing three exons. Two hsp 10 were found in the ACP genome and were 100% identical. *A. pisum* also has two hsp 10 but differ in only two amino acids (NCBI accessions: NP_001119666 & XP_003245465).

In the pairwise alignment analysis (Table 2), hsps 60, 70, and 83 have an identity of 74% or greater between the ACP and related hemipterans compared to hsps 10 and 23 which have an identity of 58% or less. Hsp β-1 was not found in other hemipterans based on BLASTp searches. However, BLASTp searches limited to hemipterans did return close matches to α-crystallin. Pairwise alignment of Hsp B-1 and α-crystallin showed a greater than 70% similarity, but only within the α-crystallin domain only.

D. citri hsps 10, 70, and 90 were analyzed with related insects hsps from the orders Isoptera, Hymenoptera, Coleoptera, and Diptera by constructing neighbor-joining trees (Figure 1, 2, 3). The psyllid hsps were consistently grouped with other hemipteran hsps, further supporting the manually curated hsps.
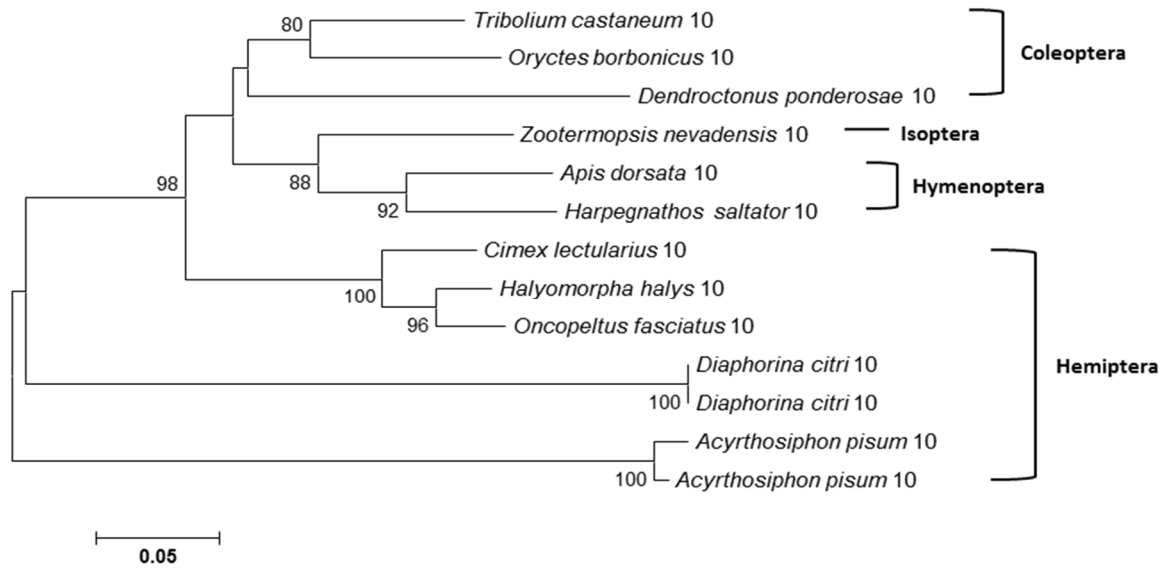
Figure 1: Neighbor-joining tree of 13 hsps 10 was constructed in MEGA6 using 1000 bootstrap replications showing 80% and greater, and the p-distance method was used to show evolutionary distance. A total of 100 amino acid positions were analyzed for the tree. NCBI accession: *T. castaneum* [XP_975179.2], *O. borbonicus* [KRT79990.1], *D. ponderosae* [ENN71973], *Z. nevadensis* [KDR14059.1], *A. dorsata* [XP_006622783.1], *H. saltator* [EFN79770.1], *C. lectularius* [XP_014258406.1], *H. halys* [XP_014273034.1], *A. pisum* [XP_003245465.1 & NP_001119666.1]. i5k accession: *O. fasciatus* [OFAS007006-RA].
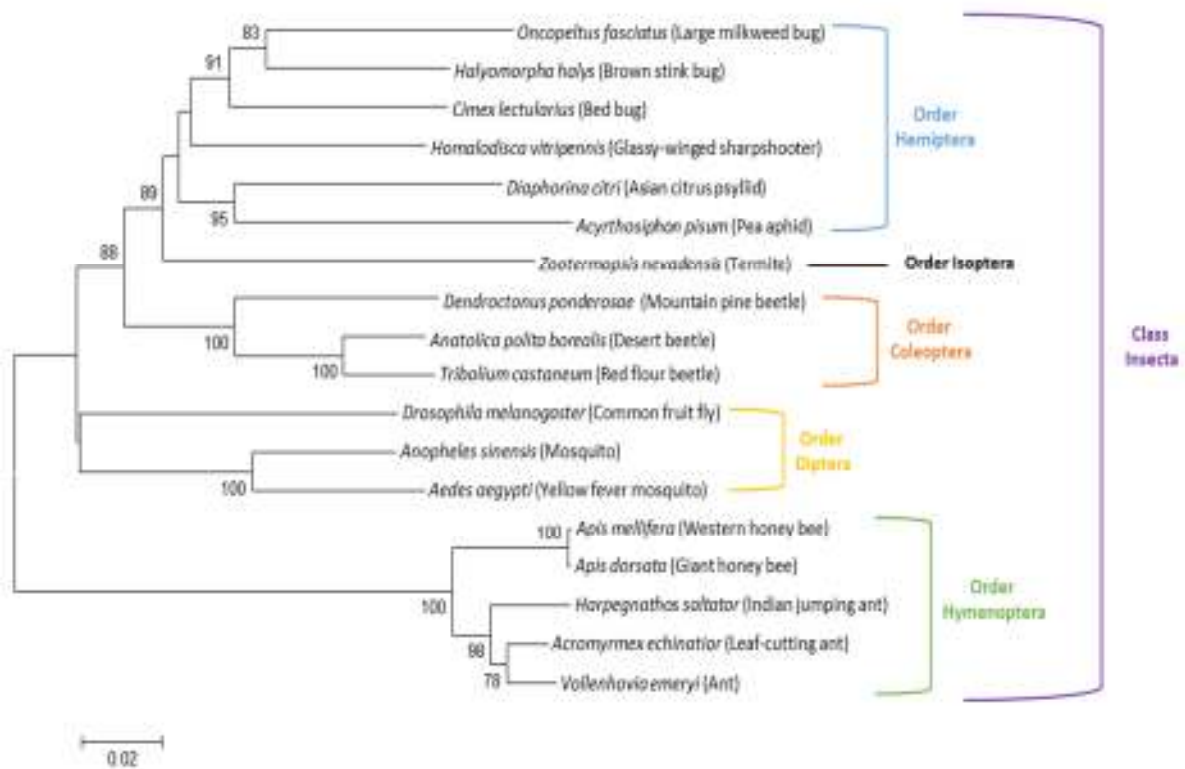
Figure 2: Neighbor-joining tree of the 18 hsps 70 was constructed in MEGA6 using 1000 bootstrap replications showing 78% and greater, and the p-distance method was used to show evolutionary distance. A total of 621 amino acid positions were analyzed for the tree. The organisms are accompanied with common name. NCBI accession: *H. halys* [XP_014293117.1], *C. lectularius* [XP_014239635.1], *A. pisum* [XP_001945786.2], *Z. nevadensis* [KDR08926.1], *D. ponderosae* [ENN76738.1], *A. polita borealis* [ABQ39970.1], *D. melanogaster* [AAG26893.1], *A. sinensis* [KFB46250.1], *A. aegypti* [ACJ64194.1], *A. mellifera* [NP_001153544.1], *A. dorsata* [XP_006620321.1], *H. saltator* [XP_011153497.1], *A. echinatior* [XP_011059681.1], *V. emeryi* [XP_011862691.1]. i5k accession: *O. fasciatus* [OFAS004738-RA], *H. vitripennis* [HVIT001451-PA].
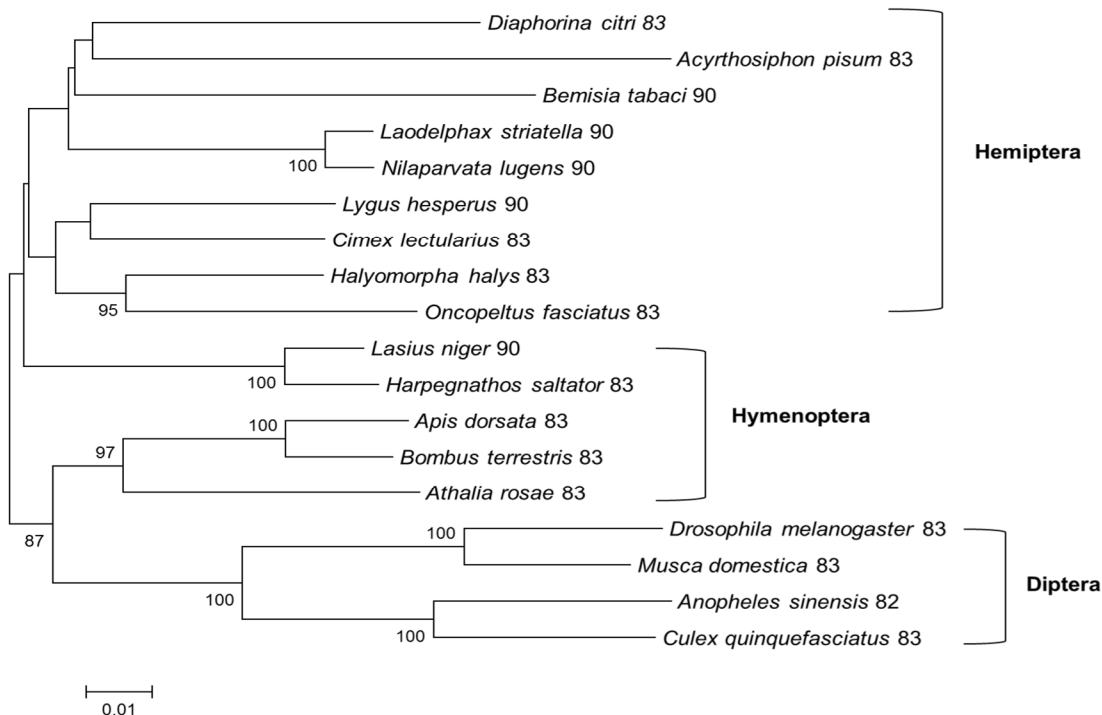

Figure 3: Neighbor-joining tree of the 18 hsps 83/90 was constructed in MEGA6 using 1000 bootstrap replications showing 87% and greater, and the p-distance method was used to show evolutionary distance. A total of 684 amino acid positions were analyzed for the tree. NCBI accession: *A. pisum* [XP_001943172.1], *B. tabaci* [AAZ17403.1], *L. striatella* [AHB63833.1], *N. lugens* [ADE34169.1], *L. hesperus* [AFX84559.1], *C. lectularius* [XP_014239772.1], *H. halys* [XP_014284945.1], *L. niger* [KMQ95531.1], *H. saltator* [XP_011154034.1], *A. dorsata* [XP_006616995.1], B. terrestris [XP_003393129.2], A. rosae [XP_012250991.1], *D. melanogaster* [NP_523899.1], *M. domestica* [XP_005176932], *A. sinensis* [KFB48798.1], *C. quinquefasciatus* [EDS36267.1]. i5k accession: *O. fasciatus* [OFAS001265-RA].

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Supplementary Note 39: Rab proteins

Introduction

Rabs are a gene family of small GTPases that act as molecular switches which regulate vesicle formation, movement, and merging (Pereira-Leal and Seabra 2001). They are involved in many key processes within all cells. They play a part in the transportation of material to and from the plasma membrane (exocytosis and endocytosis) (Seabra at al. 2002). They participate in the transport v between organelles, like the ER, Golgi apparatus, endosome, or phagosome, as seen during phagocytosis and gene expression (Seabra at al. 2002). The Rabs are phylogenetically assorted into functional groups based on their conservation (Pereira-Leal and Seabra 2001). What makes the sequence specific for Rabs are two switch regions, G box motifs (1-5), Family motifs (F1-5), and Subfamily motifs (SF1-5) (Pereira-Leal and Seabra 2000). Rabs typically consist of a domain called a GTP- binding domain. Rabs are suspected to be conserved in all branches of eukaryotes in one way or another since the last eukaryotic common ancestor (LECA) (Klopper et al. 2012).

Methods

The annotation process began with using at least three Rab sequences from closely related insects collected from the NCBI database and screening the psyllid's genome for any predicted models. The first search tool used was BLAT from Web Apollo then BLAST from I5K and the MCOT database for the psyllid. Using the available evidence tracks in Web Apollo the found predictions were manually annotated, resulting in 21 different Rabs from *D. citri* being identified including isoforms.

Following manual annotation, the models were verified using a reciprocal BLASTP search in NCBI. Orthologs from *H. sapiens, D. melanogaster, C. elegans, S. cerevisiae, and S. pombe* were collected from the supplementary materials of Pereira-Leal and Seabra 2001. MUSCLE with default settings was used to make multiple sequence alignments of the Rab protein sequences from the previously mentioned orthologs and *D. citri*. MEGA7 was used to construct a phylogenetic tree. The tree was neighbor-joining and used the Poisson Model, pairwise deletion, and 1,000 bootstrap replicate settings. Functional groups were labeled based on Figure 4 from Pereira-Leal and Seabra 2001 and Figure 3 from Klopper et al. 2012.

Table 1: This is a list of the Rab genes found in *D. citri* and the functional group they fall into, as well as the ungrouped, based on phylogeny. The purpose associated with the groups is also given (Pereira-Leal and Seabra 2001; Ackers et al. 2005).

Rab genes found in *Diaphorina citri* and their functional groups			
Functional Group	**Group members**	***D. citri* Rab family members**	**Function**
I	1,35	1A	Exocytosis

II	2,4,11,14,25	2,11A,14	Endocytosis and recycling
III	3,26,27,37	3,27A	Regulated exocytosis
IV	19,30	19,30	Golgi related
V	5,21,22	21,22B	Endocytosis
VI	6,41	6	Retrogade Golgi transport
VII	7,9	NA	Late endocytosis
VIII	8,10,13	10, 13A, 13B, 13C, 13D	Golgi related
Ungrouped	-	L3,23,28,32, 40	unknown

Table 2: Rab genes founds in *D. citri* (column 1), highest BLASTP result other than *D. citri* (column 2), corresponding E values (column 3), and their functional groups (column4).

| Rab genes found in *Diaphorina citri*			
Gene	**BLAST result**		**Functional Group**
	Name	E value	
DcRab1A	ras-related protein Rab-1A [Tribolium castaneum]	3.00E-85	I
DcRab11A	ras-related protein Rab-11A [Trichogramma pretiosum]	3.00E-115	II
DcRab14	Rab14 [Macrobrachium rosenbergii]	4.00E-125	II
DcRab2	ras-related protein Rab-2A isoform X2 [Bactrocera oleae]	3.00E-92	II
DcRabL3	rab-like protein 3 isoform X1 [Athalia rosae]	4.00E-88	III
DcRab27A	ras-related protein Rab-27A [Cerapachys biroi]	4.00E-38	III
DcRab3	ras-related protein Rab-3 isoform X1 [Halyomorpha halys]	7.00E-145	III
DcRab30	Ras-related protein Rab-30 [Zootermopsis nevadensis]	2.00E-122	IV
DcRab21	Ras-related protein Rab-21 [Zootermopsis nevadensis]	9.00E-122	V
DcRab19	putative Rab-43-like protein ENSP00000330714 [Ceratitis capitata]	2.00E-84	VI
DcRab6	ras-related protein Rab6 isoform X2 [Acromyrmex echinatior]	2.00E-100	VI
DcRab10	ras-related protein Rab-10 [Halyomorpha halys]	6.00E-77	VIII
DcRab13A	Ras-related protein Rab-8B [Zootermopsis nevadensis]	6.00E-97	VIII
DcRab22B	ras-related protein Rab-21 isoform X2 [Halyomorpha halys]	1.00E-97	Ungrouped
DcRab23	Ras-related protein Rab-23 [Zootermopsis nevadensis]	5.00E-77	Ungrouped
DcRab28	RAB-28, putative [Pediculus humanus corporis]	7.00E-25	Ungrouped
DcRab32	ras-related protein Rab-32 isoform X4 [Bombus terrestris]	9.00E-112	Ungrouped
DcRab40	Ras-related protein Rab-40C [Zootermopsis nevadensis]	2.00E-149	Ungrouped
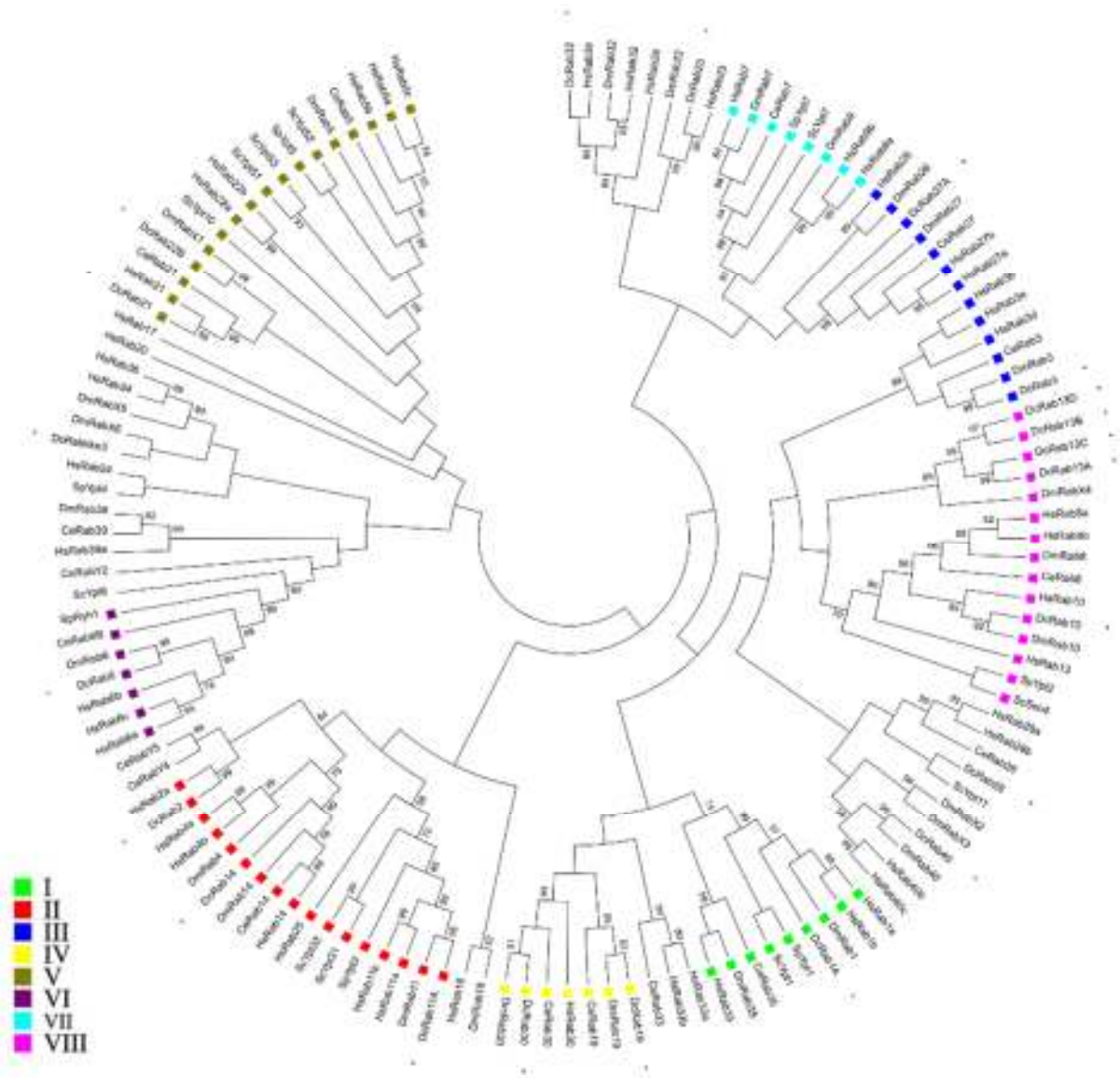
Figure 1: Phylogenetic analysis of 133 Rab amino acid sequences from *D. citri* (Dc), *C. elegans* (Ce), *D. melanogaster* (Dm), *H. sapiens* (Hs), *S. cerevisiae* (Sc), and *S. pombe* (Sp). Most Rabs grouped together in functional groups I-VIII. Groups are color-coded as shown in the legend and *D. citri* sequences are identified by an asterisk. Trees were constructed using the neighbor joining methods and bootstrap test of phylogeny. Bootstrap values >70 are placed at nodes based on 1000 replicates.
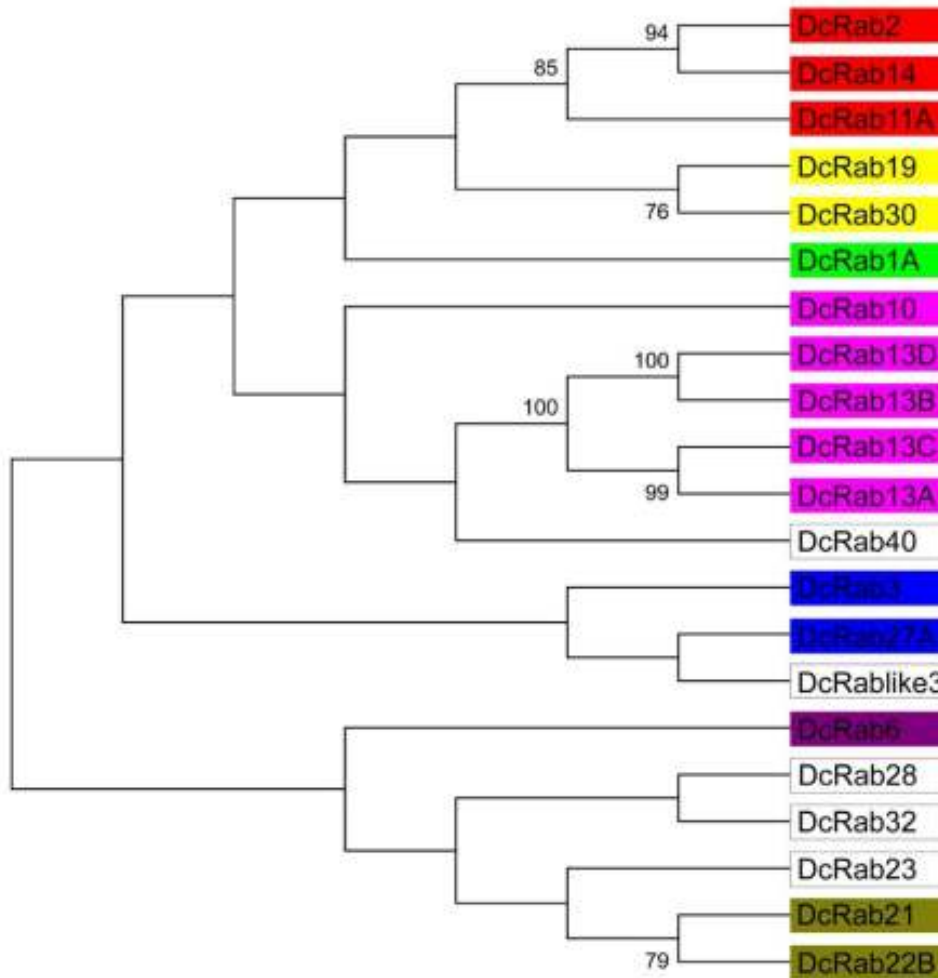
Figure 2: Phylogenetic analysis of 21 *D. citri* Rab amino acid sequences. Tree was constructed using the neighbor joining method and bootstrap test of phylogeny. Bootstrap values >70 (%) are placed at nodes based on 1000 replicates. Separation into functional groups still occurs. II, III, IV, and VIII are color coded per the same legend as in Fig.1. Clear boxes indicate ungrouped Rabs.

Table 3: This table shows the individual Rabs, their amino acid sequence length, and a general description generated during the time of manual annotation. It also shows the pairwise comparison between the *D. citri* Rabs and the sequences from *A. pisum and C. lectularius.* They have mostly high Query coverage and high bit scores.

Diaphorina citri RABs											
				Pea aphid				Bedbug			
Gene	aa	Description	Domains	Description	Accession	query	Bit Score	Description	Accession	query	Bit Score
Rab1A	204	key regulatory component for the transport of vesicles from the ER to the Golgi apparatus	Rab1_Ypt1	PREDICTED: ras-related protein ORAB-1 [Acyrthosiphon pisum]	XP_001951763.1	86%	585	PREDICTED: ras-related protein Rab-1A-like [Cimex lectularius]	XP_014260675.1	96%	647
Rab2	243	Rab2 is localized on cis-Golgi membranes and interacts with Golgi matrix proteins. Rab2 is also implicated in the maturation of vesicular tubular clusters (VTCs), which are microtubule-associated intermediates in transport between the ER and Golgi apparatus.	Rab2	PREDICTED: ras-related protein Rab-2A [Acyrthosiphon pisum]	XP_001951908.1	94%	684	PREDICTED: ras-related protein Rab-2 [Cimex lectularius]	XP_014245059.1	97%	704
Rab3	221	Controls Ca2+-regulated exocytosis. Possibly involved in hormone and neurotransmitter secretion.	Rab3	PREDICTED: ras-related protein Rab-3 [Acyrthosiphon pisum]	XP_001944695.1	100%	1034	PREDICTED: ras-related protein	XP_014257053.1	98%	1028

176

								Rab-3 [Cimex lectularius]			
Rab like 3	217	NA	RabL3	PREDICTED: rab-like protein 3 [Acyrthosiphon pisum]	XP_016660492.1	91%	522	PREDICTED: rab-like protein 3 [Cimex lectularius]	XP_014242421.1	94%	563
Rab6	378	Rab6 is involved in microtubule-dependent transport pathways through the Golgi and from endosomes to the Golgi.	Rab6	rab6-like [Acyrthosiphon pisum]	NP_001155439.1	100%	764	PREDICTED: ras-related protein Rab6 isoform X2 [Cimex lectularius]	XP_014260763.1	100%	779
Rab10	270	involved in post-Golgi transport to the plasma membrane	Rab8, Rab10, Rab13 like	PREDICTED: ras-related protein Rab-10 [Acyrthosiphon pisum]	XP_001951463.1	100%	602	PREDICTED: ras-related protein Rab-10 [Cimex lectularius]	XP_014248382.1	100%	576
Rab11A	275	regulate recycling pathways from endosomes to the plasma membrane and to the trans-Golgi network	Rab11A, Rab11B, Rab25	PREDICTED: ras-related protein Rab-11A isoform X2 [Acyrthosiphon pisum]	XP_001950094.1	100%	824	PREDICTED: ras-related protein Rab-11A [Cimex lectularius]	XP_014245308.1	99%	837
Rab13A	253	involved in post-Golgi transport to the plasma membrane	Rab	PREDICTED: ras-related protein Rab-13-like isoform X1 [Acyrthosiphon pisum]	XP_001944801.1	87%	677	PREDICTED: ras-related protein Rab-13-like [Cimex lectularius]	XP_014246087.1	80%	710
Rab13B	259	involved in post-Golgi transport to the plasma membrane	Rab	PREDICTED: ras-related protein Rab-13-like isoform X1 [Acyrthosiphon pisum]	XP_001944801.1	85%	674	PREDICTED: ras-related protein Rab-13-like [Cimex lectularius]	XP_014246087.1	78%	708
Rab13C	243	involved in post-Golgi transport to the plasma membrane	Rab	PREDICTED: ras-related protein Rab-8A, partial [Acyrthosiphon pisum]	XP_003248501.2	82%	659	PREDICTED: ras-related protein Rab-13-like [Cimex lectularius]	XP_014246087.1	97%	718
Rab13D	237	involved in post-Golgi transport to the plasma membrane	Rab	PREDICTED: ras-related protein Rab-8A, partial [Acyrthosiphon pisum]	XP_003248501.2	83%	661	PREDICTED: ras-related protein Rab-13-like [Cimex lectularius]	XP_014246087.1	92%	710
Rab14	215	Rab14 GTPases are localized to biosynthetic compartments, including the rough ER, the Golgi complex, and the trans-Golgi network, and to endosomal compartments, including early endosomal vacuoles and associated vesicles. Rab14 is believed to function in both the biosynthetic and recycling pathways between the Golgi and endosomal compartments. Rab14 has been suggested to help regulate GLUT4 translocation and play a role in the regulation of phagocytosis.	Rab14	Rab-protein 14 isoform b [Acyrthosiphon pisum]	NP_001155935.1	90%	832	PREDICTED: ras-related protein Rab-14 [Cimex lectularius]	XP_014253287.1	99%	907
Rab19	234	associated with Golgi stacks	Rab19	PREDICTED: ras-related protein Rab-40C [Acyrthosiphon pisum]	XP_001945092.1	69%	624	PREDICTED: ras-related protein Rab-1A-like [Cimex lectularius]	XP_014248369.1	92%	437
Rab21	223	Rab21 has been reported to localize in the ER. It has also been shown to colocalize with and affect the morpology of endosomes. When it is GTP-bound it has been shown to regulate phagocytosis.	Rab21	ras-related protein Rab-21-like [Acyrthosiphon pisum]	NP_001191951.1	94%	823	PREDICTED: ras-related protein Rab-21 [Cimex lectularius]	XP_014245117.1	98%	909
Rab22B	228	Rab31, also called Rab22B, is involved in the trafficking of the trans-Golgi network.	Rab	PREDICTED: ras-related protein RABF2a-like isoform X1 [Acyrthosiphon pisum]	XP_001944237.1	75%	450	PREDICTED: ras-related protein Rab-31-like isoform X2 [Cimex lectularius]	XP_014246038.1	75%	717
Rab23	375	Rab23 has been shown to regulate the sonic hedgehog pathway in mammals.	Rab23 like	GTP-binding protein YPTM1-like [Acyrthosiphon pisum]	NP_001156195.1	74%	558	PREDICTED: ras-related protein Rab-23 [Cimex lectularius]	XP_014255402.1	84%	631
Rab27A	145	Rab27A helps regulate exocytosis for different molecules and in different tissues depending on what effector molecules it has been paired up with.	Rab27A	PREDICTED: ras-related protein Rab-3 [Acyrthosiphon pisum]	XP_001944695.1	62%	225	PREDICTED: ras-related protein Rab-27A [Cimex lectularius]	XP_014244051.1	93%	347
Rab28	231	Rab28 is a late embryogenesis-abundant (Lea) protein that is regulated by the plant hormone abcisic acid (ABA).	Rab	PREDICTED: ras-related protein Rab-28-like [Acyrthosiphon pisum]	XP_003245469.1	73%	236	PREDICTED: ras-related protein Rab-28-like isoform X2 [Cimex lectularius]	XP_014252593.1	73%	269
Rab30	203	Rab30 appears to be associated with the Golgi stack and is expressed in a wide variety of tissue types.	Rab30	PREDICTED: ras-related protein Rab-30 [Acyrthosiphon pisum]	XP_001945306.2	99%	835	PREDICTED: ras-related protein Rab-30-like	XP_014250576.1	98%	914

								[Cimex lectularius]			
Rab32	318	Rab32 functions as an A-kinase anchoring protein (AKAP).	Rab32 Rab28	PREDICTED: ras-related protein Rab-38-like isoform X2 [Acyrthosiphon pisum]	XP_003241872.1	64%	774	PREDICTED: ras-related protein Rab-32 [Cimex lectularius]	XP_014246796.1	70%	797
Rab40	359	Rab40 may function as an E3 ubiquitin ligase and mediate the degradation of proteins, and it may be involved in the transport of myelin components.	Rab40; SOCS Rab40 (supressor of cytokine signaling)	PREDICTED: ras-related protein Rab-40C [Acyrthosiphon pisum]	XP_001947621.1	69%	1052	PREDICTED: ras-related protein Rab-40C [Cimex lectularius]	XP_014255234.1	74%	1091

Table 4: Rab gene family gene number in *Diaphorina citri, Drosophila melanogaster, Homo sapiens, Saccharomyces cerevisiae, Caenorhabditis elegans,* and *Trypanosoma brucei*. (Pereira-Leal e Seabra, 2001; Ackers *et al.*, 2005; Zhang *et al.*, 2007)

Rab gene family member count in *D. citri* and other species	
Diaphorina citri	21
Drosophila melanogaster	33
Homo sapiens	66
Saccharomyces cerevisiae	11
Caenorhabditis elegans	28
Trypanosoma brucei	16

Results and Discussion

With manual annotation twenty-one individual Rab models were completed within the Web Apollo editor of the *D. citri* genome. Two Rab gene-models were removed during quality control. A BLASTP search of the *D. citri* MCOT database with default settings found 60 hits above the 1e-10 e-value threshold, which includes duplicated results and sequences. When comparing the number of Rabs found in *D. citri* (21) and other organisms, like *D. melanogaster* (33), *H. sapiens* (66), *C. elegans* (28)*, S. cerevisiae* (11), and *T. burcei* (16) you see a wide range of Rab members accounted for (Table 4)*.* Since in *D. melanogaster* at least 10 more Rab genes were found it should be expected that more could be found *D. citri*. Comparison of the amino acid sequences of all Rabs in a multiple alignment revealed evolutionary conservation of the Rabs and their domains. Using BLASTP, it was shown that there was a significant match with orthologs of other insects (Table 2). Most of the *D. citri* Rabs were grouped with orthologs forming seven of eight functional groups (Figure 1) (Table 1). A pattern of inheritance helps support the identity of the sequence. The forming of the functional groups in the phylogenetic tree allows for the general function assignment to their individual group members (Pereira-Leal and Seabra 2001) (Ackers et al. 2005). Because an analysis of the Rab family has been performed we now have support of the idea that these sequences could be used for future analysis and possible therapies to help fight the spread of citrus greening. Understanding the evolution and purpose of the Rabs would help us determine where they would be best used in the process of saving citrus.

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