Annotation and analysis of *yellow* genes in the citrus greening disease vector, *Diaphorina citri*

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INTRODUCTION

The yellow gene family encodes a group of proteins important for the process of melanization in insects. *yellow* genes are of ancient lineage, as evidenced by the presence of *yellow*-like genes in several bacterial species; however, no evidence was found that these genes exist in the complete genome sequences of the worm *Caenorhabditis elegans* or the yeast *Saccharomyces cerevisiae*, suggesting that they may have been lost from many lineages and may now be largely restricted to arthropods [1]. *yellow* was first studied over 100 years ago by Alfred Sturtevant, a student of the renowned Thomas Hunt Morgan, and has been cited as the first example of a single gene mutation affecting behavior [2] [3]. *yellow*, however, was initially identified because of its role in pigmentation by T.H. Morgan during his experiments with *Drosophila* and was named for the loss of black pigment that gave mutant flies a more yellow appearance [4]. This role in melanization hints at the involvement of certain *yellow* genes in immune related responses. Melanization is a locally triggered immune effector response that is characterized by the synthesis of melanin and its cross-linking with molecules on microbial surfaces or in injured areas resulting in the killing of the invader and hardening of the wound clot [5]. Melanization is also essential for cuticle sclerotization or tanning which leads to hardening of the insect exoskeleton, an effect that has recently been confirmed in *Drosophila yellow-y* knockouts [6] [7].

While functional assignments have yet to be made for every member of this enigmatic family, research suggests that a role in melanization may be conserved for several *yellow* family members [8]. Duplications, as well as losses, are apparent at multiple levels within the *yellow* gene family and phylogenetic analysis consistently reveals a clear pattern of yellow family expansion associated with insect diversification [8]. Previous studies have shown that the *yellow-y, -c, -d, -e, -f, -g,* and *-h* genes were already present prior

to divergence of the hemimetabolous and holometabolous insects, however, some insects have not maintained all of these ancestral *yellow* genes [8]. The most notable case of *yellow* lineage duplication is the entire *Major Royal Jelly Protein (mrjp)* family which forms a distinct cluster within the yellow family phylogeny and seems to be restricted to certain species of bees (Figure 1) [1]. This relatively recent evolution is interesting for several reasons. Unlike other members of the yellow gene family which do not generally share a conserved intron/exon structure throughout the group, the *mrjp* genes share a high amount of structural similarities between themselves [1]. In *A. mellifera*, this *mrjp* group carries an intron/exon structure which resembles that of its *yellow-e3* genes (also known in other species as *yellow-d*). Microarray expression data suggest that the functions of *yellow-e3* and the *mrjp* genes have more in common than *yellow-e3* does to the rest of the yellow proteins, leading researchers to believe that the *mrjp* gene array evolved via initial duplication of *yellow-e3/d* [1]. *mrjp* genes have been found in high concentration in royal jelly, the substance fed to those larval bees that develop into queens [9]. Interestingly, however, a *mrjp*-like clade has been identified from the genome of the solitary wasp *Nasonia vitripennis* [8], and phylogenetic analysis indicates that the two hymenopteran clades have independent origins [10]. The function of the *N. vitripennis mrjp* family is not currently known [10]. While most insects do not carry this newly evolved *yellow-mrjp* gene array, the MRJP domain is conserved throughout all *yellow* genes. Due to the naming of this domain, many *yellow* genes have been improperly labeled as *mrjp*, complicating annotation of this gene family.

Here we describe the *yellow* genes of the Asian citrus psyllid, *Diaphorina citri.* Because of the multiplicity of *yellow* genes discovered in *D. citri* and the inconsistency of ortholog names, phylogenetic analysis was performed to properly classify these genes. Based on these results, we also discuss possible functions of the *yellow* genes identified in *D. citri*.

METHODS

The *D. citri* genome was annotated as part of an annotation community driven strategy [11]. Protein sequences of the yellow family were collected from the NCBI protein database and were then used for a blast search of the *D. citri* MCOT protein database, available on citrusgreening.org, to find predicted protein models. The MCOT IDs of these predicted models were then used to search the *D. citri* version 2.0 and 3.0 genomes, and regions of high sequence identity were investigated. Gene models were manually annotated in Apollo, using de novo Transcriptome and MCOT gene predictions, RNA-Seq, Iso-Seq, and ortholog data as evidence to determine and validate proper gene structure (Table 1). The gene models were then analyzed using NCBI blast to assess their accuracy and completeness. A neighbor-joining phylogenetic tree of annotated *D. citri* yellow protein sequences compared to orthologs was then created in MEGA version 7 using the MUSCLE multiple sequence alignment with p-distance for determining branch length and 1,000 bootstrap replicates [12]. Accession numbers for the sequences used in this analysis can be found in Table 2. Comparative expression levels of yellow proteins throughout different life stages (egg, nymph, and adult) in *Candidatus* Liberibacter asiaticus (*C*las) infected vs. uninfected *D. citri* insects was determined using RNA-seq data and the Psyllid Expression Network, available at http://pen.sgn.cornell.edu.

RESULTS AND DISCUSSION

Manual gene annotation performed within Apollo on the *D. citri* genome revealed the presence of nine *yellow* genes with the conserved major royal jelly protein domain. Phylogenetic analysis was conducted to determine the orthology of these *yellow* genes. Well classified *yellow* orthologs were used when available to construct this tree; however, with the exception of *yellow-y*, the yellow gene family has not been thoroughly studied in hemipterans, and therefore these orthologs are largely based on computer predicted gene models. Nevertheless, it was important to include these hemipteran sequences in order to properly

classify the *D. citri yellow* genes. The results of the phylogenetic analysis coincide well with previous studies. It is consistently shown that *yellow-f* and *yellow-c* share a close evolutionary relationship and that those two groups are also closely linked with *yellow-b*, *yellow-h*, and *yellow-y*; *yellow-e3/d* are very closely linked with *mrjp*; and *yellow-g* and *yellow-x* appear to share a relatively close relationship (Figure 1) [8] [13]. Based on this analysis, the *yellow* genes in *D. citri* are comprised of two *yellow-y* genes, two *yellow-d* genes, two *yellow-g* genes, one *yellow-h*, and one *yellow-c*, as well as one *yellow* gene (*yellow 9*) which seems to be a duplication unique to hemipterans, but is closely related to known *yellow-f* orthologs (Figure 1). Determining the *yellow* genes present in *D. citri*, and further characterizing them, is a first step towards finding possible RNAi *yellow* gene targets, which may be used to help curb the spread of citrus greening disease in the United States by this invasive insect.

yellow-y

yellow-y (also simply referred to as *yellow*), was the first *yellow* gene discovered and is the most researched member of the *yellow* family [4]. *yellow-y* encodes a secreted extracellular protein required for synthesis of black melanin pigments in *Drosophila* adult cuticle and derivative structures, as well as the larval mouth parts, and also plays a role in cuticular hardening [7] [14]. Studies suggest that the temporal and spatial profiles of *Drosophila yellow-y* and *ebony* gene together determine the pattern and intensity of melanization, but the mechanism by which the *yellow-y* gene promotes the formation of pigments is poorly understood [15]. Noh et al. (2015) proposed the yellow-y protein as the probable candidate for *Drosophila* dopachrome conversion enzyme (DCE) because its mutation leads to the formation of a yellow-colored wing and cuticle. Surprisingly, they found that the *yellow-y* gene itself is not a DCE, but that it may regulate the expression of *yellow-f*, *yellow-f2,* or other enzymes involved in melanization [16].

Many studies have also noted a role of *yellow-y* in the behavior and mating ability of *Drosophila*.

However, none of these studies were able to determine the mechanism by which behavior was affected [2] [14] [17] [18]. One study by Wilson et al. (1976) revealed that *yellow Drosophila* mutants of various backgrounds and ages showed significantly lower levels of spontaneous open field locomotor activity than their wild-type siblings. This study also found *yellow* mutant males to be severely deficient in mating speed and in competitive mating ability [17]. A recent study, however, attempted to delve into the mechanism of this decreased mating ability and found that the loss of *yellow-y* in *Drosophila* led to changes in the morphology of the structures used during mating, and showed that the reduced mating success was not due to a behavioral defect. Specifically, *yellow* mutant males showed insufficient hardening of the sex combs, thereby inhibiting them from latching on to females during mating. However, in *Drosophila willistoni*, a species which naturally lacks sex combs, *yellow* mutants showed no reduction in mating ability [7].

Phylogenetic analysis has provided insights into *yellow-y* evolution in *D. citri. yellow-y* is present in most insect species as a single copy, except phylogenetic analysis failed to find *yellow-y* in *Cimex lectularius* or *Bemisia tabaci*. Interestingly, both *yellow* and *yellow 2* in *D. citri* clade exclusively with known *yellow-y* orthologs, representing a duplication event which seems to be unique to *D. citri* (Figure 1). It is also interesting to note that these two *yellow-y* genes show inverse expression patterns in *D. citri*; that is, *yellow* (*y*) shows highest expression in the egg and nymph, while *yellow 2* (*y2*) shows highest expression in the adult (Figure 2). *yellow-y* has been found to be abundant in *Drosophila* pupae, when melanin is deposited in the adult cuticle, which would correlate with the *yellow* (*y*) in *D. citri* [14]. *yellow 2* (*y2*), on the other hand, may play a more important role in adult *D. citri* and should be studied further.

yellow-c, -f, -b, and 9

yellow-b and *yellow-c* are *yellow* genes to which no function has yet been attributed. Phylogenetic analysis does, however, reveal a close relationship between *yellow-b*, *yellow-c*, *yellow-f*, and *yellow 9* [8] [13]

(Figure 1). *yellow-f* and *yellow-f2* in *Drosophila* have been found to function as dopachrome conversion enzyme (DCE) that likely plays an important role during melanin biosynthesis in *Drosophila* larvae, pupae, and adults [15]. Interestingly, while most hemipterans seem to have one or more *yellow-c* genes, none clade exclusively with known *yellow-f* or *yellow-b* orthologs (Figure 1). Instead, hemipterans are grouped into their own separate clade, with a strong association to *yellow-f* and *yellow-c*. This distinctness of the hemipteran group is common among the other *yellow* genes in the tree, as would be expected, however, the association to a known ortholog is typically much clearer than is seen with *yellow 9*. Depending on the insects involved in the phylogenetic analysis, *yellow 9* may clade more closely with *yellow-f*, *yellow-c*, or *yellow-b*. The results seem to indicate that *yellow 9* is most closely related to *yellow-f*. This is supported by the presence of an *A. pisum* sequence in the *yellow 9* clade of figure 1 which was previously reported as grouping with *yellow-f [8]*. The addition of several other hemipteran sequences may have helped align the *A. pisum* more closely to the *yellow 9* orthologs. Since *yellow-f* has been shown to play an important role in other insects, as discussed above, this result would make sense. More studies should be conducted to conclusively determine the true identity of this hemipteran outlier.

As shown in figures 2 and 4, *yellow 8* (*c*) shows the highest expression levels of the *yellow* genes in *D. citri*. This gene is most highly expressed in the nymph whole body, but there is also considerable expression in the egg and adult. It also seems to be more highly expressed in the male abdomen versus the female, and the female antennae versus the male. There is also a noticeable downregulation of this gene in *C*Las positive *D. citri* (Figure 3). These results warrant further investigation to understand how the expression of *yellow 8* (*c*) is affected by *C*Las infection.

yellow-h

yellow-h transcripts show interesting color-related expression patterns in some species, but the

function of the encoded protein is poorly understood [8] [19]. Zhang et al. (2017) produced *yellow-h*2 and *yellow-h3* mutations using double sgRNAs in the butterfly *V. cardui*. All injected butterfly larvae that survived to pupation in this study died during late pupal pigment development. Additionally, PCR genotyping validated the existence of long deletions in all dead pupae; however, detection of possible pigment phenotypes was confounded by tissue necrosis [13].

Expression data from *D. citri* shows the highest expression of this gene in the egg and nymph (Figure 4). This data is consistent with the study by Zhang et al. (2017) which showed that mutations of *yellow-h* in larval *V. cardui* led to death in pupal stages of development, suggesting that *yellow-h* could be important during insect development [13]. Furthermore, PEN data revealed an 8.96-fold increase of *yellow-h* expression in *D. citri* nymphs raised on Citrus *spp.* and infected with *C*Las, versus uninfected nymphs (Figure 4). This differential expression might indicate that *yellow-h* could be a potential RNAi target and should be studied more closely in *D. citri*.

Overall, most insect species have *yellow-h*. However, phylogenetic analysis indicates that some species lack this gene while other species, such as the butterflies previously discussed, apparently harbor multiple copies. Of the species reviewed in this study, three are lacking this gene while the others all harbor only one copy. Phylogenetic analysis reveals that *D. citri* contains one *yellow-h* gene, *yellow 4*, (Table 3) which clusters with known *yellow-h* orthologs (Figure 1).

yellow-e3/d

A recent study on melanin pigmentation in butterfly wings, conducted by Zhang et al. (2017), shed some light on the function of *yellow-e3/d*, a previously uncharacterized, yet highly conserved and duplicated *yellow* gene family member. The results of this study indicated that y*ellow-d* shows red-specific expression in the butterfly *Vanessa cardui*, and that the loss of *yellow-d* function not only affects melanin patterns, but

also presumptive ommochrome patterns. In this study, the *yellow-d* knockout results clearly demonstrated that the activity of this single gene is sufficient to tune the color of specific pattern elements by switching pigments, although it may be possible that co-expression of *yellow-d* and two other melanin-suppressing genes, *ebony* and *black*, can be recruited together for the same purpose [13].

Phylogenetic analysis of the *yellow* genes annotated in the *D. citri* genome clearly shows *yellow 3* and *yellow 5* in a clade with known *yellow-e3/d* orthologs (Figure 1). The presence of this gene was found in nearly all examined insect species, except for the hemipteran *Sipha flava*, and duplications were found to be sporadic in species (Table 3). Expression in the *D. citri* adult antennae is similar between *yellow 3(d)* and *yellow 5(d)*, however, this is the most highly expressed region for *yellow 5(d)* whilst *yellow 3(d)* shows highest expression in the adult whole body. Expression data also reveals a slight upregulation of 29.37 TPM in the whole body of adult, *C*Las positive *D. citri*, which may indicate an immune response by *yellow 3(d)* (Figure 5).

yellow-g

The function of *yellow-g* is currently not well understood. However, the presence and duplication of this gene is quite prevalent among most insects. Of the insects reviewed in this study, three are outliers containing no copies, while all others contain two copies, including *D. citri* (Table 3). The *yellow-g* copies in *D. citri* were found to be *yellow 6* and *yellow 7* (Figure 1). *yellow 7 (g)* shows very little expression in the stages and tissues that have been assayed, with the highest expression level (7.52 TPM) seen in the whole body of *C*Las negative adults. *yellow 6 (g)*, on the other hand, shows moderate expression in the adult whole body and in the adult male abdomen. Interestingly, there appears to be no expression in the adult female abdomen. This gene is downregulated by nearly half in *C*Las positive insects and could warrant further study for this reason (Figure 6).

CONCLUSION

The *yellow* gene family is an ancient and enigmatic group of genes which is continuously evolving, with duplications and losses apparent at multiple levels. Many of these genes play a crucial role in the process of melanization. *D. citri* harbors a unique duplication of *yellow-y*, which may significantly affect cuticular hardening [7]. It is interesting that the expression of these two genes in *D. citri* are inversely related, an indication that *D. citri*'s second *yellow-y* may prove useful well into adulthood. *D. citri* and other hemipterans may also contain another unique duplication, a possible variation of *yellow-f*, which encodes for dopachrome conversion enzyme (DCE); however, this result is inconclusive and requires further investigation. Further research of the yellow gene family in *D. citri* may provide novel targets for molecular therapeutics in the fight against citrus greening disease.

Evidence Table

Gene	Identifier	MCOT	de novo	Isoseq	RNAseq	Ortholog
yellow	Dcitr06g10150.1.1	X	X	X	X	
yellow 2	Dcitr03g06860.1.1		X	X	X	
yellow 3	Dcitr07g07190.1.1	X	X		X	
yellow 4	Dcitr07g07210.1.1	X	X	X	X	X
yellow 5	Dcitr11g08710.1.1	X	X		X	X
yellow 6	Dcitr02g17880.1.1	X	X	X	X	X
yellow 7	Dcitr11g06750.1.1	X	X	X	X	X
yellow 8	Dcitr01g01210.1.1		X	X	X	X
yellow 9	Dcitr01g05760.1.1	X	X		X	

Table 1: Manually annotated *yellow* genes in *Diaphorina citri*. There are nine genes in total. Each gene has been assigned an identifier and denoted as complete or partial based on available evidence. MCOT: a de novo Oases or Trinity model from an independent transcriptome was identified and the sequence from that transcript was used to validate or modify our model; Isoseq: long RNAseq reads generated with Pacific Biosciences technology were used to help validate the exon structure of the model;. RNASeq: Illumina RNASeq reads were used to help create or validate our model;. Ortholog: orthologous sequences from related insects and information about conserved motifs or domains were used to determine the final annotation.

Accession Number Table

Gene	*Drosophila melanogaster*	*Tribolium castaneum*	*Bombyx mori*	*Apis mellifera*	*Nasonia vitripennis*
yellow-y	NP_476792.1	NP_001161919.1	NP_001037434.1	NP_001091693.1	NP_001154977.1
yellow-b	NP_523586.1	NP_001161777.1			NP_001154989.1
yellow-c	NP_523570.3	NP_001161778.1	NP_001037426.1		
yellow-c2					
yellow-f (fa) †	NP_524335.1	NP_001161780.1	NP_001037424.1	NP_001011635.1	NP_001154968.1
yellow-f2 (fb)†	NP_650247.1		NP_001037428.1		
yellow-h	NP_651912.3	XP_008196499.1		NP_001091687.1	NP_001153394.1
yellow-e	NP_524344.1	NP_001161779.1	NP_001159615.1	XP_003249426.1	NP_001154985.1
yellow-e2	NP_650289.2				
yellow-e3	NP_650288.1	NP_001161913.1		NP_001091698.1	NP_001154982.1
yellow-d	NP_523820.2		NP_001037422.1		
yellow-d2	NP_611788.1				
yellow-g	NP_523888.1	ACY71060.1		XP_396824.1	XP_008210364.1
yellow-g2	NP_647710.1	NP_001161782.1		XP_006559006.1	
yellow-k	NP_648772.1				
yellow-1 (x)‡		NP_001161783.1			
yellow-2 (x)‡		NP_001161784.1			
yellow-3 (x)‡		NP_001161785.1			
yellow-4 (x)‡		NP_001161786.1			
yellow-5 (x)‡		NP_001165862.1			

yellow-b (x)‡			NP_001037430.1		
mrjp/yellow-e3				XP_001122824.1	
mrjp 1				NP_001011579.1	
mrjp 2				NP_001011580.1	
mrjp 3				NP_001011601.1	
mrjp 4				NP_001011610.1	
mrjp 5				NP_001011599.1	
mrjp 6				NP_001011622.1	
mrjp 7				NP_001014429.1	
mrjp 8				NP_001011564.1	
mrjp 9				NP_001019868.1	

Gene	*Acyrthosiphon pisum*	*Nilaparvata lugens*	*Sipha Flava*	*Bemisia tabaci*	*Halymorpha halys*	*Cimex lectularius*	*Oncopeltus fasciatus*
yellow-y	NP_001165848.1	XP_022184245.1	XP_025405013.1		XP_014294277.1		AMW91813.1
yellow-b							
yellow-c	XP_008189794.1	XP_022204541.1	XP_025415761.1	XP_018916210.1 XP_018902711.1	XP_014273608.1	XP_014260013.1	
yellow-c2	XP_008189795		XP_025415762.1 XP_025421542.1				
yellow-f							
yellow-h	XP_016656930.1	XP_022203506.1			XP_014273783.1	XP_014247137.1	
yellow-e	XP_001948479.1	XP_022204722.1		XP_018907407.1 XP_018906454.1 XP_018907408.1	XP_014292044.1	XP_024083966.1 XP_014239663.1 XP_014247778.1	
yellow-d/e3	XP_001942700.2	XP_022204983.1 XP_022205442.1		XP_018916419.1	XP_014275903.1	XP_024083964.1 XP_024083953.1	
yellow-g	XP_001944949.2	XP_022207733.1 XP_022191720.1			XP_014292984.1		
yellow-g2	XP_001945004.2	XP_022205647.1			XP_014292982.2	XP_014244848.1	
yellow-x		XP_022206946.1		XP_018912253.1			
yellow 9	XP_001946728.1		XP_025413197.1	XP_018905078.1 XP 018901286.1	XP_014288983.1	XP_014255102.1	

Table 2: Accession numbers for genes used in phylogenetic analysis (Figure 1). XP identifiers are computer predicted models.

† Yellow-fa and -fb are the names given specifically to Bombyx Mori.
‡ Yellow-x is the name commonly used to refer to this group.

Gene Copy Number Table

Gene	*Dc*	*Ap*	*Am*	*Tc*	*Dm*	*Bm*	*Sf*	*Bt*	*Nl*	*Cl*	*Hh*
yellow-y	2	1	1	1	1	1	1	0	1	0	1
yellow-b	0	0	0	1	1	0	0	0	0	0	0
yellow-f	1†	1†	1	1	2	2	1†	2†	0	1†	1†
yellow-c	1	2	0	1	1	1	3	2	1	1	1
yellow-h	1	1	1	1	1	0	0	0	1	1	1
yellow-d/e3	2	1	1	1	4	1	0	1	2	2	1
yellow-e	0	1	1	1	1	1	0	3	1	3	1
yellow-g	2	2	2	2	2	0	0	0	2	1	2
yellow-x	0	0	0	5	0	1	0	1	1	0	0
yellow-k	0	0	0	0	1	0	0	0	0	0	0
mrjp	0	0	10‡	0	0	0	0	0	0	0	0
Total	9	9	17	14	14	7	5	9	9	9	8

Table 3: Yellow gene family ortholog numbers in *Acyrthosiphon pisum* (*Ap*), *Apis mellifera* (*Am*), *Tribolium castaneum* (*Tc*), *Drosophila melanogaster* (*Dm*), *Bombyx mori* (*Bm*), *Sipha flava* (*Sf*), *Bemisia tabaci* (*Bt*), *Nilaparvata lugens* (*Nl*), *Cimex lectularius* (*Cl*), *Halyomorpha halys* (*Hh*). Copy numbers are based on the results from the phylogenetic analysis (Figure 1). *Dc* numbers represent the number of manually annotated genes in the *D. citri* v3.0 genome.

† Represents some uncertainty in the results of the phylogenetic analysis discussed above in the *yellow-f* section.
‡ *Am* has been previously shown to only contain 9 *mrjp* genes and one pseudo *mrjp* gene. The 10th gene found in this research is a predicted sequence found through NCBI and grouped closely *mrjp*.

Phylogenetic Analysis

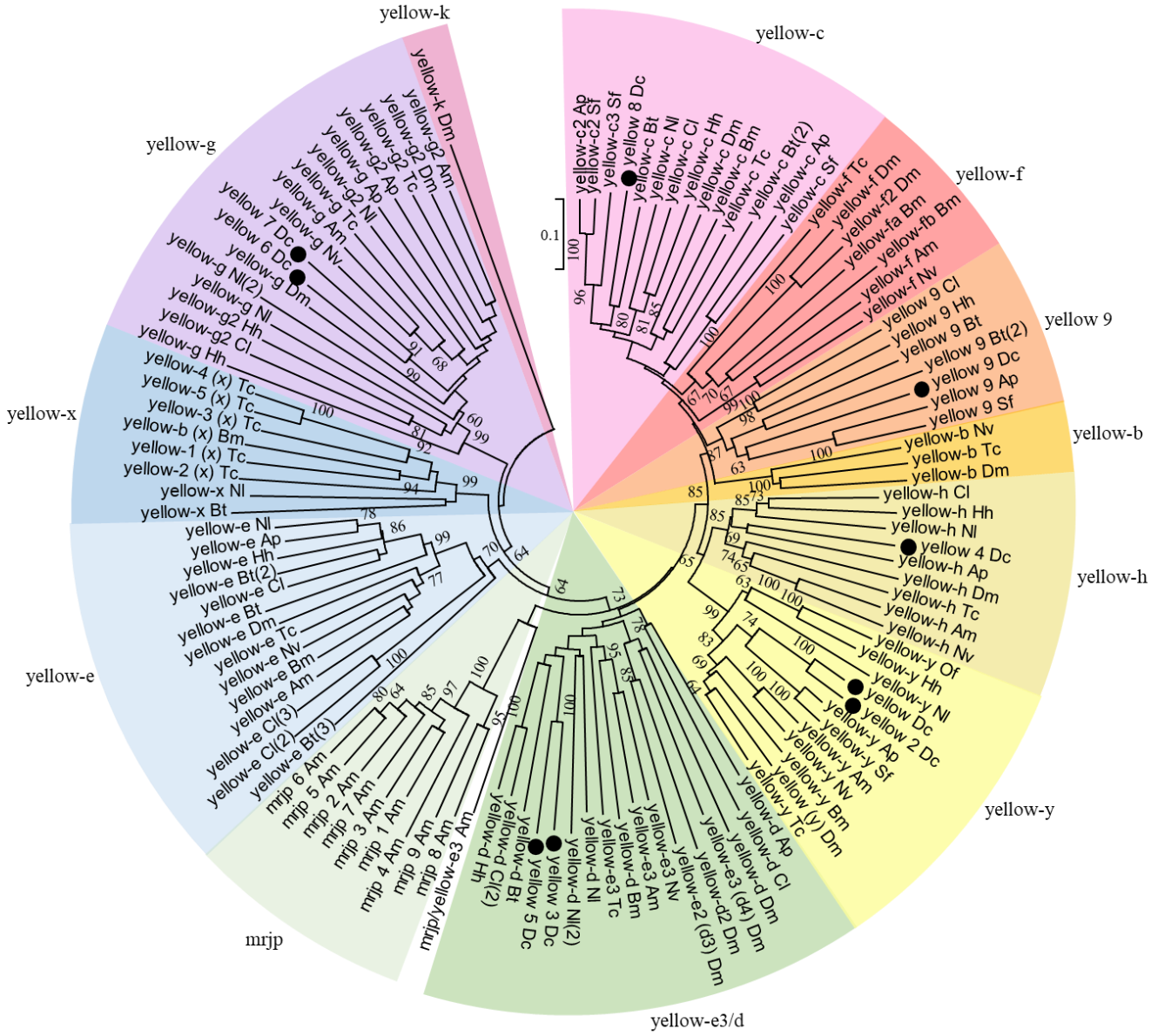
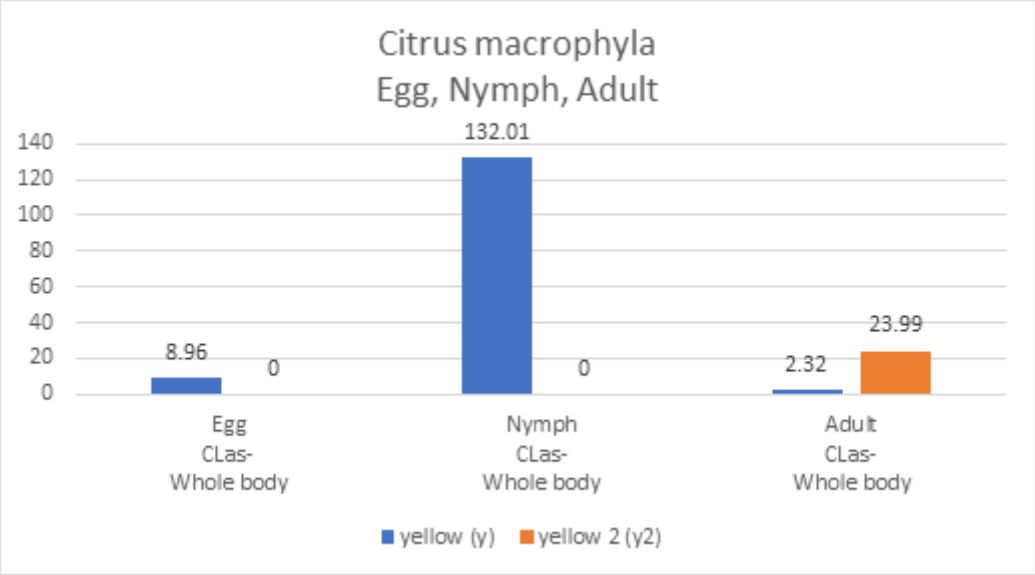
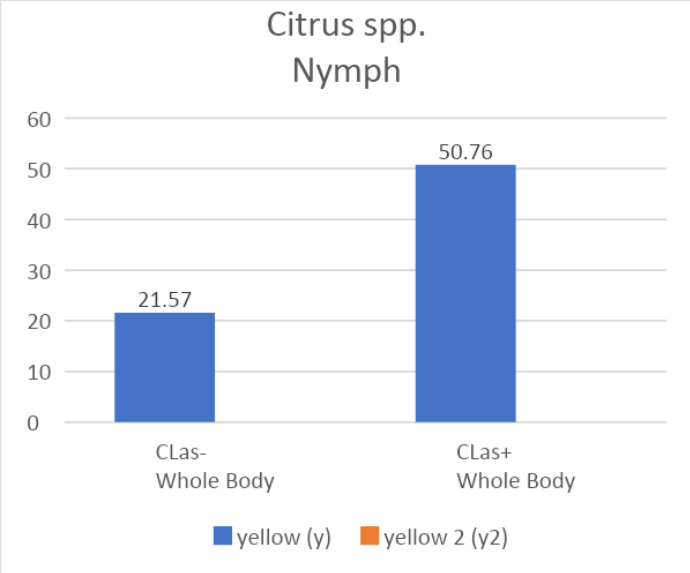
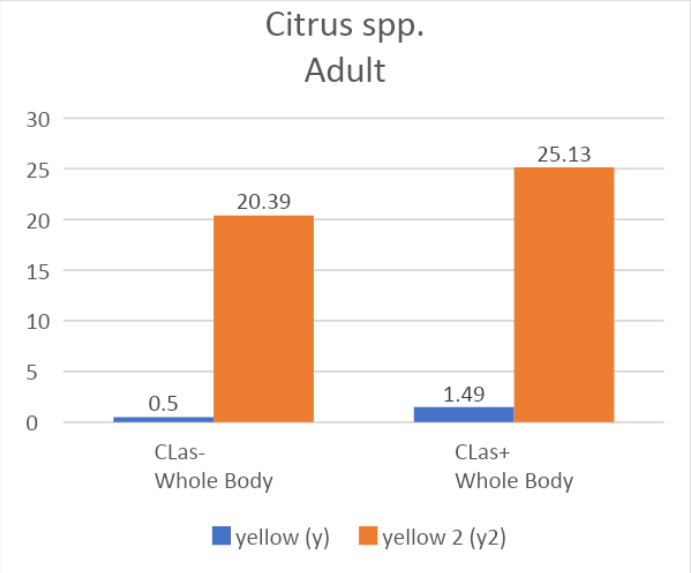


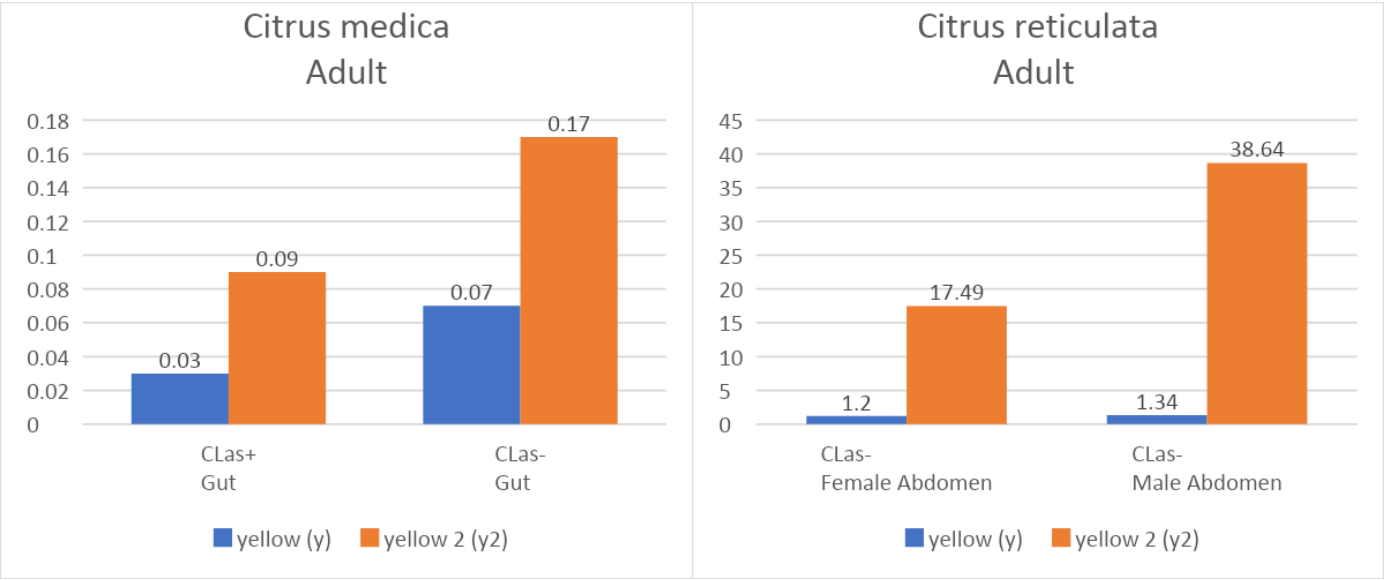
Figure 1: The yellow gene family. A neighbor-joining phylogenetic tree was generated from annotated *D. citri* (*Dc*) yellow protein sequences and the protein sequence of all predicted *yellow* genes from the insects *Acyrthosiphon pisum* (*Ap*), *Apis mellifera* (*Am*), *Tribolium castaneum* (*Tc*), *Drosophila melanogaster* (*Dm*), *Bombyx mori* (*Bm*), *Sipha flava* (*Sf*), *Bemisia tabaci* (*Bt*), *Nilaparvata lugens* (*Nl*), *Cimex lectularius* (*Cl*), *Halyomorpha halys* (*Hh*). Also included are sequences from *Nasonia vitripennis* (*Nv*) and *Oncopeltus fasciatus* (*Of*). Bootstrap analysis was performed with 1000 replicates. Values greater than 60 are shown. *D. citri* sequences are identified with a dot. Color coding indicates specific yellow clades. Genes that have been published on NCBI with a gene name are shown as published. Computer predicted models are typically improperly named and, therefore, have been named according to clade. NCBI accession numbers are shown in the Accession Number Table (Table 3).

yellow-y Expression Charts









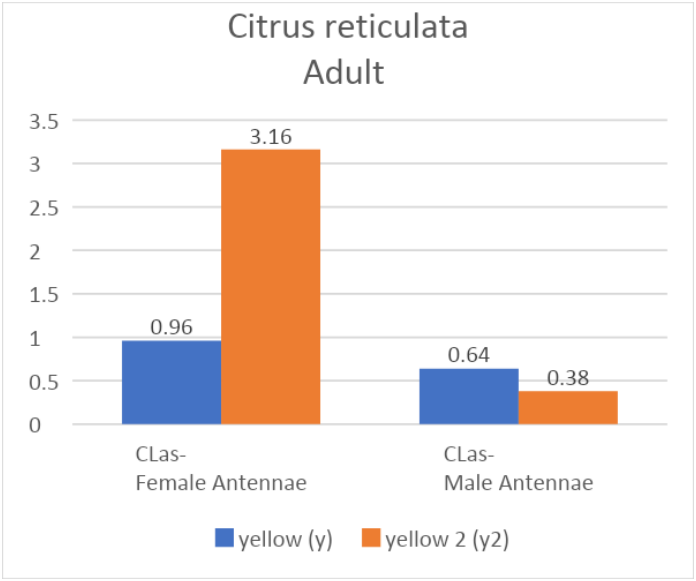
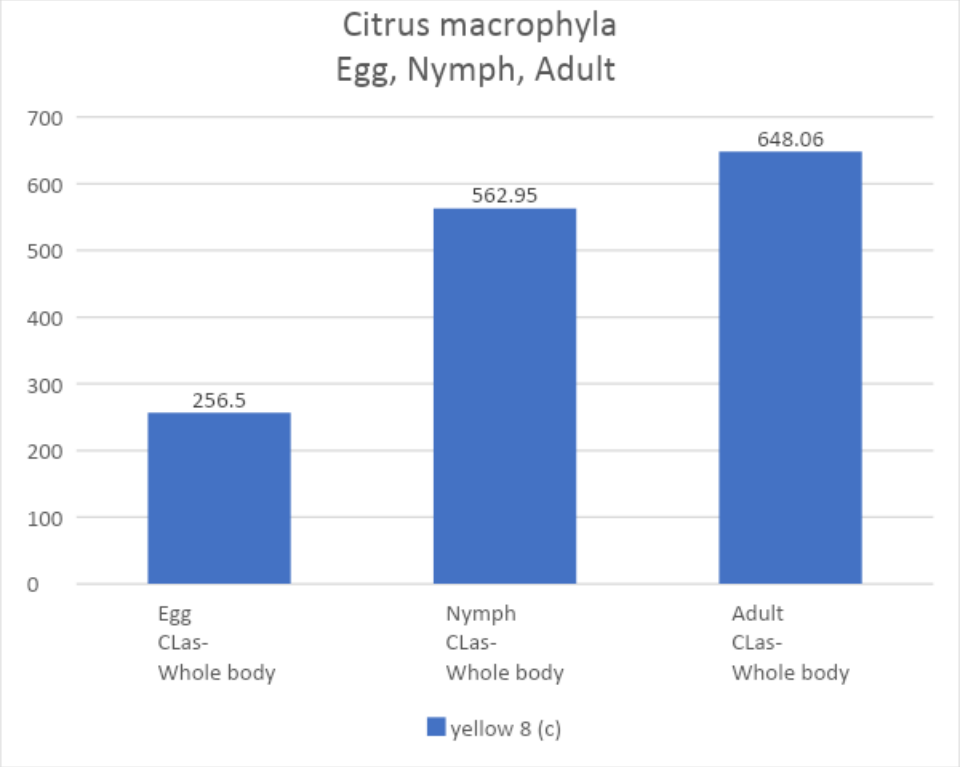
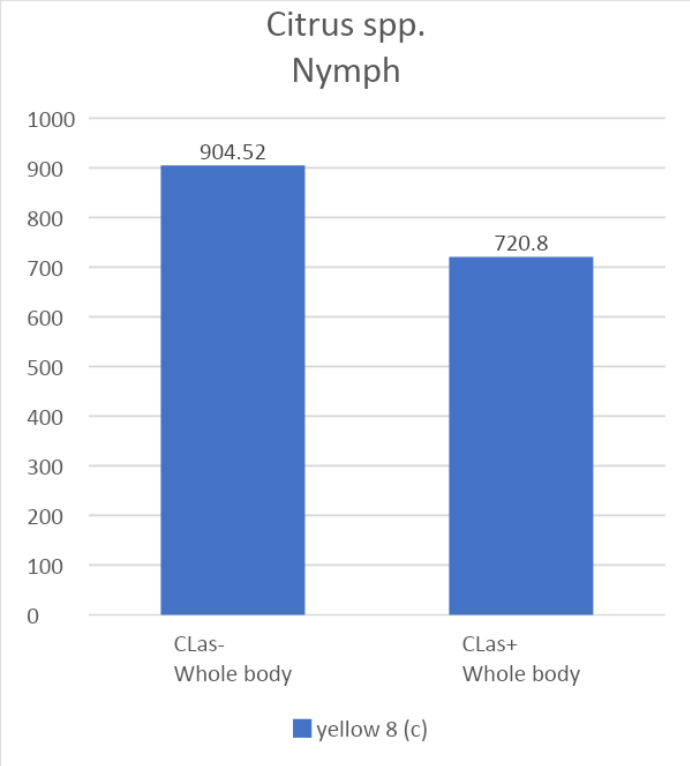
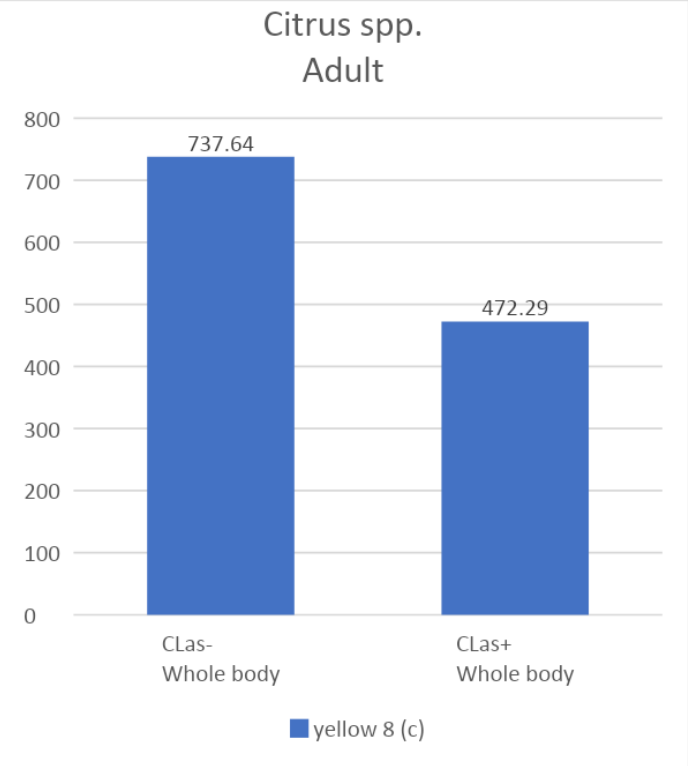


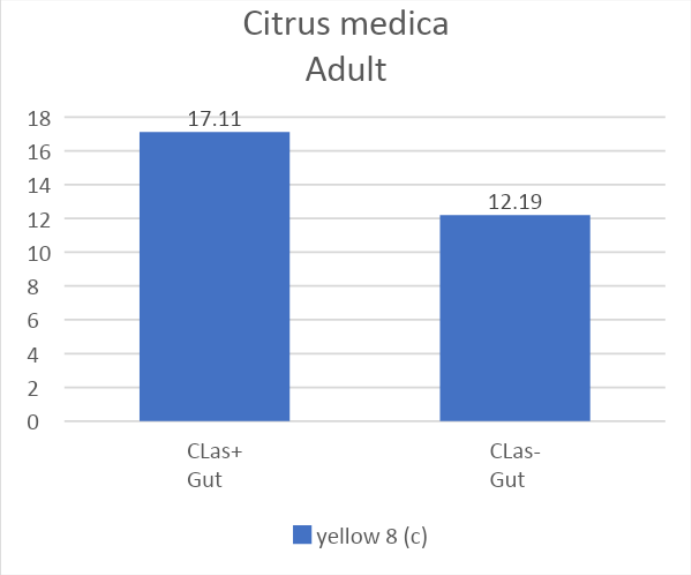
Figure 2: Comparative expression levels of the *D. citri* yellow-y proteins throughout different life stages (egg, nymph, and adult) in infected vs. uninfected *D. citri* insects grown on various citrus varieties. Values are represented in transcripts per million (TPM). Expression data obtained using the Psyllid Expression Network (http://pen.sgn.cornell.edu).

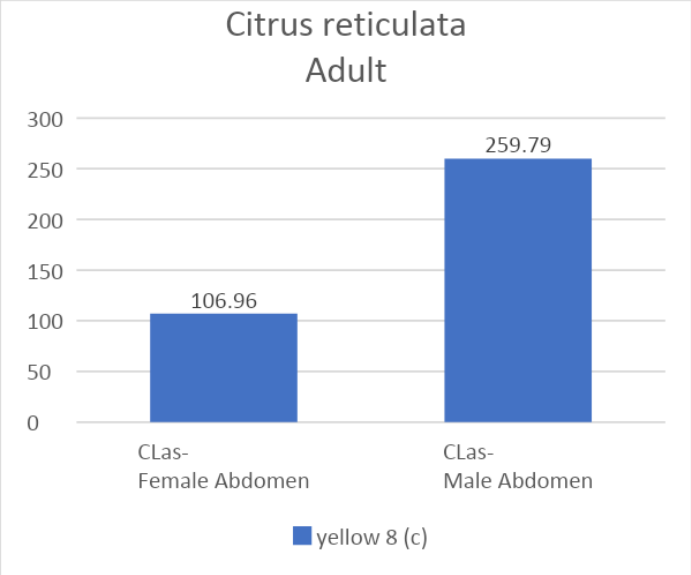
yellow-c Expression Charts











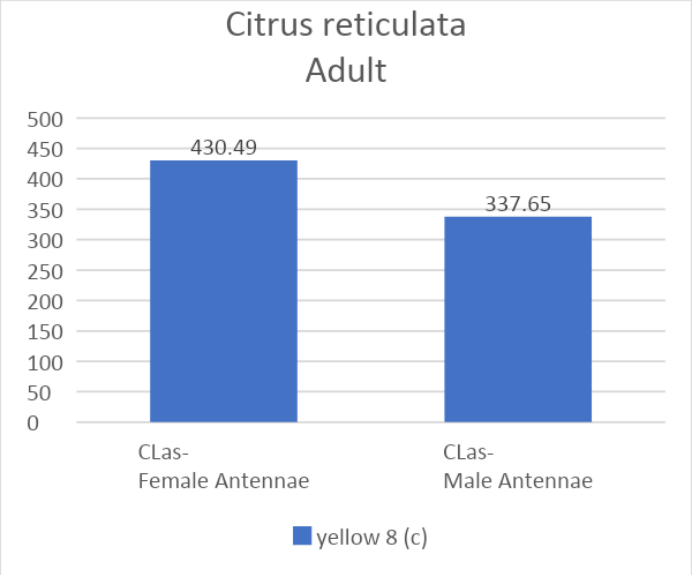
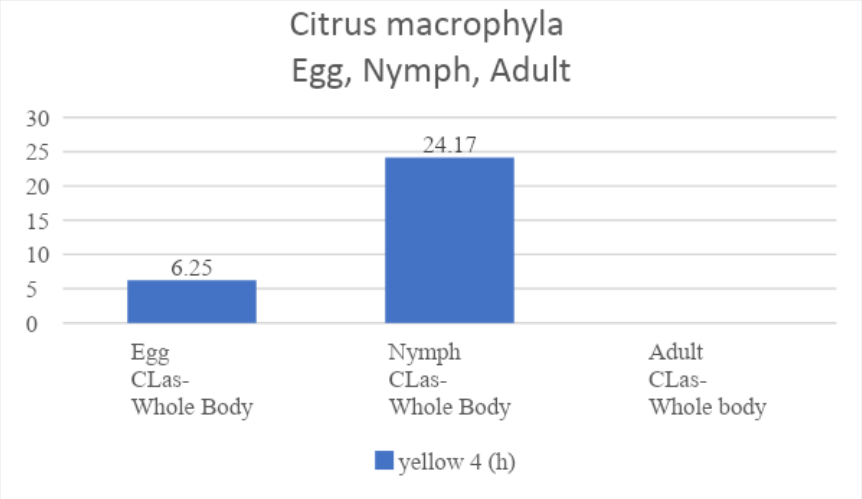
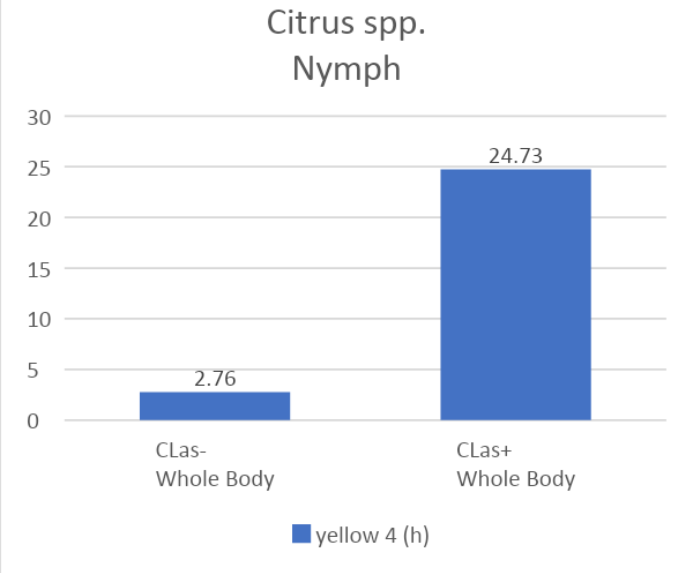
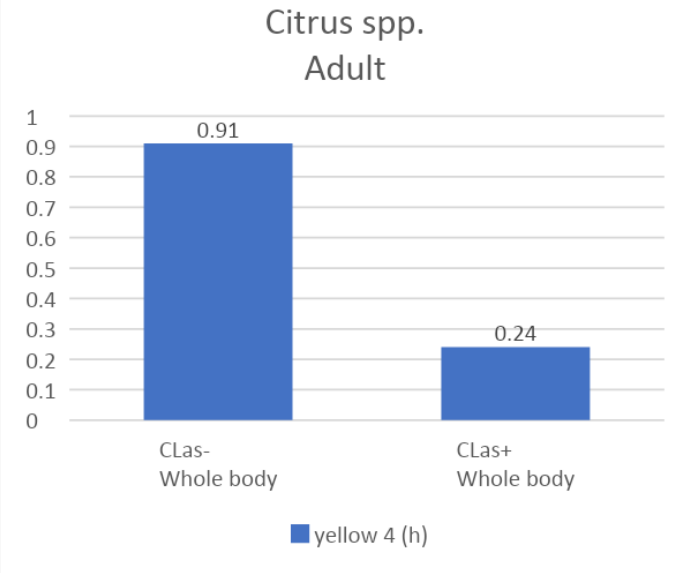


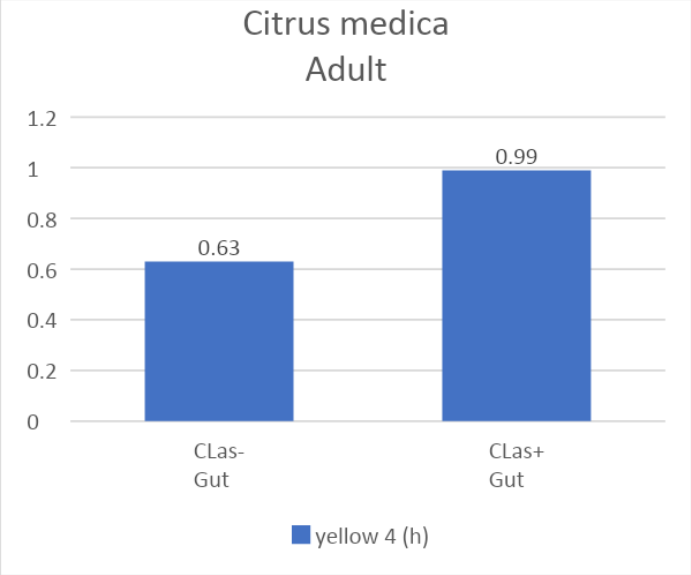
Figure 3: Comparative expression levels of the *D. citri* yellow-c protein throughout different life stages (egg, nymph, and adult) in infected vs. uninfected *D. citri* insects fed on various citrus varieties. Values are represented in transcripts per million (TPM). Expression data obtained using the Psyllid Expression Network (http://pen.sgn.cornell.edu).

yellow-h Expression Charts









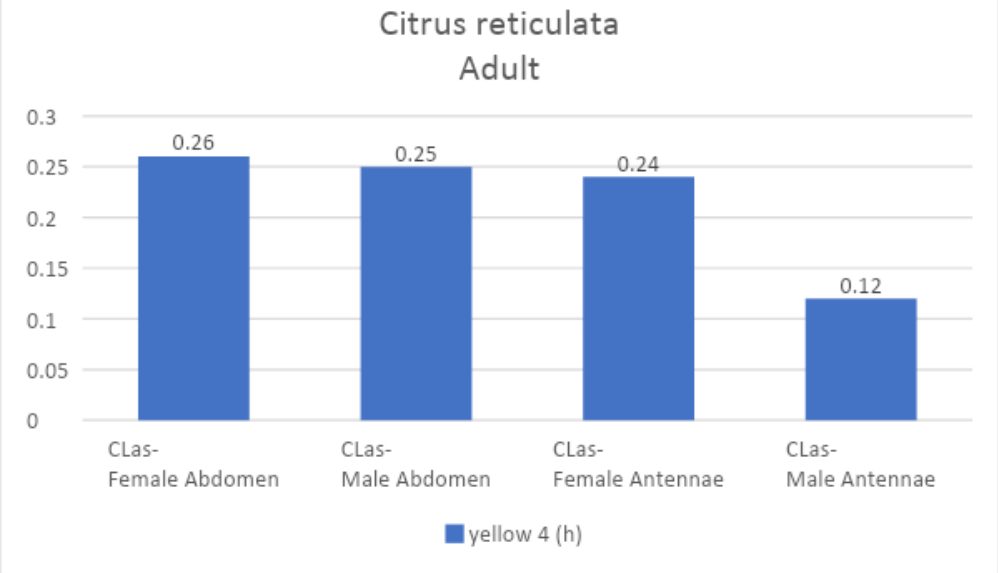
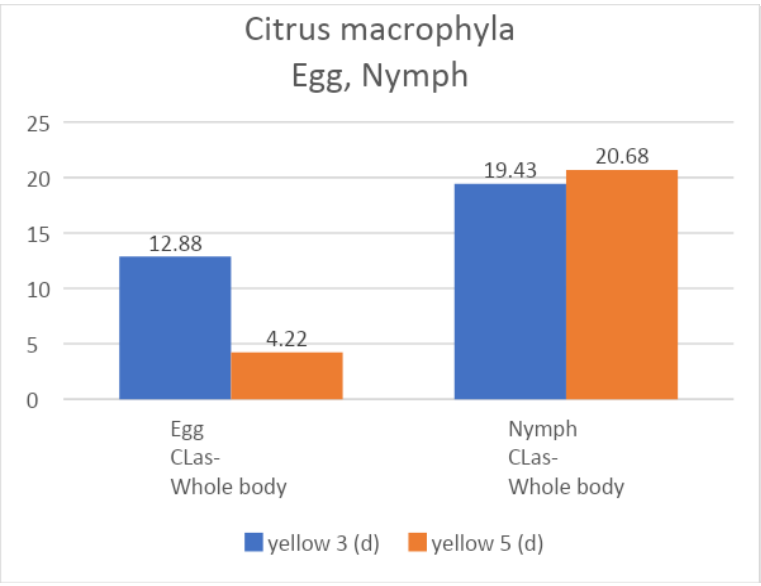
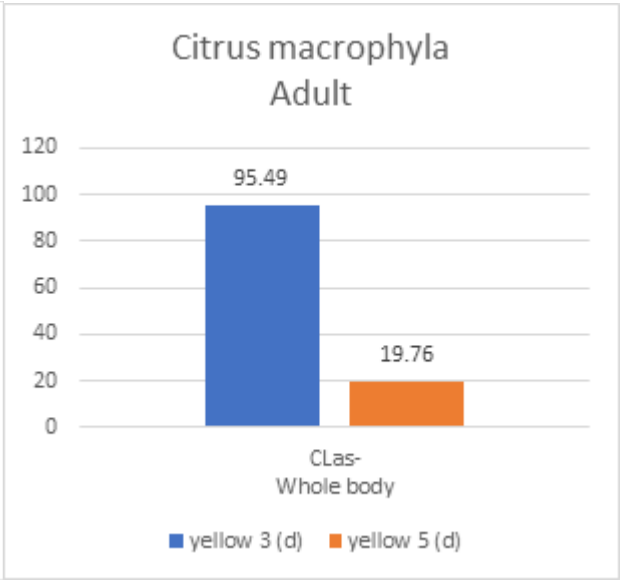
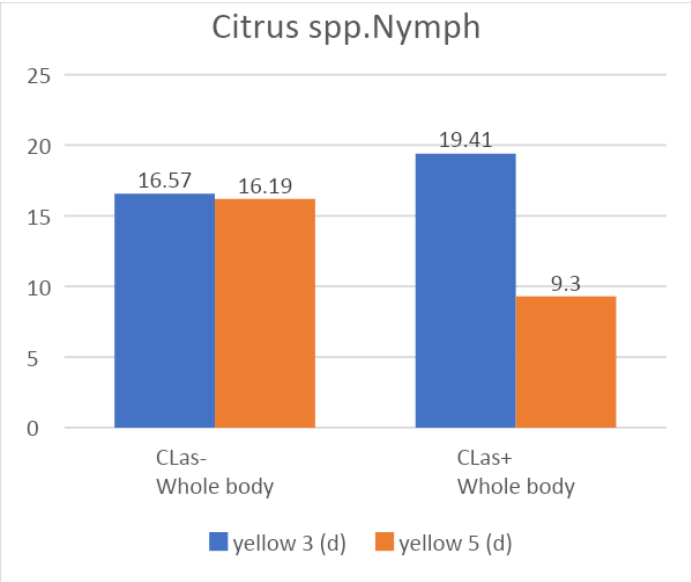


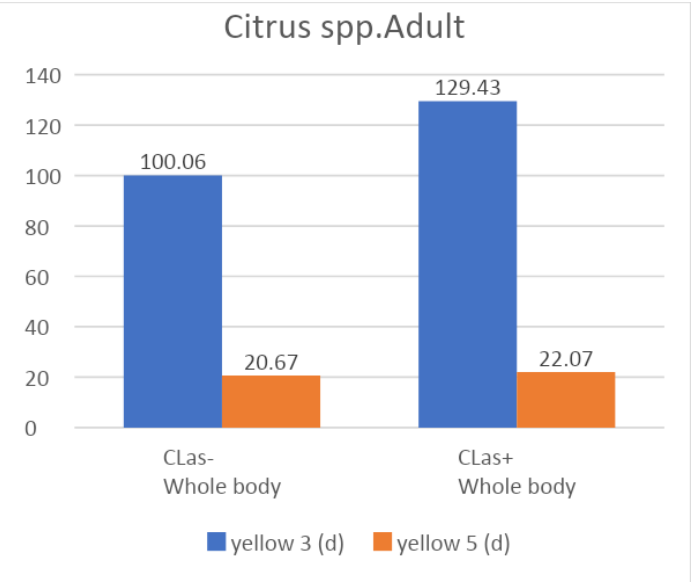
Figure 4: Comparative expression levels of the *D. citri* yellow-h protein throughout different life stages (egg, nymph, and adult) in infected vs. uninfected *D. citri* insects discovered on various citrus varieties. Values are represented in transcripts per million (TPM). Expression data obtained using the Psyllid Expression Network (http://pen.sgn.cornell.edu).

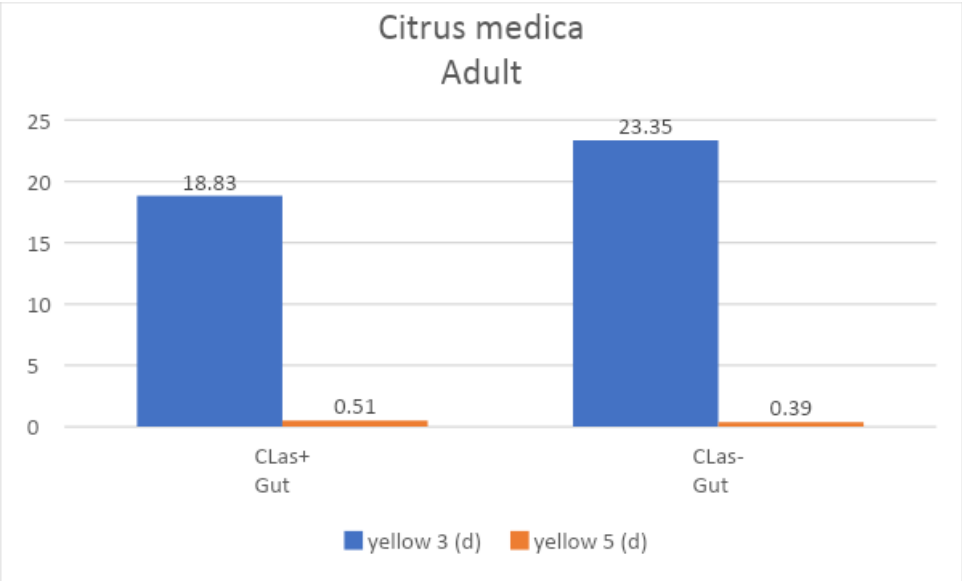
yellow-e3/d Expression Charts











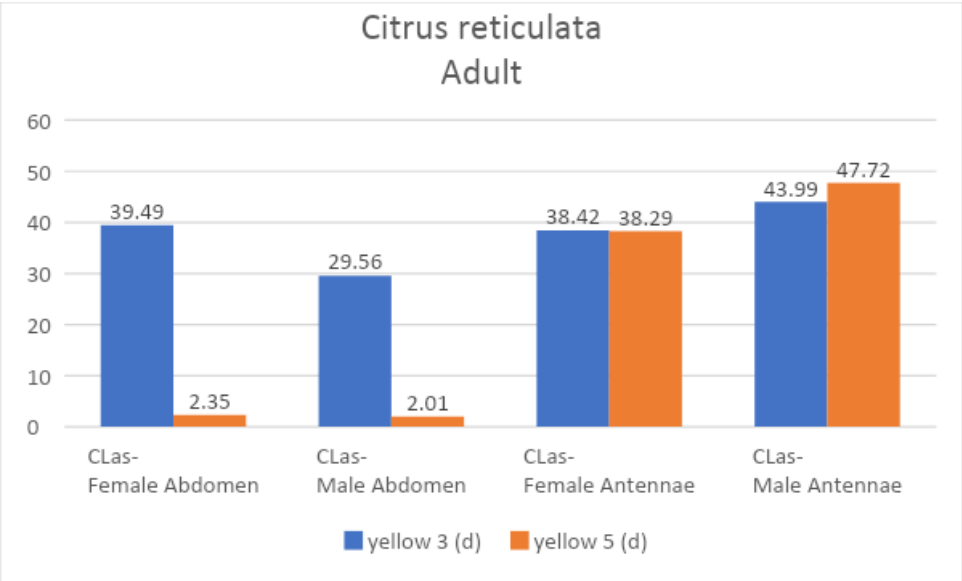
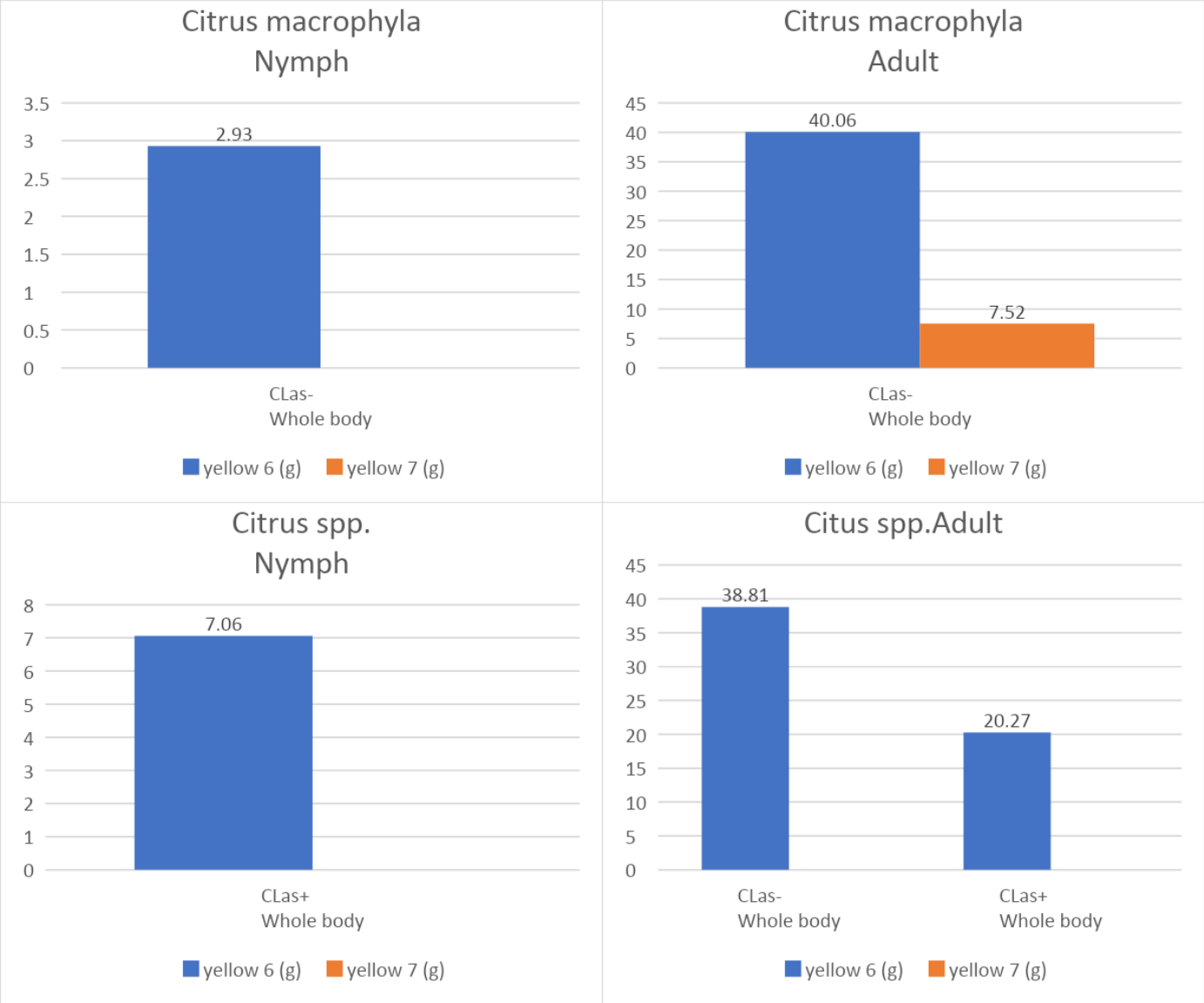


Figure 5: Comparative expression levels of the *D. citri* yellow-d proteins throughout different life stages (egg, nymph, and adult) in infected vs. uninfected *D. citri* insects discovered on various citrus varieties. Values are represented in transcripts per million (TPM). Expression data obtained using the Psyllid Expression Network (http://pen.sgn.cornell.edu).

yellow 6 and 7 (g) Expression Charts



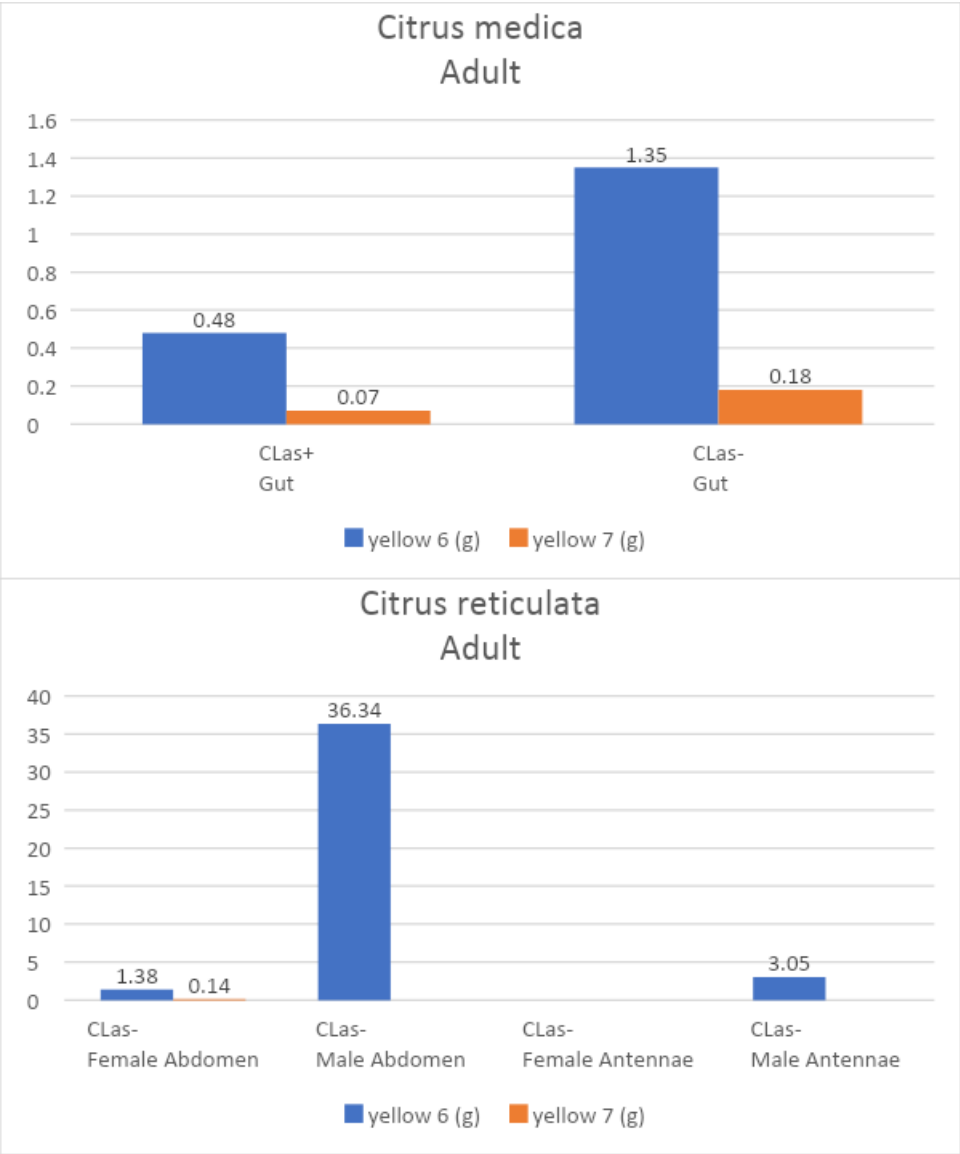


Figure 6: Comparative expression levels of the *D. citri* yellow-g proteins throughout different life stages (egg, nymph, and adult) in infected vs. uninfected *D. citri* insects discovered on various citrus varieties. Values are represented in transcripts per million (TPM). Expression data obtained using the Psyllid Expression Network (http://pen.sgn.cornell.edu).

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