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Comparative proteomic analysis between fifth-instar nymphs and adults of Asian citrus psyllid *Diaphorina citri*

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Abstract. Asian citrus psyllid *Diaphorina citri* Kuwayama is extremely problematic worldwide, particularly where Huanglongbing (HLB) disease, the most serious and devastating of citrus diseases, is found. The threat is a result of its ability to transmit the causal agent of HLB, *Candidatus* Liberibacter asiaticus (*C*Las) bacterium. Improvements in proteomics, mass spectrometry, bioinformatics tools and gene ontology annotation facilitate the mapping and large-scale identification and quantification of proteins. To date, only a few comparative proteomic studies report the developmental proteomic changes of hemimetabolous and plant–disease vector insects. Two-dimensional gel electrophoresis analysis of *D. citri* total protein is able to detect qualitative and quantitative developmental differences. Liquid chromatography-tandem mass spectrometry identifies 89 protein spots. Most proteins are metabolism and bioenergetics-related. Nineteen protein spots are found to be implicated in stress/defence/immunity; 7 in development regulation; 9 in nervous system functions; 4 in the reproductive system; 23 in cytoskeleton and muscle organization; and 4 in movement, flight and other processes. Significant increases in the level of proteins related to structural constitution of the skeleton, stress/defence/immunity, reproduction system, muscles, locomotion and flight are found in adults, consistent with the fact that *D. citri* is a hemimetabolous insect, whereas proteins involved in developmental regulation are higher in the nymphal stage. The identification of these variably expressed proteins between the nymph and adult stages, linked with the basis of their physiological roles, will lead to a better understanding of the factors influencing development in *D. citri* and the regulation of some crucial metabolic pathways. It may also help to identify targets for genetic manipulation using RNA interference or other techniques to disrupt Asian citrus psyllid development, lifespan or its ability to transmit *C*Las.

Key words. Asian citrus psyllid, *Diaphorina citri*, 2-DE electrophoresis, LC-MS/MS, metamorphosis, proteomic analysis.

Introduction

Asian citrus psyllid *Diaphorina citri* Kuwayama is a phloem sap-sucking insect belonging to Sternorrhyncha, superfamily Psylloidea and family Psyllidae (Bové, 2006). Nymphs and adults suck phloem sap from the foliage causing leaf distortion and curling. Furthermore, the psyllid produces huge amounts of honeydew, which cause the heavy development of sooty mold on honeydew-covered leaves. Also, it injects toxins that cause malformation of leaves and shoots during feeding on the phloem sap. Asian citrus psyllid transmits *Candidatus* Liberibacter asiaticus (*C*Las) bacterium, the putative causal agent of citrus greening, which is also called Huanglongbing (HLB) (Grafton-Cardwell *et al.*, 2005; Bové, 2006).

Two-dimensional gel electrophoresis (2-DE) allows separation of complex protein mixtures, visualization of these differences in the gel, and then the selection and identification of the proteins via mass spectrometry (MS). The use of a combination of these technologies provides information that

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helps to begin answering a wide range of interesting biological questions (Ong & Pandey, 2001). Although numerous successes of DNA-based approaches to monitor gene expression are reported, in many studies mRNA is not a reliable indicator of protein abundance in cells (Gygi *et al.*, 1999).

Initially, differential proteomics was used to identify changes in protein expression between the normal status compared with a specified status such, as healthy and diseased; subsequently, its use is now widely reported for identifying toxicity biomarkers and induced immunity, discovering resistance mechanisms and providing new insights into complex mechanisms in many organisms (Kennedy, 2002; Sharma *et al.*, 2004; Shi & Paskewitz, 2006; Nguyen *et al.*, 2008). In several insect studies, proteomics has emerged as a powerful method for gaining insight into many physiological changes at the cellular level (Nyström, 2005; Seehuus *et al.*, 2006; Zheng *et al.*, 2011). The technique is reported to have successfully been used with many insects, particularly silkworm, mosquito, honeybee, fruit fly and cotton bollworm (Li *et al.*, 2014). Most studies focus on insect immunity (Shi & Paskewitz, 2006), immunity-related proteins (e.g. in *Bombyx mori* and *Drosophila melanogaster*; Vierstraete *et al.*, 2004; Zhang *et al.*, 2014), and exposure to toxic compounds such as cadmium, carbamates, bio-toxins (such as cry11Aa from *Bacillus thuringiensis*) and other pesticides (Sharma *et al.*, 2004; Cancino-Rodezno *et al.*, 2012; Wu *et al.*, 2013). Also, the innate immunity of insect vectors of human disease is receiving increased attention for blocking the transmission of important diseases such as malaria (Christophides *et al.*, 2002). In the study of plant diseases, proteomic and bioinformatics sciences enable the exploration of several aspects of interactions between vector insect–plant pathogen (Elzinga & Jander, 2013).

Furthermore, with the increase of bioinformatics tools, gene ontology annotations and information from different organisms' datasets, many studies have turned away from investigating the principles of insect development via classical genetics analyses and have instead moved toward molecular biology approaches (Carmena, 2009). This facilitates the mapping and the large-scale identification and quantification of proteins, the study of their post-translational modifications, the analysis of protein–protein interactions (PPIs), as well as *in vivo* studies of protein expression, aiming to identify functional protein networks (Carmena, 2009). Metamorphosis, a process that enables insects to develop from one stage to another, consists of extensive morphological and physiological changes. The developmental proteomic changes associated with holometabolous insects receive more attention than those of hemimetabolous insects because of the additional larval and pupal stages. Developmental protein mapping of typical model insects such as the fruit fly, silkworm and honeybee is also extensively reported (Vierstraete *et al.*, 2004; de Morais Guedes *et al.*, 2005). Silkworm haemolymph proteins change markedly from the larval to moth stages. The identified proteins are involved in the processes of food digestion, nutrient storage and transport and metabolism (Hou *et al.*, 2010).

On the other hand, hemimetabolous insects, such as Asian citrus psyllid, undergo incomplete metamorphosis, passing through only three stages; eggs, nymphs and adults. The nymphal stages somewhat resemble the adult form morphologically, although adults have fully developed wings, muscles, skeletal structures and mature reproductive systems. During the nymphal stage, *D. citri* passes through five instars. Proteomic study of the last instar (fifth instar) could reveal proteomic changes associated with maturation into the adult stage. Surprisingly, to date, there are few reported comparative proteomic studies on the developmental proteomic changes of hemimetabolous and plant–disease vector insects. To understand the molecular mechanisms of development in *D. citri*, 2-DE is used in combination with liquid chromatography-tandem MS (LC-MS/MS) to analyze total proteins of the fifth-instar nymph and adult stage.

It is hypothesized that proteins involved in structural constitution of skeleton, reproduction system and flight muscles would show greater abundance in adults compared with nymphs. By contrast, proteins involved in developmental regulation would be more abundant in the nymphal stages.

Determining the differences in protein profiles between *D. citri* nymphs and adults will help to identify the proteins that are up- or down-regulated during structural development and metamorphosis, to determine their physiological roles, and possibly to identify targets for genetic manipulation using RNA interference (RNAi) or other techniques that disrupt Asian citrus psyllid development, lifespan or its ability to transmit *C*Las.

Materials and methods

Asian citrus psyllid culture

Asian citrus psyllid colonies were reared on small sweet orange (Valencia) trees inside mesh cages in controlled environment growth rooms under an LD 16 : 8 h photocycle at $25 \pm 2\,^{\circ}C$ and $60\% \pm 5\%$ relative humidity. Citrus trees were obtained from an insect-proof, temperature-controlled greenhouse (LD 16 : 8 h photocycle at 28 °C and 40% relative humidity). Nymphs of *D. citri* were collected from the plants and placed into in Petri dishes using a #4 camel hair brush. Nymphs were then classified to stages based on the morphological features (Grafton-Cardwell *et al.*, 2005). The fifth-instar nymphs were gathered in Eppendorf tubes and kept at −20 °C. For *D. citri* adults, fifth-instar nymphs were collected and placed on a new plant, then the emerged adults were collected (at the same age, approximately 15 days old) using a manual insect aspirator and stored at −20 °C.

Protein extraction and preparation

Insect samples were placed in 1.5-mL Eppendorf tubes. Each tube consisted of 50 adult psyllids or 100 *D. citri* fifth-instar nymphs. Each tube was considered as one biological replicate. Five biological replicates were analyzed for both adult psyllids and fifth-instar nymphs. The samples were homogenized using plastic pestles and then 500 µL of lysis buffer (0.2% Hepes and 10% sucrose; w/v) was added. Samples were homogenized again with the buffer for 10 min then centrifuged at 8500***g*** for

10 min at 4 °C. The supernatant was transferred to another tube and three volumes of cold 10% trichloroacetic acid in acetone were added (stored at −20 °C). Samples were kept at −20 °C at least for 2 h then centrifuged at 14 500***g*** for 20 min at 4 °C. The protein pellet was washed using cold acetone (−20 °C) three times (2 h each). The protein pellet was cleaned using a 2-DE clean up kit in accordance with the manufacturer's instructions (Bio-Rad, Hercules, California). The cleaned protein samples were stored in −80 °C.

Two-dimensional gel electrophoresis and staining

The total protein of *D. citri* adults and nymphs were separated by 2-DE in accordance with the manufacturer's instructions with some modifications (Bio-Rad). Briefly, protein samples were dissolved in rehydration solution [7 M urea, 2 M thiourea, 60 mM dithiothreitol (DTT), 65 mM Chaps, 2% TritonX-100, 0.2% IPG buffer (ampholytes pH 5–8), 0.5 µL of 1% bromophenol blue]. Protein concentration was measured with Smartspec 3000 spectrophotometer (Bio-Rad) in accordance with the Bradford method (Bradford, 1976) using the Bio-Rad protein assay kit (Bio-Rad). Each sample was adjusted to contain 500 µg of proteins for colloidal Coomassie (G250) staining in 300 µL of rehydration solution.

The solution was loaded onto a ReadyStrip 17-cm immobilized pH gradient (IPG) strip (linear, pH 5–8) (Bio-Rad) and was actively rehydrated for 14 h at 20 °C with 50 V. Isoelectric focusing electrophoresis was performed using Bio-Rad Protean II cell (Bio-Rad). The program used was: step 1: 100 V, slow, 1 h; step 2: 200 V, slow, 1 h; step 3: 300 V, slow, 1 h; step 4: 500 V, slow, 3 h; step 5: 1000 V, slow, 3 h; step 6: 5000, slow, 3 h; step 7: 10 000 V, linear, 4 h; step 8: 10 000 V, rapid, 80 000 Vh, step 9: 500 V, rapid, hold. The current was limited to 50 µA strip^{-1}. After isoelectric focusing electrophoresis, the IPG strips were equilibrated in equilibration buffer I [6 M urea, 20% glycerol, 2% sodium dodecylsulphate (SDS) and 0.375 mM Tris–HC1, pH 8.8] containing 2% (w/v) DTT for 20 min. with gentle shaking and then for another 20 min in equilibration buffer II (1% DTT replaced with 2.5% iodoacetamide). The strip and marker (15 µL of marker with 15 µL of agarose gel at 4 °C) was loaded onto a 10% SDS-polyacrylamide gel (1.5 mm gel thickness) in the Protean II xi Cell (Bio-Rad). Gels were made the day before and kept at 4 °C until use. Loaded gels were covered with melted agarose then placed into the electrophoresis tank containing 1 × running buffer (25 mM Tris, 192 mM glycine, 0.1% SDS). Electrophoresis was conducted at constant current, and the program was 5 mA gel^{-1} for 50 min, then 30 mA gel^{-1} until the bromophenol blue dye front reached the edge of the gels.

Protein visualization and gel image analysis

Protein was visualized by colloidal Coomassie brilliant blue staining, followed by silver staining (Candiano *et al.*, 2004). Gels were stored in 20% ethanol at 4 °C until the analysis.

The stained gel was scanned at a resolution of 600 dpi. Spot detection, matching and quantitative intensity analysis were performed using MELANIE, version 7.0 (Gene-Bio, Switzerland) and quantification values for each detected spot were calculated using: (i) optical density (OD), the highest calibrated pixel intensity in the spot; (ii) Area, the spot's area in mm^2; (iii) Vol, the spot's volume (Vol = OD × Area), which represents integration of OD over the spot's area; and (iv) %Vol, the relative Vol (i.e. the Vol divided by the total Vol for total spots in the image). Differentially expressed protein spots were selected and subjected to identification by LC-MS/MS.

LC-MS/MS and database searches

LC-MS/MS analysis was carried out at the Interdisciplinary Center for Biotechnology Research (University of Florida, Gainesville, Florida) using an LTQ Orbitrap XL mass spectrometer (ThermoFisher Scientific, West Palm Beach, Florida). Proteins spots of interest were excised from the gels (nymph and adult) and analyzed by LC-MS/MS as described previously (Salganik *et al.*, 2012; Chavarria *et al.*, 2014). Briefly, the samples were digested enzymatically with trypsin, and the peptides were injected onto a capillary trap (LC Packings PepMap), desalted for 5 min with 0.1% (v/v) acetic acid at a flow rate of 3 µL min^{-1} prior, then loaded onto an LC Packing® C18 PepMap nanoflow high-performance liquid chromatography column (ThermoFisher Scientific).

For the protein search algorithm, raw data were analyzed with MASCOT, version 2.2.2 (Matrix Science, U.K.). SCAFFOLD, version 3.0.9.1 (Proteome Software, Inc., Portland, Oregon) was used to validate MS/MS-based peptide and protein identifications. Mascot was searched with a fragment ion mass tolerance of 0.8 Da and a parent ion tolerance of 10 ppm. Mascot was set to search the metazoan database from NCBI assuming the digestion enzyme trypsin. Iodoacetamide derivative of Cys was indicated as a fixed modification, whereas deamidation of Asn and Gln, oxidation of Met, and isopeptide linkage to Gly-Gly were specified as variable modifications. For MS/MS-based peptide and protein identifications, SCAFFOLD, version 3.6 (Proteome Software Inc.) was used for validation. The Peptide Prophet algorithm was used to identify peptides using a 95% probability (Keller *et al.*, 2002). Similarly, proteins were identified using Protein Prophet and accepted at a 95% probability if two or more unique peptides were identified in accordance with Nesvizhskii *et al.* (2003).

Results

Qualitative analysis of Asian citrus psyllid adults and nymphs proteins

The 2-DE showed that the overall number of total protein spots increased from the fifth-instar nymph to the adult stage. Representative colloidal Coomassie brilliant blue stained 2-DE gels of total proteins extracted from adult and fifth-instar nymphs are shown in Figure 1.

Overall, comparative analysis of the 2-DE protein maps revealed 344 ± 14 and 273 ± 18 protein spots with a 70% match ratio average in adults and nymphs, respectively. The

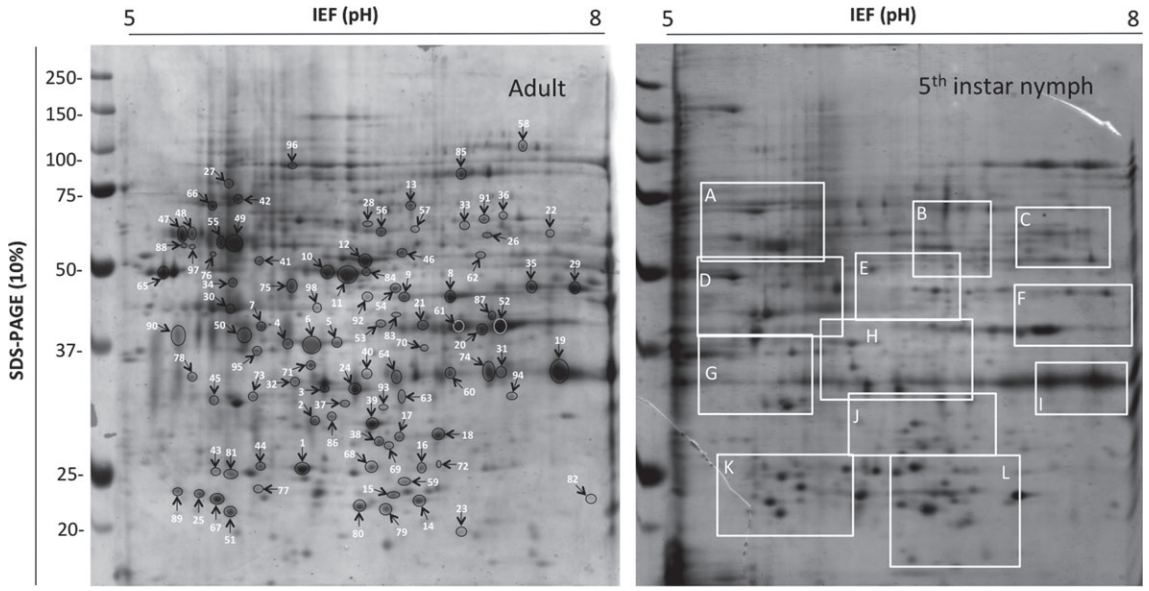

Fig. 1. Two-dimensional gel comparison of total proteins isolated from fifth-instar nymphs and adults of the Asian citrus psyllid *Diaphorina citri*. Immobilized pH gradient strips of linear pH 5–8 were used for isoelectric focusing and the sodium dodecyl sulphate-polyacrylamide gel electrophoresis was performed in 10% acrylamide. Protein molecular weight standards are shown on the left. Spots of statistically significant difference ($P < 0.05$) between nymph and adult stages were selected to be analyzed and identified using liquid chromatography-mass spectrometry.

expression levels of some proteins were dramatically changed (Fig. 1).

Class analysis indicated that the intensity of 181 protein spots was significantly different between adults and fifth-instar nymphs. Ninety-three protein spots were up-regulated from nymphs to adults from nymphs to adults and, among them, 49 spots increased by more than two-fold. Additionally, 88 protein spots were down-regulated and, among them, 43 spots decreased by more than two-fold. Furthermore, 165 and 112 protein spots were found only in adults or in nymphs, respectively. Protein spots with significant changes were selected for identification using MS analysis.

Identification of differentially expressed proteins by MS

From a total of about 181 protein spots that were significantly different between the life stages, 98 protein spots were excised and numbered as shown in Figure 1. Partial images of some of the areas containing selected proteins from the proteomic maps are shown enlarged in Figure 2. Twenty-five of the identified proteins were consistently up-regulated from the fifth-instar nymph to the adult stage. These are the spots numbered 3, 5, 13, 15, 17, 23, 24, 26, 27, 28, 34, 36, 37, 40, 42, 45, 52, 54, 56, 59, 61, 64, 72, 74 and 80 (Fig. 1 and Table 1), whereas 23 protein spots showed up-regulation (by more than two-fold) in adults compared with nymphs. In some instances, the same protein was detected in several spots (Table 1). For example, triose phosphate isomerase was identified from spots 1 and 44; glyceraldehyde 3-phosphate dehydrogenase was found in spots 3, 19, 25, 74 and 91; arginine kinase was found in spots 3, 4 and 5. Phosphoglycerate kinase was found in spots 8 and 9; and phosphoglycerate mutase was found in spots 17, 18 and 38 (Table 1). From nymph to adult stage, 12 proteins were down-regulated (by more than 0.5-fold) (Table 1). Fifteen identified protein spots existed only in adults (spots 83–98).

Functional classification of identified proteins

The identified proteins are known to participate in a variety of biological processes (Table 1). Among the 98 identified 2-DE spots, most were proteins assigned to metabolism and bioenergetics processes (protein, lipid, carbohydrate and nucleotide metabolism, oxidation-reduction process, glycolytic process and proton transport). Forty-seven proteins are putatively involved in lipid and carbohydrate metabolic processes (33 up-regulated), 12 in glycolytic processes (11 up-regulated), 22 in nucleotide metabolic processes (16 up-regulated) and 14 in proton transport (10 up-regulated). The functions of the other three proteins are currently unknown.

Nineteen proteins were found to be implicated in stress/defence/immunity (13 up-regulated), 7 in development regulation (5 down-regulated), 9 in nervous system functions (6 up-regulated), 4 in the reproductive system (3 existed only in adult Asian citrus psyllid and the fourth one was up-regulated), 23 in cytoskeleton and muscle organization (20 up-regulated), and 4 in movement and flight (3 up-regulated) and other processes (Figs 1 and 2). Functionally, most identified proteins are implicated in catalytic activity, binding, ion transport, antioxidant activity, oxidoreductase activity, cofactor or enzyme regulation, and as structural constituents of the skeleton (Fig. 3). The subcellular components of most identified proteins are cytoplasmic and cytoskeleton proteins

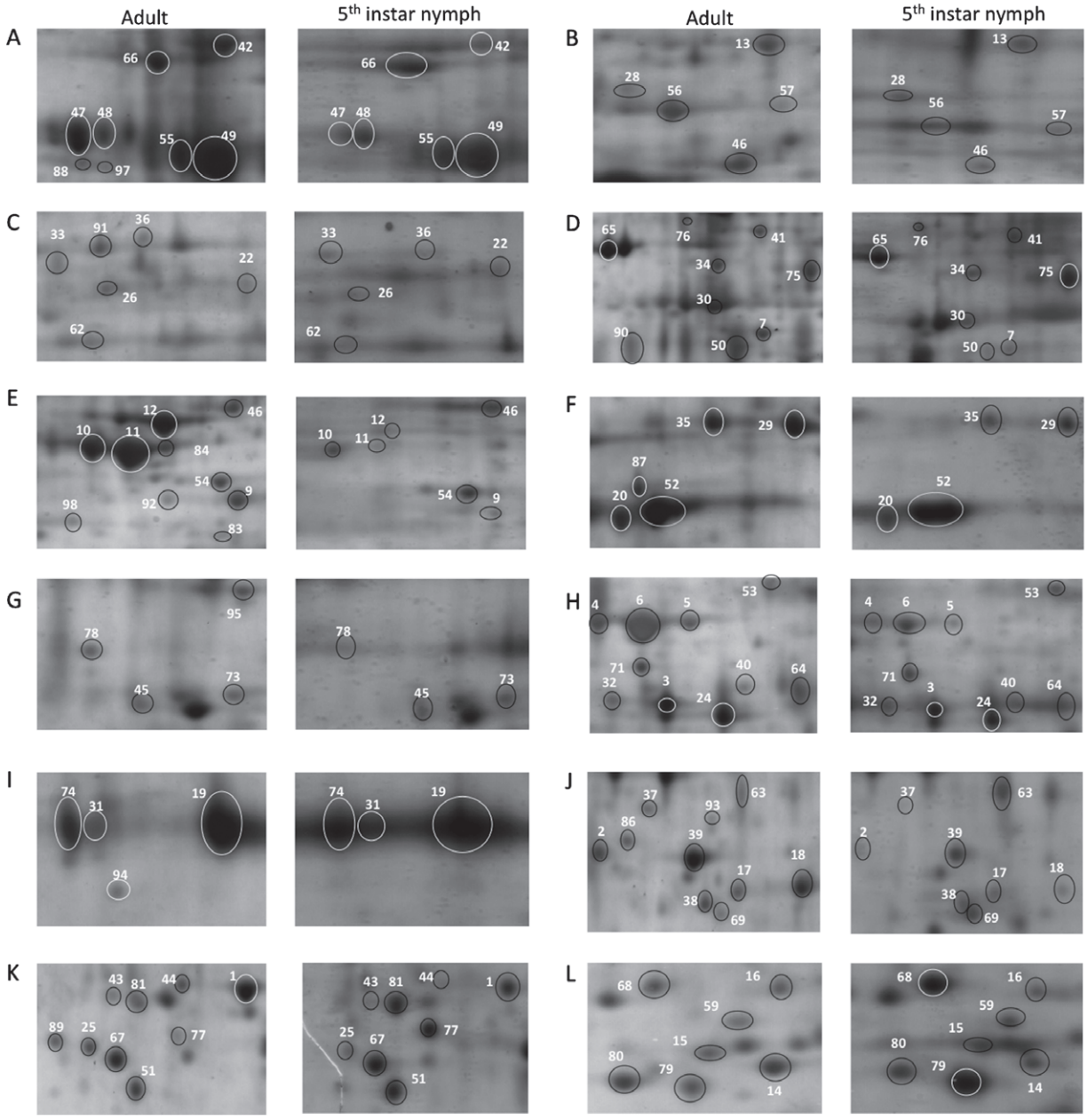

Fig. 2. Comparison between selected regions of two-dimensional gel electrophoresis gels of total proteins isolated from fifth-instar nymphs and adults of the Asian citrus psyllid *Diaphorina citri*. The original location of each selected area (A–L) is indicated on the total protein gel in Figure 1.

and 73% of them were up-regulated in adults but not nymphs (Fig. 3).

Gene ontology annotation

Gene Ontology (GO) annotation analysis of the 98 proteins (S = spot; hence S1 to S98) indicated that they are involved in several molecular functions and biological processes and belong to varied cellular components (Fig. 3). Most identified proteins were implicated in nucleotide binding, energy metabolism, glycolytic and protein processes, lipid and carbohydrate metabolic processes. Proteins such as triose phosphate isomerase (S1, S44: more than 1.7-fold), glyceraldehyde 3-phosphate dehydrogenase (S3, S19, S25, S91: up to 2.2-fold), arginine kinase (S5, S6, S7: up to 2.3-fold) and isocitrate dehydrogenase NAD-dependent (S7, S87: 7.5-fold) were increased from nymph to adult stage. Most of these proteins were mitochondrial proteins (Table 1). Phosphoglycerate kinase (S8, S9) was up-regulated by 16-fold in adults. ATP synthase subunits were up-regulated in adults in most spots (S10, S29, S30, S31, S35, S37, S45) and slightly down-regulated in adults in spot

Table 1. Proteins identified from adults and fifth-instar nymph of Asian citrus psyllid *Diaphorina citri* using a combination of two-dimensional chromatography and liquid chromatography-tandem mass spectrometry.

Spot #	Protein ID	Organism	NCBI Accession #	Exp. PI/MW	Theor. PI/MW	Protein Prob. (%)	Sequence coverage (%)	Exclusive unique peptide sequences	Intensity			Subcellular component	Functions
									Adult	Nymph	Fold change		
1	Triose phosphate isomerase, partial	*Lestes congener*	gi:46909449	6.1/25.7	5.6/22.9	100%	15%	TASPEQAQEVHAQLRK VVLAYEPVWAIGTGK	0.647	0.326	1.99	Cytoskeleton	Isomerase activity, gluconeogenesis, glycolytic process, metabolic process, catalytic activity
2	Spermine synthases	*Drosophila ananassae*	gi:194746211	6.2/29.2	5.3/32.2	100%	5%	KVLIVGGGDGGVAR VLIVGGGDGGVAR	0.106	0.060	1.76	Cytosol	Transferase activity, metabolic process, catalytic activity, bioenergetics
3	Glyceraldehyde 3-phosphate dehydrogenase	*Hypena proboscidalis*	gi:307643669	6.2/32.8	6.5/22.6	100%	28%	KVIISAPSADAPMFVCGVNLDAYDPSFK VIISAPSADAPMFVCGVNLDAYDPSFK VISNASCTTNCLAPLAK VPVANVSVVDLTVR	0.341	0.278	1.23	Cytoplasm, cytoskeleton, lipid particles	Dehydrogenase (NAD+) (phosphorylating) activity, oxidoreductase activity, metabolism and bioenergetic, muscle development
4	Arginine kinase, partial	*Diaphorina citri*	gi:296278819	5.9/37.3	5.5/27.9	100%	36%	EGDRFLQAANACR GQFYPLTGMTK LIDDHFLFKEGDR NWGDVDSFANLDPNGEFVISTR SLQGYPFNPCLTEAQYKEMEEK VSSTLSGLEGELKGQFYPLTGMTK	0.498	0.216	2.31	Extracellular, cytoplasm, plasma membrane	Kinase activity, transferase activity, catalytic activity, phosphorylation, ATP-binding, nucleotide binding
5	Arginine kinase, partial	*Diaphorina citri*	gi:296278819	6.2/37.3	5.5/27.9	100%	49%	EGDRFLQAANACR GIYHNDNK GQFYPLTGMTK KTDKHPPK LIDDHFLFKEGDR MVDQATLDKLEAGFAK NWGDVDSFANLDPNGEFVISTR SLQGYPFNPCLTEAQYKEMEEK VSSTLSGLEGELKGQFYPLTGMTK	0.359	0.295	1.22	Extracellular, cytoplasm, plasma membrane	Kinase activity, transferase activity, catalytic activity, phosphorylation, ATP-binding, nucleotide binding
6	Arginine kinase, partial	*Diaphorina citri*	gi:296278819	6.1/37.2	5.5/27.9	100%	40%	EGDRFLQAANACR GQFYPLTGMTK KTDKHPPK LIDDHFLFKEGDR NWGDVDSFANLDPNGEFVISTR SLQGYPFNPCLTEAQYKEMEEK TDKHPPKNWGDVDSFANLDPNGEFVISTR VSSTLSGLEGELKGQFYPLTGMTK	1.432	0.846	1.69	Extracellular, cytoplasm, plasma membrane	Kinase activity, transferase activity, catalytic activity, phosphorylation, ATP-binding, nucleotide binding
7	Isocitrate dehydrogenase NAD-dependent	*Drosophila ananassae*	gi|194762984	5.8/39.3	6.8/40.7	100%	20%	DLANPTALLLSAVMMLR EFNLYANVRPCR ENTEGEYSGIEHEIVDGVVQSIK GPLMTPVGK MSDGLFLR SLNLALRK	0.270	0.036	7.54	Mitochondria, cytoskeleton, lipid particles	Isocitrate dehydrogenase (NAD+) activity, oxidoreductase activity, NAD binding, magnesium ion binding, tricarboxylic acid cycle, cofactor
8	Phosphoglycerate kinase	*Aedes aegypti*	gi:15421103	6.9/44.3	6.9/43.9	100%	9%	FYVEEEGK LGDLYVNDAFGTAHR VADKIQLIENLLDK	1.574	0.096	16.39	Cytoplasm, cytoskeleton	ATP binding, kinase activity, transferase activity, glycolytic process, nervous system, muscle development
9	Unknown: phosphoglycerate kinase family	*Dendroctonus ponderosae*	gi|332376933	6.7/44	6.9/44.7	100%	9%	AHSSMLGEGFQQR ASGFLLKK GAITIIGGGDTATCAAK	0.303	0.165	1.84	Cytoplasm, cytoskeleton	ATP binding, kinase activity, transferase activity, glycolytic process, nervous system, muscle development

Table 1. Continued.

Spot #	Protein ID	Organism	NCBI Accession #	Exp. PI/MW	Theor. PI/MW	Protein Prob. (%)	Sequence coverage (%)	Exclusive unique peptide sequences	Intensity			Subcellular component	Functions
									Adult	Nymph	Fold change		
10	ATP synthase subunit β	*Camponotus floridanus*	gi|307181472	6.2/49.3	5.3/55.1	100%	27%	AHGGYSVFAGVGER AIAELGIYPAVDPLDSTSR FLSQPFQVAEVFTGHAGK FTQAGSEVSALLGR IINVIGEPIDER IMDPNIIGAEHYNIAR IPSAVGYQPTLATDMGTMQER LVLEVAQHLGENTVR TIAMDGTEGLVR	0.519	0.180	2.88	Mitochondria, membrane, catalytic domain, lipid particles	ATP synthesis from ADP, hydrogen ion transport, nucleotide-binding, metabolic process, ATP hydrolysis coupled proton transport, ATP synthesis coupled proton transport
11	Putative enolase protein, partial	*Cycloplasis panicifoliella*	gi|440210869	6.3/49.2	5.3/40.9	100%	20%	EIDEFMIKLDGTENK HLADLAGNSNIILPVPAFNVINGGSHAGNK KACNCLLLK LDGTENKSK QNGWGTMVSHR QSFEVTQQKEIDEFMIKLDGTENK	1.119	0.344	3.26	Cytoplasm, lipid particle, plasma membrane	Glycolytic process, phosphopyruvate hydratase complex, magnesium ion binding, phosphopyruvate hydratase activity, cofactor, bioenergetic
12	ATPase, V1 complex, subunit B	*Drosophila pseudoobscura pseudoobscura*	gi|125773061	6.5/51.8	5.3/54.5	100%	22%	AVVGEEALTPDDLLYLEFLTK AVVQVFEGTSGIDAK EEVPGRR HVLVILTDMSSYAEALR IPIFSAAGLPHNEIAAQICR TPVSEDMLGR TVSGVNGPLVILDEVKFPK	0.588	0.166	3.54	Mitochondria, membrane, lipid particles	Hydrogen ion transport, hydrolase activity, ATP metabolic process, proton-transporting V-type ATPase, V1 domain, ATP binding, acting on acid anhydrides, catalytic activities and metabolism
13	Serine carboxypeptidase	*Aedes aegypti*	gi|157113687	6.7/68.7	6.5/53.8	98%	2%	NESIALKR	0.166	0.118	1.40	Membrane, extracellular, cytoplasm	Hydrolase, protease, glycoprotein, zymogen, serine-type carboxypeptidase activity, structural, metabolic, stress defence and immunity
14	Glutathione *S*-transferase-like protein	*Diaphorina citri*	gi|110456486	6.7/23	5.7/21	100%	35%	AREALDFAEK GLILHEIIASPPVR GLILHEIIASPPVRAVK LCLTELGLEAEYK LHFDSGVLFSALR NEKEIPEEDKLR	0.371	0.099	3.74	Cytoplasm, cytosol	Catalytic, oxidoreductase, transferase activity, metabolic process, stress defence and immunity
15	Thioredoxin peroxidase	*Ostertagia ostertagi*	gi|18152531	6.6/23.5	6.1/21.4	100%	10%	GLFIIDPK QITVNDLPVGR	0.174	0.126	1.38	Cytoplasm, cytoskeleton, cytosol	Oxidoreductase, peroxidase, peroxiredoxin activity, antioxidant, metabolic, stress, defence and immunity
16	Heterogeneous nuclear ribonucleoprotein 87f (hrp36.1)	*Drosophila melanogaster*	gi|11036	6.7/25.8	9.1/39.7	100%	3%	KLFIGGLDYR	0.109	0.033	3.36	Cytoplasm, nucleus	Female gonad development, mitotic nuclear division, neurogenesis, regulation of alternative mRNA splicing, response to heat, response to starvation, mRNA binding, nucleotide binding, sequence-specific DNA binding
17	Phosphoglyceromutase	*Danaus plexippus*	gi|357628288	6.6/27.8	5.9/27.8	100%	23%	HGESEWNQK HYGGLTGLNK KILIAAHGNSLR RSFDIPPPAMEK TLPYWNNVIVPQIK	0.209	0.191	1.09	Cytoskeleton, Z disc, M band	Somatic muscle development, phosphoglyceromutase activity, glycolytic process, isomerase, glycolysis, metabolic process, intercellular transferase activity, phosphotransferase

Table 1. Continued.

Spot #	Protein ID	Organism	NCBI Accession #	Exp. PI/MW	Theor. PI/MW	Protein Prob. (%)	Sequence coverage (%)	Exclusive unique peptide sequences	Intensity			Subcellular component	Functions
									Adult	Nymph	Fold change		
18	Phosphoglyceromutase	*Drosophila melanogaster*	gi|111145313	6.9/28	0/28.9	100%	12%	HGESEWNQK HYGGLTGLNK ILIAAHGNSLR RILIAAHGNSLR	0.663	0.039	17.21	Cytoskeleton, Z disc, M band	Somatic muscle development, phosphoglyceromutase activity, glycolytic process, isomerase, glycolysis, metabolic process, intercellular transferase activity, phosphotransferase
19	Glyceraldhyde 3-phosphate dehydrogenase	*Hypena proboscidalis*	gi|307643669	7.7/32.7	6.6/22.6	100%	41%	KVIISAPSADAPMFVCGVNLDAYDPSFK LISWYDNEYGYSNR VIPALNGKLTGMAFR VISNASCTTNCLAPLAK VPVANVSVVDLTVR	4.709	1.661	2.83	Cytoplasm, cytoskeleton, lipid particles	Glyceraldehyde 3-phosphate dehydrogenase (NAD+) (phosphorylating) activity, oxidoreductase activity, nucleotide binding, metal ion binding, metabolic, muscle development
20	Putative acid phosphatase 1, partial	*Diaphorina citri*	gi|110456445	7.2/38.8	6.7/41	100%	17%	AQFAQGEFLR IIEDTNDKLSGR RPYDSFLGDR TPADTYPNDPYAK YQEELDNVFNSPEVR	0.582	0.366	1.59	Membrane	Acid phosphatase activity, dephosphorylation, catalytic activity, stress, defence and immunity
21	Fructose-bisphosphate aldolase-like isoform 1	*Acyrthosiphon pisum*	gi|193591901	6.8/39.5	6.9/39.9	100%	19%	ALNDHHVYLEGTILKPNMVTPGQSASK DGCHFAK GLLAADESVSTMGKR VTETVLAAVYK YASICQANR	0.252	0.067	3.75	Cytoskeleton	Glycolytic process, fructose-bisphosphate aldolase activity, lyase, metabolism, glycolytic
22	AICARFT transformylase/IMP cyclohydrolase PURH	*Drosophila ananassae*	gi|194745622	7.6/59.5	7.8/63.4	99%	2%	NGQVIGIGAGQQSR	0.115	0.069	1.67	Mitochondria, extracellular, cytosol	Purine nucleotide biosynthetic process, IMP cyclohydrolase activity, phosphoribosyl-aminoimidazolecarboxamide formyltransferase activity
23	Cyclophilin-type peptidylprolyl cis-trans isomerases	*Tribolium castaneum*	gi|91083463	7.0/19	8.1/23.1	100%	6%	DTNGSQFFITTKK TVQNFIELAK	0.092	0.064	1.43	Cytoplasm, nucleus	Protein transport, apoptosis, chaperone-mediated protein folding, protein peptidyl-prolyl isomerization, rotamase, acetylation, phosphoprotein, protein folding, Hsp70 protein binding, Hsp90 protein binding, heat shock protein binding, stress and defence
24	Predicted: hydroxysteroid dehydrogenase-like protein 2-like	*Apis florea*	gi|380029221	6.4/32	0/45.6	99%	2%	NHVAYTISK	0.626	0.556	1.12	Mitochondria	Oxidoreductase, sterol binding, metabolic process
25	Glyceraldhyde 3-phosphate dehydrogenase	*Hypena proboscidalis*	gi|307643669	5.5/23	6.5/22.6	100%	41%	LISWYDNEYGYSNR VIISAPSADAPMFVCGVNLDAYDPSFK VIPALNGKLTGMAFR VISNASCTTNCLAPLAK VPVANVSVVDLTVR	0.230	0.104	2.21	Cytoplasm, cytoskeleton, lipid particles	Glyceraldehyde 3-phosphate dehydrogenase (NAD+) (phosphorylating) activity, oxidoreductase activity, nucleotide binding, metal ion binding, metabolic, muscle development
26	Enolase-phosphatase E1 (CG12173)	*Drosophila melanogaster*	gi|24644163	7.3/57.8	5.7/30.7	84%	2%	LTPTKSASSGGGGGGGGGNSNSGGASK	0.094	0.088	1.07	Cytoplasm	Amino-acid biosynthesis, methionine biosynthesis, magnesium, metal-binding, hydrolase

Table 1. Continued.

Spot #	Protein ID	Organism	NCBI Accession #	Exp. PI/MW	Theor. PI/MW	Protein Prob. (%)	Sequence coverage (%)	Exclusive unique peptide sequences	Intensity			Subcellular component	Functions
									Adult	Nymph	Fold change		
27	NADH-ubiquinone oxidoreductase 75 kDa subunit G, putative	*Pediculus humanus corporis*	gi|242007132	5.6/79	6.5/80.7	100%	8%	AQQTLTAISPPGLAR FTDINFSGKR GSDMQVGTYVEK MCLVEVEK SPKPVAACAMPVMK	0.073	0.073	1.00	Mitochondria, membrane	NADH dehydrogenase (ubiquinone) activity, electron carrier activity, iron-sulfur cluster binding, ATP synthesis, oxidoreductase, metabolism
28	Glutamate semialdehyde dehydrogenase	*Culex quinquefasciatus*	gi|170054902	6.5/61.8	6.9/85.7	100%	5%	EALGTVGAQNAISLVSTR GPVGVEGLLTTK SKVGTGGMDSK	0.061	0.047	1.31	Mitochondria, membrane, cytoplasm	Proline biosynthetic process, glutamate 5-kinase activity, glutamate-5-semialdehyde dehydrogenase activity, protein and nucleotide binding, metabolic process
29	Predicted: ATP synthase subunit α, mitochondrial-like isoform 1	*Megachile rotundata*	gi|383860333	7.8/45.8	9.1/59.5	100%	41%	AMKQVAGSMKLELAQYR AVDSLVPIGR DNGKHALIIYDDLSK EAYPGDVFYLHSR ELIIGDRQTGK EVAAFAQFGSDLDAATQQLLNR GIRPAINVGLSVSR GMALNLEPDNVGVVVFGNDR HALIIYDDLSKQAVAYR ILGSAPKTNLEETGR LYCIYVAIGQK NIQADEMVEFSSGLK QGQYVPMAIEEQVAVIYCGVR QVAGSMKLELAQYR RSTVAQIVKR TALAIDTIINQKR TNLEETGR VLSIGDGIAR	0.667	0.224	2.98	Mitochondria, membrane, extracellular , catalytic domains and cores	ATP biosynthetic process, ATP catabolic process, ATP hydrolysis coupled proton transport, cellular metabolic process, lipid metabolic process, small molecule metabolic process, metal ion binding
30	Putative mitochondrial ATP synthase α subunit precursor	*Toxoptera citricida*	gi|52630965	5.6/42	9.1/59.8	100%	10%	EAYPGDVFYLHSR HALIIYDDLSK TGAIVDVPVGEDLLGR VVDALGNTIDGK	0.748	0.311	2.41	Mitochondria, membrane, extracellular , catalytic domains and cores	ATP biosynthetic process, ATP catabolic process, ATP hydrolysis coupled proton transport, cellular metabolic process, lipid metabolic process, small molecule metabolic process, metal ion binding
31	Putative ATP synthase subunit d	*Diaphorina citri*	gi|110671524		4.8/21.3	100%	48%	INEEEKQTMAEIKK IPVPGLVDQFQK KVSALPEAPPK KWIEESQVR LTDADRPNFNTFK	0.330	0.127	2.60	Mitochondria, membrane, extracellular , catalytic domains and cores	ATP biosynthetic process, ATP catabolic process, ATP hydrolysis coupled proton transport, cellular metabolic process, lipid metabolic process, small molecule metabolic process, metal ion binding
32	Predicted: myosin heavy chain, muscle isoform 1	*Acyrthosiphon pisum*	gi|328702403		8.8/224	100%	4%	DKELASLTAK IEELEEEVEAER LADELRAEQDHAQTQEK LDEAENNALKGGK LDEAENNALKGGKK MQDLVDKLQQK QIEEAEEIAALNLAK	0.209	0.125	1.68	Cytoplasm, cytoskeleton	Actin-binding, ATP-binding, calmodulin-binding, motor protein, muscle protein, adult somatic muscle development, epithelial cell migration, open tracheal system, flight, locomotion, structural constituent of muscle

Table 1. Continued.

Spot #	Protein ID	Organism	NCBI Accession #	Exp. PI/MW	Theor. PI/MW	Protein Prob. (%)	Sequence coverage (%)	Exclusive unique peptide sequences	Intensity			Subcellular component	Functions
									Adult	Nymph	Fold change		
33	Glycerol 3-phosphate dehydrogenase (GF13366)	*Drosophila ananassae*	gi|194756372	6.8/36	7.7/80.6	100%	18%	EIDTNMNGQVELDEYLQMMSAIK GDVLSAWSGIRPLVSDPNK GYVSINDIR GYVSINDIRR IHPEFPYIDAEIR KGYVSINDIR KGYVSINDIRR LCGAIVYYDGQQDDAR NHIVHVSPSNLVTIAGGK RGDVLSAWSGIRPLVSDPNK SSYYLSKK TALVELDDFASGTSSR TALVELDDFASGTSSRSTK	0.125	0.031	4.02	Cytoplasm, cytoskeleton, lipid particles	Glyceraldehyde 3-phosphate dehydrogenase (NAD+) (phosphorylating) activity, oxidoreductase activity, nucleotide binding, metal ion binding, metabolic, muscle development
34	Tubulin β-1 chain	*Acyrthosiphon pisum*	gi|298676439	6.1/33.7	4.6/50	100%	15%	AILVDLEPGTMDSVR FPGQLNADLR ISEQFTAMFR KLAVNMVPFPR LAVNMVPFPR LHFFMPGFAPLTSR YLTVAAVFR	0.276	0.211	1.31	Cytoplasm, cytoskeleton	Microtubule-based process, protein polymerization, GTP binding, GTPase activity, structural constituent of cytoskeleton, metabolic processes, muscles development
35	ATP synthase subunit α (GF13537)	*Drosophila ananassae*	gi|194757070		9/59.4	100%	31%	AMKQVAGSMK AMKQVAGSMKLELAQYR AVDSLVPIGR AVDSLVPIGRGQR EAYPGDVFYLHSR ELIIGDR ELIIGDRQTGK EVAAFAQFGSDLDAATQQLLNR FRVGIKAPGIIPR GIRPAINVGLSVSR HALIIYDDLSK LELAQYR LIKQGDIVK LIKQGDIVKR QTGKTALAIDTIINQK QVAGSMKLELAQYR RSTVAQIVKR RTGAIVDVPVGDELLGR STVAQIVK STVAQIVKR TALAIDTIINQK TALAIDTIINQKR TGAIVDVPVGDELLGR VGIKAPGIIPR VLSIGDGIAR	0.474	0.218	2.17	Mitochondria, membrane, extracellular , catalytic domains and cores	ATP hydrolysis coupled proton transport, ATP synthesis coupled proton transport, ATP binding, proton-transporting ATP synthase activity, rotational mechanism proton-transporting ATPase activity, rotational mechanism, protein binding, metabolic processes
36	Glycerol 3-phosphate dehydrogenase, mitochondrial	*Camponotus floridanus*	gi|307190125	5.6/46.8	5.9/65.9	100%	19%	DKIPINLTK EASEFLAQEMGQMVNR EIDTNMNGQVELDEYLQMMSAIK KGYVSINDIRR KIHPEFPYIDAEIR NYLNPDVEVRR RWPIIGKK SGHVAYSR TVKSSYYLSK	0.100	0.083	1.21	Cytoplasm, cytoskeleton, lipid particles	Glyceraldehyde 3-phosphate dehydrogenase (NAD+) (phosphorylating) activity, oxidoreductase activity, nucleotide binding, calcium ion binding, metabolic, muscle development

Table 1. Continued.

Spot #	Protein ID	Organism	NCBI Accession #	Exp. PI/MW	Theor. PI/MW	Protein Prob. (%)	Sequence coverage (%)	Exclusive unique peptide sequences	Intensity			Subcellular component	Functions
									Adult	Nymph	Fold change		
37	H^+ transporting ATP synthase β subunit	*Heliconius numata aurora*	gi|345532344	7.4/64.5	4.8/36.7	100%	12%	AHGGYSVFAGVGER LVLEVAQHLGENTVR TIAMDGTQGLVR	0.116	0.097	1.19	Mitochondria, membrane, catalytic domains and cores, lipid particles	ATP hydrolysis coupled proton transport, ATP synthesis coupled proton transport, ATP binding, proton-transporting ATP synthase activity, rotational mechanism proton-transporting ATPase activity, rotational mechanism, protein binding, metabolic processes
38	Phosphoglycerate mutase	*Acyrthosiphon pisum*	gi|187111150	6.9/26	6.1/28.9	95%	8%	FLGDEETVKK HGESEWNQK	0.122	0.029	4.27	Cytoskeleton, Z disc, M band	Somatic muscle development, phosphoglyceromutase activity, glycolytic process, isomerase, glycolysis, metabolic process, intercellular transferase activity, phosphotransferase
39	Electron transfer flavoprotein subunit α	*Acyrthosiphon pisum*	gi|193595390	6.3/30.7	8.1/35.2	100%	11%	AAVDAGFVPNDMQIGQTGK SPDTFVR TIYAGNAILTLK	0.429	0.166	2.58	Mitochondria, cytoskeleton, lipid particles	DNA repair, protein-chromophore linkage, deoxyribodipyrimidine photo-lyase activity, DNA and nucleotide binding
40	Glycerol 3-phosphate dehydrogenase, NAD-dependent	*Dendroctonus ponderosae*	gi|332376853	5.8/31	6.3/39.7	100%	18%	AEGGGIDLISHIITR LPPNVVAVPDVVEAAK LTEIINETHENVK NIVACGAGFVDGLGLGDNTK	0.051	0.042	1.21	Cytoplasm, cytoskeleton, lipid particles	Oxidoreductase activity, NAD binding, glycerol 3-phosphate dehydrogenase [NAD+] activity, protein homodimerization activity, carbohydrate metabolic process, coenzyme binding, muscles development
41	Predicted: V-type proton ATPase subunit b-like	*Acyrthosiphon pisum*	gi|328716950	5.8/35.5	5.5/55.3	100%	32%	AVVGEEALTPDDLLYLEFLTK AVVQVFEGTSGIDAK EVSAAREEVPGR IFPKEMLKR IPIFSAAGLPHNEIAAQICR KDHSDVSNQLYACYAIGK QIYPPINVLPSLSR TPVSEDMLGR VFNGSGKPIDK VFNGSGKPIDKGPPILAEDYLDIEG QPINPYSR YAEIVQLR	0.150	0.073	2.06	Mitochondria, membrane, catalytic domain, lipid particles	ATP synthesis, Ion transport, plasma membrane ATP synthesis coupled proton transport, rotational mechanism, proton-transporting ATP synthase complex, coupling factor F(o), catalytic activity and metabolism
42	Putative heat shock cognate 70 protein, partial	*Diaphorina citri*	gi|110456396	5.8/50.8	4.9/208	100%	27%	DNNLLGKFELTSIPPAPR ELEAICNPIITK LSKEDIER TQILDKCNDVIK	0.167	0.061	2.76	Cytoplasm, nucleus	Protein and nucleotide binding, response to stress
43	Peroxiredoxin	*Drosophila mojavensis*	gi|195120862	5.5/52.6	5.6/24.7	100%	6%	LGATIPNFK LGATIPNFKAETTK	0.073	0.064	1.13	Cytoplasm, cytoskeleton, cytosol	Antioxidant activity, peroxiredoxin activity, protein binding, metabolism, stress
44	Triose phosphate isomerase	*Lestes congener*	gi|46909449	5.8/88	5.6/22.9	100%	15%	TASPEQAQEVHAQLR TASPEQAQEVHAQLRK VVLAYEPVWAIGTGK	0.090	0.051	1.76		Triose-phosphate isomerase activity, gluconeogenesis, glycolytic process, metabolic process, catalytic activity, isomerase activity

Table 1. Continued.

Spot #	Protein ID	Organism	NCBI Accession #	Exp. PI/MW	Theor. PI/MW	Protein Prob. (%)	Sequence coverage (%)	Exclusive unique peptide sequences	Intensity			Subcellular component	Functions
									Adult	Nymph	Fold change		
45	H^+ transporting ATP synthase β subunit	*Heliconius numata aurora*	gi|345532344	5.5/24	4.9/36.7	100%	72%	AHGGYSVFAGVGER AIAELGIYPAVDPLDSTSR DQEGQDVLLFIDNIFR EGNDLYHEMIESGVISLKDK FTQAGSEVSALLGR GSITSVQAIYVPADDLTDPAPATT FAHLDATTVLSR IGLFGGAGVGK IINVIGEPIDER IMDPNIIGAEHYN IPSAVGYQPTLATDMGTMQER LVLEVAQHLGENTVR TIAMDGTQGLVR TREGNDLYHEMIESGVISLKDK TVLIMELINNVAK VALTGLTVAEYFR VALVYGQMNEPPGAR	0.115	0.109	1.06	Cytoskeleton	ATP biosynthetic process, ion transport, ATP synthesis coupled proton transport, proton transport, ATP metabolic process, rotational mechanism, glycolytic process, nervous system
46	Leucine aminopeptidase/peptidase B	*Danaus plexippus*	gi|357608936	5.5/25.2	7.5/55.4	100%	4%	GVTYDTGGADIK VTNTDAEGR	0.195	0.139	1.40	Mytochondria, membrane, lipid particles	Aminopeptidase activity, proteolysis protease, hydrolase, nucleotide and manganese ion binding, metalloexopeptidase activity
47	Heat shock protein 60	*Culicoides variipennis*	gi|2738077	5.5/30.5	6.5/61.9	100%	28%	AAVEEGIVPGGGTALLR AIAKEGFEK APGFGDNR DGKTLTDELQVIEGMKFDR DKFQNIGAK DRVTDALNATR EGVITVKDGK GANPIEIRR GIELKDKFQNIGAK GIIDPTKVVR GRNVILEQSWGSPK IGLQVAAVKAPGFGDNRK ISKGANPIEIR LKIGLQVAAVK LVQDVANNTNEEAGDGTTTATVLAR NVILEQSWGSPK TLTDELQVIEGMKFDR VGKEGVITVKDGK	0.242	0.045	5.41	Mytochondria, cytoplasm, cytoskeleton, lipid particles	Protein refolding, response to stress, ATP binding
48	Chaperonin Cpn60	*Drosophila simulans*	gi|195566149	6.7/52.2	5.2/60.8	100%	23%	AAVEEGIVPGGGTALLR AIAKEGFEK APGFGDNR DKFQNIGAK GIIDPTKVVR GRNVIIEQSWGSPK IGLQVAAVK LVQDVANNTNEEAGDGTTTATVLAR TLTDELEVIEGMKFDR VGGSSEVEVNEKKDR	0.173	0.098	1.76	Mytochondria, cytoplasm, cytoskeleton, lipid particles	Protein refolding, ATP binding, metabolic process, response to stress

Table 1. Continued.

Spot #	Protein ID	Organism	NCBI Accession #	Exp. PI/MW	Theor. PI/MW	Protein Prob. (%)	Sequence coverage (%)	Exclusive unique peptide sequences	Intensity			Subcellular component	Functions
									Adult	Nymph	Fold change		
49	Leukotriene a4 hydrolase	*Drosophila grimshawi*	gi|195035229	6.4/22.5	6.1/68.7	80%	6%	VATVSLPR DADKKDK	0.999	0.659	1.52	Cytoplasm	Leukotriene biosynthetic process, zinc ion binding, metallopeptidase activity, leukotriene-A4 hydrolase activity, muscle development
50	Actin 87e, isoform a	*Solenopsis invicta*	gi|17137090	5.6/25	5.3/41.8	100%	63%	AGFAGDDAPR AVFPSIVGRPR DLTDYLMK EIVRDIKEK GYSFTTTAEREIVR HQGVMVGMGQKDSYVGDEAQSKR IKIIAPPERK IWHHTFYNELR LCYVALDFEQEMATAAASTSLEK LDLAGRDLTDYLMK MQKEITALAPSTIK QEYDESGPGIVHRK RGILTLK SYELPDGQVITIGNER TTGIVLDSGDGVSHTVPIYEGYALPHAILR VAPEEHPVLLTEAPLNPK YPIEHGIITNWDDMEK	0.852	0.224	3.80	Cytoskeleton, cytoplasm, lipid particles	ATP binding, structural constitution of the skeleton, movement
51	Glyoxalase i	*Trichoplax adhaerens*	gi:196006682	5.6/21.7	5.4/19.8	95%	6%	FEQLGVEFVK	0.296	0.501	0.59	Extracellular, cytoplasm	Glyoxalase I catalyzes the isomerization of the hemithioacetal, formed by a 2-oxoaldehyde and glutathione, to S-D-lactoylglutathione
52	Fructose-bisphosphate aldolase class-I	*Drosophila mojavensis*	gi|195111400	7.3/39.2	6.9/39.9	100%	14%	ADDGTPFVQLLK ALQASVLR AQKVTETVLAAVYK GILAADESVSTMGK GILAADESVSTMGKR VTETVLAAVYK	0.875	1.032	0.85	Cytoskeleton	Glycolysis, lyase, glycolytic process fructose-bisphosphate aldolase activity
53	Glutamine synthetase 2, [GMP synthase (glutamine-hydrolyzing)]	*Acyrthosiphon pisum*	gi:237874151	6.6/39.7	6.3/41.5	100%	10%	DIVEAHYR ENNGIIEIEK HETSSIHDFSAGVANR	0.374	0.688	0.54	Cytoplasm	Catalyzes the synthesis of GMP from XMP, GMP biosynthetic process, glutamine metabolic process, ATP binding, ligase activity, pyrophosphatase activity
54	Hydroxyacid-oxoacid transhydrogenase(hot), mitochondrial-like	*Bombus terrestris*	gi:340719960	6.6/45.8	7.5/51.5	100%	11%	AVYNQDDIEAR GKPITVPLKPLIAVPTTSGTGS ETTGVSIFDYQPLKAK RAVYNQDDIEAR	0.1922	0.209	0.92	Mitochondria	Hydroxyacid-oxoacid transhydrogenase activity, metal ion binding, metabolism, oxidoreductase activity, proton transport
55	Unknown, partial	*Diaphorina citri*	gi|110456536	5.5/56.3	9.6/13.4	100%	9%	ETANAIVYLR	0.426	0.587	0.73		
56	CBN-HSP-60 protein	*Caenorhabditis brenneri*	gi|341901164	6.6/58.5	5.3/60	97%	3%	AAVEEGIVPGGGVALLR	0.296	0.327	0.91	Mitochondrion, cytoplasm, cytoskeleton, lipid particles	protein refolding, ATP binding, chaperone, response to stress, nucleotide-binding
57	Neural conserved at 73ef, isoform i	*Drosophila melanogaster*	gi|161084461	6.8/58.7	6.5/122	100%	3%	ATGFEAFLAK LSGQDVER SSPYCTDVAR	0.048	0.335	0.14	Cytoskeleton	Oxoglutarate dehydrogenase (succinyl-transferring) activity, thiamine pyrophosphate binding , tricarboxylic acid cycle, oxidoreductase

Table 1. Continued.

Spot #	Protein ID	Organism	NCBI Accession #	Exp. PI/MW	Theor. PI/MW	Protein Prob. (%)	Sequence coverage (%)	Exclusive unique peptide sequences	Intensity			Subcellular component	Functions
									Adult	Nymph	Fold change		
58	α-Actinin, sarcomeric-like isoform 3	*Acyrthosiphon pisum*	gi|328703079	7.5/94	5.6/106	100%	14%	AKLETNFNTLQTK ASFNHFDKNR DNPLQNLNTAFDVAEK ESTDTDTAEQVIDSFR FAIQDISVEEMTAK GITQEQLNEFR GKEEMLQSSDFR LETNFNTLQTK LVSIGAEEIVDGNLK MVSDIANAWK VGWEQLLTSINR	0.109	0.451	0.24	Cytoplasm, cytoskeleton, Z disc, cytosol	Actin binding, metal binding, actin cytoskeleton reorganization, movement , double-stranded RNA binding
59	Glutathione *S*-transferase s2	*Laodelphax striatella*	gi|373940157	6.7/24.7	7.3/450	96%	5%	NNGGYLANGK	0.114	0.115	0.99	Cytoplasm, cytosol	Belongs to the GST superfamily, transferase activity, metabolic process, oxidation reduction process
60	Glycerol 3-phosphate dehydrogenase, NAD-dependent	*Dendroctonus ponderosae*	gi|332376853	6.9/32.7	6.3/39.7	100%	20%	AEGGGIDLISHIITR KLTEIINETHENVK LGLMEMVK LPPNVVAVPDVVEAAK LTEIINETHENVK NIVACGAGFVDGLGLGDNTK	0.178	0.289	0.61	Cytoplasm, cytoskeleton, lipid particles	Oxidoreductase activity, NAD binding, glycerol 3-phosphate dehydrogenase [NAD+] activity, protein homodimerization activity, carbohydrate metabolic process, coenzyme binding, oxidoreductase activity, muscle development
61	Predicted: probable medium-chain specific acyl-CoA dehydrogenase, mitochondrial-like	*Megachile rotundata*	gi|383853383	7.1/39	7.3/39	100%	6%	EEIIPVAAEHDR IYQIYEGTAQIQR	0.3985	0.4396	0.91	Mitochondria, extracellular, lipid particle	Fatty acid metabolism, lipid metabolism, oxidoreductase, FAD binding, fatty acid β-oxidation, acyl-CoA dehydrogenase activity, flavin adenine dinucleotide binding
62	α-Tubulin *N*-acetyltransferase	*Aedes aegypti*	gi|157113931	7.2/52.8	5/49.9	100%	42%	AVCMLSNTTAIAEAWAR AVFVDLEPTVVDEVR AYHEQLSVAEITNACFEPANQMVK DVNAAIATIK EIVDVVLDR FDGALNVDLTEFQTNLVPYPR IHFPLVTYAPVISAEK NLDIERPTYTNLNR QLFHPEQLITGK TIGGGDDSFNTFFSETGAGK TIQFVDWCPTGFK VGINYQPPTVVPGGDLAK	0.074	0.123	0.60	Cytoskeleton	Acyltransferase, α-tubulin acetylation, neuron development, regulation of microtubule cytoskeleton organization, tubulin N-acetyltransferase activity, transferase activity, transferring acyl groups, muscle and nervous system development
63	Haemolymph juvenile hormone binding protein	*Drosophila melanogaster*	gi|442634170	6.7/31	8.5/31.1	80%	4%	LNDGIPGLTSIR	0.111	0.389	0.29	Extracellular	Ecdysteroid hormone receptor activity, protein binding, development
64	26s proteasome non-ATPase regulatory subunit 7	*Crassostrea gigas*	gi|405962439	6.7/32.5	6.7/12.7	100%	11%	DIKDTTVGSLSQR TFDHVPSEIGAEEAEEVGVEHLLR	0.423	0.470	0.90	Cytoplasm, nucleus	Catalytic activity, cofactor, metabolic process

Table 1. Continued.

Spot #	Protein ID	Organism	NCBI Accession #	Exp. PI/MW	Theor. PI/MW	Protein Prob. (%)	Sequence coverage (%)	Exclusive unique peptide sequences	Intensity			Subcellular component	Functions
									Adult	Nymph	Fold change		
65	ATP synthase subunit β	*Acyrthosiphon pisum*	gi|209915626	5.3/48.3	5/55.8	100%	43%	AHGGYSVFAGVGER AIAELGIYPAVDPLDSTSR DQEGQDVLLFIDNIFR EGNDLYHEMIESGVISLKDK FTQAGSEVSALLGR IGLFGGAGVGK IINVIGEPIDERGPIDTDK IPSAVGYQPTLATDMGTMQER IPVGAETLGR LVLEVAQHLGENTVR LVPLEETIK SLQDIIAILGMDELSEEDKLTVAR TREGNDLYHEMIESGVISLKDK TVLIMELINNVAK VALVYGQMNEPPGAR	0.6165	0.7311	0.84	Mitochondria, membrane, catalytic domain, lipid particles	ATP synthesis, ion transport, plasma membrane ATP synthesis coupled proton transport, rotational mechanism, proton-transporting ATP synthase complex, coupling factor F(o), catalytic activity and metabolism
66	Similar to V-ATPase subunit a isoform 2	*Tribolium castaneum*	gi|91081489	5.5/68	5.1/31.2	100%	14%	DMGYNVSMMADSTSR DSMSNILYQLSSMK FGYVFAVSGPVVTAEK FKDPVKDGEAK HAVESTAQSENK LAEMPADSGYPAYLGAR	0.1207	0.1748	0.69	Mitochondria, membrane, catalytic domain, lipid particles	Hydrogen ion transmembrane transporter activity, hydrogen-translocating pyrophosphatase activity, ATP hydrolysis coupled, ATP synthesis coupled proton transport cellular response to nutrient levels, vacuolar proton-transporting V-type ATPase complex assembly
67	Glutathione *S*-transferase-like protein	*Diaphorina citri*	gi|110456486	5.6/22.7	5.7/21.1	100%	7%	LCLTELGLEAEYK	0.403	0.525	0.77	Cytoplasm, cytosol	Belongs to the GST superfamily, transferase activity, metabolic process, oxidation reduction process
68	Proteasome subunit α type 6-like protein	*Xenopsylla cheopis*	gi|125809137	6.5/25.7	7.6/27.1	100%	8%	HITIFSPEGR LYQVEYAFK	0.169	0.503	0.34	Cytoplasm, nucleus	Proteasome core complex, α-subunit complex, ubiquitin-dependent protein catabolic process, threonine-type endopeptidase activity
69	Putative ribosomal protein s3	*Diaphorina citri*	gi|110456370	6.6/27	9.4/24.1	100%	6%	ELAEDGYSGVEIR	0.044	0.052	0.84	Membrane	Translation, small ribosomal subunit RNA binding, structural constituent of ribosome
70	Putative acid phosphatase 1	*Diaphorina citri*	gi|110456445	7.4/32.8	6.6/41	100%	68%	AILEANKNLLDYASK AQFAQGEFLR ELGLTLPAWTNAIFPDPLSK ESGMPIVTPDDAQSLYSTLK ESGMPIVTPDDAQSLYSTLKAER HSFEPFGWGQLTNVGK ICPWENFVSLTSSK ITAQSFVINAMTPVLQR KIIEDTNDKLSGR LIHVIFR LKGGFLLKK MSTMLFLAGLFPPK NTTSEPYLLQIPGCSK RAQFAQGEFLR RPYDSFLGDRYSPDYLK SYDEECQALNPNFVYR TPADTYPNDPYAK YQEELDNVFNSPEVR YSPDYLKVQCTDVDRTK	0.051	0.098	0.52	Membrane	Acid phosphatase activity, dephosphorylation, response to stress

Table 1. Continued.

Spot #	Protein ID	Organism	NCBI Accession #	Exp. PI/MW	Theor. PI/MW	Protein Prob. (%)	Sequence coverage (%)	Exclusive unique peptide sequences	Intensity			Subcellular component	Functions
									Adult	Nymph	Fold change		
71	Predicted: annexin-b9-like	*Acyrthosiphon pisum*	gi|193650295	7.2/63	6.1/36	100%	12%	DIKGDTSGHFK DIKGDTSGHFKR GDTSGHFKR SEIDLGDIKQVFEK SMAGLGTDDKTLIR	0.235	0.347	0.67	Membrane, plasma membrane	Calcium/phospholipid-binding, basolateral protein localization, endosome transport via multivesicular body sorting pathway, maintenance of cell polarity, regulation of multivesicular body size involved in endosome transport, actin and spectrin binding, maintenance of cell polarity, wing disc dorsal/ventral pattern formation and development
72	Hypothetical protein, partial (insect cuticle protein family)	*Hodotermopsis sjoestedti*	gi|58430712	7.5/45.7	8.8/15.2	99%	12%	TVEYTADPHNGFNAVVHK	0.032	0.034	0.95	Extracellular, cytoskeleton	Chitin-based cuticle development
73	Succinyl-CoA synthetase α subunit	*Solenopsis invicta*	gi|322787000	6.5/29.2	8.6/33.9	100%	22%	GGAQDKINALEK IGIMPGHIHQR LIGPNCPGIIAPEQCK RMGHAGAIISGGK SPAQMGNELLK VICQGFTGK	0.088	0.169	0.52	Mitochondria	Catalyzes the ATP- or GTP-dependent ligation of succinate and CoA to form succinyl-CoA. The nature of the β subunit determines the nucleotide specificity, GTP-binding, neurogenesis, cofactor binding
74	Glyceraldhyde 3-phosphate dehydrogenase	*Hypena proboscidalis*	gi|307643669	7.4/31	6.6/22.7	100%	41%	LISWYDNEYGYSNR VIISAPSADAPMFVCGVNLDAYDPSFK VIPALNGKLTGMAFR VISNASCTTNCLAPLAK VPVANVSVVDLTVR	0.865	0.954	0.91	Cytoplasm, cytoskeleton, lipid particles	Glyceraldehyde 3-phosphate dehydrogenase (NAD+) (phosphorylating) activity, oxidoreductase activity, binding, metabolic, muscle development
75	Yellow-c-like protein, partial	*Diaphorina citri*	gi|110456529	7.3/32.7	4.7/12.1	100%	71%	DAIKGTVCDV DGVGCWNSYK GLHTDEINYR HANEYSADTTDLVATDSETLVFP NDLKVDK IFQTPVKDAIK VDKEGYLWVLSDK	0.346	0.369	0.94	Extracellular	Melanin biosynthetic process
76	Predicted: V-type proton ATPase subunit b-like	*Acyrthosiphon pisum*	gi|328716950	6.0/46.3	5.1/55.3	100%	32%	AVVGEEALTPDDLLYLEFLTK AVVQVFEGTSGIDAK DHSDVSNQLYACYAIGK GQKIPIFSAAGLPHNEIAAQICR HVLVILTDMSSYAEALR IPIFSAAGLPHNEIAAQICR KDHSDVSNQLYACYAIGK QIYPPINVLPSLSR TPVSEDMLGR VFNGSGKPIDKGPPILAEDYLDIEGQ PINPYSR YAEIVQLR	0.042	0.077	0.54	Mitochondria, membrane, catalytic domain, lipid particles	ATP synthesis, ion transport, plasma membrane ATP synthesis coupled proton transport, rotational mechanism, proton-transporting ATP synthase complex, coupling factor F(o), catalytic activity and metabolism
77	Protein E	*Pediculus humanus corporis*	gi|242017402	5.8/24	5.9/24.5	100%	39%	CSDGVQHFK EGLIPSNYIEMK HDFNATAEDELSFRK HEGAFLIR KGLFPATYVTPYHS RGDVITVTDR VSESSPGDFSLSVK	0.095	0.337	0.28		Spliceosomal complex assembly, protein binding

Table 1. Continued.

Spot #	Protein ID	Organism	NCBI Accession #	Exp. PI/MW	Theor. PI/MW	Protein Prob. (%)	Sequence coverage (%)	Exclusive unique peptide sequences	Intensity			Subcellular component	Functions
									Adult	Nymph	Fold change		
78	Lactoylglutathione lyase	*Pediculus humanus corporis*	gi|242022910	5.3/58.5	6.6/32.1	100%	6%	ALHFVFK VLRHEEFK	0.147	0.357	0.41	Extracellular, cytoplasm	Lyase activity, carbohydrate metabolic process, oxidoreductase, lactoylglutathione lyase activity
79	Thioredoxin peroxidase	*Apis cerana cerana*	gi|314991296	5.3/58.7	6.0/18.0	100%	15%	GLFIIDDK GLFIIDDKQNLR QITINDLPVGR SVDETLR	0.302	0.815	0.37	Cytoplasm, cytoskeleton, cytosol	Oxidoreductase, peroxidase, peroxiredoxin activity, antioxidant, metabolic, stress, defence and immunity
80	Predicted: peroxiredoxin 1	*Apis mellifera*	gi|328777120	5.3/32.8	5.7/21.8	100%	12%	GLFIIDDKQNLR QITINDLPVGR	0.254	0.269	0.94	Cytoplasm, cytoskeleton, cytosol	Antioxidant activity, peroxiredoxin activity, protein binding, metabolism, stress
81	Transmembrane amino acid transporter protein	*Drosophila mojavensis*	gi|195112893	6.6/22	8.5/58.5	81%	6%	VARLLNR AVFIIDDKQILR	0.187	0.512	0.36	Membrane	Vitamin transport, amino acid transmembrane transporter activity
82	Nucleoside diphosphate kinase	*Drosophila melanogaster*	gi:127980	7.8/23	7.8/17.2			QMLGATNPADSLPGTIR NIIHGSDAVESAEK TFIMVKPDGVQR EIALWFNEK ELVTWTPAAK GDFCIQVGR HYADLSARPFFPGLVNYMNS GPVVPMVWEGLNVVK	0.059	0.082	0.71	Membrane, cytoskeleton, cytoplasm	Nucleoside diphosphate phosphorylation, GTP biosynthetic process, UTP biosynthetic process, CTP biosynthetic process, protein phosphorylation, microtubule-based process, wing disc development, mitotic nuclear division, open tracheal system development, epithelial cell migration, open tracheal system, nucleotide metabolic process, ATP, GTP binding, kinase activity
83	Actin	*Euagrus chisoseus*	gi|167683040	6.7/40.5	5.8/39.4	100%	12%	AVFPSIVGRPR DSYVGDEAQSKR VAPEEHPILLTEAPLNPK	0.051	–		Cytoplasm, cytoskeleton, lipid particles	Highly conserved proteins that are involved in various types of cell motility and are ubiquitously expressed in all eukaryotic cells, ATP binding, muscle development, reproduction
84	Enolase, partial	*Dryocoetoides cristatus*	gi|14161541	6.5/47.5	5.8/40.7	100%	12%	EGQYDLDFKNPNSDK FGLDATAVGDEGGFAPNILNNK KACNCILLK	0.181	–		Catalytic domain, cytoplasm, lipid particles, plasma membrane	Glycolytic process, magnesium ion binding, phosphopyruvate hydratase activity
85	Glutamate/acetylglutamate kinase	*Drosophila virilis*	gi|195379098	7.1/81.5	7.1/83.7	100%	13%	AAAAVGQSGLMSLYDAMF AQYGVK EEISDLLSMEK GPVGVEGLLTTK HIDLIIPR LAQELLMSLSMR LASIVEQVAECHLEGR LGSAVITR SKVGTGGMDSK VGTGGMDSK	0.216	–		Cytoplasm	Proline biosynthetic process, glutamate 5-kinase activity, glutamate-5-semialdehyde dehydrogenase activity, oxidoreductase activity
86	Epsilon, an isoform of 14-3-3 protein	*Tribolium castaneum*	gi|91087875	6.3/29.5	4.7/29.2	99%	10%	NLLSVAYK SASDIAMTELPPTHPIR	0.083	–			Protein binding, mitotic G2 DNA damage checkpoint , axon guidance, determination of adult lifespan, germarium-derived oocyte fate determination, imaginal disc development, oocyte microtubule, positive regulation of growth, regulation of mitosis, response to radiation, protein binding

Table 1. Continued.

Spot #	Protein ID	Organism	NCBI Accession #	Exp. PI/MW	Theor. PI/MW	Protein Prob. (%)	Sequence coverage (%)	Exclusive unique peptide sequences	Intensity			Subcellular component	Functions
									Adult	Nymph	Fold change		
87	Isocitrate dehydrogenase [NADP] cytoplasmic	*Acromyrmex echinatior*	gi|332029736	7.3/40.5	8/44.9	100%	13%	CATITPDEKR ETSTNPIASIFAWTR HAHADQYK KIWYEHR SNGGFVWSCK	0.326	–		Mitochondria	2-oxoglutarate metabolic process, NADPH regeneration, cellular lipid metabolic process, female gonad development, glutathione metabolic process, tricarboxylic acid cycle, magnesium ion binding, NADP, NAD binding, receptor binding, reproductive system development
88	Heat shock protein 60	*Polypedilum vanderplanki*	gi|303305116	5.3/55	5.3/60.5	100%	10%	AAVEEGIVPGGGTALLR LVQDVANNTNEEAGDGTTTATVLAR VGGSSEVEVNEKKDR	0.025	–		Mitochondria, cytoplasm, cytoskeleton, lipid particles	Protein refolding, response to stress, ATP binding
89	V-ATPase subunit a, partial	*Locusta migratoria*	gi|401757819	5.3/23	5.2/31.2	100%	8%	HAVESTAQSENK NMIAFYDMSR	0.205	–		Mitochondria, membrane, catalytic domain, lipid particles	ATP synthesis, ion transport, plasma membrane ATP synthesis coupled proton transport, rotational mechanism, proton-transporting ATP synthase complex, coupling factor F(o), catalytic activity and metabolism
90	Tropomyosin 1 isoform a	*Lethocerus indicus*	gi|220980842	5.3/36	4.6/32.8	100%	55%	ALQNAESEVAALNRR ANQREEEYKNQIK DANLRAEKAEEEAR DKALQNAESEVAALNRR FLAEEADKKYDEVAR IQLLEEDLERSEER IVELEEELRVVGNNLK KLAMVEADLERAEER KMQAMKLEK LAEASQAADESERQR MDALENQLKEAR RIQLLEEDLERSEER SLADEERMDALENQLK SLEVSEEKANQREEEYK	0.511	–		Cytoplasm, cytoskeleton	Muscle protein, protein binding, actin-binding, muscle contraction, oogenesis, regulation of lamellipodium assembly, pole plasm assembly, actin filament binding, movement
91	Predicted: glycerol 3-phosphate dehydrogenase, mitochondrial-like isoform 1	*Acyrthosiphon pisum*	gi|193580091	6.0/32.7	6.8/80	100%	12%	EIDTNMNGQVELDEYLQMMSALK GYVSINDIR IHPEFPYIDAEIR MCLALALTATR MVKEALHER TALVELDDFASGTSSRSTK	0.134	–		Cytoplasm, cytoskeleton, lipid particles	Glyceraldehyde 3-phosphate dehydrogenase (NAD+) (phosphorylating) activity, oxidoreductase activity, binding, metabolic, muscle development
92	Tubulin β-1	*Acyrthosiphon pisum*	gi|298676439	7.1/62.5	5.3/41.8	100%	15%	AILVDLEPGTMDSVR FPGQLNADLR ISEQFTAMFR KLAVNMVPFPR LAVNMVPFPR LHFFMPGFAPLTSR YLTVAAVFR	0.094	–		Cytoplasm, cytoskeleton	Microtubule-based process, protein, polymerization, GTP binding, GTPase activity, structural constituent of cytoskeleton, metabolic processes, muscles development
93	Thioredoxin_like	*Drosophila mojavensis*	gi|195122452	6.5/27.5	8.5/85.5	80%	6%	LKLLLTMLLAALLTR	0.093	–		Cytoplasm, cytoskeleton, cytosol	Protein binding, RNA splicing, via transesterification reactions, gene expression, mitotic nuclear division, spliceosomal complex assembly

Table 1. Continued.

Spot #	Protein ID	Organism	NCBI Accession #	Exp. PI/MW	Theor. PI/MW	Protein Prob. (%)	Sequence coverage (%)	Exclusive unique peptide sequences	Intensity			Subcellular component	Functions
									Adult	Nymph	Fold change		
94	Aldo-keto reductases	*Bombyx mori*	gi|322802295	6.6/30	6.6/36.8	100%	5%	HIDTATVYENEHVIGK	0.031	–		Cytoplasm	Lipid metabolism, steroid metabolism oxidoreductase activity
95	Enolase	*Coccotrypes dactyliperda*	gi|14161525	6.5/32.8	5.5/29.2	100%	6%	EGQYDIDFKNPNSDK	0.060	–		Catalytic domain, cytoplasm, lipid particles, plasma membrane	Glycolytic process, magnesium ion binding, phosphopyruvate hydratase activity
96	Predicted: probable aconitate hydratase, mitochondrial-like	*Acyrthosiphon pisum*	gi|328716624	5.6/71.7	7.3/86	100%	16%	ATIERDGIAQTLR EHAALEPR GKCTTDHISAAGPWLK IHETNLKK LSNPFGDELPAR VAMQDATAQMAMLQFISSGLPK	0.172	–		Mitochondria	Catalytic activity, metal ion binding, metabolic process
97	Tubulin α chain	*Aedes aegypti*	gi|157113931	5.8/26	4.9/49.9	100%	56%	AFVHWYVGEGMEEGEFSEAR AVCMLSNTTAIAEAWAR AVFVDLEPTVVDEVR AYHEQLSVAEITNACFEPANQMVK DVNAAIATIK EIVDVVLDR FDGALNVDLTEFQTNLVPYPR GHYTIGKEIVDVVLDR IHFPLVTYAPVISAEK LDHKFDLMYAK LSVDYGKK NLDIERPTYTNLNR QLFHPEQLITGKEDAANNYAR RNLDIERPTYTNLNR TIGGGDDSFNTFFSETGAGK TIQFVDWCPTGFK VGINYQPPTVVPGGDLAK YMACCMLYR	0.074	–		Cytoskeleton	Acyltransferase, α-tubulin acetylation, neuron development, regulation of microtubule cytoskeleton organization, tubulin N-acetyltransferase activity, transferase activity, transferring acyl groups, muscles development
98	Protein CBR-ACT-5	*Caenorhabditis briggsae*	gi|268574578	5.7/56	5.5/41.9	99%	16%	AGFAGDDAPR DSYVGDEAQSKR EDEIAALVVDNGSGMCK HQGVMVGMGQK IKIIAPPERK	0.037	–		Cytoskeleton, cytoplasm	ATP binding, nucleotide-binding, belongs to the actin family

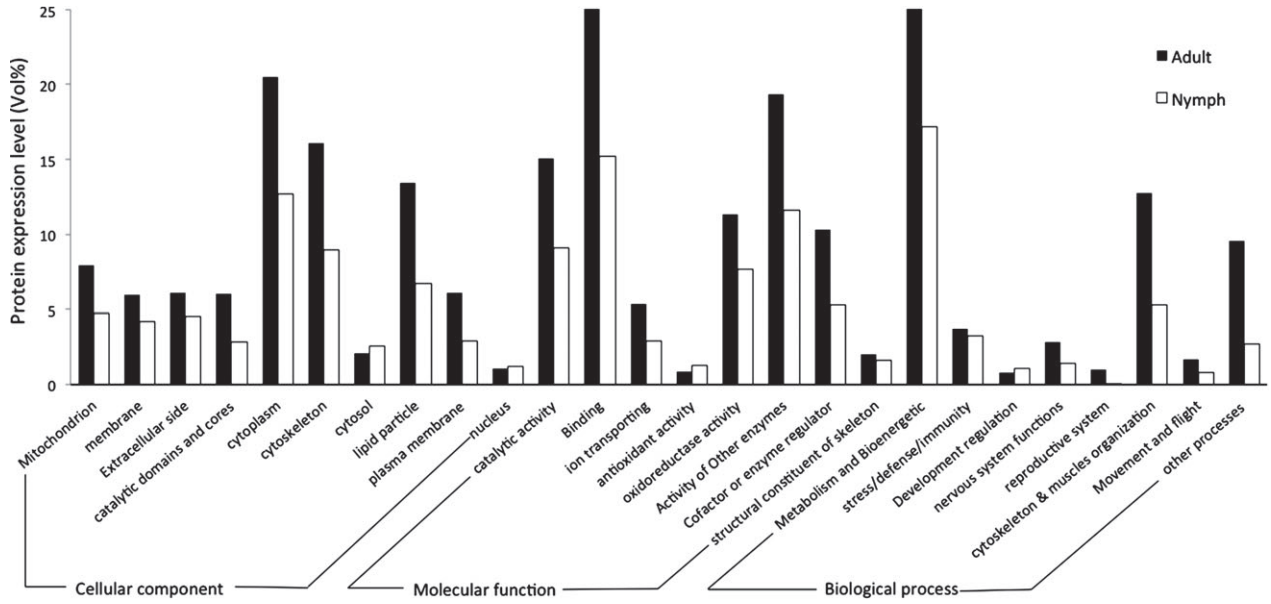

Fig. 3. Gene ontology (GO) annotations of differentially expressed proteins in fifth-instar nymphs and adults of the Asian citrus psyllid *Diaphorina citri*. All the differentially expressed proteins were first classified using the standard terminologies of the two major categories, molecular function (MF) and biological process (BP), using the GO database (http://www.geneontology.org). Protein expression level represents the total volume percentage (Vol%) for each category. The %Vol is the relative volume and represents the total volume of each category divided by the total volume for total spot volume in the image. The spot volume represents the integration of optical density for the spot area. Optical density (OD) is the highest calibrated pixel intensity in the spot. The spot area is calculated in mm^2.

65. Generally, ATP synthase subunits increased by more than two-fold. Enolase (S11, S26, S84, S95) was found at higher levels (3.3-fold in spot 11) in adults than nymphs. Phosphoglyceromutase (S17, S18) increased by up to 17-fold in adults. The enzyme 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase (EC: 2.1.2.3) (= AICARFT/IMP cyclohydrolase or bifunctional purine biosynthesis protein (gene *purH*), (S22: up-regulated 1.6-fold in adults) is implicated in nucleotide and metabolic processes and IMP cyclohydrolase activity. Glutamate semialdehyde dehydrogenase (S28: 1.3-fold) is related to the proline biosynthetic process, glutamate 5-kinase activity, nucleotide binding and metabolic processes. Electron transfer flavoprotein subunit α (S39: 2.5-fold) is related to DNA repair, protein-chromophore linkage, DNA binding, deoxyribodipyrimidine photolyase activity, nucleotide binding, enzymatic activity and ion transport. Seven protein spots implicated in somatic muscle development and differentiation, as structural constituents of the skeleton, in tracheal system development, and in movement and flight, were identified that had a significant increase from nymph to adult stage. Mainly, these proteins are suggested to be localized in the cytoplasm and cytoskeleton. These proteins are predicted as myosin heavy chain muscle isoform 1 (S32: 1.7-fold in adults), tubulin (S34, S62, S92, S97), actin and α-actin (S50, S58: 3.8- and 0.2-fold, respectively, in adults), tropomyosin 1 isoform a (S90: expressed in adults only), protein CBR-ACT-5 (S98) and serine carboxypeptidase (S13: 1.4-fold).

Proteins implicated in stress/defence/immunity were found in both stages. Most of these proteins were up-regulated in adults compared with nymphs. These proteins included triose phosphate isomerase (S1, S44: more than 1.7-fold), ATPase (S12: 3.5-fold; S41: two-fold; S66: 0.7-fold; S76: 0.5-fold; S89: only in adults), glutathione *S*-transferase-like protein (S14: 3.7-fold; S59: 0.99-fold; S67: 07-fold), heterogeneous nuclear ribonucleoprotein 87f (hrp36.1) (S16: 3.4-fold), cyclophilin-type peptidylprolyl cis-trans isomerases (S23: 1.4-fold), thioredoxin peroxidase (S15, S79) and peroxiredoxin (S43, S80), and Epsilon, an isoform of 14-3-3 protein (regulatory protein) (S86: adults only). Also, several heat chock protein spots such as putative heat shock cognate 70 protein (S42: 2.5-fold), heat shock protein 60, (S47, S56, S88: more than five-fold) and chaperonin Cpn60 (S48: 1.7-fold) were clearly up-regulated by 2.5-fold, more than five-fold and 1.7-fold in adults, respectively.

Proteins implicated in metamorphosis and development included haemolymph juvenile hormone binding protein (S63: 0.3-fold in adults), predicted annexin-b9-like (S71: 0.7-fold in adults) and nucleoside diphosphate kinase (S82: 0.7-fold in adults). Some proteins possibly involved in reproductive system functionality and mitotic division were also found. Heterogeneous nuclear ribonucleoprotein 87f (hrp36.1) (S16: 3.4-fold in adults) is implicated in female gonad development, oogenesis and mitotic nuclear division. Epsilon, an isoform of 14-3-3 protein, functions in protein binding, mitotic G2 DNA damage checkpoint, germarium-derived oocyte fate determination and the regulation of mitosis.

Other proteins implicated in neurogenesis increased in the adult stage, such as heterogeneous nuclear ribonucleoprotein 87f (hrp36.1) and tubulin (S34: 1.3-fold; S62: 0.6-fold; S92: only in adults; S97: only in adults). Succinyl-CoA synthetase α subunit

(S73), which is associated with nucleotide binding, enzymatic activity and neurogenesis, was down-regulated 0.5-fold in the adult stage. Two proteins implicated in adult life span were triose phosphate isomerase (S1: two-fold; S44: 1.7-fold) and Epsilon, an isoform of 14-3-3 protein.

Discussion

In the present study, 2-DE is combined with LC-MS/MS to identify proteins involved in the development of *D. citri* from the fifth-instar nymph to the adult stage. It is found that the total number of protein spots increases from the fifth-instar nymphs to the adult psyllids. The majority of proteins found to be common to both life stages show differences in their expression. Most proteins implicated in metabolic pathways, enzyme activities, binding and ion transport are present in both stages but show higher levels of expression in the adult stage. Significant increases are found in the levels of proteins related to structural constitution of the skeleton, stress/defence/immunity, reproduction system, muscles, locomotion and flight. These results are consistent with the fact that *D. citri* is a hemimetabolous insect; it goes through incomplete metamorphosis in which the nymph is morphologically similar to the adult and without a pupal stage. The main differences between the nymphal and adult stages are the development of wings, muscles, skeletal structures and maturity of the reproductive system. On the other hand, proteins that are involved in developmental regulation are found at a higher level in the nymph stage. Identification of these differentially expressed proteins advances our understanding of their roles in insect metamorphosis and development.

During the adult stage, the insects use more energy for metabolic processes that are required for movement, flight and reproduction. The majority of the 98 identified proteins in the present study are implicated variously in nucleotide binding, energy metabolism, glycolysis and lipid and carbohydrate metabolism. These proteins include phosphoglyceromutase, phosphoglycerate kinase, triose phosphate isomerase, glyceraldehyde 3-phosphate dehydrogenase and isocitrate dehydrogenase NAD-dependent. In *Drosophila melanogaster*, the transcripts of most of the glycolytic pathway genes initially increase, then decrease and increase again during the larval, pupal and adult stages, respectively (Roselli-Rehfuss *et al.*, 1992). Four isoforms of the glycolytic enzyme enolase are higher in adult *D. citri* compared with nymphs. Additionally, two isoforms are detected only in adults. By contrast to the results with *D. citri*, the expression of enolase is highest in the fourth instar of the blister beetle *Epicauta chinensis* (Li *et al.*, 2014).

ATP synthase subunits are more abundant in adult *D. citri* than in nymphs. Arginine kinase, which converts arginine-ATP into phosphoarginine-ADP (Cheung, 1973), plays a role in providing high amounts of ATP to flight muscles (Schneider *et al.*, 1989). In the present study, arginine kinase is up-regulated in adults, probably in keeping with the continual demand for ATP energy for use in flight and reproductive activities by adults compared with nymphs.

Many cytoskeletal protein spots are identified in the present study. These proteins include myosin heavy chain muscle isoform 1 protein, tubulin, actin, α-actin, tropomyosin 1 isoform a, protein CBR-ACT-5 and serine carboxypeptidase. The structure of *D. citri* nymphal stage exoskeleton is softer than the adult exoskeleton. Nymphs are soft bodied with many flexible membranes between sclerites and around articulation cavities (White & Hodkinson, 1985). A significant increase in these proteins is found in adults, in agreement with the fact that these proteins are involved in cytoskeleton organization.

Proteins such as triose phosphate isomerase, glyceraldehyde, glycerol 3-phosphate dehydrogenase, and phosphoglyceromutase, which are significantly increased in adults, are implicated in adult muscle development and locomotory processes and flight activities. Serine carboxypeptidase is implicated in long-term memory. In the present study, tropomyosin 1 isoform a (S90) is detected in adults only. Tropomyosin also binds protein and actin filaments, and regulates muscle contraction, lamellipodium assembly and pole plasm assembly (Cooper, 2002). Generally, an increase in skeletal proteins is found from the fifth-instar stage to adult stage, in agreement with having greater skeletal structures and muscles in the adult stage.

Some proteins that relate to stress, defence and immunity are found in both stages of *D. citri*. Most of these proteins, especially those related to heat, starvation and mechanical stimulation, are up-regulated in adults. Proteins involved in amino acid and carbohydrate metabolism are increased in the adult stage and are described as immune-responsive in haemolymph and cell line studies. Vierstraete *et al.* (2004) and de Morais Guedes *et al.* (2005) discuss the responses of cellular proteins to changes in metabolism as a result of oxidative stress. The threat for aerobic organisms, especially in adult stages, is the production of reactive oxygen species resulting in oxidative damage. Because of a higher demand for oxygen during the adult life stage, increases in reactive oxygen species and oxidative damage are assumed (Seehuus *et al.*, 2006). Subsequently, adult insects generally invest in proteins related to reactive oxygen protection to minimize this damage. The upregulation of glutathione *S*-transferase-like protein in adult Asian citrus psyllid reflects its role in detoxification and oxidative stress response. It is also up-regulated in cases of infection in other insects and may have a protective role in immunity (Vierstraete *et al.*, 2004; de Morais Guedes *et al.*, 2005). Two forms of glutathione *S*-transferase-like protein are also identified as being up-regulated in the mosquito *Anopheles gambiae* in haemolymph after bacterial infection and after wounding (Paskewitz & Shi, 2005).

Oxidative stress reduces the lifespan of most organisms if not protected by adequate defence mechanisms (Finkel & Holbrook, 2000; Nyström, 2005; Seehuus *et al.*, 2006). It is assumed that the nymphal stages must also have an oxidative stress protection system. The current proteomic data suggest that other proteins such as peroxiredoxin-like protein (PRX) and thioredoxin peroxidase (TPX) might contribute to the reactive oxygen species tolerance during the nymph stage. These proteins belong to the peroxiredoxin anti-oxidative group of enzymes and are implicated in antioxidant activity and DNA repair, and may protect mammalian muscle from oxidative damage (Powers *et al.*, 1999). In the present study, PRX and TPX are relatively equally expressed in both stages. The main functions of PRX and TPX are detoxification, resistance against oxidative

stress and immune reactions (Collins *et al.*, 2010). Thioredoxin peroxidase was identified first in the silkworm, *B. mori* and has a protective role against oxidative stress caused by temperature changes and viral infection (Lee *et al.*, 2005). It is up-regulated in aphids after parasitism treatments (Nguyen *et al.*, 2008), in larvae of *Plutella xylostella* treated with fipronil (Xie *et al.*, 2011) and in *D. melanogaster* haemolymph subsequent to fungal or bacterial challenge (Vierstraete *et al.*, 2004) or after feeding on bacterial lysates (de Morais Guedes *et al.*, 2005).

Similarly, protein spots identified as heat shock protein in the present study are clearly up-regulated in adults over the nymphal stage. Similarly, the heat shock protein, Hsc70 is down-regulated in the fourth and fifth larval instars of *E. chinensis* compared with adults (Li *et al.*, 2014). Heat shock proteins are particularly important for the transformation and thermotolerance of *Strongyloides venezuelensis* (Tsuji *et al.*, 1996). In *D. citri*, adults are able to tolerate a wider temperature range than nymphs and heat shock proteins are implicated in thermotolerance of the psyllid (Hall *et al.*, 2011).

Some proteins that have a role in insect development are found both in the nymph and adult stages of *D. citri*. Nucleoside diphosphate kinase (NDPK) protein (S82) is higher in the fifth-instar nymph stage than the adult stage (by 0.7-fold in adults). These results are consistent with a previous study from this laboratory showing that expression of abnormal wing disc gene, *awd*, which encodes for NDPK activity, increases gradually during the nymphal stages until it reaches a maximum level during the fifth-instar nymph, then decreases in the adult stage (El-Shesheny *et al.*, 2013). The *awd* gene is implicated in wing disc development in insects (Timmons & Shearn, 2000; Jiang *et al.*, 2010). Silencing *awd* using RNAi during the fifth-instar nymph stage of *D. citri* causes wing malformation in emerging adults (El-Shesheny *et al.*, 2013). The product of *awd* in *Antheraea pernyi* is also shown to contribute to insect temperature tolerance (Jiang *et al.*, 2010).

Annexin-b9-like (Anx B9) maintains cell polarity and the regulation of multivesicular body size, is involved in endosome transport, and is an actin and spectrin binding protein (Grewal *et al.*, 2000; Futter & White, 2007; Grewal & Enrich, 2009). Furthermore, it is implicated in development and wing disc dorsal-ventral pattern formation in *B. mori* (Shi *et al.*, 2013) and is steroid-induced during metamorphosis (Tanaka *et al.*, 2008). In the present study, Anx B9 and *awd* (abnormal wing disc) proteins have higher levels in the fifth-instar nymph stage than in adult stage (by 0.7-fold). A previous study from this laboratory reports similar results for ACP-*awd* expression (El-Shesheny *et al.*, 2013).

The Asian citrus psyllid reproductive system matures during the adult stage. Some proteins are found to be directly implicated in reproductive system function and mitotic divisions. Heterogeneous nuclear ribonucleoprotein 87f (Hrp36.1) (S16: 3.4-fold) is implicated in female gonad development, oogenesis and mitotic nuclear division. Epsilon, an isoform of 14-3-3 protein (S86), is found only in the adult stage. Tropomyosin, which is expressed in the adult stage, is implicated in oogenesis. Neurogenesis-related proteins are increased in the adult stage, such as Hrp36.1 and tubulin, whereas succinyl-CoA synthetase α subunit (S73) is decreased by 0.5-fold. S73 is involved in nucleotide binding, enzymatic activity and neurogenesis. The triose phosphate isomerase protein, which is involved in neurological processes and determination of the adult lifespan (Voelker *et al.*, 1979), is increased by 1.7-fold in the adult stage.

Utilization of a proteomics approach to determine stage-related proteins during the developmental of *D. citri* will help determine the molecular mechanisms of development. The identified variable proteins such as Awd and AnxB9 may provide useful targets for RNAi with respect to arresting the metamorphosis of juvenile Asian citrus psyllid and possibly interrupting transmission of *C*Las phytopathogen.

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