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Aphid biology: Expressed genes from alate *Toxoptera citricida*, the brown citrus aphid

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Abstract

The brown citrus aphid, $Toxoptera\ citricida\ (Kirkaldy)$, is considered the primary vector of citrus tristeza virus, a severe pathogen which causes losses to citrus industries worldwide. The alate (winged) form of this aphid can readily fly long distances with the wind, thus spreading citrus tristeza virus in citrus growing regions. To better understand the biology of the brown citrus aphid and the emergence of genes expressed during wing development, we undertook a large-scale 5' end sequencing project of cDNA clones from alate aphids. Similar large-scale expressed sequence tag (EST) sequencing projects from other insects have provided a vehicle for answering biological questions relating to development and physiology. Although there is a growing database in GenBank of ESTs from insects, most are from $Drosophila\ melanogaster$ and $Anopheles\ gambiae$, with relatively few specifically derived from aphids. However, important morphogenetic processes are exclusively associated with piercing-sucking insect development and sap feeding insect metabolism. In this paper, we describe the first public data set of ESTs from the brown citrus aphid, $T.\ citricida$. The cDNA library was derived from alate adults due to their significance in spreading viruses (e.g., citrus tristeza virus). Over 5180 cDNA clones were sequenced, resulting in 4263 high-quality ESTs. Contig alignment of these ESTs resulted in 2124 total assembled sequences, including both contiguous sequences and singlets. Approximately 33% of the ESTs currently have no significant match in either the non-redundant protein or nucleic acid databases. Sequences returning matches with an E-value of \leq -10 using BLASTX, BLASTN, or TBLASTX were annotated based on their putative molecular function and biological process using the Gene Ontology classification system. These data will aid research efforts in the identification of important genes within insects, specifically aphids and other sap feeding insects within the Order Hemiptera.

The sequence data described in this paper have been submitted to Genbank's dbEST under the following accession numbers:: CB814527-CB814982, CB832665-CB833296, CB854878-CB855147, CB909714-CB910020, CB936196-CB936346, CD449954-CD450759.

Keywords: Aphididae, cDNA, EST, Gene expression, Hemiptera, Development, Toxoptera

Abbreviation:

EST expressed sequence tag

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Introduction

The brown citrus aphid, *Toxoptera citricida* (Kirkaldy), is one of the most devastating pests of citrus, causing extensive crop losses worldwide. Feeding by this aphid alone can cause severe damage to citrus. However, it poses an even greater threat to citrus because of its efficient transmission of citrus tristeza closterovirus (Fasulo and Halbert, 1993).

Since the brown citrus aphid genomic sequence is not

available, expressed sequence tags (ESTs) derived from single-pass sequencing of cDNA clones prepared from the brown citrus aphid provide an invaluable resource for the identification of genes associated with the biology of the alate adult life stage. In the past, cloning of genes encoding enzymes of specific biochemical pathways by single-pass sequencing of cDNA clones has been a very successful strategy, particularly when the cDNA libraries have been prepared from tissues with high activity for the respective enzymes (Coyle-Thompson and Banerjee 1993; Newman *et al.*, 1994; Blaxter *et al.*,

1996; Cooke *et al.*, 1996; Rounsley *et al.*, 1996). This enables investigators to isolate genes derived from specific tissues and/or life stages for more detailed study, which may include developing efficient biocontrol methods.

Additionally, ESTs and their accompanying cDNAs, provide the means to construct glass or nylon based arrays that can be used for transcript profiling on a genome-wide scale (DeRisi et al., 1997; Ruan et al., 1998; Egger et al., 2002). A careful bioinformatic analysis identifying life stage-specific ESTs is a prerequisite in order to obtain a comprehensive and representative set of cDNAs for gene expression studies by arrays (Loftus et al., 1999). Given that there are only a small number of insect ESTs in public databases it was essential to build a life-stage specific library derived from aphids so that analysis of metabolism and development on a genome-wide scale could be accomplished. Even without subsequent array analysis, a relatively large number of ESTs from a specific life stage can provide clues toward the expression of specific genes important to the functions expressly connected with that life stage (Rafalski et al., 1998; Arbeitman et al., 2002). In most cases and within statistical limitations, the abundance of a specific cDNA in the EST collection is a measure of gene expression (Audic and Claverie, 1997). This technique, referred to as a"digital or electronic northern", has been utilized in several similar studies to gauge relative gene expression in various tissues. The data sets are available at GenBank, dbEST under the following accession numbers.: CB814527-CB814982, CB832665-CB833296, CB854878-CB855147, CB909714-CB910020, CB936196-CB936346, CD449954-CD450759.

Materials and Methods

Aphid rearing and collection

Alate brown citrus aphids, Toxoptera citricida, were obtained from a healthy colony maintained by WB Hunter at the USDA, ARS, U.S. Horticultural Research Laboratory, Ft. Pierce, FL. The founders were collected from a single collection site in Orlando, Florida. The colony was reared under continuous asexual reproduction for a period of 3 years on sweet orange, Madam vinous, seedlings in screen cages contained in an insectary, and held at 25° C, 16 L: 8 D. Plants free of insecticide and bearing new flush were cycled into cages on a weekly basis. Aphids and their host plants were surveyed biweekly for any incidence of contaminating insect species (e.g., mites, parasitoids, fungus gnats, shore flies, etc.). Highdensity aphid populations produced alate aphids that were collected by aspiration within two days of emergence. All alates were collected from the top of the cage so as to avoid sample contamination with other developmental forms or host plant tissue. Upon collection, alates were immediately submerged into liquid nitrogen prior to total RNA isolation. Approximately 50-100 alates were placed into 95% ethanol and stored at -80°C to be used as voucher specimens.

cDNA library construction

Approximately 4500 1-2 day old alate aphids were used in the construction of an expression library. Whole aphids were ground in liquid nitrogen and total RNA extracted using guanidinium salt-phenol-chloroform procedure as described by Strommer *et al.* (1993). Poly(A)+ RNA was purified using two rounds of selection on oligo dT magnetic beads according to the manufacturer's Downloaded From: https://bioone.org/journals/Journal-of-Insect-Science on 25 Apr 2024 Terms of Use: https://bioone.org/terms-of-use

instructions (Dynal, www.dynal.no). A directional cDNA library was constructed in Lambda Uni-ZAP® XR Vector using Stratagene's ZAP-cDNA Synthesis Kit (Stratagene, www.stratagene.com). The resulting DNA was packaged into lambda particles using Gigapack® III Gold Packaging Extract (Stratagene). An amplified library was generated with a titer of 1.0 x 109 plaque-forming units per mL. Mass excision of the amplified library was carried out using Ex-Assist® helper phage (Stratagene). An aliquot of the excised, amplified library was used for infecting XL1-Blue MRF' cells and subsequently plated on LB agar containing 100 µg/mL ampicillin. Bacterial clones containing excised pBluescript SK(+) phagemids were recovered by random colony selection.

Sequencing of clones

pBluescript SK(+) phagemids were grown overnight at 37° C and 240 rpm in 96-deep well culture plates containing 1.7 mL of LB broth, supplemented with $100\,\mu\text{g/mL}$ ampicillin. Archived stocks were prepared from the cell cultures using 75 μ l of a LB-amp, glycerol mixture and 75 μ l of cells. These archived stocks are held at the Horticultural Research Laboratory where they are kept in an ultra low temperature freezer set at -80° C. Plasmid DNA was extracted using the Qiagen 9600 liquid handling robot and the QIAprep 96 Turbo miniprep kit according to the recommended protocol (QIAGEN, www.quigen.com).

Sequencing reactions were performed using the ABI PRISM® BigDyeTM Primer Cycle Sequencing Kit (Applied Biosystems, home.appliedbiosystems.com) along with a universal T3 primer. Reactions were prepared in 96-well format using the Biomek2000TM liquid handling robot (Beckman Coulter, www.beckman.com). Sequencing reaction products were precipitated with 70% isopropanol, resuspended in 15 μL sterile water and loaded onto an ABI 3700 DNA Analyzer (Applied Biosystems).

Computer analysis

Base confidence scores were designated using TraceTuner® (Paracel, www.paracel.com). Low-quality bases (confidence score <20) were trimmed from both ends of sequences. Quality trimming, vector trimming and sequence fragment alignments were executed using Sequencher® software (Gene Codes, www.genecodes.com). Contaminating sequences such as rRNA and mitochondrial DNA were identified using BLASTN and were excluded from analysis along with sequences less than 100 nucleotides in length after both vector and quality trimming. Additional ESTs that corresponded to vector contaminants were removed from the dataset. To estimate the number of genes represented in the library and the redundancy of specific genes, ESTs were assembled into contigs using Sequencher®. Contig assembly parameters that were set using a minimum overlap of 50 bases and 95% identity match.

Functional annotation of ESTs

Putative sequence identity was determined based on BLAST similarity searches using the National Center for Biotechnology Information (NCBI) BLAST server (http://www.ncbi.nlm.nih.gov) with comparisons made to both non redundant nucleic acid (BLASTN) and protein (BLASTX) databases. ESTs that had no significant similarity to any publicly available sequences using

BLASTN and BLASTX were then screened individually using TBLASTX.

The top 5 hits for each assembled sequence were then formatted using an in-house parsing program that allowed for direct import into a Microsoft Excel® spreadsheet for further analysis. Sequence matches with E-value scores \leq -10 were considered significant and were categorized according to the Gene Ontology (GO) classification system based on annotation of the 5 'best hit' matches in BLASTX searches. All D. melanogaster matches were cataloged using FlyBase (www.flybase.org). Those sequences without a D. melanogaster hit were annotated using AmiGO (www.geneontology.org).

Results and Discussion

Generation and assembly of adult alate ESTs

An initial 5180 clones were sequenced from the 5' end. These sequences were trimmed of vector and low-quality sequence and filtered for minimum length (100 bp), producing 4267 highquality ESTs of 481 bp average length. These ESTs were analyzed with the Sequencher® assembly program to identify those that represent redundant transcripts. ESTs were assembled into 468 contiguous sequences (contigs) with 1656 ESTs remaining as singlets, suggesting a 61% redundancy. Thus, the combined set of contigs and singlets included 2124 sequences (hereafter referred to as 'assembled sequences'), putatively representing different transcripts. Only 22 contig sequences contained more than 10 ESTs.

EST quality analysis and sequence survey

Of the 2124 assembled sequences analyzed, 993 (representing 2132 ESTs) were similar to known protein sequences in the non-redundant protein database (BLASTX; $E \le -10$). Seven of these assembled sequences, representing 13 ESTs, were identified by BLASTX as contaminating vector sequences and were removed from the dataset.

Because some genes encode RNAs rather than proteins, it was necessary to run BLASTN against our dataset. Eight assembled sequences were identified as ribosomal and 2 were identified as mitochondrial DNA, representing 582 and 65 ESTs respectively, and were removed from the dataset. Although the number of ribosomal sequences appears inflated, it has been shown that several non-coding RNAs, such as rRNA, have mRNA-like modifications, such as polyadenylation and splicing. Because this EST dataset was derived from a cDNA library that was enriched for poly(A+) RNA, it is reasonable to assume that some non-coding RNAs should be present (MacIntosh et al., 2001). An additional 76 ESTs were identified as either rRNA or mitochondrial using TBLASTX, leaving 2031 assembled sequences used in subsequent functional analyses.

Of the initial 2124 assembled sequences (representing 4267 ESTs), 1045 (representing 1412 ESTs) showed no significant similarity (E>-10) to any publicly available sequence using BLASTX, BLASTN, or TBLASTX. This result suggests that a large percentage (~33%) of the ESTs sequenced here are novel. However, this estimation of potential unique sequences within the cDNA library is most likely to be an overestimation due to several factors, such as computer alignment parameters and low quality internal sequences (White et al., 2000). Moreover, assembled sequences may

have lacked an open reading frame because they were too short causing ESTs to consist mostly or entirely of a noncoding region (e.g., 3' untranslated region) (Whitfield et al., 2002).

Functional annotation of ESTs

Each Toxoptera citricida assembled sequence was tentatively assigned Gene Ontology classification based on annotation of the top 5 "best hit" matches ($E \le -10$) using BLASTX. Nearly all of these were characterized with respect to the functionally annotated genes in *D. melanogaster* using FlyBase. Of the 993 sequences demonstrating similarity to known protein sequences, 332 (33%) of these were of unknown molecular function and 685 (69%) were of unknown biological process. Tables 1 and 2 summarize assignments of *Toxoptera* sequences to major molecular functions and biological processes, respectively.

Genes of interest within the EST dataset

The BLASTX results provide useful information regarding the homology of proteins that may be critical for insect cellular communication and development. Table 3 lists sequences of the brown citrus aphid that match to D. melanogaster genes implicated in signal transduction, cell differentiation, cell fate commitment, embryonic and larval development, morphogenesis, reproduction and cuticle biosynthesis. Typically, genes involved in early development would not be present in cDNA libraries derived from adult tissues. However, many aphid species are composed entirely of viviparous parthenogenetic females. These insects telescopic generations as embryogenesis occurs in un-born daughters, producing up to three generations developing within an adult individual (Sabater et al., 2001). Therefore, genes involved in the development of several life stages may be represented simultaneously in this analysis.

For the purposes of this paper, brown citrus aphid sequences were grouped into distinct gene ontology classifications. However, it is important to recognize that many of these gene products act in concert with one another to control cell fate determination which, in turn, drives morphological changes such as eye, leg, and wing development (e.g., the *Notch* pathway) (Coyle-Thompson and Banerjee, 1993; Baonza et al., 2000).

Conclusions

We have provided a large data set of ESTs from the alate brown citrus aphid and have begun to analyze this valuable resource. The analysis of this data set is continually evolving and some of the conclusions may have to be revised as more advanced bioinformatic tools become available. Being the first EST data set for the brown citrus aphid, its preliminary examination clearly shows that it is substantially different from the aphid EST data set currently available to the public. For the most part, there is considerable congruence between conventional biochemistry regarding insect metabolism and the number of ESTs encoding metabolic enzymes. This data set provides the first experimental access to these genes and the basis for more in-depth molecular and genomic analysis. Moreover, it identifies genes that are critical in the physiology, reproduction, development, and wing morphogenesis of aphids. Genetic information is crucial to advancing our understanding of aphid

Table 1. Molecular Function

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[p] Cell Adhesion Molecule 1 0.06% 0 1 [p] Chaperone 26 1.62% 6 12 [p] Enzyme 2 0.12% 0 2 [c] Helicase 2 0.12% 0 2 [c] Histone deactylase 1 0.06% 0 1 [c] Hydrolase 1 0.06% 0 1 [i] General hydrolase 12 0.75% 3 6 [i] Acting on acid anhydrides 98 6.11% 21 21 21 [i] Acting on ester bonds 2 0.12% 0 2 2 1.37% 4 14 14 14 13 14	[c] Steroid binding	1	0.06%	0	1
[p] Chaperone 26 1.62% 6 12 [p] Enzyme		1	0.06%		
[p] Enzyme 2 0.12% 0 2 [c] Helicase 2 0.12% 0 1 [c] Histone deactylase 1 0.06% 0 1 [c] Hydrolase 12 0.75% 3 6 [i] Acting on acid anhydrides 98 6.11% 21 21 [i] Acting on ester bonds 22 1.37% 4 14 [i] Acting on ether bonds 2 0.12% 0 2 [i] Acting on elycosyl bonds 1 0.06% 0 1 [i] Peptidase 51 3.18% 11 24 [c] Isomerase 15 0.94% 5 4 [c] Isomerase 15 0.94% 5 4 [c] Kinase 23 1.43% 5 11 [c] Ligase 19 1.19% 5 5 [c] Molydopterin cofactor sulfarase 1 0.66% 6 9 [c] Small protein activating enzyme 1 0.06% 0 <td< td=""><td></td><td></td><td>1.62%</td><td></td><td></td></td<>			1.62%		
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[c] Hydrolase [i] General hydrolase [i] Acting on acid anhydrides [i] Acting on ester bonds [i] Acting on ether bonds [i] Acting on ether bonds [i] Acting on gener bonds [i] Acting on gener bonds [i] Acting on gener bonds [i] Acting on glycosyl bonds [i] Acting on glycosyl bonds [i] Peptidase [i] Acting on glycosyl bonds [i] Peptidase [i] In the standard standa					
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[i] Acting on ester bonds 22 1.37% 4 14 [i] Acting on ether bonds 2 0.12% 0 2 [i] Acting on glycosyl bonds 1 0.06% 0 1 [i] Peptidase 51 3.18% 11 24 [c] Isomerase 15 0.94% 5 4 [c] Kinase 23 1.43% 5 11 [c] Ligase 19 1.19% 5 5 [c] Lyase 27 1.68% 6 9 [c] Molybdopterin cofactor sulfarase 1 0.06% 0 1 [c] Oxidoreductase 283 17.65% 31 51 [c] Small protein activating enzyme 1 0.06% 0 1 [c] Small protein conjugating enzyme 5 0.31% 1 3 [c] Transferase 66 4.12% 10 36 [p] Enzyme regulator 21 1.31% 3 11 [p] Protein degradation tagging 18 1.12% 4 8 [p] Signal transducer 13 0.81%					
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[c] Lyase 27 1.68% 6 9 [c] Molybdopterin cofactor sulfarase 1 0.06% 0 1 [c] Oxidoreductase 283 17.65% 31 51 [c] Small protein activating enzyme 1 0.06% 0 1 [c] Small protein conjugating enzyme 5 0.31% 1 3 [c] Transferase 66 4.12% 10 36 [p] Enzyme regulator 21 1.31% 3 11 [p] Protein degradation tagging 18 1.12% 4 8 [p] Signal transducer 13 0.81% 2 7 [p] Structural molecule 2 7 [c] Cuticular protein 84 5.24% 14 6 [c] Cytoskeleton protein 42 2.62% 3 2 [c] Muscle fiber 2 0.12% 1 0 [c] Ribosomal protein 337 21.02% 65 29 [p] Transcription regulation 16 1.00% 3 10					
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[c] Small protein activating enzyme 1 0.06% 0 1 [c] Small protein conjugating enzyme 5 0.31% 1 3 [c] Transferase 66 4.12% 10 36 [p] Enzyme regulator 21 1.31% 3 11 [p] Protein degradation tagging 18 1.12% 4 8 [p] Signal transducer 13 0.81% 2 7 [p] Structural molecule 2 7 [c] Cuticular protein 84 5.24% 14 6 [c] Cytoskeleton protein 42 2.62% 3 2 [c] Muscle fiber 2 0.12% 1 0 [c] Ribosomal protein 337 21.02% 65 29 [p] Transcription regulation 16 1.00% 3 10		1	0.06%	0	1
[c] Small protein conjugating enzyme 5 0.31% 1 3 [c] Transferase 66 4.12% 10 36 [p] Enzyme regulator 21 1.31% 3 11 [p] Protein degradation tagging 18 1.12% 4 8 [p] Signal transducer 13 0.81% 2 7 [p] Structural molecule 5.24% 14 6 [c] Cuticular protein 84 5.24% 14 6 [c] Cytoskeleton protein 42 2.62% 3 2 [c] Muscle fiber 2 0.12% 1 0 [c] Ribosomal protein 337 21.02% 65 29 [p] Transcription regulation 16 1.00% 3 10	[c] Oxidoreductase	283	17.65%	31	51
[c] Transferase 66 4.12% 10 36 [p] Enzyme regulator 21 1.31% 3 11 [p] Protein degradation tagging 18 1.12% 4 8 [p] Signal transducer 13 0.81% 2 7 [p] Structural molecule 5.24% 14 6 [c] Cuticular protein 84 5.24% 14 6 [c] Cytoskeleton protein 42 2.62% 3 2 [c] Muscle fiber 2 0.12% 1 0 [c] Ribosomal protein 337 21.02% 65 29 [p] Transcription regulation 16 1.00% 3 10	[c] Small protein activating enzyme	1	0.06%	0	1
[c] Transferase 66 4.12% 10 36 [p] Enzyme regulator 21 1.31% 3 11 [p] Protein degradation tagging 18 1.12% 4 8 [p] Signal transducer 13 0.81% 2 7 [p] Structural molecule 5.24% 14 6 [c] Cuticular protein 84 5.24% 14 6 [c] Cytoskeleton protein 42 2.62% 3 2 [c] Muscle fiber 2 0.12% 1 0 [c] Ribosomal protein 337 21.02% 65 29 [p] Transcription regulation 16 1.00% 3 10	[c] Small protein conjugating enzyme	5	0.31%	1	3
[p] Enzyme regulator 21 1.31% 3 11 [p] Protein degradation tagging 18 1.12% 4 8 [p] Signal transducer 13 0.81% 2 7 [p] Structural molecule 84 5.24% 14 6 [c] Cuticular protein 42 2.62% 3 2 [c] Muscle fiber 2 0.12% 1 0 [c] Ribosomal protein 337 21.02% 65 29 [p] Transcription regulation 16 1.00% 3 10		66	4.12%	10	36
[p] Protein degradation tagging 18 1.12% 4 8 [p] Signal transducer 13 0.81% 2 7 [p] Structural molecule [c] Cuticular protein 84 5.24% 14 6 [c] Cytoskeleton protein 42 2.62% 3 2 [c] Muscle fiber 2 0.12% 1 0 [c] Ribosomal protein 337 21.02% 65 29 [p] Transcription regulation 16 1.00% 3 10	[p] Enzyme regulator		1.31%	3	11
[p] Signal transducer 13 0.81% 2 7 [p] Structural molecule [c] Cuticular protein 84 5.24% 14 6 [c] Cytoskeleton protein 42 2.62% 3 2 [c] Muscle fiber 2 0.12% 1 0 [c] Ribosomal protein 337 21.02% 65 29 [p] Transcription regulation 16 1.00% 3 10		18		4	8
[p] Structural molecule 84 5.24% 14 6 [c] Cuticular protein 42 2.62% 3 2 [c] Muscle fiber 2 0.12% 1 0 [c] Ribosomal protein 337 21.02% 65 29 [p] Transcription regulation 16 1.00% 3 10					
[c] Cuticular protein 84 5.24% 14 6 [c] Cytoskeleton protein 42 2.62% 3 2 [c] Muscle fiber 2 0.12% 1 0 [c] Ribosomal protein 337 21.02% 65 29 [p] Transcription regulation 16 1.00% 3 10			0.0170	-	,
[c] Cytoskeleton protein 42 2.62% 3 2 [c] Muscle fiber 2 0.12% 1 0 [c] Ribosomal protein 337 21.02% 65 29 [p] Transcription regulation 16 1.00% 3 10		84	5 24%	14	6
[c] Muscle fiber 2 0.12% 1 0 [c] Ribosomal protein 337 21.02% 65 29 [p] Transcription regulation 16 1.00% 3 10					
[c] Ribosomal protein 337 21.02% 65 29 [p] Transcription regulation 16 1.00% 3 10					
[p] Transcription regulation 16 1.00% 3 10					
In Translation regulation 13 0.81% 5					
					1
[p] Transporter 114 7.11% 10 23			7.11%		
Totals 1603 266 395	Totals	s 1603		266	395

a Classification is hierarchial: idented terms are children [c] of parent terms [p] listed above. All functional assignments of *Toxoptera citricida* ESTs described here are the "inferred from electronic evidence" (IEA) using the top 5 BLASTX hits with an E-value of ≤-10 generated from NCBI's nr database. The definition term associated with each sequence was entered into both FlyBase and AmiGO where the it was given a molecular function according to The Gene Ontology Consortium.

 $[^]b\%$ of total ESTs represented was calculated using only those ESTs with a BLASTX hit with an E-value of \leq -10 and of known protein function. Downloaded From: https://bioone.org/journals/Journal-of-Insect-Science on 25 Apr 2024 Terms of Use: https://bioone.org/terms-of-use

Table 2. Biological Process

Gene Ontology Term* Nymber of ESTs regressible Number of colors 1 D] Behavior			% of total ESTs		
Cell Communication	Gene Ontology Term ^a	Number of ESTs	represented ^b	Number of contigs	Number of singlets
Cell-adhesion 2	[p] Behavior	7	0.81%		4
[c] Cell-cell signaling 3 0.53% 0 3 [c] Response to external stimulus 19 2.20% 5 7 [c] Signal transduction 8 0.93% 0 8 [c] Cell Crowth and/or maintenance 1 0.12% 0 1 [c] Cell motility 10 0.11% 2 0 [c] Cell motility 10 0.16% 2 0 [c] Homestanis 60 6.94% 6 13 [c] Homestanis 16 1.88% 4 2 [c] Membrane fusion 1 0.12% 0 1 [c] Metabolism 18 2.08% 7 0 [f] Alcohol metabolism 18 2.08% 7 0 [g] Aromatic compound metabolism 18 2.08% 7 0 [g] Aromatic embodydrate metabolism 4 0.46% 0 4 [g] Catabolism 4 0.46% 0 4 [g] Catabolism 4 0.46%	[p] Cell Communication				
[c] Response to external stimulus 19 2.0% 5 7 [c] Signal transduction 8 0.93% 0 8 [p] Cell Growth and/or maintenance 1 0.12% 0 1 [c] Cell cycle 12 1.39% 0 12 [c] Cell organization and biogenesis 60 6.94% 6 13 [c] Homeostasis 16 1.85% 4 2 [c] Methabolism 1 0.12% 0 1 [e] Methabolism 1 0.12% 0 1 [i] Alcohul metabolism 2 0.23% 1 0 [i] Alcohul metabolism 2 0.23% 1 0 [i] Alcohul metabolism 3 0.55% 1 1 1 [i] Alcohul metabolism 4 0.46% 0 4 7 0 [i] Jaring metabolism 4 0.46% 1 0 1 1 1 1 1 0 1 1 0	[c] Cell adhesion	2	0.23%	0	2
Format	[c] Cell-cell signaling	3	0.35%	0	3
p Cell Growth and/or maintenance	[c] Response to external stimulus	19	2.20%	5	7
Collected Control Control Collected Collecte	[c] Signal transduction	8	0.93%	0	8
Cell cycle 12 1.39% 0 12 Cell contility 10 1.16% 2 0 Cell organization and biogenesis 60 6.94% 6 13 [c] Homeostasis 16 1.85% 4 2 [c] Membana fusion 1 0.12% 0 1 [c] Metabolism 1 0.12% 0 1 [i] Alcohol metabolism 18 2.08% 7 0 [i] Armine metabolism 2 0.23% 1 0 [i] Armine metabolism 3 0.35% 1 1 [i] Biosynthesis 18 2.08% 4 7 [i] Biosynthesis 4 0.46% 0 4 [i] Carbohydrate metabolism 4 0.46% 0 4 [i] Energy pathways 2 0.23% 1 0 [i] Energy pathways 2 0.23% 1 0 [i] Lipid metabolism 16 0.12% 0 1 </td <td>[p] Cell Growth and/or maintenance</td> <td></td> <td></td> <td></td> <td></td>	[p] Cell Growth and/or maintenance				
Cell modility	[c] General cell growh and/or maintenance	1	0.12%	0	1
Col. Cell organization and biogenesis 60 6.94% 6 13 Cel. Homeostasis 16 1.85% 4 2 C. Membrane fusion 1 0.12% 0 1 [e] Metabolism 18 2.08% 7 0 [i] Alcohol metabolism 2 0.23% 1 0 [i] Aromatic compound metabolism 3 0.35% 1 1 [i] Biosynthesis 18 2.08% 4 7 [i] Carbohydrate metabolism 4 0.46% 0 4 [i] Catabohism 4 0.46% 1 0 [i] Energy pathways 2 0.23% 1 0 [i] Lipid metabolism 1 0.12% 0 1 [i] Nucleobase, nucleoside, nucleotide and nucleic acid metabolism 36 4.17% 5 26 [i] Phosporous metabolism 126 14.58% 4 11 [ii] Phosporous metabolism 126 14.58% 4 11 [ii] Sulfur metabo	[c] Cell cycle	12	1.39%	0	12
[c] Homeostasis 16 1.85% 4 2 [c] Membrane fusion 1 0.12% 0 1 [c] Metabolism 18 2.08% 7 0 [l] Alcohol metabolism 2 0.23% 1 0 [l] Aromatic compound metabolism 3 0.35% 1 1 [l] Biosynthesis 18 2.08% 4 7 [l] Gabolydrate metabolism 4 0.46% 0 4 [l] Catabolism 4 0.46% 1 0 [l] Learny pathways 2 0.23% 1 0 [l] Learny pathways 2 0.23% 1 0 [l] Lipid metabolism 4 0.46% 1 0 [l] Lipid metabolism 1 0.12% 0 1 [l] Nucleobase, nucleoside, nucleotide and nucleic acid metabolism 36 4.17% 5 26 [l] Phosporous metabolism 16 1.458% 4 111 [l] Protein metabolism 393 45.49% 76 31 [l] Sulfur metabolism 2 0.23% 0 2 [e] Response to stress 1 0.12% 0 1 [e] Transport 5 6 6.48% 13 11 [l] Development 2 0.23% 0 2 [e] Cell differentiation 8 0.93% 2 0 [e] Cell differentiation 8 0.93% 2 0 [e] Cell differentiation 1 0.12% 0 1 [e] Embryonic development 2 0.23% 0 2 [e] Larval development 2 0.23% 0 2 [e] Lipid metabolism 1 0.12% 0 1 [e] Embryonic development 2 0.23% 0 2 [e] Lipid metabolism 1 0.12% 0 1 [e] Embryonic development 2 0.23% 0 0 2 [e] Lipid metabolism 1 0.12% 0 1 [e] Morphogenesis of an epithelium 1 0.12% 0 0 2 [e] Lipid metabolism 1 0.12% 0 0 1 [e] Embryonic development 2 0.23% 0 0 2 [e] Lipid metabolism 1 0.12% 0 0 1 [e] Responsesis 1 0.12%	[c] Cell motility	10	1.16%	2	0
C Membrane fusion	[c] Cell organization and biogenesis	60	6.94%	6	13
C Metabolism	[c] Homeostasis	16	1.85%	4	2
C Metabolism	[c] Membrane fusion	1	0.12%	0	1
Tamine metabolism	[c] Metabolism				
Taronatic compound metabolism 3	[i] Alcohol metabolism	18	2.08%	7	0
Table Tabl	[i] Amine metabolism	2	0.23%	1	0
[I] Biosynthesis 18 2.08% 4 7 [I] Carbohydrate metabolism 4 0.46% 0 4 [I] Catabolism 4 0.46% 1 0 [I] Energy pathways 2 0.23% 1 0 [I] Lipid metabolism 1 0.12% 0 1 [I] Nucleobase, nucleoside, nucleotide and nucleic acid metabolism 36 4.17% 5 26 [I] Phosporous metabolism 16 4.15% 4 11 [I] Protein metabolism 393 45,49% 76 31 [I] Sulfur metabolism 2 0.23% 0 2 [C] Response to stress 1 0.12% 0 1 [I] Sulfur metabolism 2 0.23% 0 2 [C] Response to stress 1 0.12% 0 1 [C] Transport 5 6 6.8% 13 11 [D] Development 2 0.23% 2 0 [C] Cell differentiation	[i] Aromatic compound metabolism	3	0.35%	1	1
[i] Catabolism 4 0.46% 1 0 [i] Energy pathways 2 0.23% 1 0 [i] Lipid metabolism 1 0.12% 0 1 [i] Nucleobase, nucleoside, nucleotide and nucleic acid metabolism 36 4.17% 5 26 [i] Phosporous metabolism 126 14.58% 4 11 [i] Protein metabolism 393 45.49% 76 31 [i] Sulfur metabolism 2 0.23% 0 2 [c] Response to stress 1 0.12% 0 1 [c] Transport 56 6.48% 13 11 11 [p] Development 2 0.23% 0 1 [c] Cell differentiation 8 0.93% 2 0 [c] Cell face commitment 1 0.12% 0 1 [c] Embryonic development 2 0.23% 0 2 [c] Larval development 2 0.23% 1 0 [c] Morphogenesis 1 0.12% 0 1 [iii) Histogenesis		18	2.08%	4	7
[i] Catabolism 4 0.46% 1 0 [i] Energy pathways 2 0.23% 1 0 [i] Lipid metabolism 1 0.12% 0 1 [i] Nucleobase, nucleoside, nucleotide and nucleic acid metabolism 36 4.17% 5 26 [i] Phosporous metabolism 126 14.58% 4 11 [i] Protein metabolism 393 45.49% 76 31 [i] Sulfur metabolism 2 0.23% 0 2 [c] Response to stress 1 0.12% 0 1 [c] Transport 56 6.48% 13 11 11 [p] Development 2 0.23% 0 1 [c] Cell differentiation 8 0.93% 2 0 [c] Cell face commitment 1 0.12% 0 1 [c] Embryonic development 2 0.23% 0 2 [c] Larval development 2 0.23% 1 0 [c] Morphogenesis 1 0.12% 0 1 [iii) Histogenesis	[i] Carbohydrate metabolism	4	0.46%	0	4
[i] Lipid metabolism 1 0.12% 0 1 [i] Nucleobase, nucleoside, nucleotide and nucleic acid metabolism 36 4.17% 5 26 [i] Phosporous metabolism 126 14.58% 4 11 [i] Protein metabolism 393 45.49% 76 31 [i] Sulfur metabolism 2 0.23% 0 2 [c] Response to stress 1 0.12% 0 1 [c] Transport 56 6.48% 13 11 [p] Development 8 0.93% 2 0 [c] Cell differentiation 8 0.93% 2 0 [c] Cell fate commitment 1 0.12% 0 1 [c] Cell fate commitment 2 0.23% 2 0 [c] Embryonic development 2 0.23% 0 2 [c] Morphogenesis 1 0.12% 0 1 [ii) Morphogenesis 1 0.12% 0 2 [iii) Histogenesis <th< td=""><td>[i] Catabolism</td><td>4</td><td>0.46%</td><td>1</td><td>0</td></th<>	[i] Catabolism	4	0.46%	1	0
[i] Lipid metabolism 1 0.12% 0 1 [i] Nucleobase, nucleoside, nucleotide and nucleic acid metabolism 36 4.17% 5 26 [i] Phosporous metabolism 126 14.58% 4 11 [i] Protein metabolism 393 45.49% 76 31 [i] Sulfur metabolism 2 0.23% 0 2 [c] Response to stress 1 0.12% 0 1 [c] Transport 56 6.48% 13 11 [p] Development 8 0.93% 2 0 [c] Cell differentiation 8 0.93% 2 0 [c] Cell fate commitment 1 0.12% 0 1 [c] Cell fate commitment 2 0.23% 2 0 [c] Embryonic development 2 0.23% 0 2 [c] Morphogenesis 1 0.12% 0 1 [ii) Morphogenesis 1 0.12% 0 2 [iii) Histogenesis <th< td=""><td>[i] Energy pathways</td><td>2</td><td>0.23%</td><td>1</td><td>0</td></th<>	[i] Energy pathways	2	0.23%	1	0
Nucleobase, nucleoside, nucleotide and nucleic acid metabolism 36	The state of the s	1	0.12%	0	1
[i] Protein metabolism 393 45.49% 76 31 [i] Sulfur metabolism 2 0.23% 0 2 [c] Response to stress 1 0.12% 0 1 [c] Transport 56 6.48% 13 11 [p) Development 2 0.48% 13 11 [c] Cell differentiation 8 0.93% 2 0 [c] Cell fate commitment 1 0.12% 0 1 [c] Embryonic development 2 0.23% 0 2 [c] Larval development 2 0.23% 0 2 [c] Morphogenesis 1 0.12% 0 1 [i] Morphogenesis of an epithelium 1 0.12% 0 1 [ii] Organogenesis [iii] Ectoderm development 2 0.23% 1 0 [iii] Imaginal disc development 2 0.23% 0 2 [iii] Neurogenesis 23 2.66% 4 4 [iii] Irachael system development 1 0.12% 0 1 [c] Reproduction </td <td>[i] Nucleobase, nucleoside, nucleotide and nucleic acid metabolism</td> <td>36</td> <td>4.17%</td> <td>5</td> <td>26</td>	[i] Nucleobase, nucleoside, nucleotide and nucleic acid metabolism	36	4.17%	5	26
[i] Protein metabolism 393 45.49% 76 31 [i] Sulfur metabolism 2 0.23% 0 2 [c] Response to stress 1 0.12% 0 1 [c] Transport 56 6.48% 13 11 [p) Development 2 0.48% 13 11 [c] Cell differentiation 8 0.93% 2 0 [c] Cell fate commitment 1 0.12% 0 1 [c] Embryonic development 2 0.23% 0 2 [c] Larval development 2 0.23% 0 2 [c] Morphogenesis 1 0.12% 0 1 [i] Morphogenesis of an epithelium 1 0.12% 0 1 [ii] Organogenesis [iii] Ectoderm development 2 0.23% 1 0 [iii] Imaginal disc development 2 0.23% 0 2 [iii] Neurogenesis 23 2.66% 4 4 [iii] Irachael system development 1 0.12% 0 1 [c] Reproduction </td <td>[i] Phosporous metabolism</td> <td>126</td> <td>14.58%</td> <td>4</td> <td>11</td>	[i] Phosporous metabolism	126	14.58%	4	11
[c] Response to stress 1 0.12% 0 1 [c] Transport 56 6.48% 13 11 [p] Development Figure 12 of 12		393	45.49%	76	31
[c] Transport 56 6.48% 13 11 [p] Development (c] Cell differentiation 8 0.93% 2 0 [c] Cell fate commitment 1 0.12% 0 1 [c] Embryonic development 2 0.23% 0 2 [c] Larval development 2 0.23% 1 0 [c] Morphogenesis (ii) Morphogenesis of an epithelium 1 0.12% 0 1 [ii) Organogenesis (iii) Histogenesis [iii] Ectoderm development 2 0.23% 1 0 [iii] Imaginal disc development 2 0.23% 0 2 [iii] Muscle development 2 0.23% 0 2 [iii] Neurogenesis 23 2.66% 4 4 [iii) Trachael system development 1 0.12% 0 1 [c] Reproduction 9 1.04% 0 1 [p] Physiological processes 2 0.12% 0 1 [c] Cutticle biosynthesis 1 0.12% 0 1	[i] Sulfur metabolism	2	0.23%	0	2
P Development	[c] Response to stress	1	0.12%	0	1
P Development	[c] Transport	56	6.48%	13	11
[c] Cell fate commitment 1 0.12% 0 1 [c] Embryonic development 2 0.23% 0 2 [c] Larval development 2 0.23% 1 0 [c] Morphogenesis					
[c] Cell fate commitment 1 0.12% 0 1 [c] Embryonic development 2 0.23% 0 2 [c] Larval development 2 0.23% 1 0 [c] Morphogenesis	[c] Cell differentiation	8	0.93%	2	0
[c] Embryonic development 2 0.23% 0 2 [c] Larval development 2 0.23% 1 0 [c] Morphogenesis """"""""""""""""""""""""""""""""""		1	0.12%	0	1
[c] Morphogenesis [i] Morphogenesis of an epithelium [i] Organogenesis [ii] Histogenesis [iii] Ectoderm development [iii] Imaginal disc development [iii] Muscle development [iii] Muscle development [iii] Neurogenesis [iii] Neurogenesis [iii] Neurogenesis [iii] Neurogenesis [iii] Neurogenesis [iii] Trachael system development [iii] Trachael system development [iviii] Trachael system development [iviiii] Trachael system development [iviiiii] Trachael system development [iviiii] Trachael system development [iviiii] Trachael system development [iviiii] Trachael system development [iviiii] Trachael system development [iviiiii] Trachael system development [iviiiii] Trachael system development [iviiiiiii] Trachael system development [iviiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiii		2	0.23%	0	2
[c] Morphogenesis 1 0.12% 0 1 [i] Morphogenesis of an epithelium 1 0.12% 0 1 [ii] Organogenesis	[c] Larval development	2	0.23%	1	0
[i] Morphogenesis of an epithelium 1 0.12% 0 1 [ii] Organogenesis [iii] Histogenesis [iii] Ectoderm development 2 0.23% 1 0 [iii] Imaginal disc development 2 0.23% 0 2 [iii] Muscle development 7 0.81% 3 0 [iii] Neurogenesis 23 2.66% 4 4 [iii] Trachael system development 1 0.12% 0 1 [c] Reproduction 9 1.04% 1 5 [p] Physiological processes [c] Cuticle biosynthesis 1 0.12% 0 1					
[ii] Organogenesis [iii] Histogenesis [iii] Ectoderm development 2 0.23% 1 0 [iii] Imaginal disc development 2 0.23% 0 2 [iii] Muscle development 7 0.81% 3 0 [iii] Neurogenesis 23 2.66% 4 4 [iii] Trachael system development 1 0.12% 0 1 [c] Reproduction 9 1.04% 1 5 [p] Physiological processes [c] Cuticle biosynthesis 1 0.12% 0 1		1	0.12%	0	1
[ii] Histogenesis [iii] Ectoderm development 2 0.23% 1 0 [ii] Imaginal disc development 2 0.23% 0 2 [ii] Muscle development 7 0.81% 3 0 [ii] Neurogenesis 23 2.66% 4 4 [ii] Trachael system development 1 0.12% 0 1 [c] Reproduction 9 1.04% 1 5 [p] Physiological processes [c] Cuticle biosynthesis 1 0.12% 0 1					
[iii] Ectoderm development 2 0.23% 1 0 [iii] Imaginal disc development 2 0.23% 0 2 [iii] Muscle development 7 0.81% 3 0 [iii] Neurogenesis 23 2.66% 4 4 [iii] Trachael system development 1 0.12% 0 1 [c] Reproduction 9 1.04% 1 5 [p] Physiological processes [c] Cuticle biosynthesis 1 0.12% 0 1					
[ii] Imaginal disc development 2 0.23% 0 2 [ii] Muscle development 7 0.81% 3 0 [ii] Neurogenesis 23 2.66% 4 4 [ii] Trachael system development 1 0.12% 0 1 [c] Reproduction 9 1.04% 1 5 [p] Physiological processes [c] Cuticle biosynthesis 1 0.12% 0 1		2	0.23%	1	0
[ii] Muscle development 7 0.81% 3 0 [ii] Neurogenesis 23 2.66% 4 4 [ii] Trachael system development 1 0.12% 0 1 [c] Reproduction 9 1.04% 1 5 [p] Physiological processes [c] Cuticle biosynthesis 1 0.12% 0 1		2	0.23%	0	2
[ii] Neurogenesis 23 2.66% 4 4 [ii] Trachael system development 1 0.12% 0 1 [c] Reproduction 9 1.04% 1 5 [p] Physiological processes [c] Cuticle biosynthesis 1 0.12% 0 1		7	0.81%	3	0
[ii] Trachael system development 1 0.12% 0 1 [c] Reproduction 9 1.04% 1 5 [p] Physiological processes [c] Cuticle biosynthesis 1 0.12% 0 1		23		4	4
[c] Reproduction 9 1.04% 1 5 [p] Physiological processes [c] Cuticle biosynthesis 1 0.12% 0 1	The state of the s				
[p] Physiological processes [c] Cuticle biosynthesis 1 0.12% 0 1					
[c] Cuticle biosynthesis 1 0.12% 0 1		•			•
		1	0.12%	0	1
10tais 804 143 165	Tota	ls 864		143	165

 a Classification is hierarchial: idented terms are children [c] of parent terms [p] listed above. All functional assignments of *Toxoptera citricida* ESTs described here are the "inferred from electronic evidence" (IEA) using the top 5 BLASTX hits with an *E*-value of ≤-10 generated from NCBI's nr database. The definition term associated with each sequence was entered into both FlyBase and AmiGO where the it was given a molecular function according to The Gene Ontology Consortium.

b% of total ESTs represented was calculated using only those ESTs with a BLASTX hit with an E-value of ≤-10 and of known protein function.

biology, and will play a major role in the development of future non-chemical, gene-based control strategies against these insect pests.

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Table 3. Genes of interest in the Alate BrCA EST dataset

Gene Ontology ^a	Sequence Identifier	Accession Number ^b	NCBI Descriptor	Source Organism	E- value
[p] Cellular Communication					
[c] Signal transduction	WHWTC-30_G08	NP_476884	14-3-3zeta; D14 3 3 protein; leonardo	Drosophila melanogaster	6.00E-78
	WHWTC-44_E04	NP_511144	strawberry notch; glossy-like	Drosophila melanogaster	5.00E-55
	WHWTC-50_D08	AAF57785	CG5036	Drosophila melanogaster	2.00E-52
	WHWTC-51_F11	AAF54188	CG7918	Drosophila melanogaster	9.00E-44
	WHWTC-52_H11	NP_477130	corkscrew CG3954	Drosophila melanogaster	1.00E-37
	WHWTC-27_F03	O43541	Mothers against decapentaplegic homolog 6	Homo sapiens	2.00E-19
	WHWTC-29_H02	AAF56349	CG7012 gene product	Drosophila melanogaster	2.00E-19
	WHWTC-49_B08	NP_511064	deltex	Drosophila melanogaster	5.00E-15
[p] Development					
[i] Cell differentiation	ContigWTC[0908]	NP_477122	Muscle LIM protein at 84B CG1019	Drosophila melanogaster	9.00E-50
	ContigWTC[0147]	AAF58567	guf gene product [alt 1]	Drosophila melanogaster	4.00E-17
[i] Cell fate commitment	WHWTC-09_D08	NP_523455	anterior open CG3166	Drosophila melanogaster	9.00E-51
[i] Embryonic development	WHWTC-26_C07	2115375A	snr1 gene	Drosophila melanogaster	2.00E-38
	WHWTC-01_F03	AAF49547	CG5891 gene product	Drosophila melanogaster	5.00E-15
[i] Larval development	ContigWTC[0852]	AAF53765	CG10691	Drosophila melanogaster	2.00E-72
[i] Morphogenesis					
[ii] Morphogenesis of an epithelium	WHWTC-44_E08	NP_477342	discs lost CG12021	Drosophila melanogaster	3.00E-27
[ii] Organogenesis					
[iii] Histogenesis					
[iv] Ectoderm development	ContigWTC[0527]	AAF50606	CG8624	Drosophila melanogaster	3.00E-12
[iii] Imaginal disc development	WHWTC-39_G11	NP_477444	COP9 complex homolog subunit 4 CG8725	Drosophila melanogaster	3.00E-10
	WHWTC-41_C08	A56922	transcription factor shn	Drosophila melanogaster	4.00E-22
[iii] Muscle development	ContigWTC[1198]	NP_477098	CG8416	Drosophila melanogaster	3.00E-98
	ContigWTC[0705]	A38594	troponin I	Drosophila melanogaster	6.00E-53
	ContigWTC[1037]	AAF47158	Mlp60A gene product	Drosophila melanogaster	6.00E-24
[iii] Neurogenesis	WHWTC-53_E07	AAL76026	putative calreticulin	Aedes aegypti	5.00E-75
	ContigWTC[0118]	NP_523792	FK506-binding protein 2 CG11001	Drosophila melanogaster	4.00E-40
	WHWTC-28_C11	AAD03559	failed axon connections protein	Drosophila virilis	4.00E-39
	ContigWTC[0174]	P58375	60S ribosomal protein L30	Spodoptera frugiperda	3.00E-35
	ContigWTC[1050]	AAF45520	CG7727	Drosophila melanogaster	4.00E-33
	WHWTC-42_F10	AAD43793	CDC42 protein	Drosophila melanogaster	4.00E-28
	ContigWTC[0008]	AAF47413	CG1007	Drosophila melanogaster	1.00E-12
	WHWTC-52_E04	AAB60619	neuralized protein	Drosophila virilis	3.00E-11
[iii] Trachael system development	WHWTC-24_D12	AAF50772	CG10624	Drosophila melanogaster	7.00E-12
[i] Reproduction	WHWTC-26_F08	XP_079633	CG5395 gene product	Drosophila melanogaster	5.00E-60
	WHWTC-03_C02	AAB34841	syntaxin 1, Dsynt1	Drosophila sp.	3.00E-39
	WHWTC-51_A03	AAF49765	CG6451	Drosophila melanogaster	2.00E-26
	WHWTC-33_F11	NP_477016	chickadee CG9553	Drosophila melanogaster	3.00E-24
	ContigWTC[0744]	NP_477016	chickadee CG9553	Drosophila melanogaster	5.00E-18
	WHWTC-51_H09	AAF56175	CG10367	Drosophila melanogaster	2.00E-11
[p] Physiological processes					
[c] Cuticle biosynthesis	WHWTC-04_G08	CAC34734	Yellow protein	Drosophila ananassae	3.00E-14

^aAll functional assignments of *Toxoptera citricida* ESTs described here are the "inferred from electronic evidence" (IEA) using the top 5 BLASTX hits with an E-value of ≤-10 generated from NCBI's nr database. The definition term associated with each sequence was entered into both FlyBase and AmiGO where the it was given a molecular function according to The Gene Ontology Consortium.

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^bAccession numbers correspond to the "best hit" match to Genbank's nr protein database using BLASTX.

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