EXPRESSED SEQUENCE TAGS FROM THE BLACK-WINGED SHARPSHOOTER: APPLICATION TO BIOLOGY AND VECTOR CONTROL

Project Leader:
Wayne B. Hunter
USDA, ARS
U.S. Hortic. Research Lab
Fort Pierce, FL 34945
Wayne.hunter@ars.usda.gov

Collaborators:
Russell F. Mizell III
Department of Entomology
University of Florida
Quincy, FL 32351

Christopher Tipping
Delaware Valley College
Doylestown, PA 18901

Phat M. Dang
National Peanut Res. Lab.
Dawson, GA 39842

Laura E. Hunnicutt
Genomics Sciences
North Carolina State Univ.
Raleigh, NC

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ABSTRACT
The black-winged sharpshooter, *Oncometopia nigricans* Walker, is considered a highly competent vector of several strains of the xylem-inhabiting bacterium *Xylella fastidiosa (Xf)*, the causal agent of a number of economically important plant diseases including Pierce's disease, phony peach disease, plum leaf scald and periwinkle wilt. To better understand the biology of the this leafhopper on a molecular level, our lab undertook a large-scale 5' end expressed sequence tag (EST) sequencing project of cDNA clones derived from *O. nigricans* adults. Similar EST sequencing projects from other insect pests have definitively proven their worth in answering biological questions relating to organismal behavior, development and physiology. These data enables the identification of target genes with potential for development of novel genomics-based management strategies to reduce leafhopper impacts on agricultural crops. To that end, we report on our advances on leafhopper genomic research involving a vector of Pierce's disease, the sharpshooter, *O. nigricans.* We produced a dataset containing 4,411 high-quality ESTs, representing a set of 3,301 transcripts. Assembly identified 14 full length transcripts which have important biological functions. Research centering around the annotation, characterization, and application of the genomic information is demonstrated by work on a *delta 9 desaturase* and an *Imaginal Disc Growth Factor*, IDGF, isolated from *O. nigricans.* The sequences reported in this paper have been submitted to NCBI's dbEST under the following accession numbers: DR755012-DR759538

INTRODUCTION
Sharpshooter leafhoppers (Cicadellidae) are vectors of a number of destructive plant diseases. Although the glassy-winged sharpshooter, GWSS, *Homalodisca vitripennis* Germar, is the most notorious of these vectors due to its detrimental impacts to commercial crops in California, a second leafhopper commonly referred to as the black-winged sharpshooter, *Oncometopia nigricans* Walker, has also been associated with pathogen transmission. Like GWSS, *O. nigricans* is a highly polyphagous xylem-feeding leafhopper present throughout the southeastern United States (Tipping et al., 2004). Upon feeding, bacteria are taken up into the insect's mouthparts where they attach to the walls of the cibarium (Brlansky et al., 1983). During subsequent feedings, the bacteria are released into the plant. The transfer of xylem-inhabiting bacteria, including various *Xf* Wells pathotypes, is the cause of several economically important diseases including Pierce's disease (PD) of grape, phony peach disease, plum leaf scald, and periwinkle wilt. In addition, *O. nigricans* has the ability to vector citrus variegated chlorosis (CVC) *Xylella sp*. to citrus with a transmission rate of 20.3%, a much greater efficiency than most other species tested to date (Brlansky et al., 2002). These findings demonstrate the importance of *O. nigricans* as a vector species with significant implications for the sustainability of Florida's citrus industry. The importance of having genomic information generated from expressed sequence tag, EST, studies has been definitively demonstrated through such studies as *Drosophila,* Honey bee, and other organisms. Production of genomic information on *O. nigricans* which is now available (Hunter et al., NCBI) which was derived from single-pass sequencing of cDNA clones prepared from this sharpshooter and provides an invaluable resource for the identification of genes associated with the biology of the adult life stage. Annotation of the resultant dataset will also help in the identification of the gene/enzyme pathways involved in processes such as insect pathogen and insect-plant interactions. The availability of genomic data on *O. nigricans* provides the scientific community a foundation for future studies in functional genomics, and provides the genetic basis for tool development to advance creation of novel genomics-based management strategies for this and other leafhopper vectors of plant diseases. To that end, herein we report on full-length transcripts of 14 putative proteins, and the annotation of a dataset produced from *O. nigricans* and show their potential for quantification of global gene expression patterns, functional and comparative genomics studies.

OBJECTIVES
Use a genomics approach to advance our understanding of the genetic basis of sharpshooter leafhopper biology. The genetic products produced provide the needed information to conduct further functional genomic studies. The results from this study support development of emerging management strategies to reduce leafhoppers and the spread of Pierce's disease.

RESULTS
The assembled sequences were annotated using BLASTX, TBLASTX, and BLASTn analyses. Translated proteins were analyzed with BLASTP, and EXPASY. Sequences which had an E-value ≤-10 were considered as significant. Only one table is shown, Table 1, which list the significant matches to terms related to 'Biological Processes.' The putative full – length sequences which have been annotated and published in the NCBI database are shown in Table 2. The two genes highlighted in yellow, (Table 2) have been mined from the dataset, and used to characterize genes with important functions in leafhopper biology, IDGF and Delta-9 desaturase (Figure 1, and Table 3).

Imaginal Disc Growth Factor, IDGF, protein structure. Several families of peptide growth factors are implicated in regulating cell growth and proliferation of cells in culture. Genetic studies in *Drosophila* implicate some of these factors in growth control *in vivo* and report a new family of growth factors, related to chitinase enzymes, required by *Drosophila* imaginal disc cells in culture. The importance of IDGF in insect development, and in the correct formation of wings makes them an ideal target for disruption, wherein adult insects would be unable to fly, thus reducing their ability to disperse. As research on IDGFs and their interactions continues to advance, so will our understanding of insect development at the cellular level. New IDGFs are being identified but there are still only a small handful known (Huang et al., 2006; Kawamura et al., 1999). During larval stages, the cells of the imaginal disc primordia undergo extensive growth and proliferation, increasing in number by three orders of magnitude.(Bryant 1978). The rapid proliferation is accompanied by patterning events that control the organization of cells in the growing disc. Although the application of genomics approaches in recent years has produced significant advances in identifying the molecules and mechanisms involved in patterning, and the role of the imaginal discs, there is still much to understand about how cell proliferation is regulated, and the cell-to-cell signaling in the development of specific structures, such as wings.

Expression of Δ9 Desaturase in Sharpshooters- The importance of producing and comparing multiple species of sharpshooters is demonstrated in the annotation of two cDNA libraries from *Oncometopia nigricans* and the glassy-winged sharpshooters (GWSS), *Homalodisca vitripennis* (Hemiptera: Cicadellidae). Comparisons lead to the isolation and description of a *delta 9 desaturase* transcript from each leafhopper. The desaturase gene expression data is shown for the GWSS leafhopper, covering the five developmental nymphal stages which feed on a variety of host plants. Sharpshooter leafhoppers are economically important agricultural pests due to their ability to transmit *Xf*, and other plant pathogens during feeding. Currently very little is known of leafhopper developmental physiology. Since desaturases play a key role in insect development and nutrition we chose to examine the expression of *Δ9 desaturase* in the GWSS.

CONCLUSIONS
Data mining of the genomic data produced from EST examinations provides rapid, cost effective insight into an organism's biology, pathology and development which would be difficult with any other methods. The genomic data set for *O. nigricans* has already produced valuable information on an important vector of plant diseases. This data also provides the first experimental access to these genes and builds the foundation for more in-depth molecular and functional genomic analysis by the research community. Moreover, it identifies genes that are critical in the physiology, reproduction, development, of leafhoppers. Genetic information is crucial to advancing our understanding of sharpshooter biology, and will play a major role in the development of future non-chemical, gene-based control strategies against leafhopper pests.

Table 1. Biological Process of ESTs from *O. nigricans* ESTs

Gene Ontology Term[a]	# ESTs	% of total ESTs represented[b]	# contigs	# singlets
[p] Behavior	8	0.41%	2	3
[p] Cellular Process				
[c] Cell communication				
[i] Cell adhesion	15	0.78%	2	9
[i] Cell-cell signaling	29	1.50%	2	2
[i] Signal transduction	60	3.10%	7	17
[c] Cell differentiation				
[i] Sporulation	1	0.05%	0	1
[c] Cellular physiological process				
[i] Cell death	3	0.16%	0	3
[i] Cell growth and/or maintenance				
[ii] Cell homeostasis	24	1.24%	2	1
[ii] Cell organization and biogenesis				
[iii] Cytoplasm organization and biogenesis	50	2.59%	6	13
[ii] Cell proliferation	29	1.50%	2	13
[ii] Transport	8	0.41%	2	3
[ii] General (no further information provided)	12	0.62%	1	2
[i] Cell motility	20	1.03%	2	6
[p] Development				
[c] Cell differentiation	124	6.41%	2	4
[c] Embryonic development	5	0.26%	1	3
[c] Growth	2	0.10%	0	2
[c] Larval or pupal development (sensu Insecta)	4	0.21%	1	2
[c] Mesoderm development	29	1.50%	6	4
[c] Organ development				
[i] Organogenesis				
[ii] Heart development	1	0.05%	0	1
[ii] Hemopoiesis	1	0.05%	0	1
[ii] Muscle development	12	0.62%	1	1
[ii] Neurogenesis	31	1.60%	5	4
[c] Pattern specification	1	0.05%	0	1
[c] Reproduction	2	0.10%	0	2
[c] General (no further information provided)	2	0.10%	1	0
[p] Physiological process				
[c] Coagulation	1	0.05%	0	1
[c] Death	43	2.22%	8	10
[c] Homeostasis	22	1.14%	4	3
[c] Localization	106	5.48%	11	21
[c] Metabolism				
[i] Alcohol metabolism	1	0.05%	0	1
[i] Amine metabolism	5	0.26%	1	0
[i] Amino acid and derivative metabolism	33	1.71%	5	15
[i] Biosynthesis				
[ii] Cuticle biosynthesis	2	0.10%	0	2
[ii] Nucleotide-sugar biosynthesis	1	0.05%	0	1
[i] Catabolism				
[ii] Macromolecule catabolism				
[iii] Protein catabolism	57	2.95%	9	30
[i] Cofactor metabolism	13	0.67%	3	4
[i] Electron transport	119	6.16%	6	5
[i] Heterocyte metabolism	1	0.05%	0	1
[i] Hormone metabolism	1	0.05%	0	1
[i] Lipid metabolism	72	3.72%	8	25
[i] Macromolecule metabolism				
[ii] Carbohydrate metabolism	172	8.90%	18	26
[ii] Protein metabolism				
[iii] Protein biosynthesis	232	12.00%	49	37
[iii] Protein complex assembly	1	0.05%	0	1

Table 1 (cont). Biological Process of ESTs from O. nigricans ESTs

Gene Ontology Terma	# ESTs	% of total ESTs representedb	# contigs	# singlets
[iii] Protein folding	13	0.67%	2	9
[iii] Protein modification	5	0.26%	0	5
[iii] General (no further information provided)	5	0.26%	0	5
[i] Neurotransmitter metabolism	1	0.05%	0	1
[i] Nucleobase, nucleoside, nucleotide and nucleic acid metabolism	109	5.64%	11	38
[i] Organic acid metabolism	3	0.16%	0	3
[i] Phosphorous metabolism	282	14.59%	11	15
[i] Sulfur metabolism	1	0.05%	0	1
[i] General (no further information provided)	1	0.05%	0	1
[c] Muscle contraction	78	4.04%	6	5
[c] Organismal physiological process				
[i] Organismal movement	6	0.31%	2	2
[c] Response to stimulus				
[i] Response to biotic stimulus	67	3.47%	12	21
[i] Response to endogenous stimulus	1	0.05%	0	1
[i] Response to external stimulus	1	0.05%	0	1
[i] Response to stress	3	0.16%	0	3
[p] Regulation of biological process	2	0.10%	0	2
Totals	1933	100.00%	211	395

[a]Classification hierarchial: indented terms are children [c] of parent terms [p] listed above. All functional assignments of *Oncometopia nigricans* ESTs described here are the "inferred from electronic annotation" (IEA) using the top 5 BLASTX hits with an E-value of ≤-10

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

Unknown Biological Process	481	10.90%	48	217
NSS	1307	29.63%	115	659
Virus	1	0.02%	0	1
Mt	1	0.02%	0	1
RRNA	686	15.55%	6	2
	4411	56.18%	381	1275

Table 2. Putative full-length protein sequences produced from *Oncometopia nigricans*, in silico characterization. Sequences published in the NCBI, public database. (Hunter et al., in prep).

Complete Putative Protein Sequences for WHON

Contig	NCBI Descriptor
[0010]	**IDGF-like protein**
[0015]	Actin, Muscle
[0017]	Actin, Cytoplasmic
[0018]	ADP/ATP translocase
[0021]	Rhodopsin
[0023]	**Delta-9 desaturase**
[0095]	Elongation factor 1-alpha (Posted)
[0124]	Ferritin GF2
[0158]	Fructose 1,6-bisphosphate aldolase
[0200]	Arginine kinase
[0409]	Glyceraldehyde 3-phosphate dehydrogenase
[0926]	Enolase
[1317]	CG7610
[1442]	RAB7

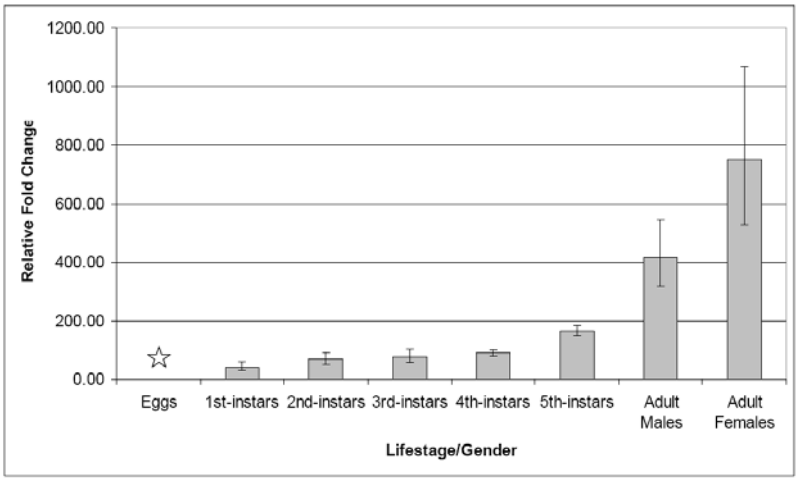

Figure 1. The isolated Δ9 desaturase cDNA from *O. nigricans* and GWSS. The cDNA encodes a 367 (aa) with 71% identity to *A. domesticus* desaturase, and significant (>50%) identity with other insect Δ9 desaturases. *In silico* analyses place GWSS Δ9 desaturase in the Family 1 of ProDomain fatty acid desaturases a Palmitoyl-CoA Δ9 desaturase-1. No detection of expression in eggs. All other instars expressed Δ9 desaturase at increasing levels for each instar. There was no significant difference in expression of Δ9 desaturase among nymphs, adults expressed significantly greater levels. Adult females showed a >7 fold increase over 1st-4th instars. There was no significant difference between adult sexes. Sequences published Acc. no. AAT01079.

Table 3. Sequences producing significant alignments:

			(Bits)	Value
gb|AAU95195.1|	delta-9 desaturase	*[Oncometopia nigrican*	744	0.0
gb|AAT01079.1|	delta-9 desaturase 1	*[Homalodisca vitripennis*	723	0.0
gb|AAK25796.1|AF338465_1	delta-9 desat.1	*[Acheta domesticus*	536	8e-151
gb|ABD72703.1|	fatty acid desaturase	*[Acyrthosiphon pisum*	518	2e-145

REFERENCES

Altschul, S.F., Madden, T.L., Schäffer, A.A, Zhang, J., Zhang, Z., Miller, W., Lipman, D.J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25:3389-3402.

Brlansky, R.H., Damsteegt, V.D., Hartung, J.S. 2002. Transmission of the citrus variegated chlorosis bacterium *Xylella fastidiosa* with the sharpshooter *Oncometopia nigricans*. *Plant Disease* 86: 1237-1239.

Brlansky, R.H., Timmer, L.W., French, W.J., McCoy, R.E. 1983. Colonization of the sharpshooter vectors, *Oncometopia nigricans* and *Homalodisca coagulata*, by xylem-limited bacteria. *Phytopathology* 73: 530-535.

Bryant, P.J. 1978. Pattern formation in imaginal discs. *In*: Ashburner M, Wright TRF, (eds.) *The genetics and biology of Drosophila*. London: Academic Press; 1978. p 230–336.

Held, LI. 2002. *Imaginal Discs. The Genetic and Cellular Logic of Pattern Formation*. Cambridge University Press. 460 pp. ISBN 0-521-58445-0.

Huang, *Z*, Hunter, WB, Cleland, CA, Wolinsky, M, Lapointe, SL, Powell, CA. 2006. A new member of the growth-promoting glycoproteins from *Diaprepes* root weevil (Coleoptera: Curculionidae). Florida Entomologist 89(2): 223-232.

Katsar, CS, Hunter, WB, Cleland, CA. 2007. Delta-9 desaturase expression and analysis in the glassy-winged sharpshooter, Homalodisca vitripennis (Hemiptera: Cicadellidae). (in prep).

Kawamura K, Shibata T, Saget O, Peel D, Bryant PJ. 1999. A new family of growth factors produced by the fat body and active on *Drosophila* imaginal disc cells. Development126:211–219.

Liu, W., Ma, P.W., Marsella-Herrick, P., Rosenfield, C., L, Knipple, D.C., Roelofs, W. 1999. Cloning and functional expression of a cDNA encoding a metabolic acyl-CoA Δ 9-desaturase of the cabbage looper moth, *Trichoplusia ni.* Insect Biochem Mol Biol 29: 435–443.

Tipping, C, Mizell, R.F., Andersen, P.C. 2004. Dispersal adaptations of immature stages of three species of leafhopper (Hemiptera: Auchenorryncha: Cicadellidae). Florida Entomologist 87(3): 372-379.

White J.A., Todd J., Newman T., Focks N., Girke T., de Ilarduya O.M., Jaworski J.G., Ohlrogge J.B., Benning C. 2000. A new set of Arabidopsis expressed sequence tags from developing seeds. The metabolic pathway from carbohydrates to seed oil. Plant Physiology 124: 1582-1594.

Whitfield C.W., Band M.R., Bonaldo M.F., Kumar C.G., Liu L., Pardinas J.R., Robertson H.M., Soares M.B., Robinson G.E. 2002. Annotated expressed sequence tags and cDNA microarrays for studies of brain and behavior in the honey bee. Genome Research 12: 555-566.

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