Gene expression in two leafhopper vectors of Pierce's Disease of Grapes: Glassy-winged sharpshooter and the Blue-green sharpshooter (Hemiptera: Cicadellidae)

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Introduction:

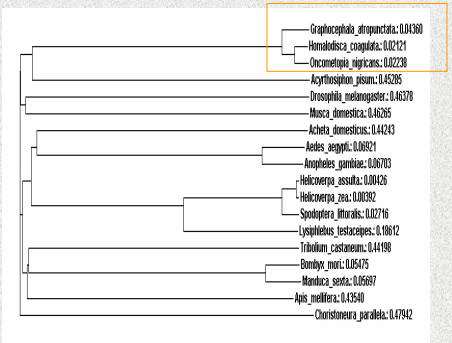
Over 350 genetic sequences were identified and determined to have significant similarity between two insect leafhopper species which transmit the plant-pathogenic bacterium, Xylella fastidiosa, known to cause Pierces Disease of grapes. Computer analyses permits comparison of new genetic sequences to those of known genes, wherein a putative function can be assigned. This first step permits the development of tools needed to conduct further functional genomic analyses on aspects of leafhopper biology and disease transmission. The glassy-winged sharpshooter, GWSS, and the blue-green leafhopper, BGSS, both spread the bacterium that causes 'Scorch-like' diseases in plants. A particularly severe type of this disease affects grapes, but these bacteria also reduce fruit production in many tree crops. Of interest is why some leafhoppers, like GWSS, can transmit this bacterium so efficiently, while other leafhoppers, like BGSS, do not. To examine this question we developed two genetic libraries to compare specific genes of interest within these two species. After computer analyses of over 13,000 sequences, we determined that 358 protein sequences isolated from these insects had significant identities. Many of these function in egg development, such as Vitellogenin proteins, and in the development of insecticide resistance, like Glutathione-s-transferase, and P450's. The results from this study on leafhopper gene expression produced a data set that advances research efforts in the identification of genes and physiological processes of leafhoppers of economic importance. The knowledge produced concerning these genes and proteins now enables functional genomic studies to be conducted, which focus on the development of new management strategies to reduce leafhopper populations and the diseases they spread.

Importance of Expressed Sequence tags (ESTs): Construction of cDNA expression libraries are an important tool for identifying and isolating insect genes. The data from these libraries will further accelerate research on Pierce's Disease interaction with its' vector species, like the GWSS and BGSS. These data are available through the public database, GenBank, National Center for Biotechnology and Information, These results lay the groundwork to develop environmentally friendly solutions to address important scientific questions related to the transmission of diseases by leafhoppers and other insects. The GWSS and BGSS libraries will help scientists understand the molecular basis of leafhopper growth and development, and will promote answer to be found for fundamental questions in insect physiology, biochemistry, cell biology, and pathology.

The GWSS (on bottom) is about three times as large as the BGSS. The GWSS flies farther and feeds on a wider variety of plants, Preferring to feed on cultivated crops which are commonly irrigated. Monitoring for these pests shows that the GWSS is more often detected within crops while BGSS detection is most often around the borders of fields.

Table 1. Subset of genes in common to GWSS and BGSS with e-values.

V-ATPase	6.67E-104
40S ribosomal protein S6	1.09E-95
60S ribosomal protein L10a	6.12E-107
90-kDa heat shock protein	1.06E-137
Acidic p0 ribosomal protein	1.03E-138
Activated protein kinase C recept	6.83E-85
ADP/ATP translocase	3.97E-93
ADP/ATP translocase	2.03E-79
AgCP10931	2.50E-104
AgCP14738	1.66E-95
AgCP15339	2.13E-159
Alpha oxoglutarate ferredoxin oxi	1.18E-109
Arginine kinase	8.81E-85
AT20865p	6.08E-88
AT21416p	1.12E-87
ATP synthase beta chain, mitoch	1.49E-103
ATP-binding component of putres	2.84E-108
Cathepsin L precursor	8.85E-132
Cathepsin L-like cysteine proteina	1.66E-95
CG11276-PA	3.16E-143
CG14934-PA	5.48E-86
CG17927-PJ	1.94E-88
Coatomer beta subunit	3.07E-110
COP9 complex subunit 6	3.72E-91
Cytochrome b	1.46E-81
Cytochrome b	2.57E-162
Cytochrome b	6.59E-95
Cytochrome c oxidase subunit I	3.39E-85
Cytochrome oxidase subunit I	6.44E-91
EbiP7022	4.88E-119
Elongation factor 1-alpha	1.86E-105
ENSANGP00000006082	6.63E-117
ENSANGP00000009989	1.96E-169
ENSANGP00000010852	1.97E-110
ENSANGP00000011432	4.69E-83
Fructose 1,6-bisphosphate aldola	3.51E-85
Glyceraldehyde 3-phosphate deh	6.27E-103
Heat shock 70 kDa protein cogna	4.63E-84
Heat shock protein 70	6.02E-88
Inositol oxygenase	1.56E-95
LD22815p	1.64E-79
Mitochondrial ATP synthase gam	2.40E-81
Mitochondrial porin	2.77E-102
Mitochondrial transcription factor	1.70E-84
Muscle actin	1.64E-120
NADH dehydrogenase 1	2.31E-92
NADH dehydrogenase subunit 5	0
Peptidyl-prolyl cis-trans isomeras	0
Phosphogluconate dehydrogenas	7.99E-91
Phosphoprotein	0
Proteasome subunit beta type 3	4.88E-142
Putative ferritin GF2	5.70E-160
Rhodopsin	0
Ribosomal protein L7	3.20E-122
Ribosome-associated protein P4(	1.22E-87
S3Ae ribosomal protein	8.25E-102
S3e ribosomal protein	3.44E-81
S8e ribosomal protein	1.78E-101
S9e ribosomal protein	2.50E-93
Ser/Thr-like protein kinase lyk4	3.63E-119
Serine/threonine protein phospha	2.97E-117
Similar to Drosophila melanogast	4.17E-86
Spectrin alpha chain	3.80E-99
Transferrin	9.59E-96
Translationally controlled tumor p	7.04E-100
Tropomyosin	3.08E-110
Tropomyosin	8.78E-105
Tubulin alpha-1 chain	1.04E-107
Vacuolar ATP synthase subunit E	5.67E-79
Zgc:56101	2.08E-113
Calmodulin 1 (CaM 1)	9.89E-111
Cytoplasmic aminopeptidase	1.16E-100
Delta-9 desaturase 1	2.25E-94
Vacuolar ATP synthase subunit D	5.07E-79



Example of amino acid alignment for Delta 9 Desaturase. Orange box shows the grouping of the three desaturase amino acid sequences from Leafhoppers forming a single clade. ClustalX, with numbers representing distances on tree.

Genetic sequences are being widely used in phylogenetic studies, development of molecular markers, and to elucidate insect biology.

Summary: As each new set of sharpshooter cDNA libraries are completed, each will be posted to GenBank for public access. The GWSS data set started with over 8,000 sequences along with an additional 5,900 from the BGSS, together these aligned to produced 4,450 quality sequences, with approximately 350 significant homologs and 2,089 unique sequences (did not match any currently known sequence in GenBank). GWSS nymphal cDNA libraries are currently being constructed to identify genes being expressed during development. Scientific Impact: The USDA, ARS, U.S. Horticultural Research Laboratory effort has developed national collaborations to establish a database of expression libraries from important vector insects of plant diseases, primarily the Hemipterans (Leafhoppers, Aphids, Whiteflies, Psyllids, Mealybugs). The ability to identify and studying insect genes at the molecular level will yield many significant advances, including discoveries about leafhopper biology, development, endosymbionts, pathology, and on pathogen interactions.