Horizontal Gene Transfer and Gene Duplication of Plant Cell Wall Degrading Enzyme Genes in an Invasive Insect Pest

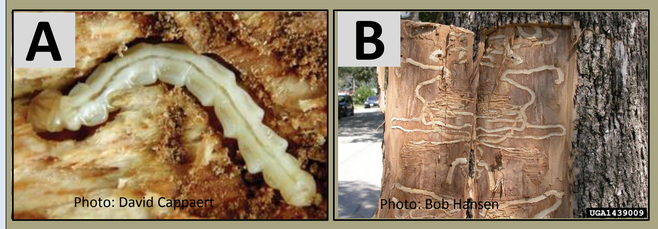
THE OHIO STATE UNIVERSITY
COLLEGE OF FOOD, AGRICULTURAL, AND ENVIRONMENTAL SCIENCES

Chaoyang Zhao* and Omprakash Mittapalli
Department of Entomology, OARDC, The Ohio State University
*E-mail: zhao.1369@osu.edu

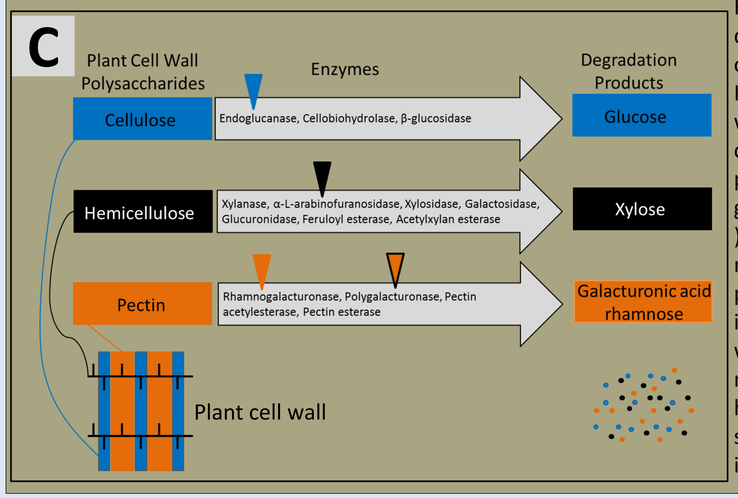
USDA APHIS

Abstract: Consumption of plant cell walls requires a suite of enzymes, including cellulases, hemicellulases, and pectinases. Using endogenous genes that encode plant cell wall degrading enzymes (PCWDEs) is less common in insects than establishing symbiosis with microorganisms for plant cell wall degradation. In this study four families of PCWDE genes were identified from the transcriptome of *Agrilus planipennis* larval midgut and subsequently confirmed in the genome sequence. Phylogenetic analysis and other evidence indicated that these genes were initially acquired by the insect through horizontal gene transfer from microorganisms and later expanded in the genome through gene duplication. Quantitative RT-PCR analysis on four selected genes in three gene families showed that they were almost exclusively expressed in the larval midgut and during larval stages of development. These results, together with the presence of N-terminal signal peptides in the deduced protein sequences, suggest that these gene products are secreted into the larval midgut, facilitating digestion of the host plant cell walls.

Introduction

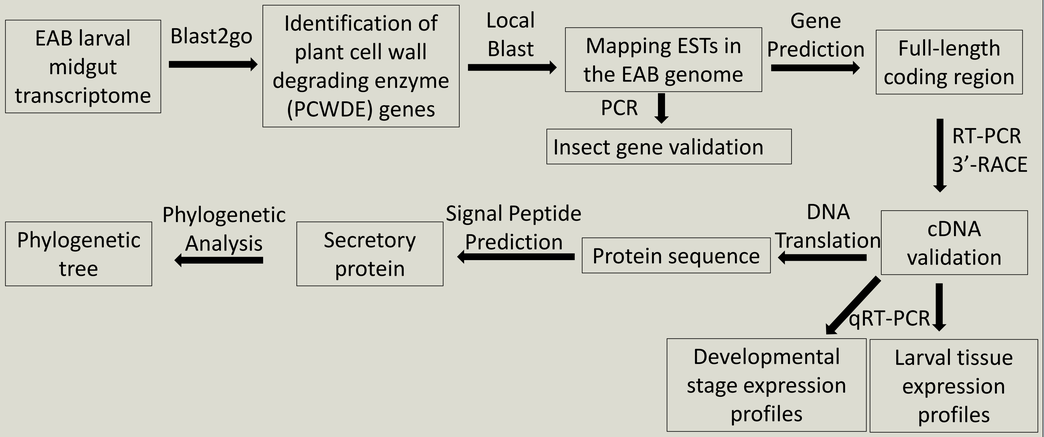


The emerald ash borer (EAB), *Agrilus planipennis*, is an invasive Coleopteran insect species originally from Asia. During the larval stage, it feeds on the phloem of ash tree (*Fraxinus sp.*) (**Fig. A**), producing galleries that eventually girdle and kill branches and entire trees (**Fig. B**).



How *A. planipennis* larvae degrade the plant cell wall and obtain energy is of high interest. It requires a suite of enzymes with diverse substrates, including cellulase, hemicellulase and pectinase (**Fig. C**). In this study, genes encoding endoglucanases (▼), α-L-arabionfuranosidases(▼), rhamnogalacturonases (▼), and polygalacturonases (▼) were identified in *A. planipennis*, which were acquired from microorganisms through horizontal gene transfer and subsequently duplicated in the insect genome.

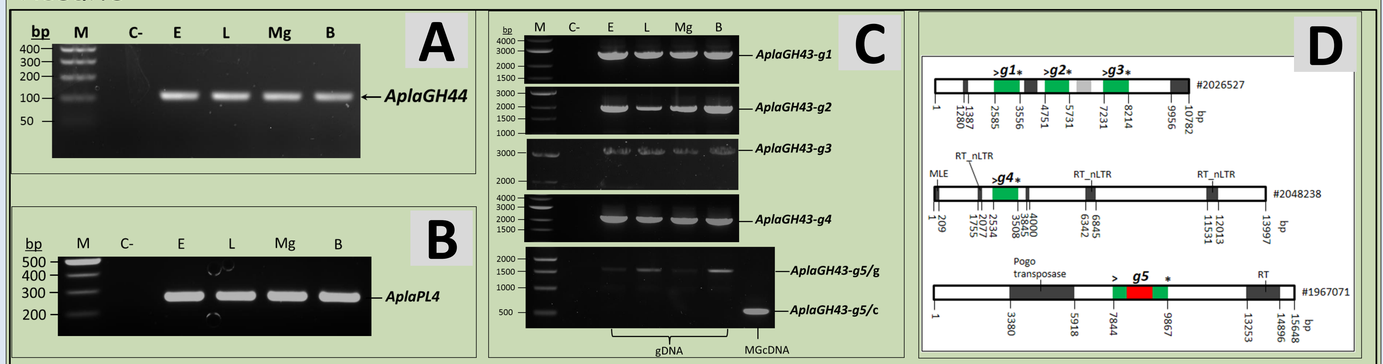
Methods



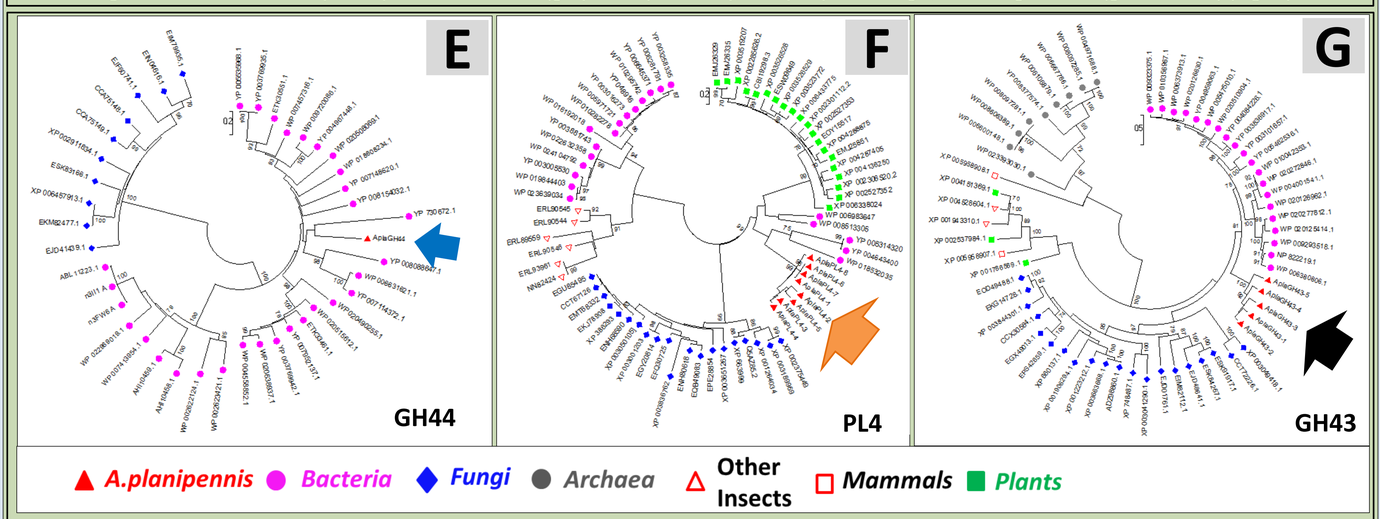
Symbol	Name	Gene Family	Copies in insect genome	Enzyme
▼	*AplaGH44*	GH44	≥10	Endoglucanase
▼	*AplaGH43*	GH43	≥5	α-L-arabinofuranosidase
▼	*AplaPL4*	PL4	≥8	Rhamnogalacturonase
* ▼	*AplaGH28*	GH28	≥35	Polygalacturonase

* Characterization of AplaGH28 genes is not shown in the results

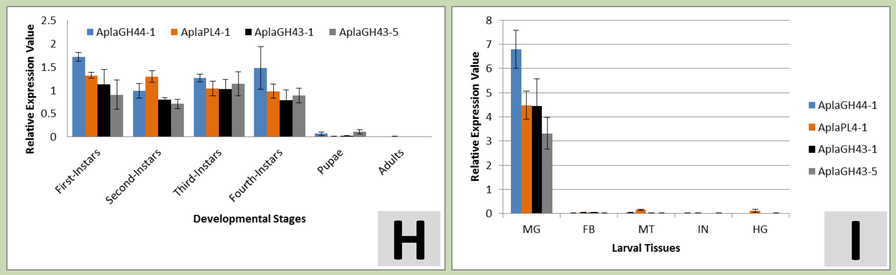
Results



Fragments of one *AplaGH44* gene (**Fig. A**), one *ApaPL4* gene (**Fig. B**) and five *AplGH43* genes (**Fig. C**) were amplified from genomic DNA isolates of *A. planipennis* adult elytra (E), adult legs (L), larval midgut (Mg), an adult whole body (B), ruling out the possibility that these genes are from larval midgut symbiont contaminant. The negative control (C-) containing no DNA template in the PCR reactions was included. Due to an intron (**Fig. D**, red box) present in *AplaGH43-g5*, the amplicon of midgut cDNA (*AplaGH43-g5/c*) has a lower size than that of genomic DNA (*AplaGH43-g5/g*, **Fig. C**). Mapping of five *AplaGH43* genes in the genome sequence showed that *AplaGH43-g1*, *AplaGH43-g2* and *AplaGH43-g3* reside in the same scaffolds. These five genes are surrounded by transposable elements (gray boxes) and they, except *AplaGH43-g5*, have single exons (green boxes) (**Fig. D**).



Phylogenetic analysis of GH44 (**Fig. E**), PL4 (**Fig. F**), and GH43 (**Fig. G**) genes showed that the *A. planipennis* genes (indicated by arrows) are clustered together and closely related to their bacterial homologs, suggesting that gene duplication post-HGT expanded these genes in the insect genome.



Quantitative RT-PCR analysis showed that four *A. planipennis* PCWDE genes, including one GH44 gene, one PL4 gene and two GH43 genes are almost exclusively expressed in larval stages of development (**Fig. H**) and midgut of larval tissues (**Fig. I**). These, together with the presence of N-terminal secretory signal peptides in the putative proteins (data not shown), indicate that they are secreted in larval midgut for plant cell wall digestion.

Conclusion

A. planipennis acquired PCWDE genes from microorganisms through HGT and duplicated them in the genome. The HGT-associated gene duplication has facilitated plant cell wall digestion in larval midgut.

Acknowledgements

USDA-APHIS for the financial support