Change in protease gene expression in an insect pest to plants in the potato/tomato family (Solanaceae)

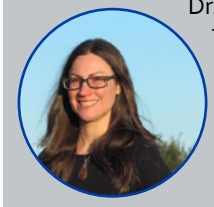
Dana Morshed, *Department of Biology*
Allison Hansen, *Ph.D., Department of Entomology*

ABSTRACT

Lineage-specific gene expansions may allow insects to adapt and diverge functional traits. The Hansen lab's chromosomal assembly of the sap-sucking potato psyllid (*Bactericera cockerelli*) previously identified three rapidly evolving genes associated with protease expression. The current study further analyzed whether or not plant diet and insect life stage might impact protease expression. We fed insects different plant diets of either potato or tomato at two insect life stages (2nd instar and young adult) to test for differences in insect protease gene expression. First, RNA extractions were conducted, followed by quantitative PCR (qPCR). The study used the Δ-Δ Ct method to analyze the relative comparison of gene expression between two genes and the ANOVA test to determine if there is a relationship between the changes in gene expression. Preliminary data revealed a significant difference between the host plant diets potato and tomato for 2nd instar nymphs for two of the three candidate genes. This project begins laying the foundation for identifying the function of species-specific protease genes that are evolving rapidly in the insect, and ultimately these genes may be involved in host plant adaptation.

KEYWORDS: *Bactericera cockerelli*, lineage-specific gene expansion, carboxypeptidase, quantitative PCR, delta-delta Ct method, ANOVA test

FACULTY MENTOR - Dr. Allison Hansen, Department of Entomology



Dr. Hansen is an Associate Professor in the Department of Entomology at the University of California, Riverside. She is interested in ecological and evolutionary genomics of insect-microbe interactions to provide novel insights into the complexity and dynamics of insect-microbe symbioses. Her research investigates the molecular mechanisms that underpin insect-microbe co-evolution using comparative and functional genomics in an evolutionary framework.

Dana Morshed

Dana Morshed is a fourth year Biology Major. She has been researching under Dr. Hansen since her sophomore year. She has received funding from the UC Riverside Undergraduate Education Mini Grant program and presented her research at the Southern California Conferences for Undergraduate Research. She is a member of the University Honors program and serves as an Honors Ambassador. In addition to being involved in various clubs on campus, she is currently a volunteer at a free clinic and also works as a medical scribe. She plans to pursue a career in medicine.

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INTRODUCTION

Lineage-specific gene expansions are the new appearance of genes in a lineage that is not in a sister lineage (Jordan et al., 2001). These lineage-specific genes are involved in pathogen interactions and stress resistance. (Lespinet et al., 2002). Lineage-specific gene expansions allow eukaryotes to adapt and are regarded as a critical reason for organizational and regulatory diversity (Lespinet et al., 2002).

A recent study from my laboratory found several gene expansion events in an insect called *Bactericera cockerelli* (Kwak et al., 2022). This insect is known to house and vector microbes (Hansen et al. 2008) and belongs to the insect group called psyllids, which are small, plant sap-sucking insects that fly and are often recognized as pests on agricultural plants because of their ability to vector bacterial plant diseases (Trumble et al., 2011). This psyllid species feeds on the *Solanaceae* or nightshade plant family, including potatoes and tomatoes (Pletsch, 1947). This study focuses on these gene expansions to ultimately better understand host-plant relations.

The first chromosomal assembly of the psyllid *B. cockerelli* from my laboratory found that *B. cockerelli* has significantly more gene expansion events than other hemipteran species examined. This study noted that 75% of these gene expansions are transposable elements found throughout *B. cockerelli's* chromosomes which may contribute to the large size of the genome relative to other psyllids sequenced (Kwak et al., 2022). For genes that could be annotated, several gene expansion events included genes associated with transcription factors, proteases, and odorant receptors (Kwak et al., 2022). For example, gene expansions of one protease family were predicted to be related to carboxypeptidase D (Kwak et al., 2022). Proteases are enzymes that break peptide bonds (Bond, 2019). Carboxypeptidases are proteases that remove the C-terminal amino acid from proteins (NIH, 1999). These carboxypeptidases play a vital role in various physiological functions in organisms (Sapio & Fricker, 2014). I hypothesize that the expansion of protease gene families in *B. cockerelli* is important because of its impact on species-specific responses to host plant defenses. For example, both tomato and potato plants, which are the psyllid's host plants, produce carboxypeptidase inhibitor proteins in response to insect feeding (Díez-Díaz et al., 2004; Graham & Ryan, 1981). Since these inhibitors can cause antifeedant effects for insects (Zhu-Salzman & Zeng, 2015), *B. cockerelli* may have an evolutionary arms race of rapidly evolving carboxypeptidase families in response to solanaceous host plant defenses (Kwak et al., 2022).

This study analyzes if there are insect life stage and host plant feeding differences in insect gene expression for three rapidly evolving genes in *B. cockerelli*. It is hypothesized that these proteases play a critical role in host-plant interactions and are significant in responding to host-plant feeding. Based on their function, certain genes are expressed in varying abundances throughout different insect life stages, so this study analyzed both nymphs and adults. Since the function of the candidate protease genes examined here is unknown, it is important to determine if there is a difference in gene expression. Furthermore, although *B. cockerelli* feeds on potatoes and tomatoes, it may exhibit different responses to each host. A significant difference in expression between life stages for all candidate protease genes on both host plants was predicted. Gene expression was quantified using quantitative polymerase chain reaction (qPCR) to test this hypothesis. Identifying and understanding these unknown genes and their functions can advance our knowledge of the psyllids' relationship with plants. Understanding this relationship is vital because psyllids impact agriculture since they are considered pests.

MATERIAL AND METHODS

This approach involved first growing colonies of psyllids from the same genetic line on plant diets of potato and tomato plants. The psyllids were obtained from the University of California (UC), Riverside, from the same genetic line

as the published genome (Kwak et al., 2022). The colony was confirmed to be free of the plant pathogen *Candidatus Liberibacter psyllaurous* (Hansen, 2008) via Real-time PCR as described in Kwak et al. (2022).

The two established lines were maintained on 12-week-old *Solanum tuberosum* (Russet potato) and *Solanum lycopersicum* (Moneymaker tomato) plants at 25°C under a 16L:8D light/dark cycle in an incubator. After three generations of rearing on either tomato or potato plants, the colony stabilized and was ready for experiments. I used mesh cages, tomato and potato seeds, soil, pots, and planting materials to grow and maintain the colonies, as detailed in Kwak et al. (2022).

Three biological samples (replicates) of 2nd instar nymphs and young adults were randomly selected per host plant treatment, totaling 12 samples. For 2nd instar nymphs, 20 insects were pooled, and for young adults, four were pooled per biological replicate. The life stages of the psyllids were confirmed under the microscope before pooling. Specifically, the psyllids were tracked daily by recording the number of psyllids and their life stage. After hatching from the egg, the nymph was in the 1st instar stage for around three days and then stayed in the second for approximately 2.5 days (Knowlton and Janes, 1931). After about 13 days, the psyllids became young adults. The stages were monitored by looking at the leaves of the plants with nymphs under a microscope.

Psyllid samples were extracted for total RNA. The extractions were performed with the Zymo Quick-RNA Miniprep kit (Zymo Research, Irvine, CA, USA) and DNase 1 treated to remove DNA contamination from samples. RNA was purified using the RNA Clean & Concentrator-5 kit (Zymo Research, Irvine, CA, USA). The concentration and quality of the RNA were checked using the SpectraMax QuickDrop Micro-Volume Spectrophotometer (Molecular Devices, San Jose, CA, USA).

Reverse transcription was then used to create cDNA from the RNA. This was done using the iScript™ Reverse Transcription Supermix with the Bio-Rad C1000 Thermal Cycler with Dual 48 Well Block machine (Bio-Rad Laboratories, Hercules, CA). The cDNA was then used for quantitative polymerase chain reaction (qPCR) to examine gene expression. Three technical replicates were run for qPCR with similar reagents and conditions for each biological replicate, as described in Kwak et al. (2022). Three plates were run to analyze each gene of interest alongside the housekeeping gene on each plate.

The Δ-Δ C_t or comparative C_t method was used to analyze the qPCR data results. The fold change of gene expression was calculated relative to the calibrator (Bookout, 2006). In this case, the calibrator was the adult treatment group to determine if expression differed in the 2nd instars for each host plant. For host plant comparisons, tomato adults were used as the calibrator to determine if there was a difference in expression among all treatment types. IBM SPSS Statistics Program (Version 28) was used to test for significance in the difference in gene expression. A two-way ANOVA test was used with *post hoc* analysis.

RESULTS

Candidate protease gene ANN00645:

When examining the fold change of gene expression relative to tomato adults among all treatment groups (**Figure 1**), using a two-way ANOVA test, there is no significant difference ($P>0.05$) in the expression of the gene (ANN00645) between host-plant treatments or life stages.

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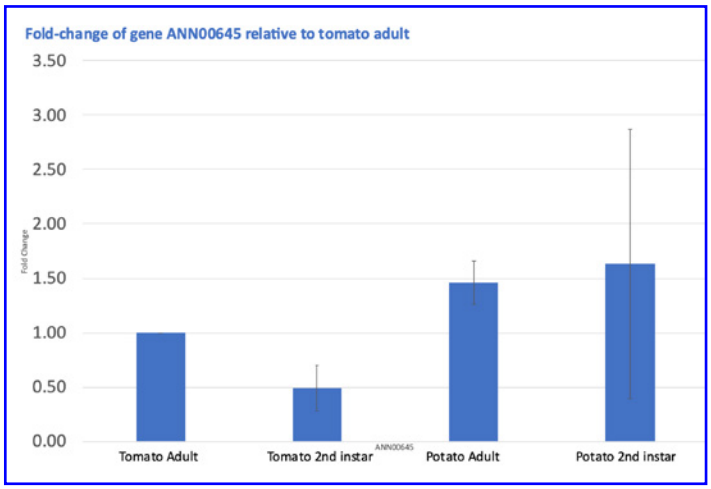

Figure 1. Fold change of Gene ANNoo645 relative to the tomato adults among all treatments

Candidate protease gene ANN17948:

When examining the fold change of gene expression relative to tomato adults among all treatment groups (**Figure 2**), using a two-way ANOVA test, there is a significant difference in the expression of the gene (ANN17948) between host-plant treatments (P=0.028), but not life stages (P>0.05). Compared to potatoes at the same life stage, a lower candidate protease gene expression level occurred when psyllids fed on tomato at 2nd instar.

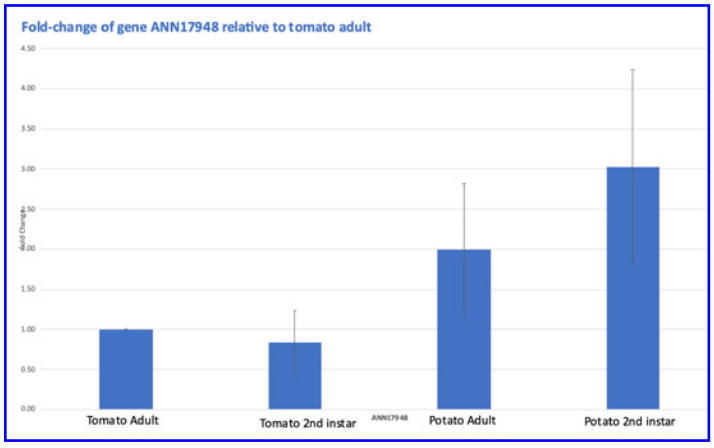

Figure 2. Fold change of Gene ANN17948 relative to tomato adults among all treatments

Candidate protease gene ANN17915:

When examining the fold change of gene expression relative to tomato adults among all treatment groups (**Figure 3**), using a two-way ANOVA test, there is a significant difference in the expression of the gene (ANN17915) between host-plant treatments (P=0.018), but not life stage (P>0.05). In sum, a lower gene expression level occurred when the psyllid fed on tomato at the 2nd instar compared to all other treatments.

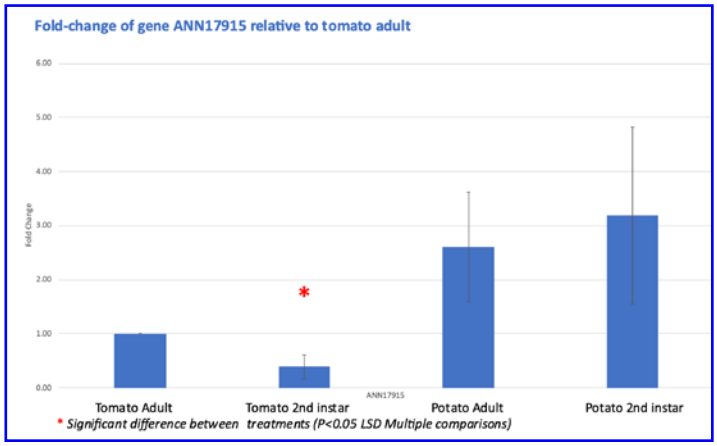

Figure 3. Fold change of Gene ANN17915 relative to the tomato adults among all treatments

DISCUSSION

When comparing the fold change of psyllid gene expression for three rapidly evolving protease gene candidates, my preliminary data suggests a significant difference between host plant treatment for candidate protease genes ANN17948 and ANN17915. The difference in gene expression between the two host plants shows that these genes may have a more specific host plant response to feeding, especially at 2nd instar.

This difference in gene expression for genes ANN17948 and ANN17915 may be attributed to the evolutionary history of tomato and potato plants. Although research is ongoing, differences in host plant history can be credited

to past hybridization events and varying speciation rates (Rodriguez et al., 2009). Furthermore, studies on tomato defense mechanisms show that regulators of gene expression are largely responsible for defensive responses to pathogens (Campos et al., 2022). Future studies can further narrow down the function of these psyllid protease genes through methods such as RNA sequencing and studies involving RNAi and CRISPR. Also, future studies should use larger sample sizes to reduce variation.

In conclusion, two candidate genes of *B. cockerelli* (Kwak et al., 2022) displayed differential gene expression (**Figures 1-3**). These gene candidates may be involved in host plant adaptation which has implications across multiple disciplines, including evolution, entomology, and agriculture. Studying these newly discovered genes adds to our understanding of the putative function of species-specific protease genes that are evolving rapidly in *B. cockerelli* (Kwak et al., 2022). In future studies, it is essential to deduce what these genes do to further elucidate insect-plant evolution and the psyllids' role as an agricultural pest.

ACKNOWLEDGMENTS

I would like to thank my wonderful faculty mentor, Dr. Allison K. Hansen, for giving me the opportunity to learn and grow throughout my undergraduate career. I would also like to thank my graduate student mentor, Younghwan Kwak, for her guidance and patience. I want to thank the Mauck Lab for providing me with psyllids to begin my project. Finally, thank you to the UC Riverside Undergraduate Education Mini-Grant committee for funding this research.

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