Gene expression of Sodium-Dependent Cation-Chloride Cotransporters (aeCCC1-3) in Adult and Larval Tissue of Yellow Fever Mosquitos (*Aedes aegypti*)

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Abstract

Yellow Fever Mosquitoes, *Aedes aegypti,* are particularly good at regulating their blood salt concentration under challenging environmental conditions such as living in salty water or when a female takes in a blood meal. Due to the importance of salt balance, a novel pesticide that blocks salt transport and is innocuous to other organisms would be appealing. Moreover, although much is known about salt transporters in vertebrate animals, transporters in invertebrate animals are less studied. Therefore, our goal is to characterize three Na^+-dependent cation-chloride cotransporter (aeCCC1-3) proteins involved in mosquito salt transport in hopes to gain insight into each protein's role in mosquito salt balance. Previous research has suggested larger amounts of aeCCC3 in larvae, particularly in anal papillae, which could suggest the need for a specialized protein during a mosquito's aquatic larval life stage. In this study, we measured the relative gene expression of aeCCC1-3 mRNA and seek to further develop previous studies of these cotransporters. We found almost 58-fold increase between the expression of aeCCC3 in larvae compared to adults and a 192-fold increase between aeCCC3 of anal papillae to Malpighian tubules. The results of this study may provide initial insights into how mosquito transporters are specialized for aquatic larval and terrestrial adult stages.

E. A. Goeldi (1905)

Background

Environmental pressures at larval and adult stage

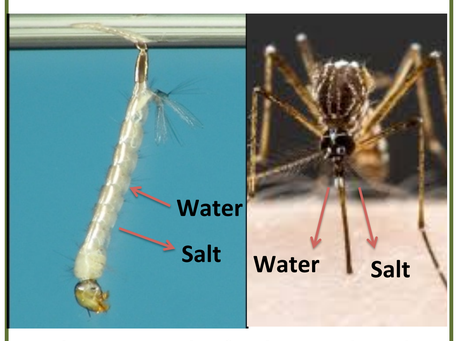


Figure 1. In the freshwater larval stage, mosquitoes gain water and lose salt.[1] Adult mosquitoes lose both water and salt to the air.[2]

Renal System of *Aedes aegypti*

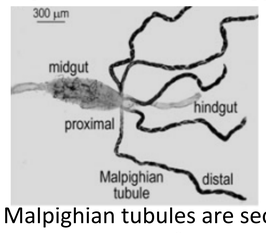
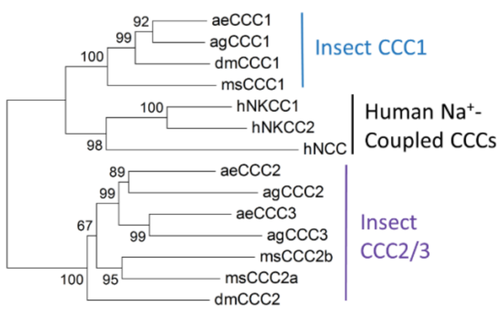


Figure 2. Malpighian tubules are secretory in function.[3]

Figure 3. Anal papillae are absorptive in function.[4]

Phylogenetic Analysis of aeCCC1-3



Gillen C. and Piermarini P. Unpublished data

Figure 3. Phylogenetic analysis of Cation Chloride Cotransporter amino acid sequences. Analysis shows Insect CCC1 is closer to human NKCC than Insect CCC2/3. Abbreviations: *Manduca sexta* (ms), *Drosophila melanogaster* (dm), *Aedes aegypti* (ae), *Anopheles gambiae* (ag), *Homo sapiens* (h).

Research Question

- Our research explores how aeCCC1, aeCCC2, and aeCCC3 function in the mosquito renal system via quantitative analysis of their respective mRNA.
- **Hypothesis**: Based on our phylogenetic analysis, we expect aeCCC1 to be involved in the excrement of salt in both larval and adult Malpighian tubules. Based on preliminary tissue distribution studies, We expect aeCCC2 to be an absorptive protein present in both adults and larvae (Akuma 2014). Finally, we expect aeCCC3 to be an absorptive protein that is highly expressed in larvae compared to adults in order to account for their aquatic larval stage.

Relative mRNA Expression between Adults and Larvae

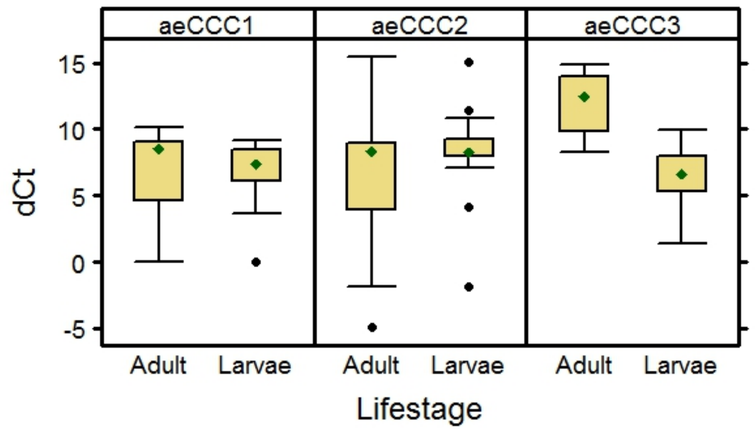


Figure 4. Quantification of aeCCC1-3 mRNA in adult and larvae *A. aegypti*. Values are expressed in dCt vales (the difference of the cycle (Ct) of target sample from the Ct of the endogenous control). Ct is defined as the first cycle at which product can be detected. Whole mosquitoes are expressed in fold differences of mRNA expression of respective aeCCC1-3. *Note:* lower dCt values indicate higher expression levels. Study shows that there is 58-fold more aeCCC3 mRNA in larvae than in adults. SEM (n=13).

Relative mRNA Expression between Larval Malpighian Tubules and Anal Papillae Tissues

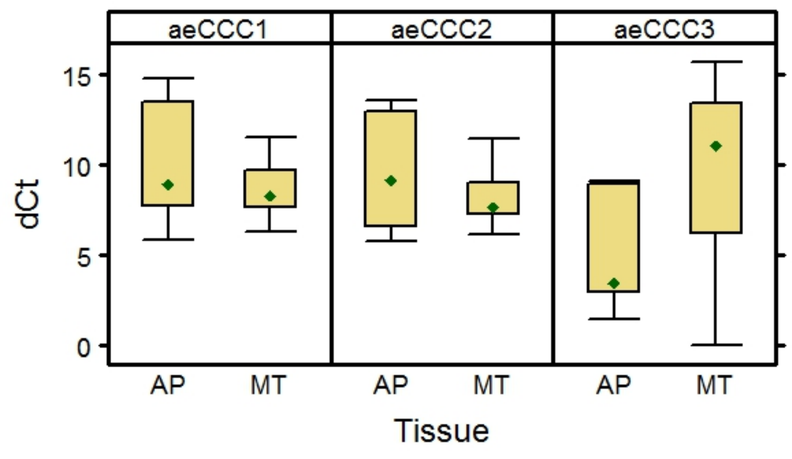


Figure 5. Quantification of aeCCC1-3 in larvae anal papillae and Malpighian tubules. *Note:* lower dCt values indicate higher expression levels. Study shows that there is 192-fold more aeCCC3 mRNA in larvae than in adults. See above figure for dCt clarification. SEM (n=9).

Methods

Animal rearing: Liverpool *Aedes aegypti* hatched and kept at 28 °C and 80% humidity on a 12:12 light:dark cycle. Larvae were sacrificed and dissected at the 4th instar stage. Adults were sacrificed 4-10 days after emerging.

Primer design: Four Primers were designed for each mRNA transcript in all areas of the transcript. Sequences found on VectorBase. (aeCCC1: AAEL006180, aeCCC2: AAEL009888, aeCCC3: AAEL009886)

Quantitative PCR: Dissected and whole tissue is placed in Trizol® and genetic material is isolated. RNA is extracted with a TURBO DNA-Free™ kit (ThermoFisher Scientific). RNA is refined with a RNA Clean & Concentrator™-5 kit (Zymo Research). RNA is reverse transcribed with a TaqMan® Reverse Transcription kit (ThermoFisher Scientific). Finally, cDNA is quantitatively compared to the endogenous control, aeRps5, with designed primers and a SYBR® GreenER™ qPCR SuperMix kit.

Amplification aeCCC1-3 mRNA in whole females

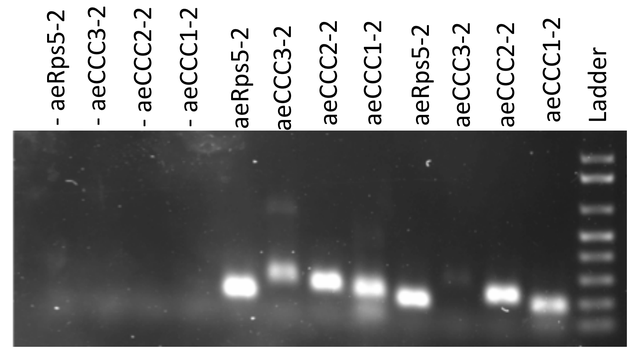


Figure 6. Agarose gel electrophoresis of PCR products for the detection of aeCCC1, aeCC2, and aeCCC3 in whole female cDNA using aeCCC-2 primer products. Control lacked Taq Polymerase.

Summary and Future Directions

- Our quantitative PCR results suggest that our hypothesis holds. aeCCC1 is equally expressed in both adults and larvae and in larval renal tissues. aeCCC2 has more variation in whole animals but is relatively equally expressed in adults, larvae, and larval renal tissues. Moreover, aeCCC3 is approximately 58 fold higher in whole larvae than adults. Even more interesting is that aeCCC3 is expressed 158 fold more in anal papillae than in Malpighian tubules.
- The results of this study suggest that aeCCC3 plays an important role in the larval stage of mosquitoes. This could be due to a mosquitoes' aquatic larval stage, in which this specific protein absorb the ions that are constantly lost to their freshwater environment. We hope to conduct more larval tissue studies in order to confirm our results.
- Additionally, we hope to conduct the same technique in adult tissue, examining their Malpighian tubule and hind gut. Finally, we would like to conduct whole and dissected tissue qPCR studies on larvae reared in 30% seawater compared to those reared in freshwater.

References

1. Photo from NSW Arbovirus Surveillance & Vector Monitoring Program.
2. Photo: http://www.forbes.com/sites/judystone/2016/02/05/smart-science-wolbachia-bacteria-might-stop-zika-and-dengue-viruses/#7f1c10b240c8
3. Photo from Beyenbach and Piermarini (2011)
4. http://entnemdept.ufl.edu/creatures/aquatic/southern_house_mosquito.htm
5. Akuma, Daniel C., "Molecular Characterization of Na-dependent Cation-Choloride Coupled Cotransporters in the Yellow Fever Mosquito *Aedes aegpyti*" (2014). *Honors Theses*. Paper 123.

Acknowledgments

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