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Expression and activity of cation cotransporters in yellow fever mosquito (Aedes aegypti) in different salinities

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Expression and activity of cation cotransporters in yellow fever mosquito (Aedes aegypti) in different salinities

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Abstract

Throughout its complex life cycle, the Yellow Fever mosquito, *Aedes aegypti*, endures varying extremes of salinity stress. Once hatched, larvae maintain ion balances while living in a primarily freshwater environment. Adults readily lose water via evaporation and must limit water loss and feed to replace the lost water. Adult females feed on blood to provide necessary nutrients for development of their eggs, and take in salt and water as a result. Larvae and adults both use cation chloride cotransporters (CCC) to osmoregulate. Larvae can osmoregulate in environments ranging from freshwater to 30% saltwater. Larvae reared in freshwater undergo greater osmoregulatory stress than larvae reared in saltwater and thus we predicted that larvae reared in deionized water would show an increase in aeCCC3 gene expression. Quantitative PCR analysis showed no significant difference between the expression of aeCCC1, aeCCC2 and aeCCC3 in larvae reared in deionized water and larvae reared in freshwater, but showed aeCCC3 tending towards statistical significance. In order to further understand the roles of the cation chloride cotransporters we exposed larvae to varying concentrations of bumetanide, a known inhibitor of mosquito efflux of sodium, calcium, potassium and ammonium. We found bumetanide increased the level of sodium ion efflux with the largest increase in larvae exposed to 2 µM. Our results suggest bumetanide is possibly inhibiting a transporter besides aeCCC3 and perhaps larvae exposed to extreme ion-deficient environments more readily use aeCCC3 than those under normal conditions.

Introduction

Gene expression:.

- Aedes aegypti larvae depend on anal papillae (AP) to absorb ions and Malpigian Tubules (MT) to secrete ions.
- By regulating ion flow between the hemolymph and outside environment, mosquitoes are able to completely adapt to changes in saline within as little as 6 hours.
- In larvae reared in 30% SW, Na+ and Cl- uptake is significantly decreased compared larvae reared in FW [6].
- We are interested in Na^+-dependent cation-chloride cotransporters (CCCs) which, are proposed to contribute to homeostasis of Na+, K+ and Cl- ions within a mosquitoes' hemolymph [7].
- aeCCC3 is suspected to contribute in ion absorption while aeCCC1 and aeCCC2 genes are responsible for the secretion of ions.

Efflux:

- Began to characterize the various roles of the NKCC transport pathway by pharmaceutically inhibiting larvae ion efflux.
- Previously bumetanide has been used to attempt to inhibit the function of NKCC but there has been no effect on the Cl^-/H^+ transporter mechanism [5].
- Bumetanide uptake is dependent on Na^+, K^+, and Cl^- ion uptake through NKCC transport.
- In order to have bumetanide taken into the larvae's body, there must be sufficient ions in the water to allow for the NKCC transport to take place and uptake the inhibitor.

Figure 1: Typical *Aedes aegypti larvae.* Photograph by Michele Cutwa-Francis, University of Florida.

Hypotheses

- Larvae raised in deionized water will have greater expression of aeCCC3 compared to larvae raised in freshwater.
- As bumetanide concentrations increase, levels of efflux will decrease.

Acknowledgements

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aeCCC1, aeCCC2, and aeCCC3 Gene Expression

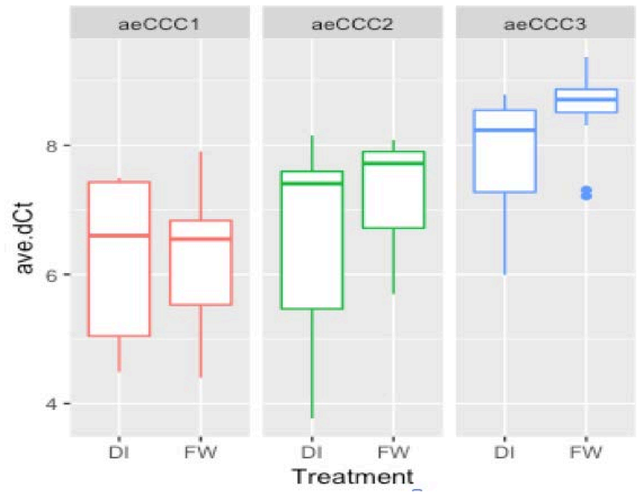

Figure 2: Gene expression of aeCCC1, aeCCC2 and aeCCC3 in larval *Aedes aegypti* reared in Freshwater or Deionized water. Lower ΔCT indicates higher expression levels. Fold differences are calculated by the following: RQ = 2-ΔΔCT. Error bars are SEM. For aeCCC1: ANOVA, F=0.0053, p=0.9428, RQ=0.98, n=11(DI), 15(FW). For aeCCC2: ANOVA, F=2.2172, p=0.1495, RQ=0.62, n=11(DI), 15(FW). For aeCCC3: ANOVA, F=5.6528, p=0.02574, RQ=0.62, n=11(DI), 15(FW).

Methods

Mosquito Rearing:

- Eggs were hatched while submerged in freshwater within a vacuum chamber and reared at 28 ˚C and 80% humidity on a 12/12 hour light/dark cycle.
- Larvae used in qPCR gene analysis were split into Deionized or freshwater 3 days after hatching.
- Water was replaced everyday after day 3. On day 7, whole body 4th instar larvae were collected in 1 mL TRIzol reagent for RNA isolation.

Quantititative PCR:

- Whole body larvae were homogenized in 150 µl Trizol®, ground up vigoriously with a pestle, and 850 µl more of Trizol® was be added.
- Sample RNA was run through clean and concentrating steps with a RNA Clean & Concentrator™-5 Kit (Zymo Research).
- RNA samples were converted into cDNA with an OneStep RT-PCR kit (QIAGEN©). Controls did not contain reverse transcriptase enzyme.
- Four primers; aeCCC1-2, aeCCC2-2, aeCCC3-2 and aeRps5 wereused to detect the relative quantification of all three proteins in comparison to aeRps5.
- Gene expression will be quantified using a comparative Ct method described by Schmittgen and Livak (2008). A threshold cycle (Ct) is the first detectable level of cDNA amplified. Expression levels of each gene were compared to a housekeeping gene, Rps5.

Cation Chromatography:

- A 200 mM stock solution of bumetanide dissolved in ethanol was used to create 0.2 µM, 2 µM, 20 µM, and 200 µM treatment solutions in deionized water.
- A pair of larvae were placed into the one of the four treatment group solutions for a 20-minute incubation period within a 24 well plate.
- 300 µL samples were taken from each experimental group at time 10, 30 and 50 minutes.
- Samples were analyzed using a 500-DX-chromatography system to measure concentrations of Na^+, Ca^{2+}, NH_4^+, and K^+ ions.

Statistics: ANOVA with Tukey comparisons via RStudio were used to compare ion concentrations and relative gene expression. Rstudio was used to calculate the rates of ion efflux for each treatment group.

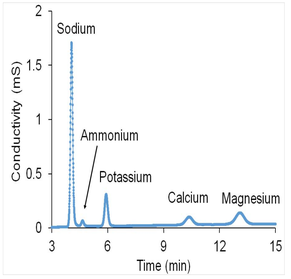

Figure 3: Example cation chromatography graph showing distribution of ions present.

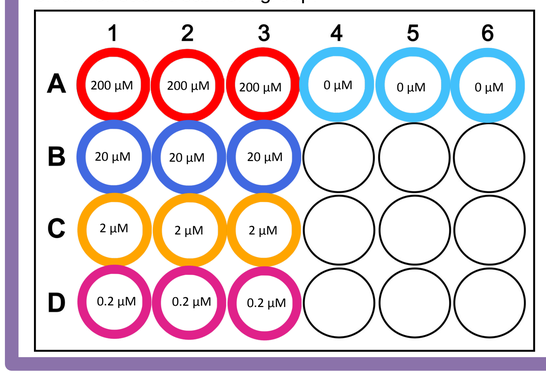

Figure 4: Diagram of varying Bumetanide treatment groups used in each experiment for ion efflux.

Ion Efflux Rate

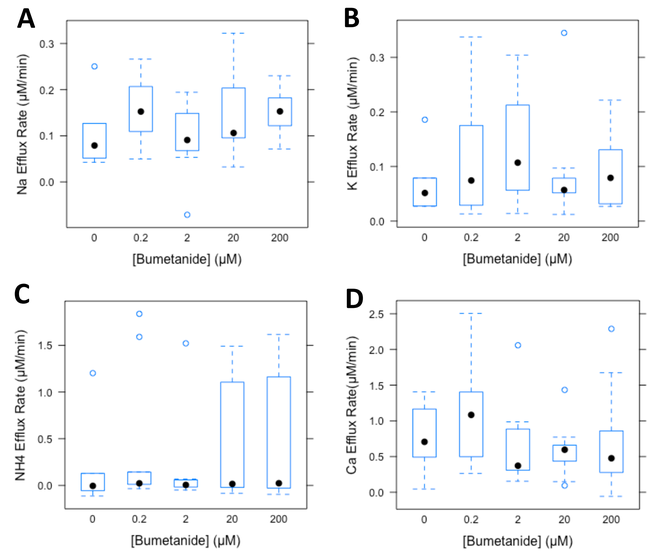

Figure 5: Cation efflux of a) Na, b) K, c) NH4, d) Ca under varying concentrations of Bumetanide treated deionized water. For sodium: ANOVA, F=1.2146, p=.3206. For potassium: ANOVA, F=0.5692, p=0.6866. For ammonium: ANOVA, F=0.2014, p=0.936. For calcium: ANOVA, F = 0.9251, p = 0.4596.

Time 50: Na+ Efflux

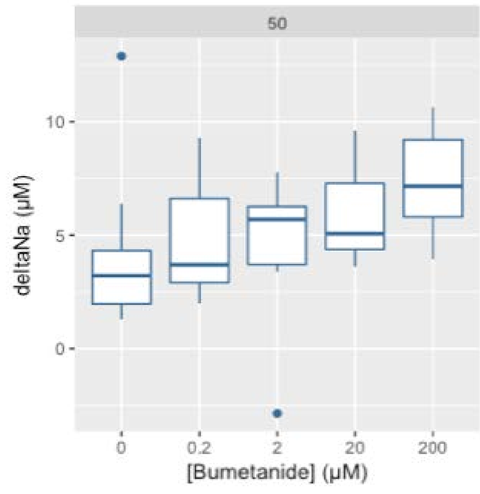

Figure 6: Larval *Aedes aegypti* sodium efflux at minute 50. All 50 minute values were compared to minute 10 values to produce deltaNa. Efflux of sodium cations increased as the concentration of bumetanide increased. ANOVA, F =1.5588 , p = 0.2051.

Conclusions

- Mosquito larvae reared in deionized water have greater expression of aeCCC3 compared to larvae reared in freshwater.
- Unlike Del Duca (2011), there is a difference in efflux between larvae exposed to bumetanide and those who were not.
- Sodium efflux increased as bumetanide concentration increased.

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