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Functional characterization of aquaporins and aquaglyceroporins of the yellow fever mosquito, *Aedes aegypti*

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After taking vertebrate blood, female mosquitoes quickly shed excess water and ions while retaining and concentrating the mostly proteinaceous nutrients. Aquaporins (AQPs) are an evolutionary conserved family of membrane transporter proteins that regulate the flow of water and in some cases glycerol and other small molecules across cellular membranes. In a previous study, we found six putative AQP genes in the genome of the yellow fever mosquito, *Ae. aegypti*, and demonstrated the involvement of three of them in the blood meal-induced diuresis. Here we characterized AQP expression in different tissues before and after a blood meal, explored the substrate specificity of AQPs expressed in the Malpighian tubules and performed RNAi-mediated knockdown and tested for changes in mosquito desiccation resistance. We found that AQPs are generally down-regulated 24 hrs after a blood meal. *Ae. aegypti* AQP 1 strictly transports water, AQP 2 and 5 demonstrate limited solute transport, but primarily function as water transporters. AQP 4 is an aquaglyceroporin with multiple substrates. Knockdown of AQPs expressed in the MTs increased survival of *Ae. aegypti* under dry conditions. We conclude that Malpighian tubules of adult female yellow fever mosquitoes utilize three distinct AQPs and one aquaglyceroporin in their osmoregulatory functions.

The yellow fever mosquito, *Aedes aegypti*, is a tropical mosquito with a worldwide distribution. It is the vector for dengue, yellow fever, and chikungunya viruses and it also transmits lesser known pathogens such as Zika virus^{1–3}.

As an anautogenous mosquito, *Ae. aegypti* females require the uptake of vertebrate blood for reproduction. Blood meals are relatively large and can more than double the mass of a mosquito within a couple of minutes. Shortly after beginning a blood meal, mosquitoes start to produce large amounts of urine in order to concentrate the nutrients taken up and to eliminate excess sodium from the blood. They begin ejecting droplets of urine while still blood feeding⁴. In order to move this large volume of water, females use their highly efficient excretion system. Water and solutes are absorbed by the midgut into the hemolymph and from there are secreted by the Malpighian tubules (MTs) back into the alimentary canal where it is excreted through the hindgut and rectum⁵. MTs are the functional equivalent of vertebrate kidneys and contain protein channels and transporters that facilitate the transport of water and other solutes across the basal and apical membrane of their cells.

Aquaporins (AQPs) are a family of membrane transporters that regulate the flow of water and in some cases other small molecules across cellular membranes in both prokaryotic and eukaryotic cells and they are important players in the mosquito excretory system⁶. There are 13 AQP genes in mammals, which consist of two subfamilies. The first subfamily is the AQPs, which are thought to pass only water, the second are the aquaglyceroporins, which in addition to water pass small, nonpolar solutes like glycerol and urea⁷. The AQP channel forms an hourglass structure with six transmembrane alpha helical domains connected by five extramembrane loops. The asparagine-proline-alanine (NPA) motif is in the center of the pore and forms a ring^{8–10}. This ring is the primary filter of the channel and regulates the flow of water through the channel. Previous studies have shown that Hg²⁺ ions can interact with a cysteine residue near the NPA motif and block the flow of water molecules through the channel of most AQPs. Aquaglyceroporins differ from AQPs in pore size and amino acid composition of the channel^{7,11}.

In earlier work, we surveyed the genome of *Ae. aegypti* and identified six putative AQP genes¹². Combining microarray, reverse transcription and qPCR data, we found that all six AQPs are expressed in distinct patterns in the female mosquito and at different time points before and after a blood meal. RNAi-mediated knockdown of

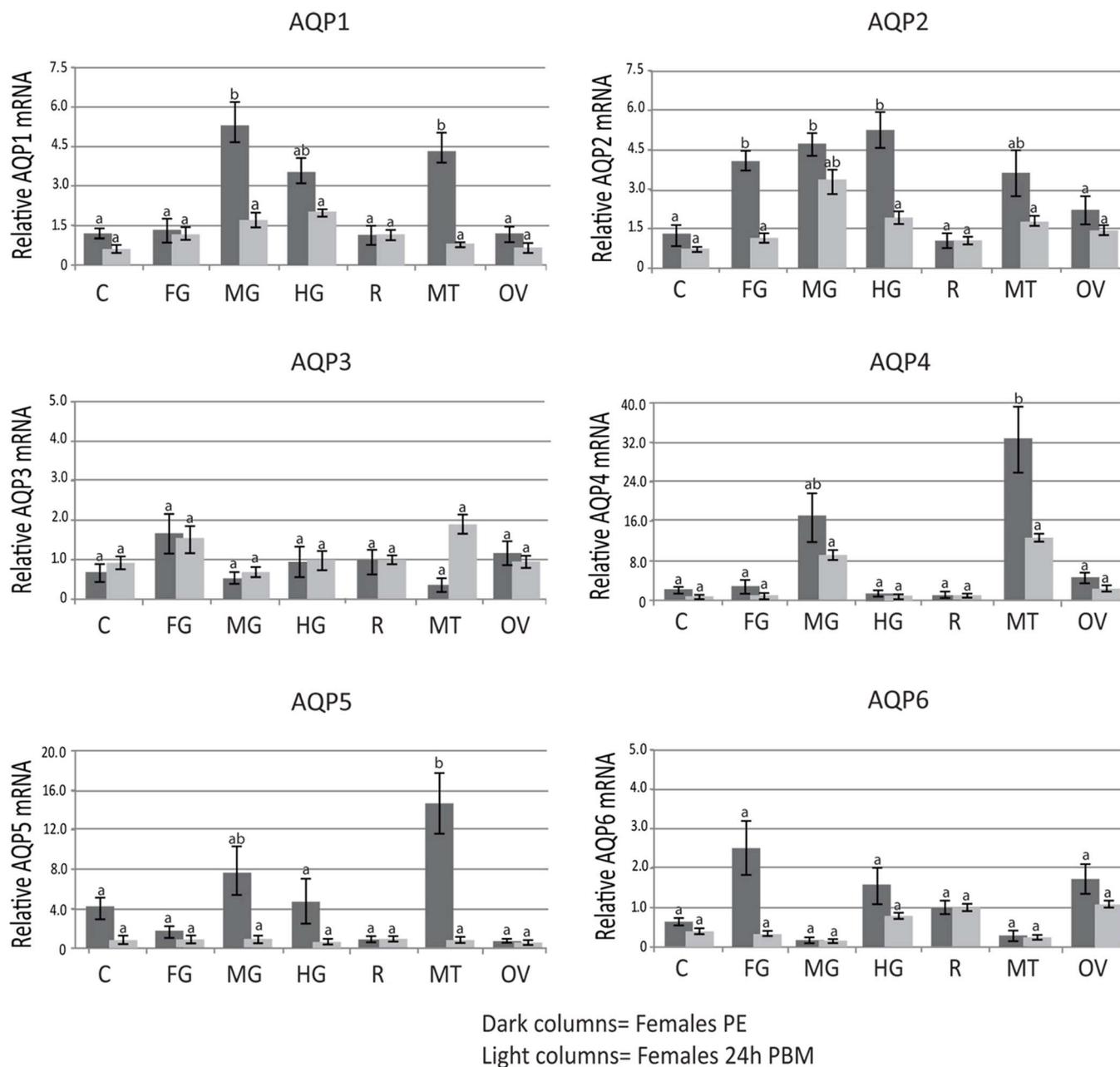


Figure 1 | *Aedes aegypti* AQP expression in various parts of the alimentary canal. Expression was assayed using qPCR. The data represent relative quantification of *Aedes* AQPs which were normalized by qPCR analysis of ribosomal protein S7 (rpS7) mRNA levels in the cDNA samples. Values are means \pm S.E. (error bars) of triplicate biological samples. The Y-axis shows expression ratios in arbitrary units with the lowest expressing tissue set as 1. Means separated by Tukey–Kramer HSD ($p < 0.05$). Means which share the same letter are not significantly different. RNA was isolated from organs/body parts of adult female mosquitoes unfed (light shaded columns) and 24 hrs after a blood meal (dark shaded columns). C-crop, FG-foregut, MG-midgut, HG-hindgut, R-rectum, MT – Malpighian tubules, OV – ovaries.

AQPs 1, 4, or 5 significantly reduced the ability of mosquitoes to excrete injected saline¹³. Furthermore, the simultaneous knockdown of AQPs 1, 2, 4, and 5 further reduced the excretion rate. This suggests that AQPs 1, 4, and 5 form the primary water channels in *Ae. aegypti* MTs.

Here we have confirmed that the AQPs expressed in the MTs (AQPs 1, 2, 4, and 5) function as water channels and mediate transcellular water transport in adult female *Ae. aegypti*. We demonstrate that these proteins are indeed water channels and that AQP 4 is a multifunctional aquaglyceroporin that is capable of transporting a wide range of solutes. Lastly, individually knocking down AQPs 1, 4, and 5 increased the resistance of mosquitoes to desiccation stress.

Results

Tissue expression. We used qPCR to evaluate relative AQP mRNA expression in different parts of the alimentary canal in female mosquitoes at two time points - 72 hrs post eclosion (PE) and 24 hrs post blood meal (PBM). We found that each AQP had a varied expression profile throughout the different organs/tissues observed (Fig. 1). AQP 1 was significantly upregulated in the midgut (MG) and the MTs PE. We observed a trend in AQP 1 expression in the hindgut (HG) PE, but it was not significantly upregulated. AQP 1 was downregulated in these tissues PBM. AQP 2 was significantly expressed in the foregut (FG), MG, and HG, and we observed a trend in the expression in the MTs PE, but this was not significant. This AQP was downregulated in these tissues PBM.

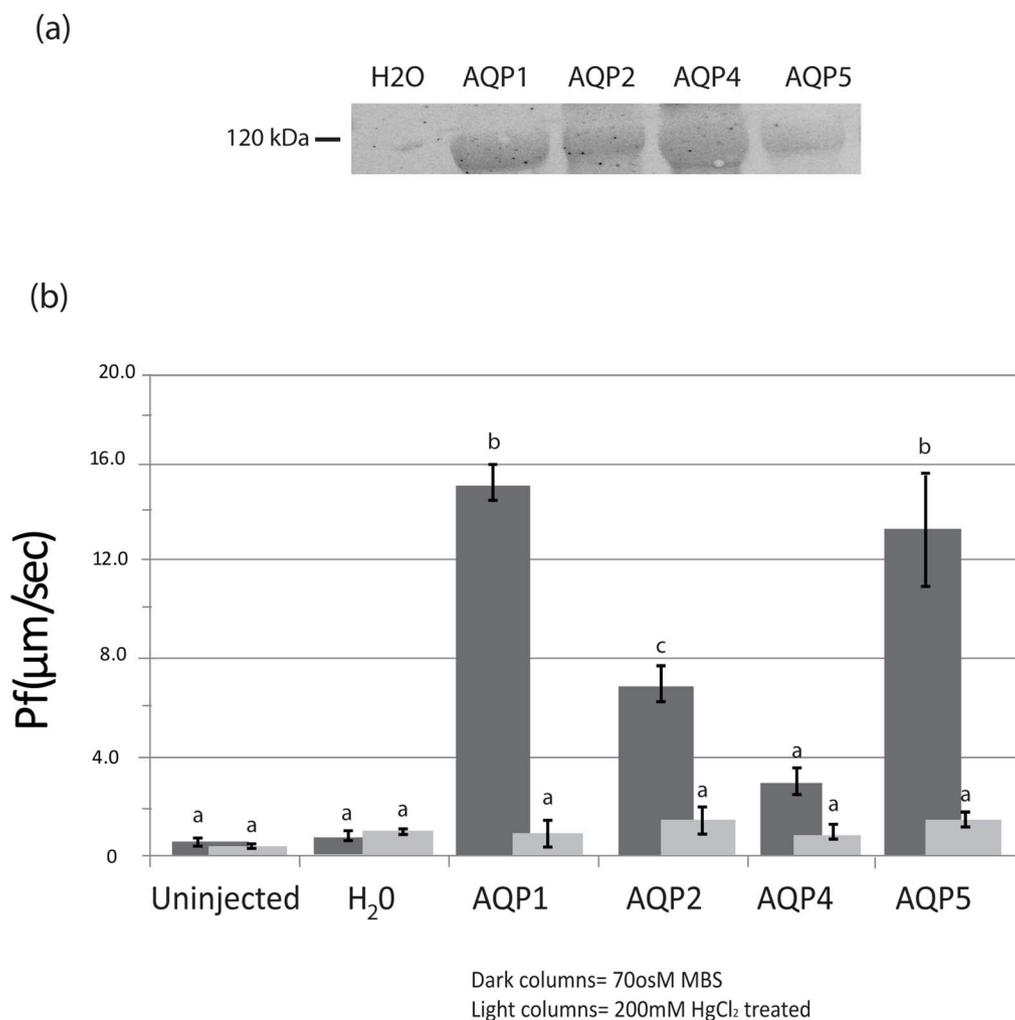


Figure 2 | Water permeability analysis of *Ae. aegypti* AQPs expressed in *Xenopus laevis* oocytes. (a) Day 3 post-injection with AQP cRNA, Western blot analyses of oocyte lysates using Myc-tag antibody (the AQP tetramer band is shown). The blots were cropped, and the full-length blots are presented in the supplementary information (see Supplementary Fig. S1). (b) Effect of heterologous expression of *Aedes* AQPs on water permeability of *Xenopus* oocytes. The light-shaded columns represent oocytes that were pre-treated with HgCl₂. Each AQP group n = 9, and their mean separated by Tukey–Kramer HSD (p < 0.05). Means which share the same letter are not significantly different.

There is no significant difference in AQP 3 expression levels in all the tissues before and after a blood meal. AQP 4 was significantly upregulated in the MTs and we observed a trend in expression levels in the MG PE, but this was not significant. These expression levels were downregulated PBM. AQP 5 is significantly upregulated in the MTs PE. We also observed a trend in AQP 5 expression in the MG PE, but this was not significant. AQP 5 expression was downregulated in these tissues PBM. There is no significant difference in AQP 6 expression levels in all the tissues before and after a blood meal.

Water uptake assays. *Xenopus* oocytes are naturally impermeable to water and thereby are an ideal model system to study the water transport properties of heterologously expressed AQPs¹⁴. Negative control water-injected oocytes swelled minimally and failed to rupture even after extended incubation times. The cRNAs encoding Myc-tagged *Ae. aegypti* AQPs 1, 2, 4, or 5 were injected into *Xenopus laevis* oocytes and AQP protein expression was confirmed by Western blot analysis (Fig. 2a). Oocytes expressing AQP 1, 2, or 5 showed significantly higher water permeability compared to the control (water-injected) oocytes; water permeation was inhibited when the oocytes were placed in 1 mM HgCl₂ solution (Fig. 2b). There were no changes in the water permeation of the control

oocytes in the HgCl₂ solution, consistent with nominal endogenous AQP expression.

Solute uptake assays. To investigate the substrate specificities of the *Ae. aegypti* AQPs, they were expressed in *Xenopus laevis* oocytes, which were subsequently subjected to non-radiolabeled solute transport analyses with six different solutes: glycerol, urea, erythritol, adonitol, mannitol, and trehalose (Fig. 3). AQP 1-expressing oocytes closely resembled the water-injected oocytes and did not demonstrate significant solute uptake. AQP 2-expressing oocytes also did not show significant solute uptake compared to the water-injected oocytes. Oocytes injected with AQP 4 cRNA consistently swelled in various test solutes to ~1.3 times their initial volume and ruptured within minutes. AQP 4 did not function as a strong water transporter (Fig. 2), but this AQP has significant transport capabilities for all other solutes tested (Fig. 3). AQP 5 demonstrated significantly high solute transport for trehalose. The results show a trend in the uptake of erythritol and adonitol via AQP 5, but the solute uptake was not significantly different than the water-injected oocytes.

Mosquito desiccation resistance assays. AQPs 1, 4, and 5 were knocked down individually in adult female *Ae. aegypti* mosquitoes

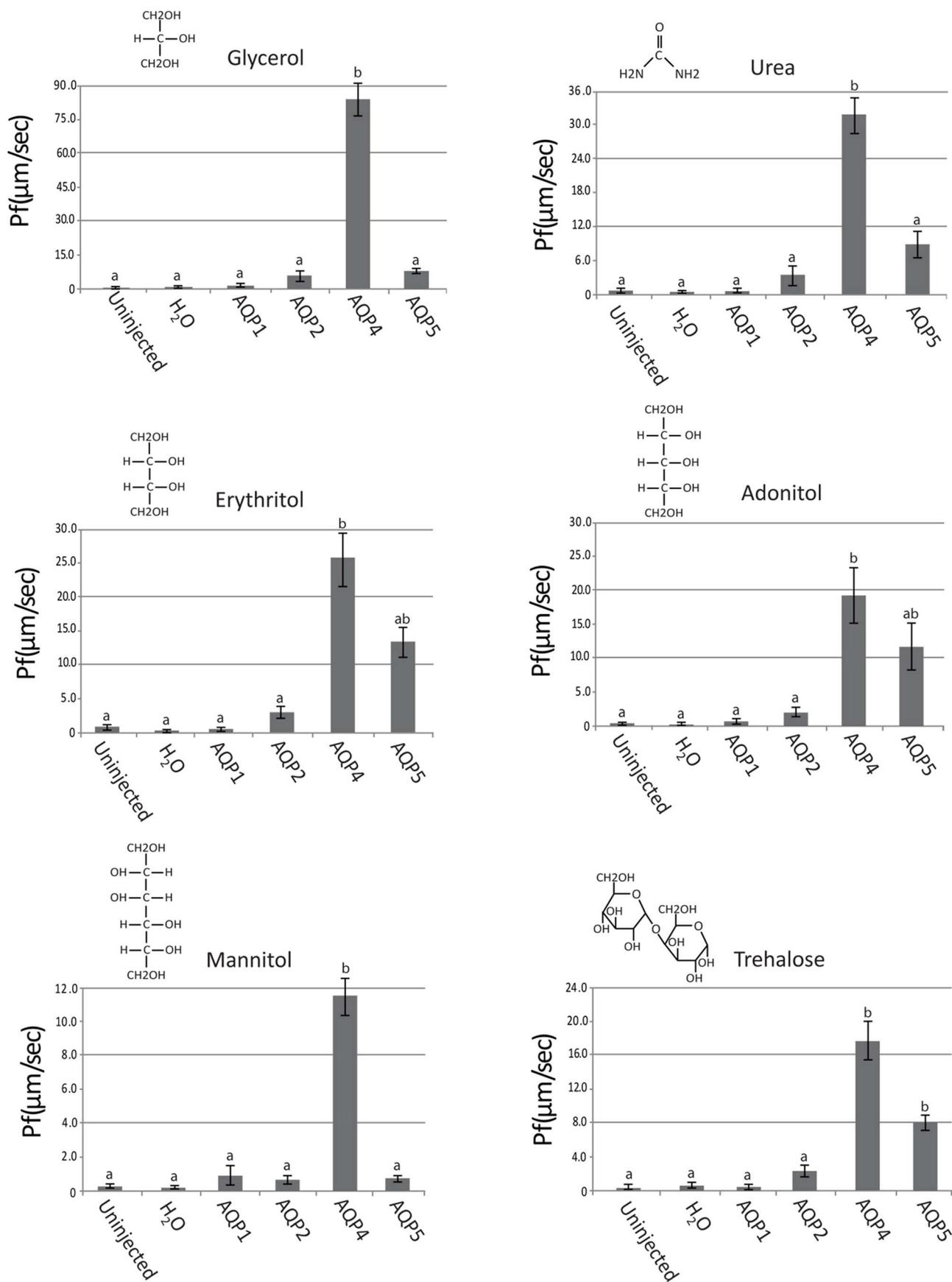


Figure 3 | Various solute transport capabilities of *Ae. aegypti* AQPs expressed in *Xenopus laevis* oocytes. Solute uptake was reported as an oocyte swelling rate $[d(V/V_0)/dt]$ and measured in $\mu\text{m}/\text{sec}$. There were six solutes tested, in each AQP group $n = 5$. The molecular structure of each solute is indicated above each graph. The means were separated by the Tukey-Kramer HSD ($p < 0.05$). Means which share the same letter are not significantly different.

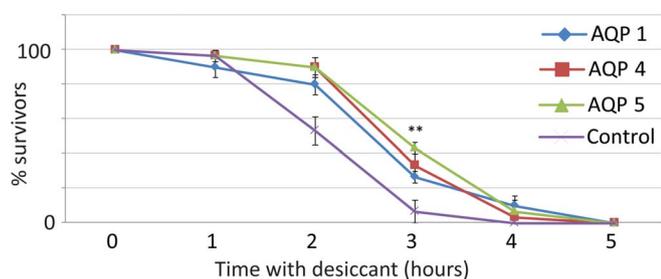


Figure 4 | RNAi-mediated knockdown of *Ae. aegypti* AQPs demonstrate desiccation resistance. Effect of RNAi-mediated AQP knockdown on *Aedes* survival under desiccation conditions. AQP 1, 4 and 5 knockdown mosquitoes have a significant increase in survival compared to the eGFP control group. Kaplan-Meier and log-rank tests were performed using Graphpad Prism software.

72 hrs PE by injecting dsRNA followed by a three day recovery period. The desiccation assay measures the viability of the mosquitoes in dry conditions. We did not include AQP 2 in this experiment since *in-vivo* diuresis assays performed in Drake et al., 2010 did not result in a significant rate of excretion for the AQP 2 knockdown group of mosquitoes¹². Therefore, AQP 2 appears to be a relatively inefficient water transporter.

Groups of five mosquitoes were placed in tubes with desiccant (<20% humidity) and the survival of the mosquitoes was recorded at 30 minute intervals until all mosquitoes died. All three AQP-knockdown groups survived significantly longer than the control group (eGFP) under these conditions (Fig. 4).

Discussion

Vertebrate and plant aquaporins have been extensively studied with a focus on their structure, substrate specificities, regulation, and roles as potential drug targets for various diseases^{15–19}. Compared to this wealth of knowledge relatively little is known about insect AQPs. Analyses of data from various genome projects suggest that the number of AQP genes in different insect species varies between six and ten^{12,20}. In haematophagous insects, AQPs have been shown to be important for regulation of water homeostasis, desiccation resistance, blood meal compaction, and general osmoregulation^{21–24}. In the case of the viviparous tsetse fly for milk production²⁰.

The first mosquito AQP was cloned from *Ae. aegypti*, AeaAQP or AaAQP 1, which is a close homologue of *Drosophila* DRIP. AaAQP 1 is localized in the tracheolar cells that are associated with MTs, and it is strictly a water channel when expressed heterologously in *Xenopus* oocytes^{22,23}. A homologous gene, AgAQP 1 has also been cloned and characterized in *Anopheles gambiae*. AgAQP 1 transports water but not glycerol and is expressed in the MG, OV and MTs and plays a role in water homeostasis^{21,25}. In the MTs, AgAQP 1 localizes to the basolateral membranes of stellate cells in the distal and proximal segments, respectively^{21,25}. Furthermore, two splice variants of AgAQP 1 are expressed in an organ-specific manner²⁵.

The alimentary canal of the mosquito consists of the primary organs/tissues involved in digestion and excretion of nutrients and fluids⁴. In *Ae. aegypti*, most water from blood meals is absorbed by the MG into the hemolymph and then secreted by the MTs. Concurring, our results revealed that AQPs 1, 2, 4, and 5 were highly expressed in the MG and MTs, which confirmed data from a previous analysis¹². AQP 2 is also highly expressed in the FG and HG (Fig. 1) which implies that these parts of the gut might also be involved in blood meal dehydration.

Blood meal dehydration is a very efficient process. After three hours, *Ae. aegypti* females can excrete 75% of the total blood meal volume as urine^{12,26}. After mosquitoes take blood, they seek out a resting place to allow vitellogenesis and egg development to take

place during the next days. Post- blood meal diuresis typically subsides around 2 hours after a mosquito takes a blood meal, which has been shown in previous studies^{26,27}. This matches the fact that we found many AQPs down regulated in the MTs and the MG at the 24 hrs PBM time point (Fig. 1). Interestingly, AQP 3 has similar expression levels before and after a blood meal in all alimentary canal tissues including the MTs. AQP 3 is most closely related to BIB-like AQPs¹². BIB was first characterized in *Drosophila melanogaster* and named big brain (BIB) (CG4722 in FlyBase)²⁸. This AQP does not function as a water channel in *Drosophila*, instead, it is involved in the regulation of cell to cell adhesion²⁹.

Our results confirm that AQP 1 is a strict water channel^{21,23}, and this is also true for AQP 2. These observations suggest that AQPs 1, 2, and 5 are the main water channels in the MG and the MT and raise the possibility that these AQPs might be functioning together for efficient water permeability in these organs. This redundancy of function has been observed in previous studies in which multiple AQPs act together in the same tissue. For instance, in tsetse flies, the combined knockdown of three AQPs significantly extended larval pregnancy and ultimately led to dehydration of the developing larva while knockdown of a single AQP gene had a much weaker effect^{30,31}.

To date, only a handful of aquaglyceroporins have been identified in insects^{32–36}. RhoprMIP from the kissing bug, *Rhodnius prolixus* facilitates the transport of H₂O₂³². In the pea aphid, *Acyrtosiphon pisum*, there is another identified aquaglyceroporin, ApAQP 2, which has been shown to be a multifunctional transport channel capable of passing a wide range of linear polyols, including glycerol, mannitol, and sorbitol³³. Here we present the first characterization of aquaglyceroporins in a mosquito. *Ae. aegypti* AQP 4 did demonstrate the lowest water permeability from all AQPs in our study, but significantly enhanced the uptake of all other solutes we tested - linear polyols, urea, and trehalose. The solute with the strongest uptake by AQP 4-expressing oocytes was glycerol, which is a precursor for the synthesis of triacylglycerols and of phospholipids and has also been shown to be important for insect cold tolerance and diapause^{37,38}. *Ae. aegypti* AQP 5-expressing oocytes also demonstrated significant solute permeability for trehalose. Trehalose is the primary sugar circulating in the hemolymph of an insect and can be considered insect 'blood sugar'^{39,40}. This molecule is the primary energy store in the mosquito and it is up regulated in the event of environmental stresses such as temperature changes and dehydration. In *Anopheles gambiae*, a trehalose transporter, AgTreT1, is highly expressed in the fat body and transports trehalose into the hemolymph^{41,42}. Interestingly, both, *Ae. aegypti* AQP 4 and 5, demonstrated significant transport of trehalose in the uptake assays. This is the first study that suggests transport of trehalose via a multifunctional aquaglyceroporin channel and proposes potential novel roles of these AQPs in regulation of mosquito hemolymph trehalose levels or uptake of circulating trehalose into tissues.

In 2005, *Ae. aegypti* mosquitoes were distributed throughout 23 of the United States in South and South-eastern regions including Southern Florida, Gulf coast of Texas and Louisiana. Recently, this distribution has spread to more arid and dry regions, such as Arizona and New Mexico. This raises the question as to how this tropical mosquito manages water conservation in the desert. Previous studies have shown that AQPs play a role in insect desiccation resistance and cold hardiness. For example, RNAi-mediated knockdown of AQP 1 in *Anopheles gambiae* resulted in significantly longer survival of the knockdown group compared with the control group under desiccating conditions²¹. In the goldenrod gall fly, *Eurosta solidaginis*, AQPs promote larval cell survival during freezing conditions⁴³. Our results confirm the role that AQPs play in maintaining water homeostasis in adult mosquitoes. More specifically, the ability of *Ae. aegypti* to conserve water in an extremely dry environment was significantly enhanced after RNAi-mediated knockdown of AQPs 1, 4, and 5



(Fig. 4). This suggests that the regulation of AQP expression in the alimentary canal could be an adaptive strategy to attenuate excretory water losses when *Ae. aegypti* are exposed to an arid environment. How this is balanced with the need for rapid excretion after a blood meal is an interesting topic for future studies.

In summary, our current findings confirm that *Ae. aegypti* AQPs 1, 2, and 5 transport water and are involved in the regulation of mosquito water homeostasis. AQP 4 is a multifunctional aquaglyceroporin with a wide range of solute transport capabilities. AQP 5 also functions as a limited aquaglyceroporin in *Ae. aegypti*. Down regulation of AQPs 1, 4, and 5 enhanced mosquito desiccation resistance. Our data provide a basis for further investigation into the transport capabilities of mosquito AQPs. Further research is necessary to determine how expression and activity of these AQPs are regulated in mosquitoes.

Methods

Maintaining mosquito strains. The *Ae. aegypti* mosquito Rockefeller strain was used in all experiments. Mosquito culture was performed as previously described by Hays and Raikhel⁴⁴. Mosquito larvae were hatched in large larval trays, and adult mosquitoes were maintained in Bugdorm-1 insect rearing cages (Bioquip, Rancho Dominguez, CA). Mosquitoes were maintained at a temperature of 28°C with 80% humidity and a photoperiod of 14:10 hrs Light:Dark. Larvae were fed on a diet of dry cat food. The adult mosquitoes were fed on a 20% sucrose solution.

qPCR analysis of AQP transcript abundance. Primer BLAST was used to develop gene-specific primers: AaAQP1f: TAA TAC GAC TCA CTA TAG GGA GCA CTA TGG GCT GGG GCG GAG ACT; AaAQP1r: TAA TAC GAC TCA CTA TAG GGA CGG CTG GTC CGA AAG AGC GAG CTG; AaAQP2f: TAA TAC GAC TCA CTA TAG GGA CTG CTG GCT TAC TTG CGG CTG GCA; AaAQP2r: TAA TAC GAC TCA CTA TAG GGG TCA CAC GGT AGC GCT CTG AGG CGG; AaAQP3f: TAA TAC GAC TCA CTA TAG GGC CTT CCT AAC CCG GTT AAC CAC; AaAQP3r: TAA TAC GAC TCA CTA TAG GGT GGG CCA TTG GGTAGC CCC CTG GAT; AaAQP4f: TAA TAC GAC TCA CTA TAG GGG CGG CAT CGG GTT CGG CTT CAC AGT; AaAQP4r: TAA TAC GAC TCA CTA TAG GGT GGC CGG GTT CAT ACT CGC TCC GGT; AaAQP5f: TAA TAC GAC TCA CTA TAG GGT ACG TTG CGG CCC AGT GCA TCG GAG; AaAQP5r: TAA TAC GAC TCA CTA TAG GGG GAA CCT CGC GCC TGA ACA CCG TCT; AaAQP6f: TAA TAC GAC TCA CTA TAG GGG ATC GCG GCA GTT GCT CGC CGA GTG; AaAQP6r: TAA TAC GAC TCA CTA TAG GGA GGC CAA GCG ACA GCA CAG AGT GGC. Crop, foregut, midgut, hindgut, rectum, MTs, and ovaries were dissected from adult females pre- blood meal and 24 h. post- blood meal. RNA was isolated using the phenol/chloroform extraction using Trizol[®] Reagent (Invitrogen, Carlsbad, CA). First-strand cDNA synthesis was performed on 1 µg of total RNA in 20 µL reaction using Qiagen Omniscript[®] RT Kit (Valencia, CA). The functionality of the primers and quality of the cDNA was confirmed via RT-PCR. The PCR products were visualized on a 1% agarose gel with ethidium bromide DNA staining dye for electrophoresis. Transcripts were normalized by qPCR analysis of ribosomal protein S7 (rpS7) levels on an Eppendorf Mastercycler ep realplex[®] (Eppendorf, Hamburg, Germany) using iQ Supermix (Biorad, Hercules, CA). Reactions were run in triplicate with 25 µl volumes. Each experiment was done with three independent biological replicates. PCR conditions were as follows: an initial incubation at 95°C for 2 min; followed by 95°C incubation for 15 sec; then 40 cycles of 60°C for 15 sec, 72°C for 20 sec.

Xenopus expression vector construction. Myc-tagged *Ae. aegypti* AQP 1, 2, 4, and 5 were cloned in pUNIV vector using cDNA from all the AQPs. Capped RNAs were synthesized from NotI linearized pUNIV vector by using T7 RNA polymerase in the mMessage-mMachine[®] (Ambion Inc., Carlsbad, CA) and procedures similar to those previously used⁴⁵.

***Ae. aegypti* AQP overexpression in *Xenopus laevis* oocytes and swelling assay.** Defolliculated *Xenopus laevis* oocytes were ordered through ecocyte-us.com. Each oocyte was injected with 10 ng of cRNA or 50 nl of nuclease-free water. The injected oocytes were incubated at 18°C for 3 days in 200 milliosmolar modified Barth's solution. The oocytes were transferred to 70 milliosmolar modified Barth's solution. Images were acquired of the oocyte silhouette every 30 seconds using an Olympus SZX12 stereomicroscope with a Lumen 200 light source and an Ample Scientific TCC3.3 ICE supercooled CCD camera up to 4 minutes. The permeability coefficient for each oocyte was calculated using a method previously described²¹.

Western blot analysis for oocyte protein expression. AQP cRNA-injected and water-injected oocytes were lysed in 200 µl of breaking buffer (50 mM Tris, pH 7.4; 1% IGEPAL; 0.25% sodium deoxycholate; 150 mM NaCl; 1 mM EDTA; 1 mM phenylmethylsulfonyl fluoride; 1 mM protease inhibitor mixture; 1 mM phosphatase inhibitor mixture 1, all purchased from Sigma-Aldrich Co. Oocyte protein extracts were resolved on 7.5% gradient sodium dodecyl sulfate-polyacrylamide gels and electrotransferred to polyvinylidene difluoride membranes by the use of Trans-Blot SD semi-dry electrophoretic transfer cell (Bio-Rad, Hercules, CA). The membranes

were blocked overnight in Starting Block T20 Blocking buffer (Thermo scientific, Waltham, MA) at 4°C. The membranes were washed with TBS and incubated in blocking buffer containing 1:1000 dilution mouse anti-myc tag monoclonal antibody (Cell Biolabs Inc., San Diego, CA) at room temperature for 1 hr. After extensive washing with TBS, the membranes were incubated with an alkaline phosphatase-labeled secondary antibody (Millipore, Billerica, MA) in blocking buffer for 2 hrs at room temperature. The bands were visualized using 1 step NBT/BCIP suppressor (Thermo Scientific, Waltham, MA). This procedure was similar to one previously described⁴⁶.

Nonradio-labeled glycerol uptake assay. To analyze solute transport activity, AQP 1, 2, 4, and 5 cRNA and H₂O (negative control) were injected into *Xenopus* oocytes and incubated for 3 days at 18°C. The oocytes were incubated in modified Barth's solution with NaCl replaced with 200 mM of glycerol, urea, mannitol, trehalose, erythritol, and adonitol as the test solutes. Images were acquired of the oocyte silhouette every 30 seconds using an Olympus SZX12 stereomicroscope with a Lumen 200 light source and an Ample Scientific TCC3.3 ICE supercooled CCD camera. Solute uptake was reported as an oocyte swelling rate [d(V/V₀)/dt]. This procedure is similar to those previously used^{33,47}.

RNAi-mediated knockdown of *Ae. aegypti* AQPs and desiccation assay. Generation of double-stranded RNA (dsRNA) was performed using AQP 1, 4, and 5 primer sets with T7 sequence attached (TAATACGACTCACTATAGGG). The PCR product was used as a template for dsRNA synthesis using the MEGAscript T7 Kit (Ambion, Austin, TX). eGFP dsRNA was used as a control. *Ae. aegypti* mosquitoes 5–6 day post-emergence were collected by aspiration. 1000 ng of dsRNA in 268 nl of RNAase-free H₂O was injected into the thorax of CO₂-anesthetized female *Ae. aegypti* mosquitoes three days after emergence. The injected mosquitoes were allowed to recover for three days before the desiccation assay was performed. The knockdown was confirmed with qPCR at 3 days post-injection of dsRNA with knockdown efficiencies between 60–95%. On day 3 post-injection, groups of five mosquitoes for each treatment were placed in 50 ml falcon tubes (six replicate tubes for each treatment) with 16 g of 8-mesh Drierite in each tube and a cotton ball on top of Drierite. The tubes were capped and sealed with parafilm. The tubes were incubated at room temperature (22°C). Surviving mosquitoes were recorded every hour until all mosquitoes were dead as previously described²¹. The experiment was repeated in triplicate. ***P* < 0.05 Kaplan-Meier and log-rank tests were performed using GraphPad Prism software.

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Author contributions

I.H. and L.D. conceived the experiments. L.D. and S.R. carried out the experiments and data analysis. L.D. and I.H. wrote the manuscript. All authors reviewed the manuscript.

Additional information

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