Supporting Information

**Preimaginal development of *Aedes aegypti* L. (Diptera: Culicidae) in brackish watergives rise to adult mosquitoes with thicker cuticles and greater insecticide resistance**

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**Table S1. Other countries where *Aedes aegypti* and *Aedes albopictus* have been shown to develop in brackish water habitats in the field**

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| **Country** | **Species** | **Habitats** | **Reference** |
| Brazil | *Ae. aegypti* and *Ae. albopictus* | Discarded household containers, used tyres, water tanks, drains and phytotelmata with BW up to 13.5 g/L salt for *Ae. aegypti* and BW up to 9.2 g/L salt for *Ae. albopictus* in coastal areas of Sao Paulo state.  | de Brito Arduino et al., 2010 |
| Brunei Darussalam | *Ae. albopictus* | Discarded plastic containers and empty coconut shells with up to 8 g/L salt BW in beaches and estuarine villages | Idris et al., 2013 |
| India | *Ae. albopictus* | Shallow ponds, plastic containers and discarded bottles with up to 4.5 g/L salt BW in coastal areas of Kerala state | Shamna et al., 2022 |
| Indonesia | *Ae. aegypti* and *Ae. albopictus* | Discarded household containers, used tyres, abandoned boats and wells with up to 5 g/L salt BW in coastal beach areas of Sulawesi | Ratnasari et al., 2020 |

**Legend to Table S1.** BW: brackish water.

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| **Table S2. Differences between brackish and fresh water developing *Ae. aegypti* larvae** |
| **Characteristic** | **Differences** | **References** |
| LC50 for salt | Significantly higher LC50 in BW *Ae. aegypti* for the larva to adult transition which was an inheritable characteristic | Ramasamy et al., 2011;Ramasamy et al., 2014;Sivabalakrishnan et al., 2023 |
| Osmoregulatoryanal papillae in L3 larvae | Significantly larger anal papillae in JBW *Ae. aegypti* which was an inheritable characteristic | Surendran et al., 2018b |
| Haemolymph osmolarity in L4 larvae  | (i) Increased concentration of amino acids in haemolymph for osmoconformation on limited exposure to BW of approximately 10 g/L salt(ii) Haemolymph osmoconformation on 24 h exposure to BW of up to approximately 10 g/L salt | (i) Edwards, 1982(ii) Kengne et al., 2019 |
| Voltage-gated ion channels in osmoregulatory epithelia of larval Malpighian tubules and anal papillae  | Differential expression in larvae exposed to 10 g/L salt for 24 h | Farrel et al., 2024 |
| Aquaporins and ion transporters in L4 anal papillae | Differential expression in L4 exposed to 10.5 g/L salt for 24 h | Durrant et al., 2021 |
| Aquaporins in L4 anal papillae | Differential expression of aquaporins in L4 anal papillae epithelium in 7.5 g/L salt | Akhter et al., 2016 |
| Ion transport and ion transporters in the L4 gastric caecum  | Differential localization and activities of Na+/K+ ATPase and V-type H+ ATPase in BW and FW reared L4 associated with differential ion fluxes | D’Silva et al., 2017 |
| Ion transport in the anal papillae and haemolymph ion concentrations in L4 | Decreased uptake of Na+ and Cl- by anal papillae within 5h and increased haemolymph Na+, Cl- and H+ concentrations within 6 h on transferring FW-adapted L4 to BW of approximately 10 g/L salt | Donini et al., 2007 |
| Gene expressionin mid-L4 larvae | Marked increases in transcripts for specific cuticle proteins (notably of the RR-2 family), signalling proteins, moulting hormone-related proteins, membrane transporters, enzymes involved in cuticle metabolism, enzymes concerned with synthesizing long chain hydrocarbons and cytochrome P450 monooxygenases in JBW L4. Also marked changes in long non-coding RNAs between JBW and JFW L4 | Ramasamy et al., 2021 |
| Protein composition of L4 cuticles | Marked differences between JBW and JFW compatible with gene expression data | Ramasamy et al., 2021 |
| Cuticle structure by TEM | Thicker endo-, exo- and whole procuticles with a tendency for more prominent lamellae and bouligands in JBW L4 larval bodies. Thinner procuticles in anal papillae of JBW L4 | Ramasamy et al., 2021 |
| Surfaces of shed L3 and L4 cuticles | More pronounced surface undulations in JBW *Ae. aegypti* cuticles by AFM and SEM | Sivabalakrishnan et al., 2023 |
| Egg sizes | Significantly smaller eggs in JBW *Ae. aegypti* | Sivabalakrishnan et al., 2023 |
| Surfaces of eggs by AFM and SEM | JBW *Ae.* *aegypti* egg surfaces were significantly less elastic by AFM, with more undulating surfaces seen by AFM and SEM | Sivabalakrishnan et al., 2023 |
| Hatchability of eggs and preimaginal development to adults | Hatchability of eggs and preimaginal development to adults of JFW *Ae. aegypti* is decreased in 10 gL-1 salt BW. These properties were maternally inherited in genetic crosses between JBW and JFW colony *Ae. aegypti* | Sivabalakrishnan et al., 2023 |
| Susceptibility of L3 and L4 to the common larvicide temephos | JBW *Ae. aegypti* were significantly more resistant in a 24h assay | Sivabalakrishnan et al., 2023 |

**Legend to Table S2.** BW: brackish water, FW: fresh water, JBW: Jaffna brackish water colony, JFW: Jaffna fresh water colony, L1-L4: first to fourth instar larval stages, AFM: atomic force microscopy, SEM: scanning electron microscopy, TEM: transmission electron microscopy, LC50: concentration producing 50% lethality.

**Table S3. Calculated mean and median values for thicknesses of exocuticles, endocuticles and whole procuticles for T1 tarsomere in different *Aedes aegypti* populations**

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| **Mosquito population**(number of mosquitoes sampled) | **Exocuticle** Thickness in nm mean ± sd (median) | **Endocuticle** Thickness in nm mean ± sd (median) | **Whole procuticle** Thickness in nm mean ± sd (median) |
| **JFW** G75 (n=9)  | 1473 ± 238 (1452) | 984 ± 270 (958) | 2483 ± 486 (2397) |
| **JBW** G75 (n=9) | 1736 ± 227 (1722) | 1276 ± 333 (1272) | 3031 ± 527 (3010) |
| **JFWR** G10 (n=3) | 1597 ± 23 (1557) | 1002±250 (895) | 2638 ± 514 (2480) |
| **JBWR** G10 (n=3) | 1465± 237 (1401) | 1156 ± 34 (1159) | 2533± 560 (2388) |
| **BW field** (n=9) | 1724 ± 295 (1691) | 1245 ± 329 (1146) | 2953 ± 515 (3014) |

**Legend to Table S3.** BW: brackish water,JBW: Jaffna brackish water colony, JFW: Jaffna fresh water colony, JBWR: Jaffna brackish water reversal colony, JFWR: Jaffna fresh water reversal colony.

**Table S4. Calculated mean and median values for thicknesses of exocuticles, endocuticles and whole procuticles of abdomen in different *Aedes aegypti* populations**

**Legend to Table S4.** BW: brackish water,JBW: Jaffna brackish water colony, JFW: Jaffna fresh water colony, JBWR: Jaffna brackish water reversal colony, JFWR: Jaffna fresh water reversal colony.

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| --- | --- | --- | --- |
| **Mosquito population**(number of mosquitoes sampled) | **Exocuticle** Thickness in nm mean ± sd (median) | **Endocuticle** Thickness in nm mean ± sd (median) | **Whole procuticle** Thickness in nm mean ± sd (median) |
| **JFW** G75 (n=4) | 677 ± 90 (628) | 714 ± 212 (732) | 1372 ± 207 (1306) |
| **JBW** G75 (n=4) | 903 ± 182 (876) | 1124 ± 173 (1142) | 2058 ± 393 (1925) |
| **JFWR** G10 (n=4) |  775 ± 176 (730) |  711±99 (686) | 1526± 267 (1544) |
| **JBWR** G10 (n=4) |  821± 63 (831) | 553±29 (550) | 1316± 44 (1298) |
| **BW field** (n=6) | 1044 ± 166 (1059) | 902 ± 152 (857) | 1912 ± 290 (1902) |

**Table S5. Viable stages produced from eggs in the G1 to G7 generations when salinity was reversed in the JBWR and JFWR colonies**

|  |  |  |
| --- | --- | --- |
| **Colony & Generation** | **Approximate starting number of eggs** | **Estimated percent viable stages resulting from eggs**  |
| **JBWR** |  | **L1** | **L2** | **L3** | **L4** | **Pupa** | **Adult** |
| G1 | 600 | 85 | 82 | 81 | 81 | 80 | 80 |
| G2 | 500 | 97 | 96 | 96 | 95 | 94 | 94 |
| G3 | 400 | 89 | 87 | 86 | 86 | 85 | 85 |
| G4 | 450 | 97 | 96 | 95 | 95 | 95 | 95 |
| G5 | 600 | 96 | 95 | 95 | 94 | 94 | 94 |
| G6 | 500 | 96 | 96 | 96 | 96 | 96 | 96 |
| G7 | 500 | 97 | 96 | 96 | 95 | 95 | 95 |
| **JFWR** |  |
| G1 | 600 | 35 | 16 | 15 | 12 | 11 | 09 |
| G2 | 450 | 43 | 20 | 17 | 14 | 13 | 11 |
| G3 | 600 | 46 | 19 | 17 | 15 | 15 | 14 |
| G4 | 500 | 71 | 58 | 56 | 55 | 55 | 54 |
| G5 | 600 | 76 | 72 | 70 | 70 | 70 | 70 |
| G6 | 550 | 77 | 75 | 74 | 74 | 74 | 74 |
| G7 | 500 | 90 | 89 | 89 | 89 | 89 | 89 |

**Legend to Table S5.** The approximate starting number of eggs used for hatching and the percent of different stages formed from the eggs are shown. JBWR: Jaffna brackish water reversal colony, JFWR: Jaffna fresh water reversal colony, L1-L4: first to fourth instar larval stages.

**Table S6. Reversibility of characteristics associated with preimaginal stages of Jaffna brackish water colony on transfer to fresh water**

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| --- | --- | --- |
| **Characteristic** | **Rate of reversal after transfer to fresh water** | **Reference** |
| LC50 for salt in the L1 to adult transformationwhich was an inheritable trait | Significant decrease in LC50 compared with JBW colony within five generations in FW | Ramasamy et al., 2014 |
| LC50 and LC99 of L3 and L4 for the larvicide temephos in a 24 h exposure assay | Significant decrease in LC50 and LC99 in 11 to 13 generations in FW to values not different from JFW and NFW colonies | Sivabalakrishnan et al., 2023 |

**Legend to Table S6.** FW: fresh water,JBW: Jaffna brackish water colony, JFW: Jaffna fresh water colony, NFW: Nawalapitiya fresh water colony, L1-L4: first to fourth instar larval stages, LC50: concentration producing 50% lethality, LC99: concentration producing 99% lethality.

**Additional references for supporting information**

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