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*FELLOWSHIP FINAL REPORT*

# Role of chemosensory proteins in taste sense in the mosquito *Aedes aegypti*, vector of dengue and other arboviruses

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## ABSTRACT

*Aedes aegypti is an urban mosquito vector of emergent arboviruses, with a huge impact on public health, the economy, and the environment. Mosquitoes use taste organs to guide behaviors such as feeding, biting, and egg-lying. Therefore, taste sense is crucial for their survival and reproduction, and consequently for the spread of arboviruses. It could be a source of targets for new-generation repellents for personal protection. Despite this, the taste system remains understudied in mosquito species. Chemosensory proteins are a family of globular proteins from insects, mainly characterized in olfaction. Recent evidence suggests that the role of these proteins goes far beyond the olfactory system, in processes such as detoxification, insecticide resistance, development, nutrition, etc. Evidence from different species suggests that they could have an understudied role in taste perception. The objective of this project was to describe new components in the mosquito taste molecular machinery, and recognize targets for new-generation repellent compounds. In particular, the experiments aimed to generate evidence on the involvement of CSPs in the taste recognition in adult females of A. aegypti. Progress in understanding the molecular machinery of mosquito taste sense will provide tools for vector control, such as new-generation repellents, with an impact on the spreading of infectious diseases.*

## 1- Introduction

*Aedes aegypti* is an urban mosquito, vector of emergent arboviruses such as dengue, yellow fever, zika, and chikungunya. Almost half of the population in the world lives at risk of being infected with arboviruses, with a huge impact on public health, the economy, and the environment. Mosquitoes use taste organs located in mouthparts and legs to guide behaviors such as feeding, biting, and egg-lying. Therefore, taste sense is crucial for their survival and reproduction, and consequently for the spread of arboviruses. It could be a source of targets for new-generation repellents for personal protection. Despite this, the taste system remains understudied in mosquito

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species [1].

Membrane proteins involved in chemoreception in insects belong to different families: gustatory receptors, ionotropic receptors, pickpocket channels, and odorant receptors. Besides, two families of small soluble proteins have been studied in the context of olfaction : odorant binding proteins (OBP) and chemosensory proteins (CSP). It has been proposed that OBP and CSP act in the transport of hydrophobic odor molecules through hemolymph. Recent evidence suggests that the role of these protein families goes far beyond the olfactory system, in processes such as detoxification, insecticide resistance, development, nutrition, etc (reviewed in [2]). It has been demonstrated that OBP members have a role in taste perception in the fly *Drosophila sechellia* [3]; more recently, high expression of OBP genes was described in the labellum of the mosquito *Aedes albopictus*, a structure directly related with tasting [1]. The role of CSP in the taste system in insects has not been addressed to date, to the best of our knowledge, but the high expression of CSP in legs and mouthparts in insects [4,5] suggests that they could have a role in taste perception. Identification of new components in the mosquito taste molecular machinery can contribute in the search or targets for new-generation repellent compounds.

RNAi-mediated gene silencing (RNAi) is a powerful tool for studies on insect physiology; is is also one of the more active areas in biotechnology applied to controlling insect pests [6]. This technique allows the transcriptional repression of a specific gene by administering double-strand RNA (dsRNA) encoding a gene fragment. Small fragments of dsRNA recognize the specific sequence of a target gene mRNA by base complementarity, promoting the cleavage of the mRNA target by an enzymatic complex. In *A. aegypti*, RNAi is particularly challenging, due to lack of effectiveness and reproducibility among different mosquito strains (see for example [7]). To overcome these difficults, cost-effective systems for the *in vivo* synthesis of dsRNA by modified bacteria were developed [8].

In this project, we designed a 300 bp dsRNA fragment that allows for the simultaneous silencing of 34 conserved CSP sequences from the 42 CSPs encoded in *A. aegypti* genome [9]. We synthesised this dsRNA, together wit a dsRNA fragment from a reporter lethal gene, used as a positive control (IAP ; [10]), and an unspecific dsRNA (β-*lac* gene) used as a negative control. We compare two synthesis methods (*in vitro* and *in vivo*). These tools will allow us to test he Hypothesis of an involvement of *A. aegypti* CSPs in taste perception of aversive and/or appetitive compounds from the food source, in an ongoing stage of the project. For this, a refined protocol was designed, that will allow us to improve the behavioural test initially proposed, to better understand the involvement of CSP in mosquito taste sense. Progresses in this field has an application field in the development of new-generation repellents, with an impact on the spreading of infectious diseases.

## 2- **Experimental details**

2.1 Mosquito rearing: *A. aegypti* of the Bora strain were reared in the laboratory from eggs obtained from INFRAVEC (IRD—Montpellier, France) at 26 ± 1 °C and 60—70% RH. After their imaginal moult, the mosquitoes were kept with free access to sugar and water (imbibed cotton).

2.2 dsRNA preparation by bacteria: A fragment of *aedaecsp* or *aedaeiap* genes were amplified with specific primers (Table 1) from cDNA. A *β-lac* each gene was amplified by PCR from cDNA prepared as detailed above, using a specific primers (Table 1). PCR products were cloned into the commercial plasmid pGEM-T (Promega), following the manufacturer's instructions. The recombinant plasmids were transformed into competent *Escherichia coli* strain HT115 (DE3) cells. Transformed bacteria were cultured in LB medium supplemented with ampicillin, and dsRNA expression was induced by adding IPTG (1 mL per liter of culture). The bacteria producing dsRNA were centrifuged and the pellets were directly used for feeding larvae.

**Table 1**

| Primer name | Primer sequence |
|---|---|
| AeCSP1999F | TTCTTCATCGTTGTTCTGGCCCTG |
| AeCSP1999R | TAATACGACTCACTATAGGGA-GTACTTGGCCTTCAGCATGGTCC |
| AeIAPF | CTTCTGCCGAGTGGAAATCGG |
| AeIAPR | TAATACGACTCACTATAGGGA-ATATTCCGGTAGCTTCTGTTG |
| ARNiAMPF | CCAGTGCTGCAATGATAC |
| ARNiAMPR | TAATACGACTCACTATAGGGA-GCTGAATGAAGCCATAC |

2.3 dsRNA preparation *in vitro*:

Total RNA was extracted from adult mosquitoes with Tri-reagent (Sigma-Aldrich). cDNA was prepared using MMLV-retro transcriptase (Promega) according to manufacturer protocol. The cDNA was used for the amplification of *aedaecsp* (dsCSP) or *aedaeiap* (dsIAP) genes with specific primers (Table 1). A *β-lac* gene fragment was amplified from pGemT plasmid (ds*β-lac*) (see primer sequences in Table 1). *In vitro* transcription was carried out using the T7 polymerase enzyme (Thermo Scientific), following the manufacturer's instructions. After isopropanol precipitation, the dsRNAs were resuspended in sterile ultrapure water. To assess their integrity and size, 1.5% (w/v) agarose gel electrophoresis was performed. Quantification was carried out via gel imaging by comparison with a standard of known concentration using ImageJ software. Samples were stored at -20 °C until use.

2.4 Feeding experiments:

Ten second-instar *A. aegypti* larvae were placed in 10 mL of deionized water. Larvae were fed with bacteria expressing dsIAP or ds*β-lac*. Mortality was recorded daily until the larvae reached the pupal stage. The experiment was replicated 6 times.

2.5 Micro injections :

Male and female adult mosquitoes were collected from the colony and placed for 10 min at 4°C. Mosquitoes were placed in a cold plate, and 80 nl of either water or *in vitro* synthesized dsIAP were abdominally injected using a NanoJet III microinjector (Drummond) coupled to a glass capillaire.

## 3- Results and discussion

3.1 dsRNA treatments:

Previous results from our lab indicate that feeding *A. aegypti* larvae with bacteria producing dsRNA induced specific gene silencing in CEPAVE strain, originated from wild Argentinean mosquitoes 10 years ago. However, the efficiency of dsRNA-mediated gene silencing is challenging, and depends on the *A. aegypti* strain used [7]. In order to test Bora strain for its sensitivity to oral-delivered dsRNA, we fed larvae with bacteria expressing high levels of either dsIAP or ds*β-lac* (controls); dsIAP was used as a reporter gene, because its silencing cause a lethal phenotype in *A. aegypti* [10]. We did not observe significant differences between dsIAP and control groups in larval mortality, indicating a lack of sensitivity of Bora strain to the oral-delivery dsRNA.

In order to improve gene silencing, we set up a system for dsRNA injection in adult mosquitoes. A high mortality due to injections was observed. For this reason, we have designed a behavioral protocol that, different to the original proposal, allows the testing of individual mosquitoes instead of pools. In this way, the number of individuals necessary for obtaining statistical differences can be drastically reduced.

3.2 Improved experimental design for testing taste perception in mosquitoes:

The feeding responses of individual mosquitoes under different conditions will be assessed. A female mosquito (either from dsCSP or ds*β-lac* group) will be placed in a

plastic tube covered with a mesh, in contact with the membrane of a feeder containing artificial blood meal (110 mM NaCl, 20 mM NaHCO3, ATP 1mM) collored with a blue dye. Each female will be exposed to a feeder where the membrane and the artificial meal will be treated with one of three solutions :

a) Quinin 1 mM (bitter and aversive; [11].

b) Lysine 4 mM (appetitive; [1]).

c) distilled water.

The behavior of the mosquitoes will be monitored and its behavior will be recorded for 5 min. After this period, each female will be checked for feeding success, by examining the color of its abdominal content. A score of 1 will be assigned to fed and 0 to unfed females. Twenty individuals will be tested for each condition. Statistical analysis will be performed with a binomial GLM with a logit link function to model the probability of feeding in individual mosquitoes. The ratio between the insects fed under each stimulus (appetitive or aversive) and the control condition (water) will be calculated. It is expected that, if the Hypothesis of an involvement of CSPs in taste is corroborated, this ratio will not significant differ between stimulus and control. Conversely, for ds*β-lac* group, mosquitoes will prefere the appetitive and reject the aversive compounds, compared to water.

Regarding the constraint in the obtention of a high number of treated mosquitos, the improved protocol will allow the obtention results by reducind the number of insects used.

## 4- Conclusion

Our work allowed to determine the lack of sensitivity of Bora strain to dsRNA by oral administrarion in the conditions assayed. A protocol for microinjection coupled with an improved behavioural assay was designed, joining together scientific capabilities of the international collaboration. Further assays will be performed in order to test hypothesis related to taste sense in mosquitoes, by using dsRNA and refined behavioural and statistic tools.

## 5- Perspectives of future collaboration with the host laboratory

The productive scienfific discussion mantained by Drs. Ons and Lazzari during the stage at IRBI, allowed for the design of an optimized protocol. The experiments are planned to be performed in the Argentinean lab, in collaboration with Dr. Romina Barrozo. A facility for behavioral experiments in mosquitoes its being organized; the laboratories will collaborate in this research line in the years to come.

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