Report on the outcomes of a Short-Term Scientific Mission[1]

Action number: CA17108

Grantee name: Alexandros Belavilas-Trovas

Details of the STSM

Title: Characterization of reproductive lncRNAs of Aedes aegypti

Start and end date: 12/09/2022 to 15/10/2022

Description of the work carried out during the STSM

During the STSM the grantee successfully completed the work packages and program's goals.

WP-1: LncRNA expression analysis

He conducted a comprehensive analysis of genomic data derived from DNA and RNA sequencing of *Ae. aegypti* and created a shortlist of lncRNAs that were differentially expressed in reproductive-related conditions. He focused on a lncRNA that shared common features with a published reproductive-related lncRNA of *Ae. albopictus* (Belavilas-Trovas et al, 2022) and performed functional experiments to characterize its role in *Ae. aegypti*. Moreover, he shortlisted protein-coding genes with potential role in reproduction of *Ae. aegypti* that could be regulated by the lncRNA. He reared a colony of Ae. aegypti Liverpool strain and extracted RNA from ovaries of non-blood fed and blood-fed mosquitoes in serial time-points post blood meal (PBM): 12h, 24h, 36h, 48h, 60h, 72h. He synthesized cDNA and generated the expression profile of the selected lncRNA and other protein-coding genes via qPCR.

WP-2: RNAi assays and phenotypic impact of knockdown

Afterwards, he used genomic DNA as a template to create dsRNA against the lncRNA, a protein-coding gene (Mucin C.1) and an eGFP control. He administered the dsRNA through adult microinjection into groups of female mosquitoes 5 days post their eclosion to trigger an RNAi-mediated gene silencing effect. Two days post-injection he provided fresh blood to the mosquitoes and assessed the effect of gene silencing in specific reproductive phenotypes. 1) He measured the average ovarian follicle length of 30 individual mosquitoes from each group 48h and 72h PBM. 2) He counted the eggs laid by 50 individual mosquitoes of each group to compare their oviposition rate. 3) He hatched the eggs that were collected from the previous assay and calculated the number of larvae that were produced.

[1] This report is submitted by the grantee to the Action MC for approval and for claiming payment of the awarded grant. The Grant Awarding Coordinator coordinates the evaluation of this report on behalf of the Action MC and instructs the GH for payment of the Grant.

Fig.1: dsRNA-treated mosquitoes placed individually into cups for fecundity bioassay

Fig.2: Ovaries dissected from mosquitoes 48h PBM

WP-3: Post-RNAi expression analysis

Finally, he extracted RNA from the ovaries of 10 individual dsRNA-treated mosquitoes of each group 48h and 72h PBM and used it as a template for cDNA synthesis. He performed qPCR assays to assess the expression level of the targeted genes and validate the expression drop provoked by RNAi in all dsRNA-treated groups. Moreover, he collected data regarding the effect of gene silencing of the lncRNA in other protein coding genes that are located in its vicinity, such as Mucin C.1 (located 120kb upstream).

Description of the STSM main achievements and planned follow-up activities

The main achievements of the STSM are the following:

1: Detection of novel reproductive-related genes of Ae. aegypti

The project produced strong preliminary results regarding the influence of a species-specific lncRNA in reproductive phenotypes of *Ae. aegypti*. Its RNAi mediated silencing resulted in a statistically significant reduction of laid eggs in dsRNA-treated mosquitoes, compared to the eGFP control. In addition, silencing of its neighbouring Mucin C.1 produced an effect in the ovarian development as observed 48h post blood meal that will be further investigated. Specifically, the average length of the ovarian follicles of dsMucin.1-treated mosquitoes was significantly smaller compared to the eGFP control. Ongoing experiments that investigate the potential interplay between the lncRNA and Mucin C.1 and other genes aim to explain the parallel effect of on different reproductive traits. Neither lncRNAs nor mucins have been reported to affect reproductive phenotypes of *Ae. aegypti* so far.

2: Exchange of knowledge and access to infrastructure

During this STSM there was a significant exchange of knowledge between the grantee and the members of the hosting group. The grantee was trained in rearing *Ae. aegypti* mosquitoes and learned how to perform reproductive and fitness bioassays of the species. *Ae. aegypti*, is a mosquito that is not

allowed to be reared in his current academic environment in Greece, due to special regulations that apply.

Moreover, he exchanged knowledge and gained skills on adult and embryonic microinjection of mosquitoes and familiarized himself with the facilities and equipment that he made use at the University of Giessen.

3: Future follow-up collaborations

Through the project the impact of lncRNAs in both *Aedes albopictus* and *Aedes aegypti* was highlighted indicating a potential functional conservation. The grantee generated strong preliminary results that will serve as the basis for a collaborative publication. The next steps of the project include the validation of the interplay of the lncRNA, Mucin C.1 and other protein-coding genes of the mosquito and the generation of knock-out (KO) strains of mosquitoes whose lncRNA and Mucin C.1 genes will be deducted. Our goal is to prove that the KO mosquitoes will lose their ability to reproduce. If our hypothesis is confirmed, then a research article will be published presenting all produced data. The expected publication will justify why lncRNAs should be considered ideal species-specific targets with prospects for pest control.