

## Abstract

Long non-coding RNAs (lncRNAs) constitute a type of RNA molecules that emerged during the last decades as important regulators of cellular and metabolic processes. Through their interaction with the genomic DNA, other RNA transcripts or proteins, they can modulate vital biological pathways of eukaryotic organisms. Several studies in insect models like *D. melanogaster* and *B. mori* have revealed the role of lncRNAs in important processes like development, reproduction, and immunity. However, limited research had been conducted, up to now, to elucidate the functional roles of lncRNAs in disease vectors, like mosquitoes.

The present thesis aimed to fill this gap by investigating the features and functions of lncRNAs in two major *Aedes* mosquitoes: *Ae. albopictus* and *Ae. aegypti*. These two species are the main vectors of several arboviruses, like Zika, dengue, chikungunya, yellow fever that have been responsible for multiple disease outbreaks throughout the world. The goal was to unravel the role of specific lncRNAs in three biological systems that have great potential for mosquito control: reproduction, immunity, and development.

A similar pipeline was applied for the detection of lncRNAs in each system. First, a comprehensive analysis of genomic and transcriptomic data was conducted to unravel lncRNAs that were differentially expressed in *Ae. albopictus* or *Ae. aegypti* under conditions, related to reproduction, immunity, or development. Then, a shortlist of lncRNAs was generated, their expression profiles were validated by qPCR, and gene expression was inhibited through RNAi-mediated silencing. RNAi was triggered through microinjection of dsRNA in adult mosquitoes. The phenotypic effect of lncRNA silencing was assessed to determine their potential role in the studied pathways.

Reproductive analysis of *Ae. albopictus* focused on the elucidation of the role of ovary-specific lncRNAs that were enriched after a blood-meal. From several targeted one (Norma3) was highlighted due to its pronounced effect on fecundity and fertility. Silencing of Norma3 provoked an intensive reduction of oviposition (43%) and hatching rates (53%), along with other defected phenotypes, such as retarded ovarian growth and defective embryonic development.

Immune analysis of *Ae. aegypti* focused on the investigation of the influence of lncRNAs that were differentially expressed upon infection with Zika virus, in immunity and reproduction. Three lncRNAs (Zinc1, Zinc2, Zinc22) had an impact on immunity, as their silencing enhanced immune response and reduced significantly viral intensity. Moreover, targeting of two lncRNAs (Zinc22, Zinc9) affected fecundity and fertility of *Ae. aegypti* indicating their potential role in trade-offs.

Developmental analysis of *Ae. albopictus* relied on the detection of larval-specific genes that could affect larval molting or its metamorphosis to pupae. Various genes that were enriched in larvae were detected and an RNAi pipeline was developed. Instead of injecting dsRNA, a methodology based on feeding of larvae with dsRNA-expressing *E. coli* bacteria was preferred, to reduce fitness cost. Results from these assays indicated that bacterial feeding was an insufficient method to trigger RNAi response, contrary to what is stated in published research articles.

In addition to the identification of specific lncRNAs that influence reproduction and immunity of *Aedes spp.*, our computational study confirmed the high species-specificity of lncRNAs that has already been reported in other species. lncRNAs of *Ae. albopictus* presented extremely high species-specificity as 7,827 out of the 7,900 annotated lncRNAs (99%) did not share sequence homology with other insect species. The respective number of species-specific lncRNAs of *Ae. aegypti* was 3,6403 out of the 4,709 annotated genes (77.4%).

Overall, the present study introduced species-specific lncRNAs that have a great potential for pest management approaches, especially Norma3 and Zinc22, two lncRNAs that modulate fecundity and fertility of female *Ae. albopictus* and *Ae. aegypti* respectively. Species-specificity is a crucial matter for insecticide development, due to the serious off-targeting side effects on beneficial insects, including pollinators and natural predators. Species-specificity along with high efficiency are the ultimate goals when developing pest control approaches. The high species-specificity of lncRNAs in combination with their functional significance render them ideal candidates as molecular targets of novel pesticides.