Experimental selection of *Plasmodium berghei* adapted to *Nyssorhynchus darlingi* and investigation of molecular determinants of parasite-vector compatibility

Amanda Caroline de Jesus Alves[1]; Elaine Hellen Nunes Chagas[1]; Rosa Amélia Gonçalves Santana[2]; Stefanie Costa Pinto Lopes[2,3]; Gerhard Wunderlich[1]; Daniel Youssef Bargieri[1]

[1]Programa de Pós-graduação em Biologia da Relação Patógeno-Hospedeiro, Departamento de Parasitologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, SP, Brasil.
[2]Fundação de Medicina Tropical Dr. Heitor Vieira Dourado, Manaus, AM, Brasil.
[3]Instituto Leônidas e Maria Deane, Fiocruz-Amazônia, Manaus, AM, Brasil.

In Brazil, *Nyssorhynchus darlingi* is the main vector of *Plasmodium*. Experimental models are essential for understanding parasite-vector interactions. However, the murine model with *P*. *berghei* traditionally employs *Anopheles stephensi*, a species not native to Brazil. Recent evidence indicates that *P. berghei* can complete part of its development in *Ny. darlingi*, and the recent colonization of this mosquito in Brazil makes it a promising alternative as an experimental vector, including for other *Plasmodium* species. Thus, the aim of this study is to experimentally select a lineage of *P. berghei* capable of infecting *Ny. darlingi* and to investigate genetic alterations associated with parasite adaptation, as well as to evaluate the infectivity of *P*. *falciparum* isolates in *Ny. darlingi* and analyze the variability of the *P47* gene in these parasites. Mosquitoes originating from a colony maintained in Manaus, Brazil, were adapted to the insectary at the University of São Paulo and will be fed on mice infected with *P. berghei* GFP@HSP70. Parasites recovered from infected mosquitoes will be retransmitted to new mice, allowing successive cycles of selection of lineages potentially adapted to the vector. Parasite development will be monitored by fluorescence microscopy and by dissection of the midgut and salivary glands. Lineages before and after selection will be compared by genomic sequencing. In parallel, mosquitoes will be infected with *P. falciparum* isolates, including the NF54 strain and Brazilian isolates. The *P47* gene will be amplified and sequenced to analyze genetic variability and its possible association with susceptibility to infection in *Ny. darlingi*. The colony is currently being maintained in the USP insectary and, after an initial adaptation period, shows population growth. This study may contribute to the development of an experimental model involving Amazonian vectors and expand the understanding of the molecular determinants of parasite-vector interactions.