Developing new genetic tools to manipulate the mosquito olfactory system

Introduction

The mosquito *Anopheles gambiae* is the major Afrotropical vector of malaria. Female mosquitoes target humans, as they need blood to produce eggs, causing ~435 000 deaths per year (WHO, 2018). Current efforts to control malaria are insufficient, mainly because mosquitoes quickly develop resistance to commonly used insecticides. Effects of climate change also enhance malaria transmission. Therefore, innovative strategies are needed to control the disease.

Genetic manipulation of mosquito neurons is a potentially promising yet unexplored strategy (Riabinina *et al.*, 2016, Afify *et al.*, 2019). Mosquitoes rely heavily on their sense of smell to find humans. Thus, reprogramming the mosquito olfactory neurons is likely to change their preference for human smell and divert them from biting humans. Better understanding of the mosquito olfactory system will lead to the development of more effective insect repellents and traps, while release of mosquitoes with modified olfaction into the wild could be considered in future.

Aims

This project aims to develop new genetic tools to manipulate the function of olfactory sensory neurons in *Anopheles gambiae*. In Part 1, I will make two DNA constructs for mosquito transgenesis. In Part 2, I will test two existing constructs in the fruit fly *Drosophila melanogaster* by olfactory behavioural assays. Once the functionality of these constructs in *Drosophila* is confirmed, they will also be used to create transgenic mosquitoes.

Methods

For transgenesis, I will use the Q-system, a recently developed genetic tool (Potter *et al.*, 2010; Riabinina *et al.*, 2015). It has two components: a driver (X-QF2) and a responder (QUAS-Y). In my case, the driver ORCO-QF2 will limit manipulations to olfactory sensory neurons. Responders will enable three different neuronal modifications, widely used in *Drosophila*: neuronal silencing (Baines *et al.*, 2001), optogenetics (Klapoetke *et al.*, 2014) and chemogenetics (Chin *et al.*, 2018). Responders will be cloned (Part 1), functionally tested in *Drosophila* (Part 2), and used to create transgenic mosquitoes. Due to time constraints, the last step (takes >1 year) is not included in this project.

Olfactory neurons will be silenced via expression of the inward-rectifying K^+-channel Kir2.1 (Baines *et al.*, 2001). It is expected to reduce responses to smells. Optogenetic activation will be achieved via expression of CsChrimson, a cation channel that opens under red light, depolarising olfactory neurons (Klapoetke *et al.*, 2014). Finally, olfactory neurons will be activated via endogenous expression of the mosquito larval-specific olfactory receptors OR40 and OR52. OR40 responds to the widely used repellent DEET, while the ligand specificity of OR52 is unknown and will be studied (Xia *et al.*, 2008). I expect to see increased repellence by DEET in flies and mosquitoes that overexpress OR40.

Part 1: Creation of new DNA constructs

I will create two DNA constructs: QUAS-CsChrimson and QUAS-OR52. I will use the QUAS-GFP vector plasmid (Riabinina *et al.*, 2016), replacing the GFP gene with CsChrimson/OR52 genes. The CsChrimson sequence will be PCR-amplified from an existing plasmid (https://www.addgene.org/111544/). The OR52 sequence will be PCR-amplified from the genomic DNA of *Anopheles gambiae* (1164bp). The PCR products will be InFusion-cloned into the vector plasmid.

Part 2: Behavioural testing of DNA constructs in Drosophila

Existing QUAS-Kir2.1 and QUAS-OR40 flies will be crossed with existing Orco-QF2 flies. The progeny (larvae and adults) will express Kir2.1 or OR40 in olfactory receptor neurons.

Larvae will be given a choice between two odorants (Xia *et al.*, 2008). Their movements will be video-recorded for 10 minutes, quantified (using ImageJ, BioImage and Prism), and compared with wild-type controls. Adult flies will be tested in a trap assay. They will be placed in a box with two traps: one containing an attractive (e.g. ethyl acetate, isoamyl acetate) or repellent (e.g. benzaldehyde) odorant and one control. Flies in traps will be counted after 24 hours, and transgenic lines will be compared with wild-type controls.

Project timeline

Weeks 1-2: Clone constructs, send for sequencing. Set up fly crosses.

Week 3: Generate high-volume, high-concentration DNA via maxiprep using the correct plasmids. Send for injection into *Drosophila*. Collect progeny of correct genotype from fly crosses.

Weeks 4-5: Behavioural assays. Start data analysis and write-up of results.

Week 6: Finish data analysis and write-up.

Summary

This project will create two new DNA constructs and verify the functionality of two other constructs by behavioural assays in *Drosophila*. As the next step, if their functionality is confirmed, these constructs will be used to create transgenic mosquitoes. This project will make new genetic modifications possible, contributing to our understanding of the olfactory system of the major malaria vector.

References

Afify, A. *et al.* (2019) 'Commonly used insect repellents hide human odors from Anopheles mosquitoes', *Current Biology*. Elsevier, 29(21), pp. 3669–3680.

Baines, R. A. *et al.* (2001) 'Altered electrical properties in drosophilaneurons developing without synaptic transmission', *Journal of Neuroscience*. Soc Neuroscience, 21(5), pp. 1523–1531.

Chin, S. G. *et al.* (2018) 'Olfactory neurons and brain centers directing oviposition decisions in Drosophila', *Cell reports*. Elsevier, 24(6), pp. 1667–1678.

Klapoetke, N. C. *et al.* (2014) 'Independent optical excitation of distinct neural populations', *Nature methods*. Nature Publishing Group, 11(3), p. 338.

Potter, C. J. *et al.* (2010) 'The Q system: a repressible binary system for transgene expression, lineage tracing, and mosaic analysis', *Cell*. Elsevier, 141(3), pp. 536–548.

Riabinina, O. *et al.* (2015) 'Improved and expanded Q-system reagents for genetic manipulations', *Nature methods*. Nature Publishing Group, 12(3), p. 219.

Riabinina, O. *et al.* (2016) 'Organization of olfactory centres in the malaria mosquito Anopheles gambiae', *Nature communications*. Nature Publishing Group, 7, p. 13010.

World Health Organization (2018) 'World Malaria Report', Geneva, Licence: CC BY-NC-SA 3.0 IGO.

Xia, Y. *et al.* (2008) 'The molecular and cellular basis of olfactory-driven behavior in Anopheles gambiae larvae', *Proceedings of the National Academy of Sciences*. National Acad Sciences, 105(17), pp. 6433–6438.