Drosophila as a model for mosquito: olfactory signals and host seeking behaviour

*Indira Paddibhatla and Rakesh Kumar Mishra**

Olfactory cue dependence of insects such as mosquitoes is beneficial in understanding insect behaviour during host seeking. Understanding of the host-seeking behaviour, particularly, the olfactory process involved in it at molecular level is likely to provide new means of controlling insects. A number of studies have shown Drosophila as a successful model system to decipher and delineate the role of olfactory genes. Such studies can be extended to understand the host-seeking behaviour of mosquitoes. We discuss recent progress in this area and an emerging new approach that can utilize Drosophila, genetically modified to carry mosquito receptors, in identifying compounds that can be subsequently used to control mosquitoes.

Keywords: *Drosophila*, host-seeking behaviour, mosquito, olfactory signals.

INSECTS form one of the most versatile groups among animals and have the greatest species diversity. Due to their unparalleled strength of variety, insects show the most extensive range of interactions in the ecosystem. From the human perspective, insects play an important role in decomposition, pollination, transmission of human and cattle pathogens, and as crop pests with the most visible significant consequences. Mosquito bite leads to severe health problems and as vectors, mosquitoes transmit a number of diseases. Female mosquitoes feed on blood and transmit parasites such as *Plasmodium*, *Wuchereria bancrofti* and also viral infections to their hosts (Table 1). While the harmful effects of mosquitoes are many, the methods used to tackle them are few and inefficient. Insecticides, pesticides, repellents and mosquito traps are some of the ways of controlling the mosquito population. Pesticides used for controlling mosquitoes such as pyrethroid or organophosphate insecticides have various side effects that include acute and chronic problems. Even with this compromise, there is clear risk of mosquitoes developing resistance to insecticides[1]. The common ingredient in most of the insect repellents is DEET (*N*,*N*-diethyl-meta-toluamide), which repels but does not eliminate mosquitoes. Besides, most of the chemicals and drugs against mosquitoes in any form can be harmful to other harmless or beneficial insects, and disturb the ecosystem on extensive usage. In spite of continuous efforts, research has not delivered an effective method to control mosquitoes.

One of the approaches to create a 'mosquito-free surrounding' can be to disrupt the communication system that mosquitoes use in identifying their hosts. A recent finding showed that the receptors expressed in the CpA neurons of the mosquitoes interpret both CO_2 and odour to locate their hosts, which opens a new possibility in this direction[2]. This study also showed that fruit flies could be used for analysing the behavioural aspects of mosquitoes, which opens a new front to tackle this major public health issue, especially in a country like India.

First reported in 1922, mosquitoes were shown to be attracted to CO_2 (ref. 3). Chemosensory neurons involved in detecting CO_2 are found in the antennae or the appendages (maxillary and labial) in many insects. Maxillary palps of *Aedes aegypti* mosquito have CpA neuron cells

Table 1. List of diseases and mosquitoes as the vectors

Disease	Mosquito
Malaria	*Anopheles*
Dog heart worm	*Anopheles*
Dengue	*Aedes*
Yellow fever	*Aedes*
Chikungunya	*Aedes*
La Cross encephalitis	*Aedes*
Murray Valley encephalitis	*Culex*
Barmah Forests virus infection	*Culex*
Japanese encephalitis	*Culex*
Filariasis	*Culex*
Rift Valley virus	*Culex* and *Aedes*
Ross River virus infection	*Culex* and *Aedes*
West Nile disease	*Culex* and *Aedes*
Western equine encephalitis	*Culex* and *Aedes*
St Louis encephalitis	*Culex* and *Aedes*
Eastern equine encephalitis	*Culiseta*, *Aedes*, *Coquillettidia* and *Ochlerotatus*

The authors are in the Centre for Cellular and Molecular Biology, Council of Scientific and Industrial Research, Uppal Road, Hyderabad 500 007, India.
*For correspondence. (e-mail: mishra@ccmb.res.in)

(Figure 1) that co-express the three CO_2 receptors (Gustatory receptors – Gr1, Gr2, Gr3)[4,5]. In *Anopheles gambiae*, CpA neurons co-express conserved receptors Gr22, Gr23, and Gr24. To date, CO_2 receptor neuron CpA is the only known olfactory receptor neuron (ORN) activated in response to CO_2 and host odour signals in mosquitoes. In *Drosophila*, Gr21a (ortholog of Gr1 and paralog of Gr2) and Gr63a (ortholog of Gr3) ORNs, all of which respond to CO_2 levels are expressed in ab1C neurons. Sharing the receptors and their functions with those of mosquitoes, *Drosophila* offers an excellent model to identify the ligands interacting with such receptors to interfere with the communication system in mosquitoes.

Drosophila can be used as a mosquito model for olfaction mechanism due to comparable olfactory system between the two insects[6]. A recent study showed *Drosophila* to be an effective paradigm to determine if the three receptors Gr1, Gr2 and Gr3 in the mosquito CpA neurons that respond to CO_2 can also respond to human odour and to other ligands[2]. In this study a new method was first followed by shutting down CpA responses using butaryl chloride, a volatile compound, and then exposing mosquitoes to CO_2 and to human foot odour. Results showed that CpA neuron, after being blocked, did not show any firing response, while the other two CpB and CpC neurons were not affected by the exposure to butaryl chloride and showed low responses, implicating the role of CpA alone. In order to elucidate the strongest activators and inhibitors of the CpA neurons, an initial list of compounds was established. Based on computational similarity to known ligands of the CO_2 receptor, >440,000 compounds were screened. Eventually a final number of 138 odorant-like compounds were selected based on their smell, their presence in natural sources, human safety (by the Flavor and Extract Manufacturer's Association) and also for being cost-effective. Selected compounds were tested on *Aedes aegypti* and *Culex quinquefasciatus* for strongest activators and inhibitors of CpA neurons. In both species, the CpA neuron responses to a set of selected odorants were similar. Finally, two compounds, cyclopentanone and ethyl pyruvate, were shown as the strongest activator and strongest inhibitor of CpA neurons respectively.

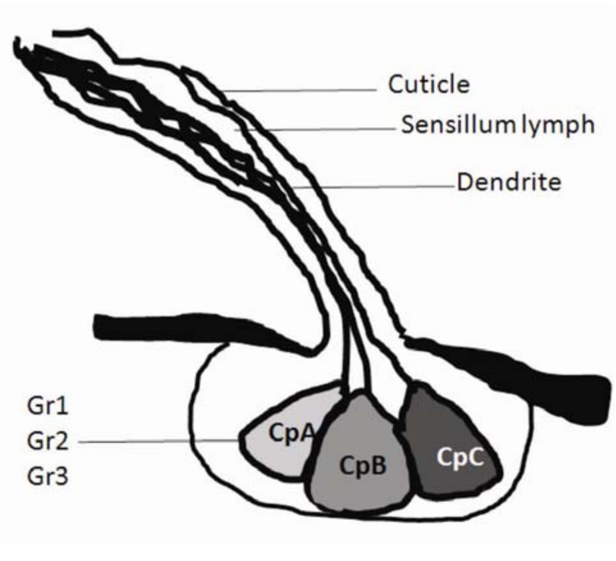

Figure 1. Schematic showing the maxillary palp of mosquito with three neurons (CpA, CpB and CpC) and the gustatory receptors in the CpA neurons.

Tauxe et al.[2] further exploited *Drosophila* to determine if the ligands under test evoked responses only by the mosquito Gr1, Gr2 and Gr3 receptors of the CpA neurons and not the other receptors present in the same cell such as the *Or* or *IR* families. *Drosophila orco* mutants were used for this purpose. Homozygous *Or83b* null (*orco* mutants) flies show reduced general olfactory capabilities and yet sense CO_2 normally[7]. Hence these *orco* mutants in *Drosophila* were used to exclude the *Or*-expressing neuron responses and define the specific role of the Gr21a and Gr63a receptors in ab1c neuronal (similar to mosquito CpA neurons) response to a set of odorants, including cyclopentanone and ethyl pyruvate. These experiments confirmed the role of mosquito CpA neuron expressing the Gr receptor in both detecting CO_2 and also responding to the other ligands.

This study provides strong evidence in support of using *Drosophila* as a model to decipher the mosquito olfactory system. It opens an approach to screen for compounds or plant extracts/preparations that can act as ligands, both attractants and repellents of mosquitoes and *Drosophila*. A further extension of this approach can be that of using transgenic *Drosophila* genetically adopted to mosquito olfaction – mutants or knockdown flies with widely used UAS-GAL4 system of endogenous receptors (loss of receptors sensitive to CO_2 in ab1c neurons) at the same time carrying the mosquitoes CO_2 receptor. These transgenic flies are expected to behave like mosquitoes to a great degree and, therefore, can be used for screening of odorants/ligands obtained from the chemical library to determine the strongest attractant and strongest repellent (Figure 2). Such screens can provide the most responsive attractants and repellents signalling the CpA neurons in mosquitoes. These data can be eventually utilized in understanding the responses of mosquitoes for the regulation of CpA neurons expressing the gustatory receptors. This approach helps construe further hypothesis in mosquito research taking us one more step forward towards understanding olfactory cue dependence of mosquitoes and the genes involved. This knowledge can help identify and compare the downstream pathways and signalling molecules responding to gustatory receptors, and use powerful tools of *Drosophila* to understand molecular aspects of this process. The new approach discussed here opens a possibility to identify strongest mosquito repellents and attractants in fruit flies carrying mosquito CO_2 receptor. Attractants to trap in and repellents to repel off mosquitoes can offer effective control in any surrounding.

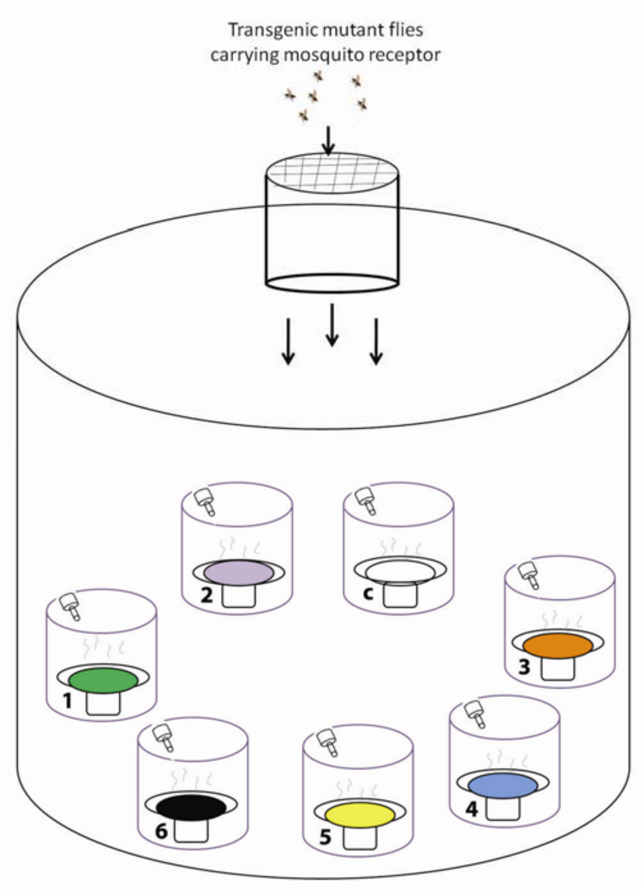

Figure 2. Screen to search for attractants and repellents from the chemical library. Transgenic *Drosophila*, which is mutated for its own receptor and carries mosquito receptor, is exposed to a set of compounds from a common entrance. Greater or smaller number of files in different sub-compartment can be taken as indicator of attractant or repellent property, respectively, of the corresponding compound.

Genetics and genome of *Drosophila* can be further utilized to explain the molecular basis of insect behaviour and the underlying mechanisms.

Even though Tauxe *et al.*[2] used *Drosophila* to decipher the exact role of gustatory receptors in CpA neurons, the study involved only uninfected *Aedes aegypti*, *Anopheles gambiae* and *C. quinquefasciatus*. Evidences from the earlier literature suggest that mosquito vectors behave differently when carrying the pathogen involved in disease transmission. Understanding the responses of CpA neurons in mosquitoes infected with pathogens can lead to interesting information on pathogens manipulating host response[8]. Yet to be answered is the effect of these ligands, cyclopentanone and ethylpyruvate, on pathogen-infected disease-causing mosquitoes. It also remains to be addressed if the effect of the identified compounds using other species of mosquitoes will have similar and as effective interruption of the olfaction process of malarial parasite *Anopheles stephensi* that affects the Indian population. The answers to these questions can provide effective means for developing repellents/attractants against the desired mosquitoes species.

1. Liu, N., Insecticide resistance in mosquitoes: impact, mechanisms, and research directions. *Annu. Rev. Entomol.*, 2015, **60**, 537–559.
2. Tauxe, G. M. *et al.*, Targeting a dual detector of skin and CO_2 to modify mosquito host seeking. *Cell*, 2013, **155**, 1365–1379
3. Crumb, S. E., A mosquito attractant. *Science*, 1922, **55**, 446–447.
4. Jones, W. D., Cayirlioglu, P., Kadow, I. G. and Vosshall, L. B., Two chemosensory receptors together mediate carbon dioxide detection in *Drosophila*. *Nature*, 2007, **445**, 86–90.
5. Lu, T. *et al.*, Odor coding in the maxillary palp of the malaria vector mosquito *Anopheles gambiae*. *Curr. Biol.*, 2007, **17**, 1533–1544.
6. Ramdya, P. and Benton, R., Evolving olfactory systems on the fly. *Trends Genet.*, 2010, **26**, 307–316.
7. Larsson, M. C. *et al.*, Or83b encodes a broadly expressed odorant receptor essential for *Drosophila* olfaction. *Neuron*, 2004, **43**, 703–714.
8. Smallegange, R. C. *et al.*, Malaria infected mosquitoes express enhanced attraction to human odor. *PLoS ONE*, 2013, **8**, e63602.

ACKNOWLEDGEMENT. I.P. thanks the Council of Scientific and Industrial Research (CSIR), New Delhi for the CSIR Nehru postdoctoral fellowship.

Received 12 January 2015; revised accepted 3 August 2015

doi: