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Genetic Decoding of the African Malaria Mosquito Olfactory System: New Insights into Responses to Human Odors

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The paper "An expanded neurogenetic toolkit to decode olfaction in the African malaria mosquito Anopheles gambiae," authored by Diego Giraldo, Andrew M. Hammond, Jinling Wu, et al., was published in *Cell Reports Methods* on March 27, 2024, from institutions such as Johns Hopkins Malaria Research Institute, Johns Hopkins Bloomberg School of Public Health, and Department of Life Sciences, Imperial College London. In this research, Giraldo and colleagues developed a neurogenetic toolkit to decode the olfactory system of the African malaria mosquito, *Anopheles gambiae*. By creating cell-type-specific driver lines, the research team successfully encoded genetic access to specific olfactory sensory neuron populations and validated the application of these tools in decoding mosquito responses to human odors. The method integrates the driver-responder-marker (DRM) system using CRISPR-Cas9 technology, thereby enabling rapid identification of the expression patterns of target chemoreceptor genes by screening GFP+ olfactory sensory neurons.

1 Experimental Data Analysis

In this study, scientists successfully achieved cell-type-specific expression in olfactory sensory neurons of the African malaria mosquito, *Anopheles gambiae*, by applying CRISPR-Cas9 gene editing technology. This technological breakthrough allows the research team to precisely manipulate the activity of specific chemoreceptor genes, thereby exploring how mosquitoes recognize and respond to human odors through olfaction. Furthermore, the introduction of calcium imaging techniques enabled researchers to observe in real-time the activity changes in these olfactory sensory neurons upon exposure to human odor molecules, revealing the differential responses of various chemoreceptor genes to specific olfactory substances. These experimental results provide important biological insights into understanding the olfactory host-seeking mechanism of mosquitoes.

Figure 1 illustrates the driver-responder-marker (DRM) system constructed using CRISPR-Cas9 mediated homology-directed repair (HDR) technology, which is designed for rapid reporting of gene expression patterns and has developed a binary T2A-QF2 driver for the Anopheles gambiae chemoreceptor genes *Gr22*, *Ir25a*, and *Ir76b*. The diagram shows the three components of the DRM module: the T2A-QF2 driver, *QUAS-mCD8*::GFP responder, and *Act5C-ECFP* transgenic marker. These components are surrounded by homology arms to facilitate the correct insertion of the DRM module into the coding exons of *Gr22*, *Ir25a*, and *Ir76b*, generating the corresponding DRM lines. Part B explains that once the DRM lines are established, the *QUAS-mCD8*::GFP responder component can be removed via Cre-loxP mediated excision to produce standard *T2A-QF2* driver lines suitable for binary use, resulting in the $Gr22^{QF2}$、$Ir25a^{QF2}$ and $Ir76b^{QF2}$ lines. This system provides an efficient tool for rapidly and precisely manipulating and identifying specific olfactory neurons.

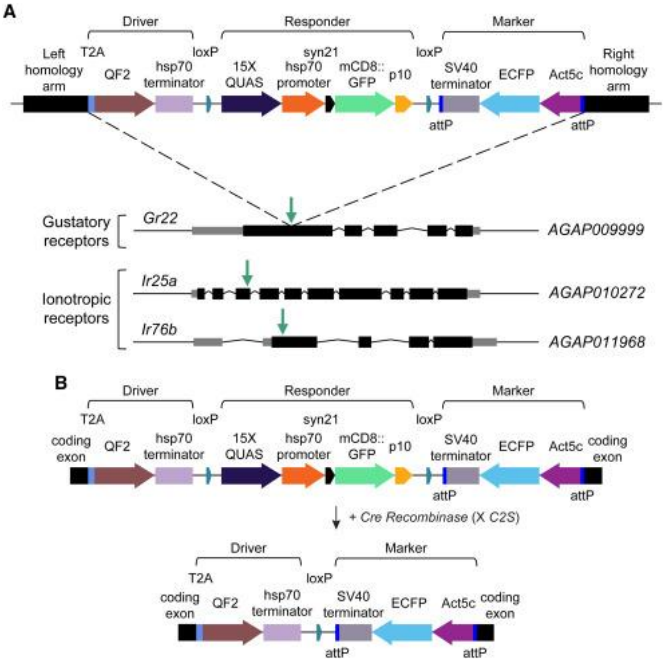

Figure 1 Schematic of the DRM system for rapid reporting of gene expression patterns and development of binary T2A-QF2 drivers for the Anopheles gambiae chemoreceptor genes *Gr22*, *Ir25a*, and *Ir76b*

Figure 2 displays the expression patterns of the chemoreceptor genes *Gr22*、*Ir76b* and *Ir25a*, modified through T2A-QF2 technology, in the olfactory organs of the African malaria mosquito Anopheles gambiae. Part A shows the labeling of the mosquito's head's primary olfactory appendages, with the right panel summarizing the expression patterns of *QUAS-GCaMP6f* activated by *Gr22* ^{QF2}, *Ir76b* ^{QF2} and *Ir25a* ^{QF2} within these appendages. Part B reveals that *Gr22+* olfactory sensory neurons are confined to the antennae in the *Gr22* ^{QF2} genotype. Part C indicates that *Ir76b+* neurons are expressed solely in the antennae and the labial palps. Part D shows a broader expression of *Ir25a+* neurons in the antennae, maxillary palps, and labial palps. The scale bar represents 30 mm, providing a reference for observation. These results reveal the distribution and response characteristics of specific olfactory neurons within the olfactory organs, which is of significant importance to the study of olfactory navigation in malaria mosquitoes.

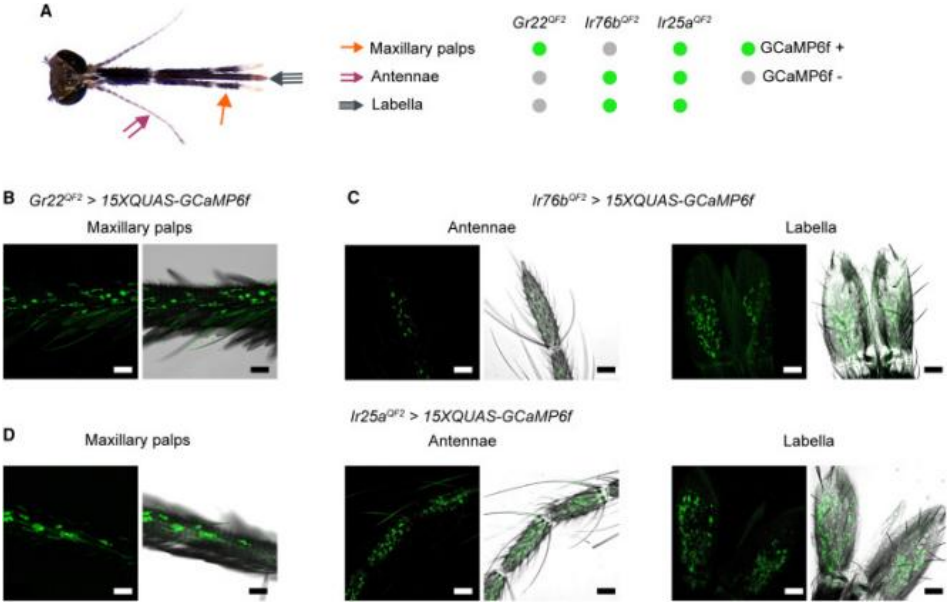

Figure 2 T2A-QF2 in-frame fusions for binary use report expression patterns of the chemoreceptor genes *Gr22*, *Ir76b*, and *Ir25a* in Anopheles gambiae olfactory appendages

Figure 3 reveals the integration of the driver-responder-marker (DRM) system into the olfactory gene *orco* in the African malaria mosquito, *Anopheles gambiae*. Part A displays a diagram of the DRM system integrated into the *orco* gene using CRISPR-Cas9 mediated homology-directed repair (HDR) technology, and the construction of a binary-use *orco*^{QF2} driver line through excision of the responder gene by Cre-loxP technology. Part B shows the expression of *orco*+ olfactory sensory neurons in the antennae, maxillary palps, and labial palps. Part C exhibits the heterologous expression of the *Act5C-ECFP* marker in different T2A-QF2 integration lines, highlighting the positional effects of specific gene loci on responder and marker transgene expression. It shows the expression of *Act5C-ECFP* marking T2A-QF2 driver integration in the midgut of larvae, and the expression of *3xP3-ECFP* marking *QUAS-GCaMP6f* responder integration in the ventral nerve cord and optic lobes. These results not only show the locality dependence of gene expression but also emphasize the complexity of gene expression regulation that must be considered when implementing gene editing strategies in complex biological systems.

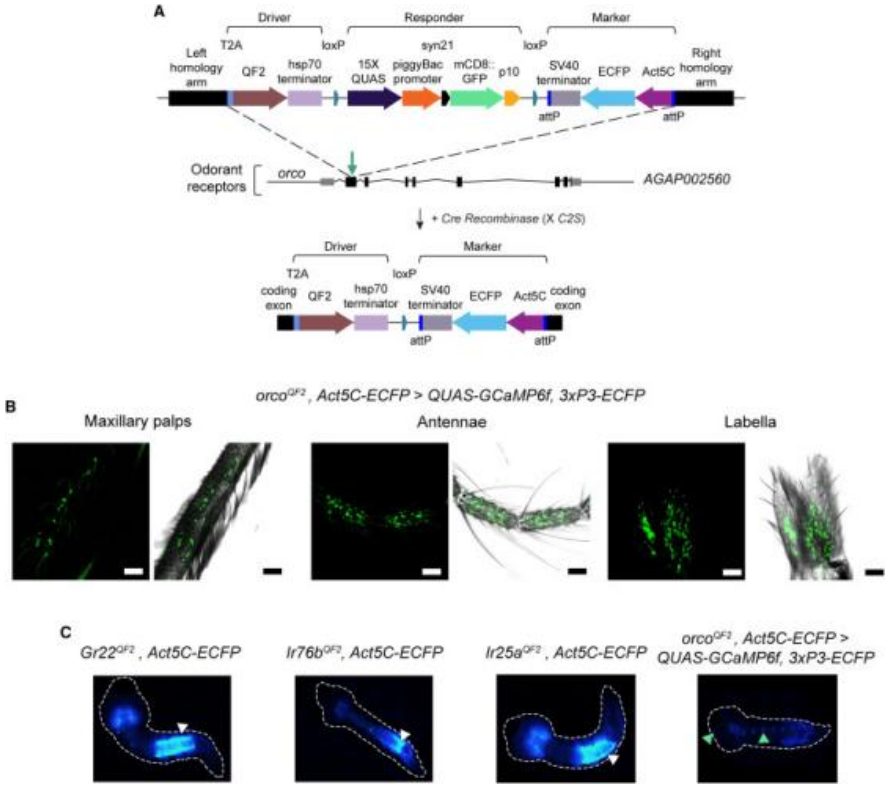

Figure 3 A DRM integration into the Anopheles gambiae orco gene reveals positional effects modulating responder and marker transgene expression

Figure 4 displays the response of *Gr22*+ olfactory sensory neurons on the antennae of Anopheles gambiae to carbon dioxide (CO_2). Part A shows the activity of *Gr22*+ neurons before and after exposure to 1% CO_2 stimulus, indicated by changes in GCaMP6f fluorescence. Under a 1% CO_2 environment, a marked increase in fluorescent response reveals the neurons' sensitivity to CO_2. Part B tracks the activity changes of *Gr22*+ neurons following stimulation with different concentrations of CO_2, with mean ± standard error of the mean (SEM) illustrating an increase in neuronal response intensity as CO_2 concentration rises. Part C quantifies the maximum amplitude of fluorescence change under a 1-second CO_2 pulse, with mean ± SEM again confirming the neurons' activity dependence on CO_2 concentration changes. Bilateral Wilcoxon signed-rank tests indicate that responses at higher CO_2 concentrations are significantly stronger than at baseline levels. These data clearly indicate that *Gr22*+ olfactory neurons can specifically respond to changes in CO_2 concentration, a finding crucial for understanding how mosquitoes utilize olfaction to locate hosts.

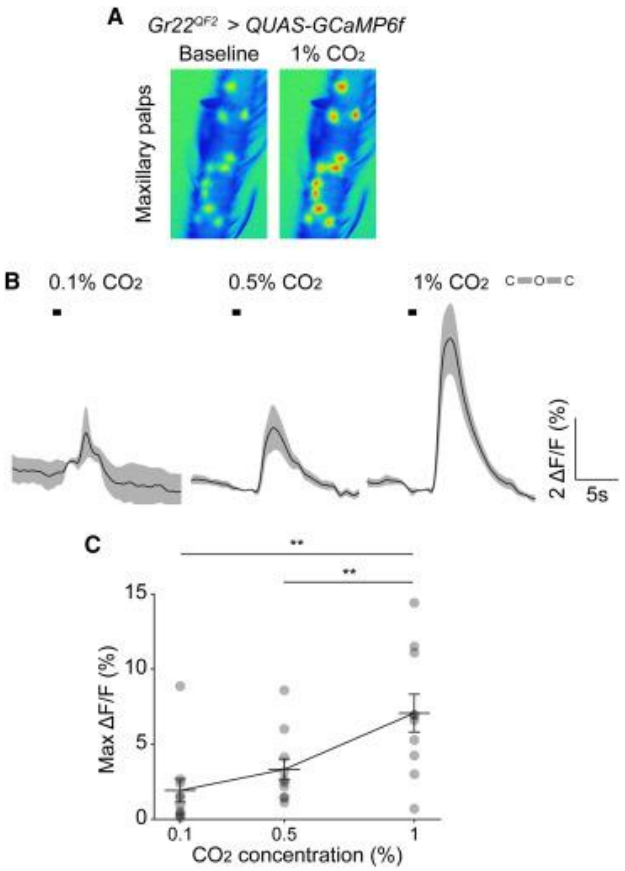

Figure 4 *Gr22*+ OSNs on the *Anopheles gambiae* maxillary palps respond to CO_2

Figure 5 shows the response of *Ir76b*+, *Ir25a*+ and orco+ olfactory sensory neurons (OSNs) on the antennae of Anopheles gambiae to specific human-related odor stimuli. Part A presents the response of *Ir76b*+ neurons to 0.28% trimethylamine and its comparison with a water control; Part B displays the response of *Ir25a*+ neurons to 1% pyridine and its comparison with a water control; Part C shows the response of *orco*+ neurons to 1% hexanal and its comparison with a paraffin oil control. On the left side of each figure are the GCaMP6f activity traces following stimulation, and on the right side is the quantification of the maximum ΔF/F values derived from these traces. Orange and black bars indicate the start and duration of the stimulus, respectively. All charts are plotted with mean ± standard error, and bilateral Wilcoxon signed-rank tests show p-values less than 0.01, indicating that the responses after stimulation are significantly higher than controls. This indicates that these OSNs can specifically respond to odor molecules related to humans, providing important insights into how mosquitoes use olfaction to identify humans.

2 Analysis of Research Findings

By delving into the olfactory system of *Anopheles gambiae*, this study unraveled the complex mechanisms behind this malaria mosquito's response to human odors, demonstrating how scientists can precisely insert the driver-responder-marker (DRM) system into the mosquito genome using CRISPR-Cas9 technology. This enables precise manipulation and tracking of specific chemoreceptor genes in olfactory sensory neurons. Such innovative gene editing methods not only enhance the precision of research but also pave new avenues for experimental design and olfactory studies. Utilizing calcium imaging techniques, the research team further validated the efficiency and accuracy of the created gene expression lines in capturing the mosquito's response to human odor molecules. The combined use of these techniques allows scientists to observe and analyze in real-time the activity of olfactory sensory neurons upon encountering specific odorants, deepening our understanding of how mosquitoes identify human hosts through their olfactory system. These findings not only deepen our knowledge of mosquito olfactory behavior but also provide a valuable scientific basis for developing targeted mosquito control

strategies and preventing malaria transmission. By revealing the subtle differences in the response of specific olfactory sensory neurons to human odors, this study offers important clues for future exploration of more effective mosquito repellents and attractants.

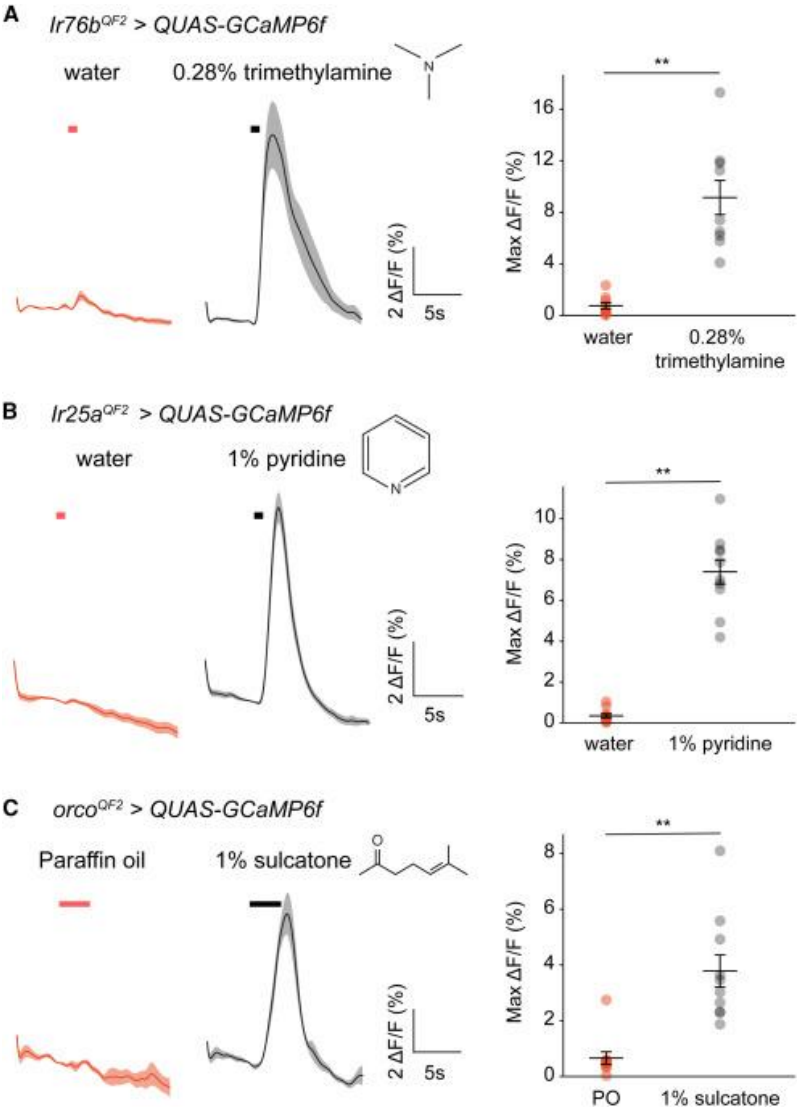

Figure 5 Odor-evoked activity of *Ir76b*+, *Ir25a*+ and *orco*+ OSNs in the *Anopheles gambiae* antennae to select human-related odorants

3 Evaluation of the Research

This study represents a significant breakthrough in the field of malaria mosquito olfactory mechanisms, offering an in-depth analysis of the mosquito's olfactory coding process through the introduction of an advanced neurogenetic toolkit. Utilizing CRISPR-Cas9 gene editing and calcium imaging techniques, scientists were not only able to track the mosquito's precise responses to human odors but also provided new perspectives and a scientific basis for developing effective mosquito control strategies. Additionally, the research findings enhance our understanding of mosquito behavioral habits, offering key insights for innovation in malaria prevention and control strategies. While this study is technically innovative, its complexity demands a deep understanding of genetics and molecular biology, which may limit its application outside specialized fields. However, its contribution to advancing mosquito research and the public health sector cannot be underestimated, providing valuable data and methods for future research and malaria prevention practices.

4 Conclusion

Through the research of Giraldo and colleagues, we now have a deeper understanding of the workings of the *Anopheles gambiae* olfactory system, especially in how they respond to human odors. Utilizing a combination of CRISPR-Cas9 gene editing technology and calcium imaging techniques, the study not only successfully tracked the neurophysiological response of mosquitoes to specific odor molecules but also revealed specific response patterns of olfactory sensory neurons. This work provides us with powerful tools to study mosquito olfactory behavior more precisely, laying a solid foundation for developing new mosquito repellent strategies and malaria prevention measures. These findings are not only scientifically groundbreaking but also have significant implications for public health practice, offering new strategies and hope for future malaria control and elimination. In summary, the research by Giraldo and others greatly enriches our understanding of the olfactory mechanisms of malaria mosquitoes and opens up new avenues for formulating more effective mosquito management and malaria control strategies.

5 Access the Full Text

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I want to express my gratitude to *Cell Reports Methods* for giving me the opportunity to write a review, allowing me to share the latest academic progress of this research with a wide readership. Additionally, I am thankful for the magazine's open and professional attitude towards scientific research and academic exchange. Their efforts make such knowledge dissemination possible, further promoting public understanding and interest in science.