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Novel Methodological Perspectives In The Study Of Mosquito Biology

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Editorial

Mosquito-transmitted pathogens are responsible for millions of human infections each year. To fight diseases that are caused by these pathogens such as malaria and dengue[1,2,3], researchers are strengthening the understanding of mosquito biology and developing novel mosquito control tools[4,5,6]. This collection facilitates new research in these areas through methodological advances involving mosquito biology, mosquito control, pathogen infection, and mosquito transgenesis.

Jensen et al.[7] describe an insecticidal activity assay in mosquitoes and fruit flies involving direct topical application of insecticides and a dosage-determination procedure based on insect mass. The article covers preparation of the workspace and the chemical solutions, insecticide application, and assaying insect survival post-exposure. The topical application methodology facilitates consistent exposure to defined insecticide doses, reducing inter-specific and inter-assay variability, which will be of great interest to those involved in pest insect control or investigating insecticide resistance in mosquito populations.

Chen et al.[8] highlight a simple, tractable, high-throughput DNA extraction protocol of mosquito samples for whole genome sequencing, utilizing magnetic DNA-binding beads. The protocol includes sample homogenization and the complete extraction process. This approach promotes a high yield, yet highly reproducible, DNA extraction method, which is suitable for resource-limited laboratories. It will interest those working with mosquito genomics applications, especially with field mosquito DNA preparations.

Several articles in the collection cover methodological advances linked to the study of mosquito biology. Jové et al.[9] describe high-throughput, fluorescence-based quantification of meal volumes for artificial diet-fed mosquitoes. It encompasses meal preparation, adding the fluorescein dye, delivering the meal on a low-cost feeder system, followed by the quantification of meal volume in fed mosquito samples. The described methodology permits the effective assessment of mosquito feeding, with relevance for feeding

assays involving artificial diets, blood, sugar, or nectar. It will be valuable for those studying insect feeding behavior. Tsujimoto and Adelman[10] outline a novel technique for assaying mosquito fecundity and fertility using agarose-filled 24-well cell culture plates. The protocol details plate preparation, mosquito feeding, oviposition, plate imaging, and data acquisition. It reduces time, space, and labor compared to conventional methodology, and will interest researchers assessing mosquito fitness or the molecular basis of mosquito reproduction. Lajevardi et al.[11] utilize the Ramsay assay to study ion transport and fluid secretion in mosquito excretory tissues. The protocol adapts the Ramsay assay for use with a small amount of insect tissue, and also discusses preparing and calibrating electrodes, preparing mosquito tissues for the assay, and recording readings from malpighian tubules and digestive tissues. This protocol will interest those working with digestion or excretory systems and neuropeptide/endocrine regulation of insect tissues. Wang et al.[12] describe heterologous expression of *Ae. aegypti* odorant receptors in *Xenopus* oocytes to assess their response to human odors. It covers cloning odorant receptor genes, cRNA synthesis, dissection and isolation of *Xenopus* oocytes, heterologous expression of the genes, and then single sensillum recording to gauge receptor activity. This protocol provides a unique system for assessing the genetic and functional basis of mosquito olfaction, with potential applications linked to mosquito behavior research.

Four articles in the collection discuss genetically modified mosquitoes and the process of transgenesis. Carballar-Lejarazú et al.[13] outline detailed procedures for producing transgenic *Anopheles gambiae* mosquitoes *via* embryonic microinjection, while Bui et al.[14] demonstrate microinjection of *Culex quinquefasciatus* embryos for CRISPR/Cas9-based genome editing. The former is a key vector of human malaria parasites, while the latter is a key vector of arboviruses, including West Nile virus, and is understudied in the context of transgenesis. These protocols cover pulling and loading needles, egg preparation and alignment followed by injection, and embryo recovery and screening for transgene phenotypes. The *An. gambiae* protocol details the oviposition and egg harvesting procedures, and visually depicts successful injections. The *Cx. quinquefasciatus* protocol outlines specific parameters for needle pulling and tips for increasing mutagenesis rates. The article by Meuti and Harrell[15] provides an in-depth focus on transgenesis in *Culex pipiens*, particularly the injection process. The video shows the entire injection process in great depth; it outlines backfilling and attaching the needle, positioning the eggs and the injector, as well as the specifics about how to successfully inject. It provides rigorous technical instruction and troubleshooting about the injection process and offers tips to maximize the egg hatching rate and larval fitness. These three articles will interest those seeking to develop novel vector control strategies, as well as those studying *Anopheles* and *Culex* genetics and host-pathogen interactions. Carballar-Lejarazú et al.[16] describe the process for running small-cage trials to assess whether gene drive systems can spread specific genes into naïve mosquito populations under different conditions. The protocol examines the mechanics of inundative non-drive release trials, and gene drive trials with either overlapping or non-overlapping mosquito generations. It provides precise details on wildtype-transgenic addition ratios, screening processes, and adapting experimental parameters to include fitness assays or for modeling gene drive allele spread. This protocol will benefit researchers in the vector control field who are developing novel gene drive systems and examining gene drive efficacy.

The final three articles in the collection discuss pathogen culturing and exposure. Liu et al.[17] demonstrate the protocol for reintroducing bacteria into adult mosquitoes after antibiotic treatment, in order to help understand the biological roles played by the microbiota. It covers culturing and isolation of bacteria, antibiotic treatment, and bacterial feeding assays. The protocol provides a good synthesis of a complex, multi-faceted procedure. It will be useful for those interested in the study of mosquito microbiota or developing adult gnotobiotic mosquitoes. Grigsby et al.[18] highlight the protocol for the integration of culturing the *Edhazardia aedis* microsporidian parasite into standard *Aedes aegypti* rearing. It details the isolation, identification, and enumeration of infectious spores, before reinfection of mosquitoes and maintenance of the infected mosquito eggs. It gives troubleshooting advice on detecting spores and coordinating mosquito hatching with spore preparation. This protocol will interest those seeking to culture microsporidian parasites for experimental purposes, and those examining the impact of parasite infection on mosquito immunity or vectorial capacity. Tripathi et al.[19] detail the protocol for culturing *Plasmodium falciparum* gametocytes, performing infections of *Anopheles* mosquitoes *via* the membrane-glass feeder system, and counting oocysts and sporozoites. It is an essential protocol for *P. falciparum* culture, which can be difficult to achieve. The protocol will be highly valuable for those investigating *Plasmodium* infection and transmission in mosquitoes, for those looking to develop anti-malarial strategies, including transmission blocking tools, and for those examining the vector competence of *An. gambiae* populations, including transgenic lines and gene-drive strategies.

The article collection deals with many research topics that are important to the future of mosquito and mosquito-borne pathogen control. Understanding mosquito resistance to pesticides is critical as it reflects the success of current control programs; moreover, detecting resistance can be a signal that changes in control strategies are required. Genetic engineering technologies, including mosquito transgenesis, genome editing, and gene-drive, will likely play a key role in mosquito control over the coming decade and beyond due to their high tractability and adaptability. Improved knowledge of mosquito biology, specifically in the topics of population genomics, host-pathogen interactions, mosquito behavior, and the molecular basis of key biological traits, will provide valuable insights that can be utilized to develop new control tools. For instance, identifying genes involved in olfaction could help researchers to develop novel repellents, while identifying genes involved in host-pathogen interactions could eventually lead to the development of transgenic mosquitoes carrying anti-pathogenic effectors. This collection touches on all of these topics, and in the hands of vector biologists from around the world, the methods described here will help facilitate new and unique insights into many aspects of mosquito biology. A key challenge for the future will be leveraging the knowledge generated in these subject areas to produce new control strategies or to complement or optimize current strategies.

Disclosures

The authors have nothing to disclose.

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