

Preface

Mosquitoes are the vectors of a large number of infectious diseases, such as malaria, dengue fever, Japanese encephalitis, filariasis and chikungunya. Mosquito-borne diseases are a major threat to human health, and they infect 700 million and kill over one million of persons globally each year. The World Health Organization (WHO) estimates that there were about 390 million persons to be infected with dengue fever worldwide each year, and there were 228 million to be infected with malaria and 405,000 to be died from malaria in 2018. Dengue is emerging as a new threat, with 22,599 dengue cases in China in 2019. The control of mosquito-borne diseases mainly depends on the control of vector mosquitoes, and insecticides are still the main measure of vector mosquito control.

Anopheles sinensis is the main vector of malaria parasites in China, East Asia and South-east Asia, and it also transmits filariasis and Japanese encephalitis. Since the 21st century, malaria has broken out again in many parts of China, and the malaria outbreak only appears in the area where *Anopheles* mosquitoes are distributed. *Anopheles sinensis* has a wide distribution, high population density and enhanced malaria transmission energy, making it the most important vector of malaria transmission in China. In recent years, *Anopheles sinensis* has developed resistance to pyrethroids, which has seriously hindered *Anopheles sinensis* and malaria control.

Lasting insecticide nets (LLIN) and indoor residual spraying (IRS) are the most important means for vector mosquito control. Pyrethroid insecticides are highly effective and less toxic. They are the only LLIN insecticide and the main IRS insecticide approved by WHO. Pyrethroids are also used for agricultural pest control in China. However, with the widespread use of pyrethroids around the world, mosquitoes have developed resistance to pyrethroids. The continuous spread of resistance has greatly reduced the effectiveness of pyrethroids, and has become a serious obstacle to the control of mosquito-borne diseases internationally. WHO has launched a global program on insecticide resistance management, calling for the development of new methods to further understand the molecular mechanism of insecticide resistance and strengthen resistance monitoring.

In recent years, we have systematically conducted the study on the genome biology and pyrethroid resistance mechanism using *Anopheles sinensis* as a model species. We sequenced and annotated the genome, transcriptome, sialotranscriptome and non-coding RNAs of *Anopheles sinensis*. We have analyzed the gene expression profiles of pyrethroid-resistant

and -susceptible mosquitoes from three geographical populations in Chongqing, Yunnan and Anhui in China to identify the genes potentially associated with pyrethroid resistance, and the gene expression profiles at different developmental stages, issues, sexes and periods after blood feeding at pyrethroid-resistant in comparison of susceptible mosquitoes using RNA-seq technology for the species. We have also re-sequenced and analyzed 36 individuals with six resistant and susceptible ones from each of the three populations to identify the mutation associated with pyrethroid resistance.

We have established a series of experimental technology and platform for genomic and functional genomic researches, including bioinformatics analysis, comprehensive omics database, *piggyBac*-mediated germline transformation, electroporation-mediated nucleic acid delivery during non-embryonic stages, RNAi- and CRISPR/Cas9-based gene-function, ReMOT control-based gene editing, and protein expression, purification and activity analysis system of mosquitoes. We constructed the exogenous DNA delivery system based on ovary-delivering peptide and Gal4-USA binding property, which made the genetic transformation more operatable. We have conducted a lot of researches surrounding the genome biology and pyrethroid resistance mechanism of *Anopheles sinensis*, particularly the genome-wide gene identification, characterization, evolution, expression, activity, function and regulation of a large number of genes, gene families or super families, including P450, CCE, GST, ABC, UGT, CP, OBP, CSP, Trypsin, OCT, HSP, OR, iGluR and IR, as well as simple sequence repeat, miRNAs and mitochondrial genome. We have also conducted a lot of researches regarding the *kdr* mutations and their functions, the relationship between the symbiotic microbial community and insecticide resistance, and the relationship among knockdown resistance, metabolic detoxification and organismal resistance to pyrethroids.

These researches show that there are four main pyrethroid molecular resistance mechanisms for mosquitoes: target site resistance, metabolic resistance, cuticular resistance and behavioural resistance. These mechanisms involve target site variation leading to insensitivity, increased enzyme expression due to gene repetition and amplification events, upregulation of *cis*- and *trans*-factor regulatory genes, and enhanced enzyme activity due to structural changes. Multiple mechanisms often occur simultaneously to further enhance insect resistance. These conclusions are of important theoretical and applied value for understanding the molecular mechanism of pyrethroid resistance, developing molecular techniques for resistance monitoring, preventing the extension of resistance, developing new insecticides, and finally controlling vector mosquitoes and mosquito-borne diseases.

Up to now, we have published 71 papers regarding the genome biology and pyrethroid resistance mechanism of *Anopheles sinensis* in Prof. Dr. Bin Chen's Laboratory. This book

includes 25 chapters, which are rewritten from 25 most representative publications, and aims to partially summarize our work and motivate further research in this field. All of these achievements stem from joint efforts of all members in our laboratory, including teachers and postgraduates participating in this field of research. We are especially grateful to these teachers for their contributions: Prof. Dr. Zheng-Bo He, Prof. Dr. Liang Qiao, Prof. Dr. Yu-Juan Zhang, Prof. Dr. Ting-Jing Li, Dr. Feng-Ling Si, Dr. Zhen-Tian Yan, Dr. Wen-Bo Fu, Dr. Yong Zhou, Dr. Qi-Yi He, Dr. Shu-Lin He, Dr. Shuang Ren, Dr. Yi-Yun Wei, Dr. Cao Zhou.