DEVELOPMENT AND CALIBRATION OF AN EXPOSURE-FREE BEDNET TRAP FOR THE SAMPLING OF AFROTROPICAL MALARIA VECTORS .

BY

EVAN MURIMI MATHENGE (B.Ed. (Sc), M.Sc.)

A thesis submitted in fulfilment of the requirement for the degree of Doctor of Philosophy (Medical Entomology) of the University of Nairobi.

Faculty of Science, Department of Zoology.

2004.

DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

Evan Murimi Mathenge

University of Nairobi

Signature: _____

Date: 25/10/2004

We confirm that the candidate, under our supervision, carried out the work reported in this thesis and it has been submitted with our approval

Prof. Lucy W. Irungu

University of Nairobi

Signature: _____

Date: 26/10/2004

Dr. Paul N. Ndegwa

University of Nairobi

Signature: _____

Date: 27/10/2004

Dr. ir. Bart GJ Knols,

Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratory,

Signature: _____

Date: 20 - 9 - 2003

Dedication

To the entire family of Mathenge Kimange

TABLE OF CONTENTS

PREFACE

When Dr. William A. Hawley, my former supervisor at CDC/KEMRI, organized for me to attend a WHO workshop on population genetics of malaria vectors that was held at the ICIPE headquarters-Duduville in 1998, he gave me a good word of advice. He said, 'Evan, although you might not understand much of what will be discussed in that workshop, you should keep your eyes open as you are likely to meet people who matter in science. Market your ideas'. Thank you Bill for this word of advice. True to your word, I met one scientist who later became one of my mentors. This was a bald man smoking a pipe. He was very friendly and on one social evening, over a bottle of Tusker®, we came to know each other. His name was Dr. Bart G.J. Knols. He told me that he had been working on mosquito behaviour in Tanzania and at that moment he had an idea in mind. He wanted to develop some bednet traps for mosquitoes and was to forward a proposal to WHO/TDR. Having worked on bednets I told him that I would be very interested in his worthwhile endeavour. We exchanged email addresses and kept on communicating.

When Dr. Knol's proposal got funded, he got me on board as a Ph.D. student under the Dissertation Research Internship Programme at ICIPE (DRIP). We got to work and started brainstorming. On one occasion, I asked Bart what he thought the trap will look like and his answer was: ' Evan, I also don't know but I am confident we will have it by the end of it all'. Thank you Bart for your optimism that kept the fire burning in me. At times, when your imaginative mind caught fire, we really came up with nice ideas and tried them out. You have created endless opportunities for me to become a true scientist. Thank you for that. I also miss the social side of you especially in Mbita. Your company, guidance and generosity are unmatched.

In the picture came two very crucial personalities. These were Prof. Lucy W. Irungu and Dr. Paul N. Ndegwa, my University supervisors. I will comment on their rules that made them very crucial. Prof. Irungu, thank you for facilitating all the university processes and bureaucracy that would otherwise have hindered my progress. Your welcoming nature and your sincere concerns of my welfare and progress are appreciated. Finally, your visit to Mbita and your encouragements kept me going at a time that I was

overwhelmed by problems stemming from the departure of Bart against his will. Dr Ndegwa, thank you for your deep concern on my work, thank you for your grammar lessons when you read my manuscript not to forget some nice emails you sent to me.

A couple of scientist also came into the real picture of my work. An Irish scientist, Dr. Gerry F. Killeen played a crucial role in my thesis. He was my lead advisor on statistics and modelling. Thank you Gerry for killing a couple of my brain cells in the process of making me understand the conceptual basis of modelling. Dr. Ulrike Fillinger from Germany, volunteered her salary to assist me complete my studies. Uli, thank you for turning a blind eye when your work suffered at the expense of my being busy on my thesis. In the company of Uli was Aruna (Hindi), my fellow scholar, who made sure that I ate even without a single penny in my pocket. Thank you Hindi, I will miss your cooking. Dr. Heather Ferguson from Scotland was very instrumental on the statistical part of this thesis as she made complex concepts look simple. Thank you Heather for this and for your constant encouragement and valuable company. Elizabeth Walczak tirelessly guided me in statistical analysis even after her departure from ICIPE-Mbita. Thank you very much Elizabeth. Dr. Luna Kamau allowed me use her laboratory, reagent and staff. Thank you Luna for this was a very important assistance. Joseph Mwangi and Lucy Njeri carried out PCR and ELISA on my samples. Lucy and Joseph, I will always cherish you. Dr. Andrew K. Githeko from KEMRI Kisumu provided valuable reading materials and reprints. Thank you Andrew for this and also for welcoming me in Kisumu on several occasions.

The members of the strong team alias '*Unbwogables and Sapphire Crew*', need a special mention. These were the technicians and the field workers who were the lifeblood and the backbone of this work. My reliable and prudent technicians Gideon Misiani, David Oulo, and George Omweri made sure that things went well all the time. Thank you very much for this. Gideon and David stood by me even at a time when our project went bankrupt. "*Kaeni hivyo hivyo na Mungu atawabariki.*" To Christian Abuya, James Nderitu, Sammy Maina, Cannon Maina, Steve Abong'o, Peter Khaemba, John Mahani, Ken Mahani, Shem Kisero, Douglas Omukuba, Geoffrey Nyang'au, Geoffrey Ondara, thank you for accepting to become my nocturnal colleagues. To the people of the entire

Luo Nyanza, you are a great community and above all welcoming and generous. Receive my gratitude and "*Nyasaye ogwethu duto*"

In Mbita, many people played an important role to ensure that I succeed. These included the insectaries staff namely Jackton, Hassan, Peter and Samuel. Without them, these studies would not have started. My fellow scholars, R. Mukabana, L. Sumba, B. Oketch, B. Njiru, T. Guda and H. Manda shared their resources with me at all times. Thank you all. Dr. L. C. Gouagna taught me a lot of practical entomology and provided reading materials. Thank you Louis. The company of Peter (*Shetani*'), Lawi (*lufwombo*), Mr. Gitau, Mr. Sese, the two James (Tall & Short) is appreciated. You made my life in Mbita very enjoyable. My friend George Sonye was very instrumental in contact setting.

My friends in Nairobi gave moral and financial support. These were Jama, Faith, Waweru, Jeff, Cannon, Kim, Nduta, and Kate. Thank you all. One person in Nairobi needs a very special mention. That is my girlfriend Mercy. Dearest, you made my studies bearable as I always had someone to turn to at times of intense stress and despair. Your visits to Mbita made science more interesting . *Asante Sana*!!

Last but not by any means least, I gratefully acknowledge the enduring support given to me by my brothers, Njogu, Wamai , Gatere & Ngari, and my sisters Joyce, Ann, Wandia & Nyaguthii. To my father who thought it wise to take me to school and was always prompt in paying school fees, I say, " *Asante sana Baba*"!!

ABSTRACT

Interventions aimed at reducing malaria vector populations through impregnated bednets, environmental management, residual indoor spraying etc., require monitoring of mosquitoes before, during and after the intervention. Various sampling methods have been developed, but collections by these do not reflect a true cross-section of the host-seeking population and are biased in various ways. The commonly accepted best estimate of the entomological inoculation rate (i.e. an indicator for malaria risk over a certain time period) relies upon human volunteers collecting biting mosquitoes from exposed feet and limbs. As some of these mosquitoes are inevitably infected with human malaria parasites, this represents an increased and undesirable risk to the volunteers. Therefore, effective and economical monitoring tools for African malaria vectors are urgently needed to assess the impact of malaria interventions on mosquito populations. The development of improved sampling systems based on an improved understanding of host-oriented behaviour is needed. The possibility of modifying bednets into traps for sampling malaria vectors would be a worthwhile endeavour.

To pursue this possibility of modifying bednets into mosquito sampling devices, various objectives were designedformulated to: (i) Study the behaviour of mosquitoes around human-occupied bednets, (ii) to modify bednets into mosquito traps and (iii) To evaluate the efficacy and applicability of these traps aganist existing trapping techniques at different sites with different mosquito species and densities thereof. Results obtained from the study of mosquito behaviour demonstrated that temperature and relative humidity inside a human-baited bednet play a crucial role in the behaviour of mosquitoes

around such nets. Mesh size of the material making the net was found to be a direct determinant of these parameters.

From these studies of mosquito behaviour around human-baited bednets, an exposure-free bednet trap (the 'Mbita trap') for sampling of Afrotropical malaria vectors was developed. Its mosquito sampling efficacy was compared to the CDC miniature light-trap and human landing catches under semi-field conditions in a screen-walled greenhouse using laboratory reared *Anopheles gambiae* Giles *sensu stricto* (Diptera: Culicidae). When compared in a competitive manner (side by side), the Mbita trap caught 4.1 times as many mosquitoes as the CDC light-trap, hung beside a human occupied bednet, and 43.2 % the number caught by human landing catches. The ratio of Mbita trap catches to those of the CDC light trap increased with decreasing mosquito density. Mosquito density did not affect the Mbita trap catch to human landing catch ratio. In a non-competitive comparison (each method independent of the other), the Mbita trap caught 89.7 % the number of mosquitoes caught by human landing catches and 1.2 times more mosquitoes than the CDC light trap. Differences in Mbita trap performance relative to the human landing catch under non-competitive versus competitive conditions were explained by the rate at which each method captured mosquitoes.

The mosquito sampling efficiency of the new bednet trap (the Mbita trap) was compared with that of the CDC miniature light trap (hung adjacent to an occupied bednet) and the human landing catch in two different epidemiological settings (Lwanda and Ahero) in western Kenya. In Lwanda, the Mbita trap caught 48.7 % the number of *Anopheles gambiae* Giles *sensu lato* caught in the human landing collections and 27.4 % of the number caught by the light trap. The corresponding figures for *An. funestus* Giles

were 74.6 % and 39.2 % respectively. Despite the clear differences in their sampling efficiencies, both the Mbita trap and light trap caught mosquitoes at rates directly proportional to human landing catches regardless of mosquito density. No significant differences in parity or sporozoite prevalence were observed between mosquitoes caught by the three methods for either *An. gambiae* s.l. or *An. funestus*. PCR identification of the sibling species of the *An. gambiae* complex indicated that the ratio of *An. gambiae* Giles *sensu stricto* to *An. arabiensis* Patton did not vary according to the sampling method used. In Ahero where *An. arabiensis* predominates, the Mbita trap caught only 17% of this species compared to the human landing catches but there was good proportionality between the two methods. The Mbita trap caught about 60% of the *An. funestus* caught in human landing catches. The CDC light trap, in contrast, caught about 60% of the *An. arabiensis* caught by the landing catches, with good proportionality and more *An. funestus* than in the landing catches. It is concluded that the Mbita trap is a promising tool for sampling malaria vector populations since its catch can be readily converted into equivalent human biting rates, can be applied more intensively, requires neither expensive equipment nor skilled personnel, and samples mosquitoes in an exposure-free manner. Such intensive sampling capability will allow cost-effective surveillance of malaria transmission at much finer spatial and temporal resolution than has been previously possible.