EFFECTS OF PYRETHROID RESISTANCE ON THE FITNESS AND LIFE HISTORY TRAITS OF *Anopheles gambiae s.s* IN BUNGOMA, WESTERN KENYA

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A Thesis submitted to the School of graduate studies in partial fulfillment for the award of the Masters of Science in Public Health of Masinde Muliro University of Science and Technology

JULY, 2024

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DEDICATION

This work is dedicated to my parents and family for the great support they granted me throughout my studies.

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to my supervisors Dr. Christine Wanjala, Dr. Eric Ochomo, Dr. Elizabeth Omukunda and Prof. Yaw Afrane for the continuous support and guidance they accorded me during my master's study both in research and thesis writing.

I would also like to thank the Masinde Muliro Medical Laboratory sciences department fraternity for the academic input in my master's degree project work through reviews and providing an audience during my presentations.

My appreciation also goes to the CGHR-KEMRI Entomology staff members for offering a laboratory space for my master's research work and also for the support during my masters project work.

ABSTRACT

Pyrethroid based vector control tools have played a major role in malaria control. However, *Anopheles gambiae s.s* from Bungoma has shown increased insecticide resistance to pyrethroids. Mosquito life traits such as larval development, gonotrophic cycle, fecundity and longevity are an integral part of the vectorial capacity of malaria vectors that influence malaria transmission. Studies have reported effects of pyrethroid resistance on the life history traits of *Aedes* mosquitoes but its effects on the biological fitness of *An. gambiae* a major malaria vector remain largely understudied yet knowledge of these traits is important for improving insecticide resistance management strategies. The main objective of this study was to determine the effect of pyrethroid resistance on *An. gambiae* fitness as measured by life-history traits. Established colonies of *An. gambiae s.s* collected from Bungoma were used in the study. The insecticide resistant selected colony was exposed to 0.05% deltamethrin while the unselected colony was not exposed to any insecticide. The susceptible *An. gambiae s.s* Kisumu laboratory reference strain was used as the control. The study applied an experimental design involving the construction of larval and adult life tables so as to evaluate the fitness and biological traits of the selected and unselected colonies. The larvae and adult mosquitoes used were randomly selected from the insectary. The first larval instars were reared while recording the surviving larvae and their development stage until the adults emerged. The larval development time, pupation rate and adult emergence rate was then determined. Gravid females were individually allowed to lay eggs which were counted and recorded and their gonotrophic cycle measured. The adult longevity was also measured by rearing female mosquitoes in a cage in the malaria sphere then the dead mosquitoes were recorded, recorded daily. The average female adult survival rate, fecundity and longevity was calculated. Turkey HSD test compared the significance of insecticide resistance on development time, gonotrophic cycle, fecundity and longevity. Kaplan-Mier survival analysis determined the effect of insecticide on adult mosquito survivorship. The mean pre-marginal development time for the females in selected colony (12.1 ± 0.3) was significantly longer than that of the unselected colony (9.6 ± 0.2 days; $p<0.05$). The gonotrophic cycle for the selected colony (6.07 ± 0.83 days) was also significantly longer than that of the unselected colony (4.27 ± 1.14 days; $p<0.05$). On the contrary the female longevity of the selected (39.68 ± 1.6 days) was significantly longer than that of the unselected colony (29.86 ± 1.1 days; $p<0.05$). This study showed parathyroid resistance delayed the development, elongated the gonotrophic cycle, reduced fecundity and increased longevity of *An. gambiae s.s*. The study findings are important in understanding the benefits and demerits of pyrethroid resistance on malaria which could be useful in shaping tailor made resistant management strategies with the target of eliminating malaria..

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Malaria affects over 3 billion people globally in about 100 developing countries (WHO, 2016). In 2020, there were around 245 million cases and about 625 thousand malaria related deaths in the globe (WHO, 2021) with Africa region carrying a large portion of the global malaria burden. The Kenya Domestic Health survey report of 2021 reported 3,302, 189 malaria cases and 11,768 malaria deaths in the year 2020 which indicates that approximately 70% of Kenya's population was at a risk for malaria with an incidence rate of about 10.5 per 1000 population (KDHS, 2021).

Anopheles gambiae, *Anopheles funestus* and *Anopheles arabiensis* are the main African malaria vectors (Kyalo *et al*., 2017) . In Kenya these three species of malaria vectors are mostly found in the Lake Victoria region and the western part of the country. *An. arabiensis* are found in the lowlands surrounding the lake region. *An. gambiae* is majorly found in malaria endemic areas, and is highly resistant to pyrethroids in the highlands of the western region while *An. funestus* are distributed in both the western lowlands and highlands (Wanjala & Kweka, 2018).

The fitness of a mosquito is a crucial component of its vectorial capacity to transmit a pathogen. Larval development time that determines the adult emergence time and affects the survivorship of the mosquito species which is associated with the density vectors at a time and high densities of the vectors enhance malaria transmission (Vézilier *et al.,* 2012). The gonotrophic cycle dictates the need and time of the next blood meal by a vector after laying eggs (Yaw A Afrane *et al*., 2006; Lardeux *et al*., 2008). This influences the biting rate of the mosquitoes as they search for the source of blood meal hence will facilitate malaria transmission in case of infectious bites (Vézilier *et al.,*

2012). Fecundity on the other hand brings forth continuity in a species population and is therefore directly linked to the population densities of the vector species. The adult survivorship more so the female longevity affects the external cycle of *Plasmodium spp* parasites since they need enough time in order to develop into the infectious sporozoites. Successful malaria transmission calls for an infected female mosquito to live longer than the sporozoite development time. The female survivorship also dictates the number of bites by a given female hence influencing malaria epidemiology (Alout *et al.*, 2017))

Long lasting insecticide nets (LLINS) and Indoor residual sprays (IRS) have been extensively used for the control of malaria vectors (Greenwood and Hygiene, 2009). The six chemical classes that are employed in the control of mosquito vectors include: pyrethroids, carbamates, organophosphates, organochlorines, pyrroles and neonicotinoids. Currently, pyrethroids are the only ones accepted for application in LLINs and account for 68% malaria cases reduction in Africa (Bhatt *et al*., 2015). However, reported pyrethroid insecticide resistance poses a threat on the efficacy of LLINs (Hemingway *et al*., 2016). The principal causes for insecticide resistance are: target site modification, rendering it less sensitive or increased metabolism of the insecticide molecules by the vectors. The extensive use of insecticides through LLNs, IRS and application of agricultural pesticides have also imposed an intense insecticide resistance selective pressure on mosquito vectors (Diabate *et al*., 2002); (Stump *et al*., 2004); (Mathias *et al*., 2011)). Selection of insecticide resistance has contributed to the development and widespread of resistant alleles in many insects (Hemingway *et al*., 2016). This has also led to selection of phenotypes that confer ability to survive the insecticides use (Ranson *et al*., 2011)

Insecticide resistance genes show an advantage in the availability of a respective chemical environment hence resistance levels (García *et al.*, 2009)Nevertheless, the absence of these

chemicals result to very low frequencies of resistance alleles (Berticat *et al.*, 2002)). Thus, a relationship between resistance alleles and fitness costs is expected (Coustau & Chevillon, 2000) Insecticides used for malaria vectors control are designed to negatively affect the longevity and survivorship of malaria vectors because competent vectors require higher daily survival rates; Alout *et al.*, 2013b; Alout *et al.*, 2014). Studies have confirmed alleles responsible for insecticide resistance to influence some life-history traits of insects. These traits include the biting frequency, larval development, adult survivorship, male mating success, blood feeding and the egg laying capacity (Rivero *et al.*, 2011)). Doubly-resistant *C. quinquefasciatus* females harbouring both the *kdr* and *ace-1R* alleles showed a less likelihood to reach adulthood (Berticat *et al*., 2008)while the pyrethroid resistant males exhibited low mating success as compared to the susceptible individuals with the *ace-IR* (Berticat *et al.*, 2002). On the contrary, resistance has shown a fitness benefit in untreated areas such as greater mating success (Arnaud & Haubruge, 2002). Insecticide resistance negates the benefits of vector control interventions in eradicating malaria.

Many studies have concentrated on the resistance mechanisms of these insecticides but little is known of how these mechanisms affect the biology of malaria vectors particularly the life history traits and fitness. The successful implementation of the management of insecticide resistance strategies is hampered by the finite knowledge on the fitness cost imposed on the life history traits of malaria vectors including: larval development, reproductive fitness and adult longevity. To fill this gap, established colonies of resistant selected *An. gambiae s.s* and resistant unselected *An. gambiae s.s* with the same origin were evaluated for the effect of pyrethroid insecticide resistance on the larval development time, gonotrophic cycle, fecundity and adult survivorship.

1.2 Problem statement

Long- lasting insecticidal nets (LLINs) remain a core intervention in the control of malaria vectors for most National malaria programmes due to their success in malaria reduction in the last decade. However, the occurrence and progression of insecticide resistance has really threatened these rewards achieved (Hemingway et al., 2016; Ranson & Lissenden, 2016) The WHO malaria report of 2018 records a rise in increased malaria incidence rates in the years 2016 and 2017 that are thought to result mainly from insecticide resistance by malaria vectors that change the dynamics of transmission capabilities of the vector by impacting physiological processes of the mosquito (WHO, 2018). Changes in any of the biological traits including the time taken for the immature forms of mosquitoes to develope, gonotrophic cycle, fecundity and female longevity have an impact on the vectorial capacity of malaria vectors which may in turn affect the transmission dynamics of malaria by *An. gambiae s.s.* Insecticide resistance involves energy cost in either expression of resistance genes that account for increased detoxification of insecticides or mutations on the target site (Rivero *et al*., 2011). These alleles have been reported to affect the fitness cost and life history traits of insects including other mosquito vectors. These trait parameters include the larval development time, male mating success, blood feeding capacity by females, longevity and egg laying capacity of *Aedes aegypti* (Viana-Medeiros *et al*., 2017). Therefore, this necessitated the need to establish the outcomes of insecticide resistance on the fitness cost and life history traits of *An. gambiae* in order to establish proper implementation of instruments for containing insecticide resistance in malaria vectors effectively for the purpose of achieving successful malaria control.

1.3 Objectives of the study

1.3.1 General objective

To determine the effects of pyrethroid insecticide resistance on *An. gambiae* fitness and life-history traits in western Kenya.

1.3.2 Specific objectives

- To determine larval development time of pyrethroid selected/ resistant and unselected/ susceptible colonies of *An. gambiae* sensu stricto
- To determine the gonotrophic cycle of pyrethroid selected / resistant and unselected/ susceptible colonies of *An. gambiae*.s.s adult females.
- To determine the fecundity of pyrethroid selected/resistant and unselected/susceptible colonies of *An. gambiae s.s* adult females

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1.4 Research Questions

- What is the effect of pyrethroid resistance on the larval development of the *Anopheles gambiae sensu stricto?*
- What is the effect of pyrethroid resistance on the gonotrophic cycle of the *Anopheles gambiae s.s?*
- What is the effect of pyrethroid resistance on the fecundity of the *Anopheles gambiae s.s*

1.5 Justification

Insecticide resistance has been found to affect the fitness cost in terms of reproduction and physiological processes of insects (Msangi et al., 2020; Riveron et al., 2018). Changes in the larval development time affect the mean generation time hence affecting the vector population. The adult

mosquito survivorship is also an important epidemiological variable since it impacts the development of malaria parasites and determines the frequency of infectious bites to hosts(Vézilier *et al*., 2012). Changes in the reproductive fitness and larval development attributed to deltamethrin resistance have been reported in the *Aedes aegypti* (Brito *et al*., 2013) while permethrin resistant *Aedes albopictus* females were found to have an elongated life than their susceptible counterparts(Chan & Zairi, 2013). However, there are limited studies that have been done on the assessment of the impact of insecticide resistance on the life history traits of *An. gambiae s.s* from the same genetic background. Bungoma county is a malaria endemic area in western Kenya and has been documented to have *An. gambiae s.s* as the predominant malaria vector with a high pyrethroid resistance. This therefore, justifies the need for this study that assessed the effects of insecticide resistance on the larval development, gonotrophic cycle, fecundity and female survivorship of established colonies of pyrethroid selected and unselected *An., gambiae s.s* with a common genetic background.

1.6 Significance of the study

The results of the study highlight the dvantages and disadvantages of pyrethroid resistance to the fitness of *An. gambiae s.s* . This could be crucial in modelling interventions that will help to curb the dangers caused by pyrethroid resistance especially in the areas where the biological traits redeemed benefits. The knowledge obtained will be resourceful devising tailor-made novel vector control strategies to manage survival of insecticide resistant immature stages and the extended life time of the adult resistant female vectors. Therefore, these results will also be fundamental in developing strategies for the management and control of insecticide resistance towards malaria elimination by 2030 as articulated by the WHO global technical strategy for malaria 2016-2030

(WHO, 2016) and also support the Global Plan for Insecticide Resistance Management (GPIRM) (WHO,2012)

1.7 Scope of the study

This study evaluated the impact of pyrethroid resistance on the larval development, gonotrophic cycle, fecundity and longevity of established colonies of selected/ resistant and unselected /susceptible *An. gambiae s.s* mosquitoes with the same origin that were reared in the insectary at the Centre for Global Health Research (CGHR)-KEMRI, Kisumu. The metabolic resistance and target site resistance statuses of these colonies were known and documented by (Machani *et al.*, 2020). The study also used the Kisumu susceptible reference strain as the control.

1.7.1 Limitations

The main limitations of this study was the generation differences between the insecticide selected and unselected populations arising from the delayed development in the selected resistant colony and the loss of resistance in the unselected population. Therefore, the study used different generations of the selected and unselected mosquitoes.

1.8 Theoretical framework

The study adopted the principles evolutionary consequences of insecticide selection pressure on malaria transmission as postulated by Aluot et al (Alout *et al*., 2017). This theory proposes that mutations that mediate insecticide resistance modify physiological processes by influencing availability and utilisation of resources. This threatens the performance and fitness of body organs of the vector. The fitness costs caused could affect broad aspects of the vector's life history traits such as the life span and host seeking behavior that equip the vector for the task of transmitting malaria which might end up changing the dynamics of malaria transmission.

Fitness advantages or disadvantages depend on parasite infection or elements in the environment which may maximize or minimize the potential of a mosquito to transmit the pathogen. A parasite can inflict a cost to mosquitoes having resistance alleles which may further affect the life- history traits involved in promoting the capacity of the vectors to spread malaria and the expression of phenotypic insecticide resistance. Therefore, interaction between insecticide resistance, selection pressure and physiological processes introduce contrasted effects on mosquito traits resulting to complications in the control and epidemiology of malaria in places with dominant resistant vectors. This therefore formed the basis for carrying out studies that evaluate the impact of insecticide resistance on the life history traits of the malaria vectors and to ascertain whether they affect the transmission of malaria.

1.9 Conceptual framework

This study simply describes the relationship between insecticide resistance and the life history traits and fitness which finally influence malaria transmission. Larval development resulted to pupation time, emergence time, pupation rates and emergence rates of the experimental mosquitoes that increased the population densities of the mosquitoes. The gonotrophic cycle and fecundity were outcomes of the reproductive fitness. Adult female longevity was also measured. All these variables have an impact on malaria transmission dynamics.

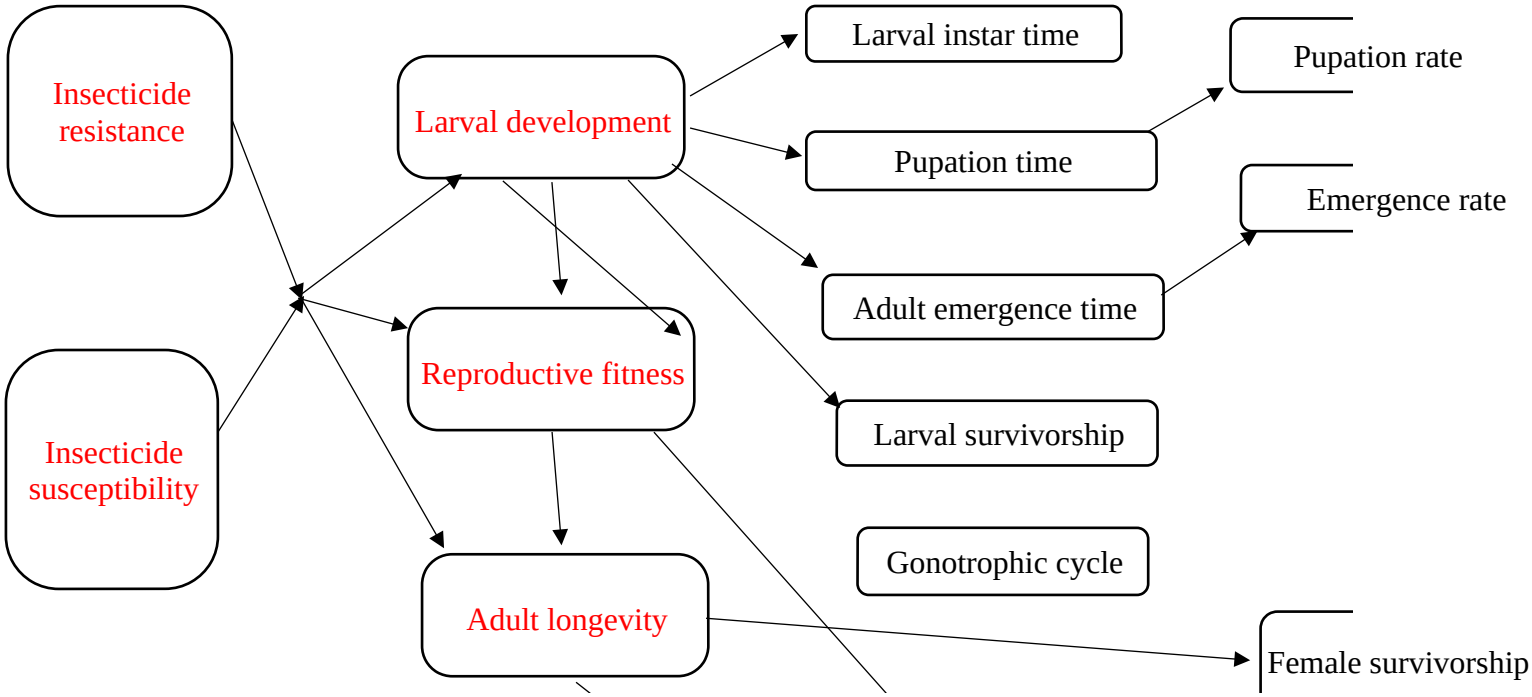


Figure 1.1 Conceptual framework

CHAPTER TWO

LITERATURE REVIEW

2.1 Malaria Burden

Malaria, a tropical infectious disease is spread by the female mosquitoes from the *Anopheles* species. In 2018, there was an estimated 228 million malaria cases globally. Africa had the highest malaria cases (213 million or 93%), South-East Asia at 3.4%, while Sub-Saharan Africa and India were at about 85% of the malaria burden (WHO, 2019). *Plasmodium falciparum* is more prevalent in Africa accountable for up to 99.7% of the 2018 malaria cases while South-East Asia, Eastern Mediterranean were 50% and 71% respectively. *P. vivax* infections were in India (47%) and America representing 75% of malaria cases (WHO, 2019). *P. malariae* and *P. ovale* contribute minimal infections (Sutherland *et al*., 2010). *P. knowlesi* (White, 2008) is a primate malaria species of Southeast Asian countries (Eede *et al*., 2009). *Plasmodium falciparum* is responsible for 99% of malaria cases in Kenya (PMI malaria report, 2018). In Kenya, malaria is a still a great burden and remains endemic in the western and coastal region with a prevalence of 27% (about 6 million people) (Machini *et al*., 2016).

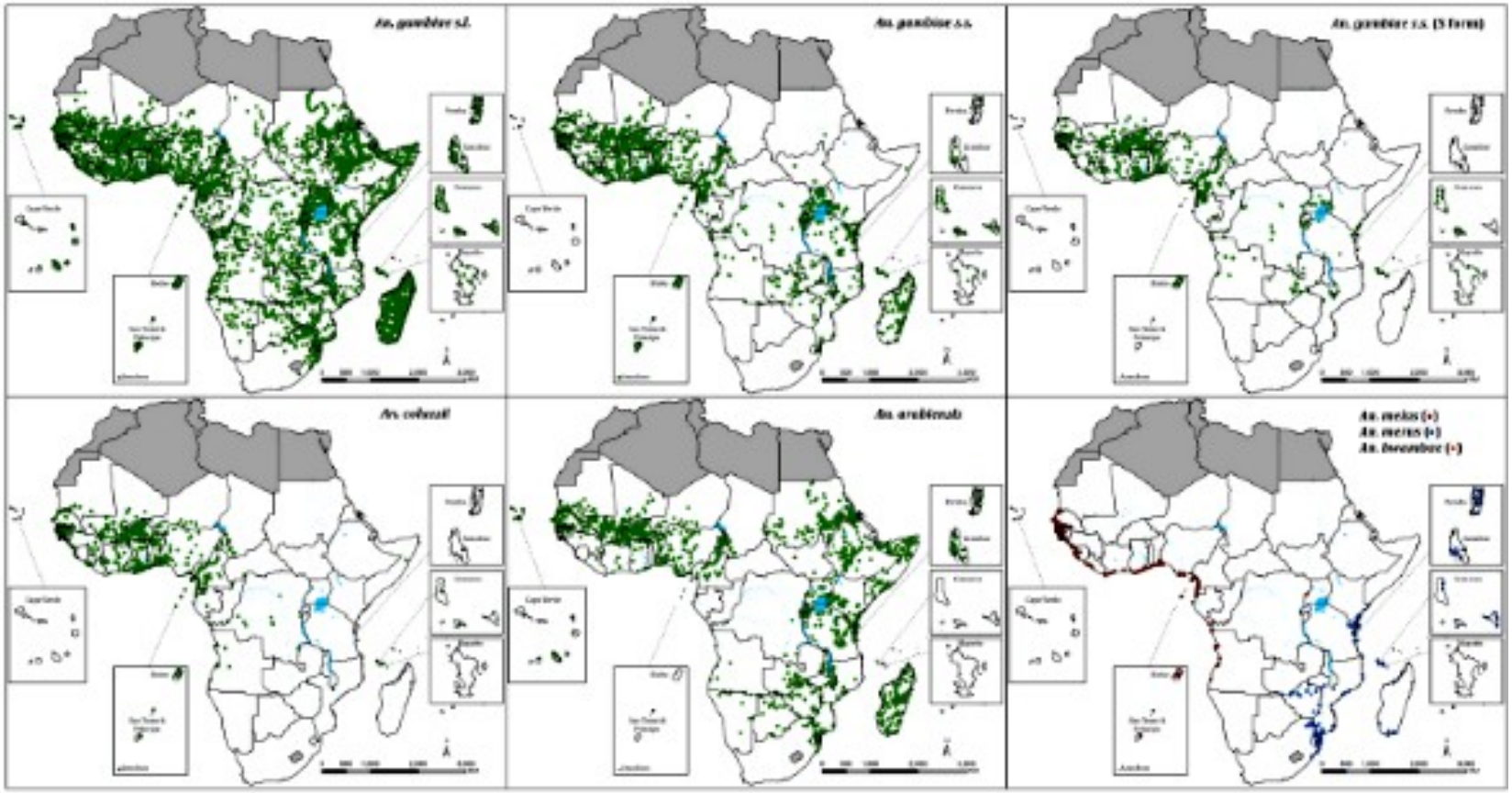


Figure 2.1 A map showing distribution of malaria vectors in Africa
(Kyalo *et al*., 2017).

2.3 Insecticide resistance in Malaria vectors

Insecticide resistance in malaria vectors takes four basic forms i.e. faster sequestering of the insecticidesi , target site insensitivity, behavioral resistance and cuticular thickening (Liu, 2015). Increased metabolic detoxification and target site insensitivity are the principal forms off insecticide resistance. These were attributed to the mutations in the genome of malaria vectors. Resistance to carbamates and organophosphates results from the alteration of target site by substitution of glycine by serine at the acetylcholinesterase enzyme (Weill *et al*., 2003). A single point mutation gene involving alanine-serine replacement in the GABA receptor leads to dieldrin resistance (R dl)(Wondji *et al*., 2011). Extensive use of pyrethroids cause mutations in the voltage –gated sodium ion channels(vgsc) a phenomenon referred to as knock-down resistance/*kdr* mutation. Two different forms of these mutations caused by pyrethroid resistance have been documented in west and East Africa (vgsc-1014S and the vgsc-1014F alleles) and are characterized by replacing leucine with either phenylalanine or serine respectively (Hemingway & Ranson, 2000)

Metabolic resistance through increased activities of monoxgenase and esterase enzymes have been confirmed in *An. gambiae s.s* mosquitoes from Bungoma county(Ochomo et al., 2015; Wanjala & Kweka, 2018). However, there are no reports of heightened detoxification reactions of glutathione-s-transferase enzyme in the *An. gambiae s.s* from the study area(Wanjala & Kweka, 2018). This resistance has been associated with the prolonged application of insecticides in addition to the use of agricultural pesticides by the farmers resulting to the expression of insecticide resistance alleles which in turn dilute the intended insecticides role on the life and population densitye of vectors (Alout *et al*., 2017). The western region in Kenya has recorded both forms of kdr mutations (vgsc-1014S and the vgsc-1014F alleles) (Ochomo *et al*., 2015). Metabolic resistance characterized by

increased activities of detoxifying enzymes such as monooxygenases and esterases have also been confirmed while there no reports of any heightened reactions of glutathione-s-transferase (GST) (Ochomo *et al*., 2015; Wanjala & Kweka, 2018).

Behavioral resistance which is the phenomenon by which the mosquitoes modify their behavior as a way of avoiding the lethal effects of insecticides. For example, resistant malaria vectors change their blood feeding behavior as a form of avoiding exposure to insecticide. The changes include; shift of the biting time, change of their resting location and host preferences enabling them to feed on humans at odd hours when they are not protected thus jeopardizing the effectiveness of ITNs because they target malaria vectors that feed indoors (Gatton *et al*., 2013; Pates & Curtis, 2005). Shift of the mosquitoes tending to search for a blood meal early in the evening (1800hours- 2000 hours) or late in the morning(0500-0800 hours) and outdoors negates the effectiveness of pyrethroid based insecticide treated nets and residual sprays (Abong'o et al., 2020; Doucoure et al., 2020; Reddy et al., 2011; Russell et al., 2016). In west Africa, reports of the biting behavioral resistance have been documented in the *An. funestus* mosquitoes that bite up to 11 h (Moiroux *et al*., 2012). This could be a great advantage to the resistant malaria vectors because studies have proved that residual biting occurring outdoors or early in the evening are adequate to maintain malaria transmission (Huho et al., 2013; Monroe et al., 2020).

The above mechanisms of insecticides resistance have been reported to impact the life history traits of other mosquito vectors yet there is inadequate information on the effects of the insecticide resistance on the biology of *An. gambiae* . Therefore this study explored the impact of knock down and metabolic resistance on life history traits and fitness of *An. gambie s.s* from western Kenya.

2.4 The burden of insecticide resistance in Africa

Pyrethroid and DDT resistant Anopheles populations are dominant in Africa while organophosphate and carbamate resistance was found in western Africa and east Africa (Knox *et al*., 2014). Pyrethroid resistance in *An. gambiae s.s* has also been recorded in Tanzania (Protopopoff *et al*., 2013) while *Anopheles funestus* are resistant to pyrethroids in Kenya, Uganda, Benin and Cameroon (Ranson & Lissenden, 2016). The L1014S and L1014F forms of *kdr* mutations are majorly in *An. gambiae s.l.* while metabolic resistance such as the Ace-1R is mainly detected in western Africa (Knox *et al*., 2014)

Kenya has reported Insecticide resistance in the four groups of insecticides used as interventions in the control of malaria (Hemming-Schroeder *et al*., 2018; Ochomo *et al*., 2013; Ondeto *et al*., 2017; Wanjala *et al*., 2015) Pyrethroid resistance has been found in *Anopheles gambiae* (*s.s.*), *An. funestus* and *An. arabiensis* while resistance to carbamate is restricted to *An. arabiensis* and *An. gambiae s.s* . Organochlorine resistance has been found in and *An. funestus* and *An. gambiae (s.s))* while resistance to organophosphates has been established in *An. gambiae (s.l.)* only. Elevated activities of carboxylesterases and glutathione- S-transferases (GST) (*An. Arabiensis),* monooxygenases (*An. arabiensis* and *An. gambiae* (*s.s.*) while the L1014S and L1014F forms of *kdr* mutations in *An. Arabiensis An. gambiae (s.s.)* and have also been confirmed (Ondeto *et al*., 2017; Wanjala & Kweka, 2018)

2.5 Effects of insecticide resistance on the fitness of mosquito vectors

Insecticide resistance is associated with energy costs that can impact the biology of a mosquito in terms of reproductive fitness and physiological processes (Rivero *et al*., 2011). Fitness costs are suspected to arise from phenotypic changes that can be lost within an insecticide-free environment (Coustau & Chevillon, 2000), reallocation of energy from other physiological processes, e.g., egg production by biochemical pathways compatible with resistance status (Chevillon *et al*., 1999) and

changes in the insecticide receptor proteins that may influence fitness disadvantage or advantage in resistant mosquitoes (Brito *et al*., 2013; Grossman *et al*., 2018). Insecticide resistance in mosquito is reported to interfere with malaria transmission by influencing vector feeding and longevity (Alout *et al*., 2017)

In the mosquito life, larval development duration is a major determinant of change in adult mean generation time. This is a basic component of fitness in increasing a species population which is a primary factor in the effective transmission of a vector borne disease. Therefore, slow larval development lowers survival of a vector by either increasing the larval risk to adverse climatic conditions, natural predation and pathogens which may end up negatively affecting the competence of mosquito vectors. Pyrethroid resistance has been linked to increased larval development time in *Culex quinquefasciatus* and *A. aegypti* (Hardstone *et al*., 2009; Jaramillo-O *et al*., 2014; Li *et al*., 2018; Martins *et al*., 2012) *Culex pipiens* harbouring the $Ace1^{R}$, $Ester^{1}$and $Ester^{4}$ alleles presented delayed larval development. Permethrin resistant *Aedes aegypti* with k*dr* mutation exhibited reduced adult emergence rates (Brito *et al*., 2013) as well as laboratory reared *Bti* resistant *Aedes aegypti* (Paris *et al*., 2011). Permethrin resistant *Aedes albopictus* expressed longer larval development time, low rate of adult emergence and low larval survivorship rates as compared to their susceptible counterparts (Chan & Zairi, 2013). Effects of pyrethroid resistance in the larval development of *An. gambiae* in western Kenya are poorly understood therefore, this knowledge could be important in designing novel ways for malaria vectors control.

Gonotrophic cycle is the time interval taken by a mosquito from blood feeding to the oviposition of eggs and thus dictating the biting rate of a given vector (Y. A. Afrane *et al*., 2006; Lardeux *et al*., 2008). This depends on the search and availability of a blood meal, ability to blood - feed, digestion, maturation of the eggs, search for a suitable site for oviposition and the ability to lay eggs

(Lardeux *et al*., 2008). This implies that gonotrophic cycle determines the biting frequency of a vector that will in turn affect the vectorial capacity and malaria transmission(Afrane et al., 2007; Lardeux et al., 2008). Previous studies done report that resistant *Aedes aegypti* showed prolonged gonotrophic cycle in comparison to their susceptible counterparts (Mebrahtu *et al*., 1997).

The blood meal engorged by a vector is directly proportional to the loads of pathogens ingested thus affecting the vector competence. This also affects fecundity since a blood meal is necessary for the egg nourishment. Behavioural aspects such as ability to sense the favourable host for a blood meal can also be affected by insecticide resistance. Organophosphate resistant *Aedes aegypti* showed reduced response to blood stimuli contrast to their susceptible counterparts (Belinato *et al*., 2012). Diflubuzorone a chitin synthesis inhibitor and resistance to organophosphate temephos by the *Aedes aegypti* led to a low egg laying capacity, reduced male mating success and also impaired blood-feeding in *Aedes egypti* (Belinato & Valle, 2015). Organophosphate resistance in *Culex pipiens* has been associated with a male mating disadvantage as compared to the susceptible individuals (Raymond *et al*., 2001)Pyrethroid resistant *Aedes aegypti* have showed a lower egg laying capacity in females (Brito *et al*., 2013).

Longevity is a fundamental parameter for any mosquito vector because it dictates the time for parasite development in a vector and hence affects malaria transmission cycle. Resistant insects tend to survive longer even in high resistance ratios of insecticides as demonstrated by the Brazilian field populations of organophosphate and pyrethroid resistant *Aedes aegypti* (Belinato & Valle, 2015),. However, other studies have reported that insecticide resistance has negatively affected longevity of vectors such as observed in *Culex pipiens pallens* that were selected for the pyrethroid resistance in the laboratory (Belinato & Valle, 2015; Martins *et al*., 2012) and

Permethrin –resistant *Aedes albopictus* starved females survived longer as compared to their susceptible adult females (Chan & Zairi, 2013).

Studies done on the effects of insecticide resistance on insects suggest that insects can modulate their survivorship, reproductive fitness and larval development in the presence and absence of insecticides. However, the effects of pyrethroid resistance and especially metabolic resistance on the life-traits and fitness of the *Anopheles gambiae* are understudied with the existing studies concentrating on the impact of target site resistance. Moreover, existing studies used mosquito samples from different genetic backgrounds which could be having other factors that are not related to insecticide resistance affecting the life history traits and fitness of mosquitoes. The results of this study will be of a great benefit in combating insecticide resistance.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study site

The parental colony of *An. gambiae s.s* mosquitoes were previously collected from Bungoma county at Kimaeti village (00.54057°N, 034.56410°E, altitude 1545 m above the sea level) a highland in western Kenya (Machani *et al.*, 2020). Studies have documented the existence of *An. funestus* and *An. gambiae s.s.* and their dominance is dependant on the season (Maxwell G Machani et al., 2020). Deltamethrin is the main insecticide used for the malaria vector control in (Bonizzoni *et al*., 2012; Ochomo *et al*., 2013). The main economic activity of the people in this locality is small scale mixed farming (crop and livestock) that employs the use of xenobiotics and pesticides. The experimental mosquitoes used for the study were reared in the insectary of the Entomology section at the Kenya Medical Research Institute (KEMRI), Kisumu.

3.2 Study design

The study applied an experimental design and was done in a semi field set up, which was achieved through the use of malaria sphere (a screen house consisting of natural vegetation and a traditional hut) at the Entomology section of KEMRI, Kisumu in western Kenya.

3.3 Mosquito population used in the study

The population of mosquitoes used for the experiment in the study consisted of established colonies of resistant *Anopheles gambiae sensu stricto* selected through exposure to deltamethrin insecticide and a susceptible colony of *An. gambiae s.s* that was not exposed to any insecticide over several generations. Both resistant and susceptible colony originated from Bungoma, western (M. G. Machani et al., 2020).

3.3.1 Selected/Resistant colony

The resistant colony was selected by exposing *An. gambiae s.s* mosquitoes to 0.05% deltamethrin at every generation. This resistant colony used was characterized by an average mortality rate of 20% when exposed to 0.05 deltamethrin, loss of the wild type allele (L1014) and high frequencies of kdr mutations L1014S and L1014F at 0.77 and 0.23 respectively. Metabolic resistance of this colony was mainly defined by cytochrome P450 detoxification enzyme (M. G. Machani et al., 2020)

3.3.2 Unselected/Susceptible colony

The susceptible colony was reared without selection at any of its generation but was checked for resistance after every generation. This colony had an average mortality rate of 97.3% when exposed to 0.05% deltamethrin. The *kdr* mutation detected was L1014S which was already fixed in the parent population. This colony also recorded a reduction in cytochrome P450 detoxification enzymes in comparison to their parent population (M. G. Machani et al., 2020)

3.3.3 Kisumu reference laboratory strain

The Kisumu reference *An. gambiae s.s* laboratory strain was colonized and is reared at the KEMRI insectary laboratory since 1954 (Shute, 1956). It has no records of any detectable insecticide resistance mechanism. Therefore, it was used as a control susceptible strain in all bioassays.

The *An. gambiae s.s* mosquitoes that were used in this study were reared in the insectary at KEMRI/CGHR under standard conditions for rearing *An. gambiae* mosquitoes (25 ± 2 °C; 80% ± 4% relative humidity with a 12 h: 12 h light/dark cycle).

3.3.4 Inclusion criteria

Only two (2) hours old larvae were used in the larval development experiment. The gonotrophic cycle and fecundity experiment used three (3) days old blood fed female mosquitoes only since gonotrophic cycle is measured from the time of blood feeding. The adult longevity utilized one day old female mosquitoes only.

3.3.5 Exclusion criteria

Larvae that were less than or older than two (2) hours were not used in the study**.** Female mosquitoes that were less than or older than three (3) days old were not used in the gonotrophic cycle and fecundity experiment. Also female mosquitoes that were older than a day old were excluded in the longevity experiment.

3.4 Study variables

This study had two independent variables; insecticide resistance and insecticide susceptibility. The dependent variables were; the length of larval development time in days, duration of the female gonotrophic cycle in days, quantity of eggs laid by females in numbers and the length of lifespan of adult female mosquitoes in days.

3.5 Sampling design

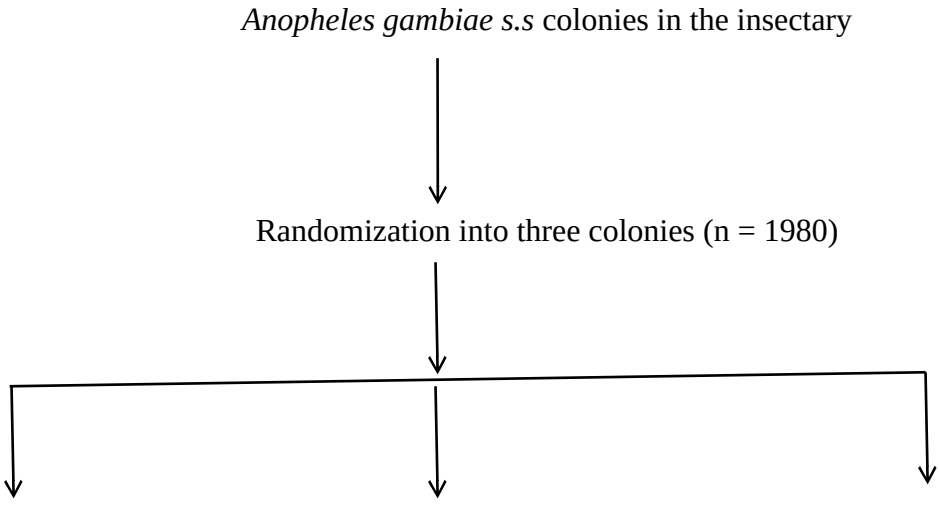
Mosquito samples used in this study were purposively sampled on the basis of their known metabolic and target site resistance (kdr) resistance status.

3.5.1 Mosquito sampling procedure

The established colonies of *An. gambiae s.s* used in the study included the resistant colony selected for deltamethrin resistance using the WHO tube Bioassay, the unselected colony that was reared without exposure to insecticide and the Kisumu laboratory reference strain that was raised in the insectary at KEMRI, Kisumu. *Kdr* (1014S and 1014F) and metabolic resistance mechanism through increased activities of cytochrome P450 enzymes were implicated in the selected population. In the unselected population only *kdr* 1014S was detected (M. G. Machani et al., 2020)

3.6 Sample size determination

Thirty (30) larvae were randomly selected per a basin in the larval development time experiment as described by Afrane and others(Afrane *et al*., 2007; Minakawa *et al*., 2006).There were a total of 1980 larvae (660 per a colony) for this experiment.



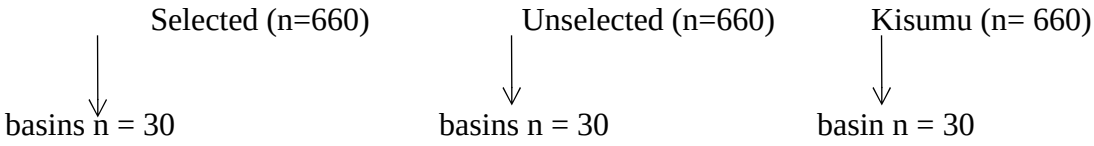


Figure 3.6.1: sample size determination for the larval development experiment

The sample size of larvae to be used was determined as stipulated in the WHO manual on larval control operations in malaria programmes (Organization, 1973). Three hundred (900) adult females mosquitoes were used in the reproductive potential and longevity experiment and were also randomly selected from the insectary as described by Afrane and others (Y. A. Afrane *et al*., 2006; Afrane *et al*., 2007; Minakawa *et al*., 2006). These two experiments involved the use of 900 females (300 per colony).

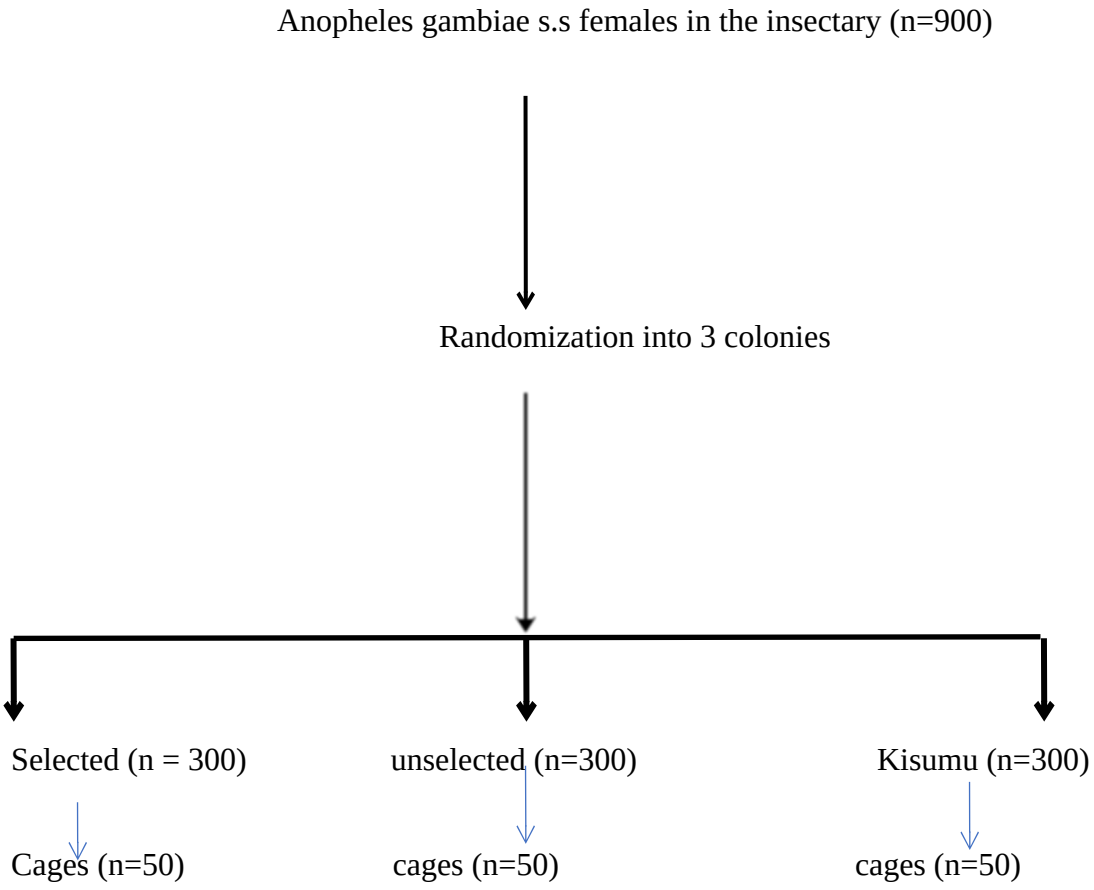


Figure 3.6.2: sample size determination for the reproductive fitness experiments

3.7 Data Collection Procedure

This study was carried out in three experiments; the larval development experiment, gonotrophic cycle experiment and the adult longevity experiment. The mosquito samples used were from three groups; the selected *An. gambiae s.s,* the unselected *An. gambiae s.s* and the Kisumu susceptible reference strain that was the control.

3.7.1 Determination of larval development time of resistant selected and unselected *An. gambiae s.s*

The determination of larval development time experiment was carried out according to the WHO manual on larval control operations in malaria programmes (Organization, 1973). To achieve this objective, two Kilograms of soil from breeding sites of the parent mosquitoes were put into clean basins and 5 litres of rainwater added into the basins containing the soil so as to imitate the natural habitat of mosquito larvae. Thirty larvae that were two-hour were picked from the three groups and were placed separately in the basins containing water and soil and then allowed to grow and pupate (Afrane *et al*., 2007; Hawaria *et al*., 2021; Minakawa *et al*., 2006). Each basin was covered using an insect-proof nylon netting to prevent predators. The number of larvae alive was recorded on a daily basis while noting their stage of development. This was useful in the determination of larval development times, pupation rates and emerge rates. There were several replicates for each colony so as to clarify differences in the larval life trait parameters.

3.7.2 Gonotrophic cycle and fecundity of resistant selected and unselected *An. gambiae s.s*

To achieve this objective, the following experiment was carried out;

One hundred pairs of newly emerged females and males from the three established colonies of selected and unselected *An. gambiae s.s* were placed separately in 30cm*30cm*30cm metal-framed cages for 3 days to allow time for mating and were maintained on 10% sugar solution. After

three (3) days, the female mosquitoes were fed on the arm of a human volunteer. Fifty gravid mosquitoes from the three groups were then transferred into an individual oviposition cups as demonstrated in a previous study done using *An. coluzzi(Nkahe et al., 2020)*. The total number of eggs laid in each oviposition cup was recorded while noting down the duration in terms of days taken for the eggs to be laid from the time of blood meal ingestion. in order to determine the length of gonotrophic cycle and fecundity. The experiment was done in six replicates for each colony.

3.7.3 Adult female vector longevity of resistant selected and unselected *An. gambiae s.s*

To achieve this objective, one hundred newly emerged (3 days old) mosquitoes from the three colonies were put separately in a (30 by 30 by 30)-cm^3 cages made of metal frames that were covered with a netting. These cages were suspended 2M above the ground from the roof of the hut in the malaria sphere using wooden twines. The twines were greased to prevent ants from feeding on mosquitoes (Afrane *et al.*, 2007a). Mosquitoes were fed daily on cattle blood from an abattoir and were also maintained on 10 % sugar. Dead mosquitoes were counted daily and their numbers recorded to determine the daily survival rates.

3.7.4 Data Collection Instruments

The data collected from the three experiments was recorded in data collection forms.

3.7.5 Reliability and validity

Validation was achieved by using the Kisumu laboratory reference strain that has been reared in the insectary since 1954 as a control for all the experiment.

3.8 Data Presentation and Analysis

Data was entered in Microsoft Excel sheets and analysis was done using the R software (version 4.0.0). The level of significance for each test was set at $\alpha < 0.05$.

Mean generation time was defined as the average time for first-instar larvae to develop into the subsequent second, third and fourth instar larvae, pupae then emerge as adults. The mean larval development time for the male and female mosquitoes was computed separately since they have different development times. The pupation rate was calculated as pupae formed out of the total number of first instar larvae at the start of the experiment (N_P/N_0*100) N_p was the number of pupae formed; N_0 was the sum of larvae subjected to the experiment. Adult emergence rate was calculated as the percentage of the number of adults that emerged out of the total number of pupae formed (N_A/N_P*100) where N_A is the number of emerged adults. Analysis of variance (ANOVA) determined the effects of insecticide resistance on larval life trait parameters of the three colonies. Tukey HSD test was used in comparing the difference in the larval development time, pupation rates and emergence rates acrsoss the three colonies of *An. gambiae s.s*. The larval development time together with the other larval life parameters data was presented in a table.

Gonotrophic cycle was defined as the duration calculated in days taken for a mosquito to lay eggs after ingestion of the blood meal. ANOVA was used in the determination of the effects of insecticide resistance on the mosquitoes from the three colonies. Tukey HSD test was used in gauging the difference in the gonotrophic cycle length among the three colonies. The gonotrophic cycle data was presented in a table. Fecundity was calculated as the average number of eggs oviposited by each female mosquito per day and the data was presented in tables.

Longevity of the female mosquitoes was calculated as the median of the number of days lived by the mosquitoes per group. ANOVA determined the difference in the longevity among the three colonies. Kaplan–Meier survival analysis was applied in determining the level of significance in the female survivorship of the mosquitoes across the three colonies. Longevity data was presented using tables while the female survival probability was presented using curves.

3.9 Logistical and ethical considerations

Ethical approval was obtained from National Commission for Science, Technology and Innovation (NACOSTI), Masinde Muliro University of Science and technology Institutional Ethical Review Committee (MMUST-EIREC) and the Kenya Medical Research Institute Scientific and Ethical Review Unit (KEMRI- SERU) under approval number SSC 3434. All experimental protocols were approved by these institutional ethics committees. All experiments and methods were carried out in accordance to the regulations of KEMRI- SERU.

CHAPTER FOUR

4.0 RESULTS

4.1 Effects of insecticide resistance on larval development time

The mean development time from first instar (L1) to second instar (L2) in the selected colony was 4.9 ± 0.2, while the unselected colony was 3.4 ± 0.1 and 3.4 ± 0.1 for the Kisumu strain ($F_{2,63}$=44.43, P<0.05; Table 1). The average length of larval development time (L1-L2) for the selected colony was 1.5 days longer compared to the unselected colony. The time for the selected colony to develop from L1 to third instar (L3) was 6.9 ± 0.2 days while the unselected colony was 4.9 ±0.2 and 4.8 ± 0.2 days for the Kisumu colony. The development time (L1-L3) for the selected colony was 2 days longer compared to the other colonies ($F_{2,63}$=44.61, P<0.05). The mean pre-imaginal development time from L_1 to fourth instar (L_4) of the selected colony was 8.8 ± 0.2, while the unselected colony was 6.6 ± 0.2 and 6.3 ± 0.2 for Kisumu laboratory susceptible mosquitoes. The selected colony took a significantly longer period (2 days) to develop from L_1-L_4 with respect to the unselected colony ($F_{2,63}$=47.06, P<0.05) as shown in table 4.1

Table 4.1 Larval instar development time among the selected, unselected and Kisumu colonies

	Larval instar development time (days)		
Population	L2[i]	L3[ii]	L4[iii]
	Mean ± SE	Mean ± SE	Mean ± SE
R Group	4.9 ± 0.2[b]	6.9 ± 0.2[b]	8.8 ± 0.2[b]
S Group	3.4 ± 0.1[a]	4.9 ± 0.2[a]	6.6 ± 0.2[a]
Kisumu strain	3.4 ± 0.1[a]	4.8 ± 0.2[a]	6.3 ± 0.2[a]

4.1.2 Pupation and emergence times between the selected and unselected colonies

The selected colony reached pupal stage at 10.28 ± 0.3 days after hatching as L1, whilst the unselected colony took 7.5 ± 0.29 days. Development time from L1 to pupal stage was significantly longer (2.78 days) in selected colony than in the unselected colony ($F_{2,63}$=39.45, P <0.05, Table 2). The Kisumu strain took 7.9 ± 0.2 days to pupate.

The pupation rate in the selected colony was 80% (95% CI: 77.5-83.6), while it was 83.5 % (95% CI: 80.6-86.3) for the unselected colony. Although the selected colony took a longer time to develop, the difference in the pupation rate between selected and unselected colonies was not significant ($F_{2,63}$=0.084, P > 0.05). The emergence time for males and females in selected colony was 11.9 ± 0.3 and 12.1 ± 0.3 respectively while the unselected colony was 9.2 ±0.2 and 9.6 ± 0.2 days respectively. The male emergence time for the Kisumu strain was 9.4 ± 0.2 days and 9 ± 0.2.8 days for females. The emergence time for males and females in the unselected colony was significantly shorter compared to unselected colony (Males; $F_{2,63}$=38.4, P< 0.05; Females, $F_{2,63}$=35.81, P<0.05; Table 4.2).

Table 4.2 Larval - life trait parameters of the colonies

Colony	Pupation time (days)[1]	Pupation rate (%)[2]	Mean development time of Male (days)[3]	Mean development time of females (days)[4]	Emergence rate (%)[5]
Selected	10.28 ± 0.3[b]	80 ± 0.0[a]	11.9 ± 0.30[b]	12.1 ± 0.3[b]	86.3 ± 0.0[a]
Unselected	7.5 ± 0.2[a]	83.5 ± 0.0[a]	9.2 ± 0.2[a]	9.6 ± 0.2[a]	92.8 ± 0.0[a]
Kisumu strain	7.9 ± 0.2[a]	84.5 ± 0.0[a]	9.4 ± 0.2[a]	9.8 ± 0.2[a]	85.7± 0.0[a]

4.2 Duration of the gonotrophic cycle

The mean number of days for the gonotrophic cycle measured from the blood feeding to egg laying for the selected females was longer (6.1± 0.8 days) than the unselected females 4.3 ±1.1 days and 3.7 ± 0.2 days for the Kisumu strain. The average duration of gonotrophic cycle for the selected females was significantly longer by 2.2 days than the unselected colony ($F_{2, 13}$ =9. 836, P <0.05; Table 4.3).

Table 4.3 Gonotrophic cycle length of the Selected, unselected and the Kisumu strain

COLONY	No. tested (N)[1]	Laying mosquitoes[2]	cycle length(days)[3]
Resistant	300	94	6.1 ± 0.0.8[a]
Susceptible	300	161	4.3 ± 1.1[b]
Kisumu	300	197	3.7 ± 0.2[b]

4.2.1 Fecundity of the experimental *An. gambiae s.s*

In absolute numbers overall, the egg laying rate for the resistant females was significantly lower at 31.30% (94/300) compared to 54% (162/300) for the susceptible colony and 65.7% (197/300) for the Kisumu strain ($F_{2,11}$ = 0.461, P< 0.05). The mean number of eggs laid by an individual after the first blood meal in the selected colony was lower (88.02 ± 20.0) contrasted to the unselected colony (104.9 ± 28.8) and Kisumu strain (97.6 ± 34.8). Therefore, fecundity of the resistant colony was significantly reduced than the unselected colony by 16 eggs per mosquito ($F_{2,449}$ = 6.786, p < 0.05); Table 4.4

Table 4.4 Fecundity of the Selected, unselected and the Kisumu strain

COLONY	No. tested (N)[1]	Laying mosquitoes[2]	Mean ± SE Fecundity[3]
Resistant	300	94	88.02 ± 20[a]
Susceptible	300	161	104 ± 28[b]
Kisumu	300	197	97 ± 34[a]

4.3 Adult survivorship

The average lifespan time for the selected colony was 39.7 ± 1.6 days while the susceptible colony was 29.9 ± 1.7 days and the Kisumu strain had 29.6 ± 1.1 days ($F_{2,8}$ =45.05, $p<0.05$; Table 3). The mean survival time recorded by the resistant colony was significantly longer (10 days) than the susceptible colony and Kisumu strain mosquitoes ($F_{2,8}$ =45.05, $P < 0.05$; Table 4.4).

Table 4.4 Means and median survival times for the adult *An. gambiae s.s* females of the selected, unselected and Kisumu colonies.

Colony	Mean ± SE(days)	median (days)
Resistant	39.7 ± 1.6	43
Susceptible	29.9 ± 1.7	34
Kisumu strain	29.6 ± 1.1	33

4.3.1 Survivorship among the selected, unselected and Kisumu strain *An. gambiae s.s* females

Resistant females survived slightly longer (85 days), with a median survival length of 43 days than the susceptible colony that survived for 67 days having a median of 34 days and Kisumu strain that survived for 65 days with a median length of 33 days (Figure 4.1).

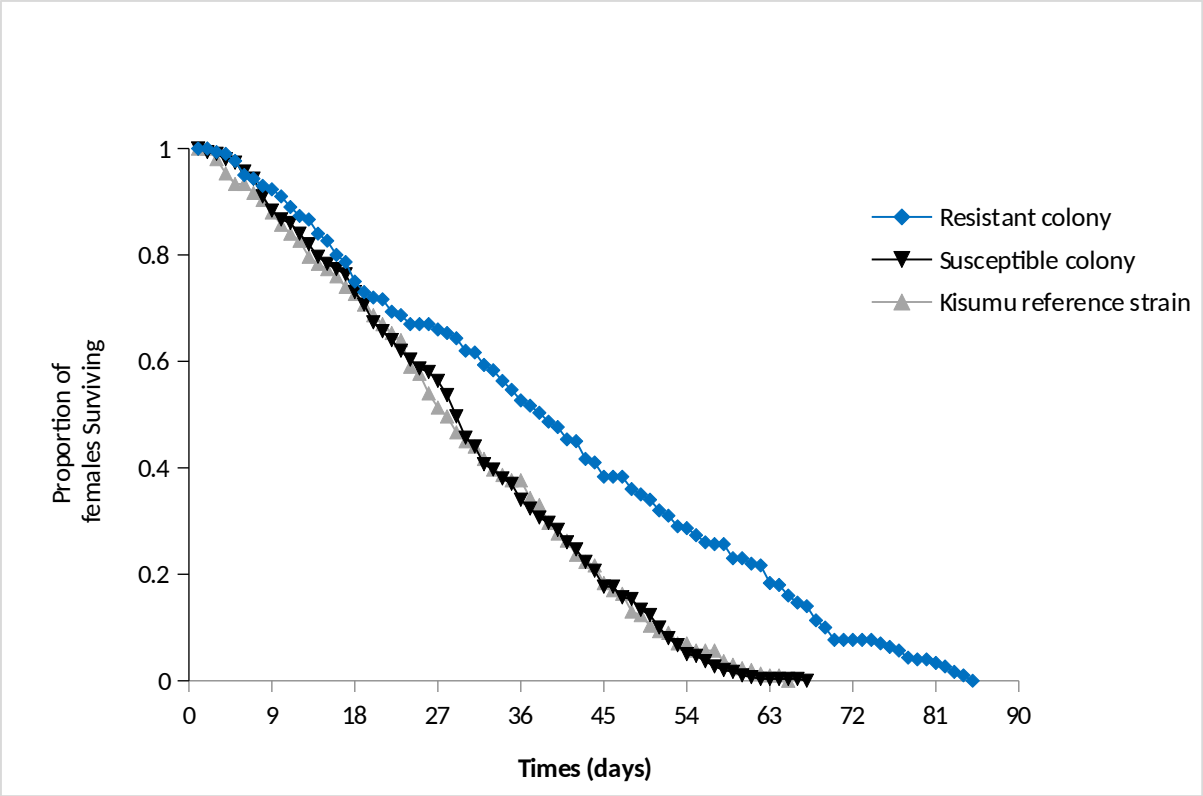


Figure 4.1 survival probability curve for the selected, unselected and the Kisumu *An. gambiae s.s.*

CHAPTER FIVE

5.0 DISCUSSION

Insecticide resistance in malaria vectors of Sub- Saharan Africa has greatly strained the control of malaria (Ranson & Lissenden, 2016). Although the resistance status of these mosquito vectors has been studied and reported, there is limited information on how it influences larval development, reproductive fitness and female survivorship of the vector. Understanding the mechanisms and effects of insecticide resistance on the biological traits of the vector will help to enhance insecticide resistance management strategies. In an evolutionary perspective, genetic changes arising as a result of insecticides' selective pressure can foster a fitness cost to resistant insects bearing negative effects on their biological traits (Kliot & Ghanim, 2012). This study assessed the larval development time, gonotrophic cycle, fecundity and adult female survivorship of established colonies of two progenies of *An. gambiae* with the same origin exhibiting different pyrethroid insecticide resistance profiles.

The results of this study demonstrate the existence of a fitness cost on *An. gambiae sensu stricto* that is linked to pyrethroid resistance. Overall larval development time and reproductive fitness (gonotrophic cycle and fecundity) was compromised in the pyrethroid- resistant selected colony as opposed to the unselected colony originating from the same background. On the contrary, the female longevity of the pyrethroid-selected colony was advantaged compared to the unselected colony. The development time, reproductive fitness parameters and lifespan of the unselected susceptible colony was closely similar to that of the susceptible Kisumu reference strain.

5.1 Larval development time of the experimental *An. gambiae s.s*

The study observed prolonged development time from one larval instar to the other in the selected resistant colony when compared with the unselected colony whose development time was similar to the Kisumu colony. Majority of individuals from the unselected colony and the Kisumu strain reached the pupal stage in about 7 days after the hatching of the first instar whereas the selected colony took additional 3 days before pupation. These findings present adaptive disadvantage on the resistant individuals as the amount of time spent in the natural breeding habitats in the field may impact their survival rates due to exposure to natural predations. They are also likely to risk loose of their habitats in before emerging to adults during seasons of short rains. This could reduce their survivorship which may in turn have a negative influence on the vectorial capacity (Rivero *et al*., 2011)

It is important to highlight that monooxygenase enzyme was majorly implicated in the pyrethroid resistance of the selected colony even though kdr mutations were observed at high frequencies(M. G. Machani et al., 2020). It is likely that the overproduction of monooxygenase would have committed resources important for primary biological functions such as development to maintaining secondary functions i.e insecticide detoxification(Kliot & Ghanim, 2012). For instance, some studies have linked the staggered larval development time of resistant individuals with spending more time in the accumulation of nutrients to achieve the development threshold that triggers growth to the next stage as most of the resources are used to maintain resistance (Diniz *et al*., 2015)

Pyrethroid-resistant *Aedes albopictus, An. funestus, Culex quinquefasciatus,An. Coluzzii* and *Aedes aegypti* have reported longer periods of development in comparison to their susceptible counterparts in already existing studies (Hardstone et al., 2009; Jaramillo-O et al., 2014; Martins et

al., 2012; Nkahe et al., 2020; Tchouakui et al., 2018). The findings of this study are similar to reports on *An. funestus* from West Africa harboring 119F-GSTe2 resistant alleles which exhibited delayed larval development compared to the population without the resistant alleles (Tchouakui *et al*., 2018). The *kdr* mutation has been associated with a delay in the larval development of *A. aegypti,* (Macoris *et al*., 2018). The observed negative effects are attributed to insecticide resistance that could affect the spread of insecticide resistance genes in a population, as the resistant individuals are likely to take a longer time to develop and emerge as adults unlike the susceptible ones. Based on this, innovation of new approaches to the management of insecticide resistance may rely on this reduced fitness disadvantage to design integrated vector control management strategies with an aim of limiting the spread of insecticide resistance and making the existing vector control tools adequate.

5.2 Effects of pyrethroid resistance on the gonotrophic cycle of *An. gambiae s.s*

The findings of this study suggest that pyrethroid resistance prolonged the first gonotrophic cycle length of the resistant mosquitoes in comparison to their susceptible counterparts suggesting a lower human biting rate by a resistant mosquito which could result to a lower malaria transmission rate in areas with pyrethroid resistant mosquitoes. This findings coincides with the findings of Mebrahtu who observed that permethrin resistance delayed the time interval between blood ingestion and egg oviposition in resistant *Aedes aegypti* (Mebrahtu *et al*., 1997)in comparison to their susceptible females.

The longer gonotrophic cycle reported in the resistant female mosquitoes could be due to lower blood meal digestion rates that may have resulted from altered physiology of the resistant mosquitoes from the continued exposure to pyrethroids (Yun *et al*., 2017). Studies have documented Insulin- like peptidases and ovarian ecdysteroidogenic hormones released by the

neurosecretory cells in the brain of *Aedes aegypti* to being involved in the activation of blood meal digestion (Gulia-Nuss *et al*,2013). This implies that any substance that may affect or interfere with the central nervous system could slow or inhibit the release of these hormones that are crucial for the stimulation of the process of digestion of the ingested blood hence inhibiting or delaying the maturation of eggs of the resistant females. Therefore the prolonged gonotrophic cycle in the resistant females could be attributed to the target site resistance mechanism documented (M. G. Machani et al., 2020) that affects the nervous system of the mosquitoes that are resistant to pyrethroids.

Previous studies have reported a relationship between Insecticide resistance and reduced blood intake in resistant females as documented in the following studies; (Gulia-Nuss et al., 2011; Mebrahtu et al., 1997; Nkahe et al., 2020). Reduced blood intake can lead to delayed gonotrophic cycle resulting from the mosquito need for more bites necessary for egg maturation (Oliver & Brooke, 2014). The longer gonotrophic cycle exhibited by the resistant mosquitoes could imply reduced biting frequencies due to insecticide resistance hence suggesting less rates of malaria transmission in areas with a high population of pyrethroid resistant vectors. However, mosquitoes with delayed gonotrophic cycles due to slow egg maturation would tend to take multiple blood meals before laying eggs as reported in studies carried out in *Anopheles pseudopunctipennis* and *Aedes albopictus*(Lardeux *et al*., 2008). This could increase the opportunities of ingesting malaria parasite hence increasing their potential of transmitting malaria.

5.3 Effects of pyrethroid resistance on the fecundity of *An. gambiae s.s*

The fecundity of pyrethroid resistant mosquitoes was also reduced as recorded in the results of this study. Fecundity of the resistant mosquitoes was characterized by less number of egg laying females and the batches of eggs laid by an individual mosquito was lower than what was observed

in the unselected susceptible colony that laid more eggs. The decreased fecundity in the pyrethroid resistant *An. gambiae* as compared with the unselected counterparts implies that pyrethroid resistance has a disadvantage on the fecundity which is a measure of the reproductive fitness of malaria vectors. The findings on the reduced reproductive fitness as a result of insecticide resistance in this study agree with other previous studies done on insecticide resistant *Anopheles funestus, Anopheles coluzii* and *Aedes aegypti* (Nkahe *et al*., 2020; Tchouakui *et al*., 2018; Tchouakui *et al*., 2020)

The reduced fecundity in the resistant females could be resulting from the re-allocation of nutrients obtained from the blood meal to the maintenance of processes necessary for the survival of the female mosquitoes other than egg production. Low egg production could also be attributed to suspected reduced rates of insemination in resistant mosquitoes as opposed to the susceptible individuals or the reduced blood feeding rates in resistant mosquitoes as previously documented (Brito *et al.*, 2013; Tchouakui *et al.*, 2020). Blood meal digestion could also have been slowed down because of the implication of target site insecticide resistance in the resistant colony used (M. G. Machani et al., 2020). The consequence of this is the interference of the central nervous system(CNS) resulting to suppression in the levels of hormones necessary for the regulation of the blood meal digestion in mosquitoes (Gulia-Nuss et al., 2011). The long gonotrophic cycle length would also make the resistant mosquitoes to take blood meals less often reducing the egg laying frequency hence explaining the reduced fecundity of the resistant colony.

The reduced fecundity will tend to result to a lower net reproductive rate in the resistant selected colony than in the unselected mosquitoes. Net reproductive rate is a measure of the proportion of females reproduced from each original parental female. This will in turn lead to a lower intrinsic growth rate in the resistant individuals than in their susceptible counterparts. Although this study

did not evaluate the viability of the eggs laid, some studies report reduced viability of eggs laid by deltamethrin resistant *Aedes aegypti* in contrast to susceptible individuals. The diminished reproductive potential in the resistant colony could compromise the number of progenies of the resistant mosquitoes leading to less mosquito populations which will lower the vectorial capacity of resistant mosquito vectors. Therefore, in nature mosquitoes carrying resistant genes could result in reduced populations which will limit malaria transmission. This negative impact of pyrethroid resistance on the reproductive fitness of the resistant *An. gambiae s.s* could facilitate insecticide resistance management strategies.

5.3 Effects of pyrethroid resistance on the female longevity of the selected and unselected *An. gambiae s.s*

The life span of a mosquito vector is the primary life trait that directly impacts disease transmission. The vector has to live long enough in order to enable ingestion of the infected blood meal ,hosting of the parasite and allowing it to develop into the infective stage (Alout et al., 2017) The resistant colony recorded a significantly higher survivorship than the susceptible colony in this study. These results concur with reports from studies on pyrethroid resistant *An. funestus* (Tchouakui *et al.*, 2018b). On the contrary, there are studies that reported shorter lifespan in resistant females such as pyrethroid resistant *An. coluzzii* (Nkahe *et al.*, 2020) and *Aedes aegypti* with kdr mutations (Martins *et al.*, 2012) which were associated with decreased longevity than their susceptible counterparts. Increased mortality rates was also reported in dieldrien resistant *An. gambiae* and *An. stephensi* (ROWLAND, 1991).

The prolonged longevity in the resistant mosquitoes could be a result of the implication of the extended duration of larval development, creating an opportunity for aggregation of nutritional and metabolite reserves in order to compensate for the losses that arise from the costs of maintaining the resistance mechanism (Telang et al., 2007). The enhanced longevity could also be

attributed to the metabolic resistance implicated in the resistant selected colony since metabolic resistance has been previously associated with the ability to protect tissues of plant hoppers and fruit flies from oxidative stress and elongating the life of fruit flies exhibiting high levels of glutathione transferase enzymes (McElwee et al., 2007). This is achieved through the benefit of the resistant alleles that give protection against poisonous oxygen species produced by metabolic reactions during the development of the insects (Mittapalli et al., 2007).

The increased survivorship of resistant *An. gambiae* will boost the gonotrophic cycle of the resistant malaria vectors since it provides the mosquitoes with more days that will enable them to achieve their long gonotrophic cycle. This will in turn enhance fecundity owing to the fact that the resistant females will have enough time for digestion, maturation and egg oviposition. Therefore, the long life of pyrethroid resistant *An. gambiae* females implies a better vectorial capacity through enhanced reproductive fitness and also increased chances of mosquito bites. This will as well enable completion of saprogenic cycle of malaria parasites hence enhancing malaria transmission by the resistant mosquitoes. These results are an indication of the negative impact of pyrethroid insecticide resistance that could compromise the existing malaria vector control measures that include the LLINs and IRS which could result to high malaria transmission.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The findings of this study on the impact of pyrethroid resistance on the life history traits and fitness of *An. gambiae s.s* has revealed significant fitness costs on larval development time, fecundity and gonotrophic cycle of resistant mosquitoes resulting to their delayed development and reduced fertility. On the other hand, the unselected mosquitoes reported an advantage on the development time of immature stages and reproductive fitness parameters.

This study also reported a fitness advantage on the longevity of the resistant colony while the life span of the unselected mosquitoes was shortened.as opposed to that of the selected *An. gambiae s.s* resulting to delayed emergence of adults. This suggests that pyrethroid resistance disadvantages the development of *An. gamble s.s.*

Therefore, pyrethroid resistance increases larval development time, elongates the gonotrophic cycle duration and diminishes the ability of resistant mosquitoes to lay eggs as well as lessening the size of egg batches. Pyrethroid resistance also extends the longevity of resistant female *An. gambiae s.s*.

6.2 Recommendations

6.2.1 Recommendations for action

This study recommends that larval source management for malaria vectors should be reconsidered and incorporated into the malaria control programmes so as to curb the resistant immature stages

that survive through the extended development time. This will supplement the existing vector control mediations by controlling the resistant larvae that take long time to develop and prevent adult emergence in the resistant populations in areas having pyrethroid resistance.

This study postulates that future insecticide management strategies should consider implementation of rotational insecticide usage where non-pyrethroid based LLINs will be used so as to reduce the selection pressure, contain the spread of pyrethroid resistance and probably shorten the lifespan of resistant *An. gambiae s. s*

The study also recommends that policy makers should adopt novel malaria vector control tools that do not apply insecticides targeting the resistant adult females so as to complement the existing vector control tools.

6.2.2 Recommendations for further studies

Future studies should be done on the effects of pyrethroid resistance on the life history traits and fitness of *An. funestus and An.arabiensis* being other malaria vectors in western Kenya.

Studies in the future should explore the effects of pyrethroid resistance on other factors that influence the reproductive fitness of the mosquitoes but were not covered in this study. This includes; the blood feeding rate, male mating success, female engorgement rate and insemination rates so as to exhaust the causes of reduced fecundity of the resistant mosquitoes.

More studies should be done on the effects of insecticide resistance on the survivorship of *An. gambiae s.s* in the field by using the LLINs that exist in the field in order to achieve effective and adequate management and control of spread of insecticide resistance in malaria vectors.

Studies assessing the impact and role of climate change on the life history traits and fitness of insecticide resistant malaria vectors should also be carried out in future so as to provide information on the role of extreme weather conditions and changing rainy seasons which is a

rising threat in the control of vector borne diseases. This will help to equip malaria programmes so as to come up with measures that can support communities in times of climatic calamities.

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APPENDICES

Appendix I: Larval Survivorship Data Form

Date of start of experiment_____ Replicate_____

		Development stage of alive larvae					Emerging adults		
Day	**Date**	**I**	**II**	**III**	**IV**	**Pupae**	**Males**	**Females**	**Total**
0									
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									

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Appendix II: Gonotrophic Cycle Data Collection Form

Colony_____ Replicate_____ Date of feeding _____

Mosquito ID	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Remarks
1											
2											
3											
4											
5											
6											
7											
8											
9											
10											

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Appendix III: Longevity Data Form

Date									
Colony									
Replicates	**Rep.1**			**Rep.2**			**Rep.3**		
N-tested									
Days	**Male dead**	**Female dead**	**No. egg**	**Male dead**	**Female dead**	**No. egg**	**Male dead**	**Female dead**	**No. egg**
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									