The Demonstration of Larval Polytene Chromosomes of Anopheles Gambiae

AJU-AMEH
Onyawoibi Celina and MAFUYAI, Hayward Babale
Department of Zoology,University of Jos,
UniJos.
Jos,Plateau State,Nigeria.
celinaaju@gmail.com

***Abstract*- Anopheles gambiae (Diptera: Culicidae) larvae were found in large numbers in the open ground pools at Rayfield, Jos and a few from along the stream edges formed by some pool. Within the pool mixed populations of both culicine and Anopheline larvae were found. A total of four hundred and ninety-seven Anopheline larvae were brought into the insectary from the wild. Four hundred and forty-four were reared to adult stage successfully. Fifty were fixed for dissection and three died. Laboratory colonization of the Anopheles gambie failed due to, among other factors, that they could not blood feed. Consequently only the salivary gland polytene chromosome squashes of wild caught larvae were examined. Dissections of the salivary gland of the Anopheles yielded chromosomes which spread successfully as expected. A Least Significance Difference test (LSD) showed a highly significant interaction between seasons and sites.**

***Index Terms*— Anopheles gambiae, Larval instar, Polytene chromosomes,Salivary gland.**

I. INTRODUCTION

Malaria is caused by plasmodium parasites transmitted to people through the bites of infected Anopheles mosquitoes called “malaria vectors” and it is a life threatening disease[1].In Nigeria only 3% of the total population live in malaria free zone while 97% live within malaria risk zones. It is estimated that over 300,000 deaths occur in Nigeria [2] as a result of malaria. Proper identification of the vectors is vital in studying and combating mosquito-borne diseases. Balbiani [3] first described polytene chromosomes in 1881,but it was not until about fifty years later that more detailed study revealed their true significance. Polytene chromosomes arise from normal somatic chromosomes which undergo several rounds of DNA replication (repeated chromosome duplication) with accompanying nuclear cell division. The cells are therefore polyploid but the chromatids remain laterally associated to produce the polytene type of organisation. They are very good and have been used for clarifying species complexes, the capacity for disease transmission and different behaviors are associated with genetic evolution over the years for both the vector and parasite. Oyewole reported that modern molecular techniques have been used to discover that the Anopheles complex consist of five species which are vectors of human malaria with different degrees of efficiencies [4]. In an attempt at resolving the *Anopheles gambiae* evolutionary history, Kamali reported that an African mosquito species with a deadly capacity to transmit malaria has a perplexing evolutionary history. These Virginia Tech scientists' discoveries suggest that this species is actually genetically linked to an older, ancestral lineage. These scientists identified breaks in DNA that lead to new chromosomal arrangements, and used these rearrangements to demonstrate the repeated evolution of the ability to transmit a parasite, in a back-and-forth fashion. In the same report Sharakhov said "This curious stop-and-go flexibility could help us to better understand the nature of the mosquito's capacity to transmit malaria, and calls into question what is driving the genetic flexibility,"[5].This study is aimed at providing possible links (data) that will provide more scientific insight into links in the evolutionary dynamics (past, present and perhaps future connections) in relation to vector control.

II. MATERIALS AND METHODS

The keys used for the identification of the mosquitoes in this project work were adapted from Gilles and De Meillon [6] and Gilles and Coetzee [7]. Also used are the reviewed concepts by Zahar [8] and White [9] for discriminating species and subspecies . According to their report taxonomist routinely examine the senilae on antennae and perform cross-mating experiments, polytene chromosomes, and comparisons of electrophoretic patterns of enzyme systems [10].Successful cytological studies have led to the identification of species complexes and sibling speciation within the *Anopheles* species. A very good example is the Anopheles gambiae complex. Kitzmiller[11]stated that, the presence of several kinds of inversion on different chromosomes at different frequencies has led to the collection of strains of *Anopheles gambiae* in different areas [12,13,8and 14].Anopheles larvae were collected from an open water body in the Rayfield area of Jos. The water was very turbid, with no living vegetation but with dead plant debris. A small, but adequate quantity of dry yeast was used to feed the larvae twice daily in the morning and evening. The method by Hunt [15] as reported by White [9] was adopted. The technique employed using carnoy's fixative as preservative for whole larva. After preservation at room temperature for 24 hours, the material was held at about 4^0 C in a refrigerator.

LARVAL DISSECTION: The larva was placed on a slide with a droplet of 50% propionic acid for 30-60 seconds. The larval abdomen was then cut off and discarded; the thorax was then slit open dorsally by running a needle over it to sever the layers between. Gentle traction on the head usually pull the glands free. The salivary glands were then macerated, clearing excess tissue from the chromosomes.

STAINING AND EXAMINATION OF CHROMOSOMES:A droplet of diluted aceto-lactic orcein (2% aceto-lactic orcein diluted 1:10 with 50% propionic acid) was then added and mixed with the tissues. Excess stain should be blotted away after half a minute and the tissues washed with

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successive droplets of 50% propionic acid. The preparation was then covered with a clean cover slip and squashed. The squashing was done using a flat bottom pen used for the tapping, while checking for adequate spreading of the chromosomes.

MOUNTING: The squash preparations were made permanent as soon as the chromosomes were judged to be spread adequately. There were sealed using rubber solution all around the edge of the cover slip and kept in the fridge.

III. RESULTS

Results of the seasonal abundance revealed that the *Anopheles gambiae* species increased in density reaching a peak in August (appendix 1 & 2). Thereafter the population declined from September to November. The larvae were collected from a ground pool in Rayfield south of Jos. The highest number of larvae were recorded from the open ground pool followed by its associated stream edge. There were no larvae found in the artificial containers during the period of study (table 1).A Least Significance Difference test (LSD) showed a highly significant interaction between seasons and sites (table 2).A total of four hundred and ninety-seven(497) Anopheline larvae were brought into the insectary from the wild. Four hundred and forty-four (444) were reared to adult stage successfully. Fifty (50) were fixed for dissection and three(3) died (table 3). Larval development from one instar to the other, and to the pupa and adult stages; within any one twenty-four hours ranged from zero (none) to eight (8) during the period of study. The males emerged before the females. Successful dissections were obtained after several attempts and practice (Plate 1).

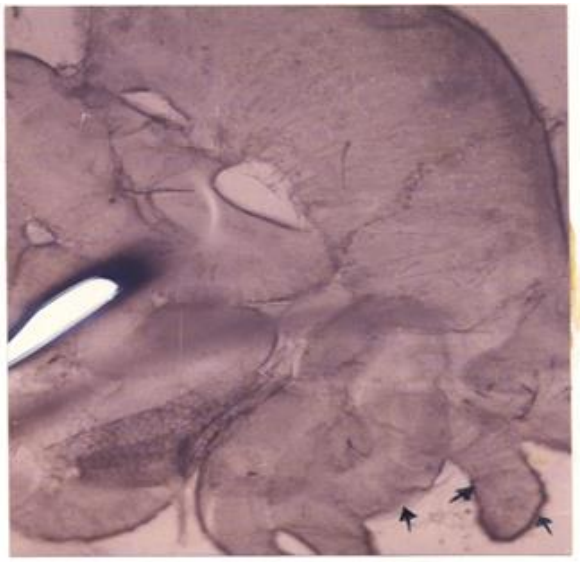

Plate 1: *Anopheles ganabiae* Larval salivary gland chromosom preparation X40.

TABLE I. MONTHLY LARVAL COLLECTIONS AT DIFFERENT HABITATS ANOPHELES GAMBIAE

SAMPLING OCCASIONS

Season/Month	1	2	3	4	HT	MT
June SE	0	0	0	0	0	
June GP	8	5	6	15	34	34
June AC	0	0	0	0	0	
July SE	17	9	8	16	50	
July GP	24	22	24	18	88	138
July AC	0	0	0	0	0	
August SE	11	16	25	18	70	
August GP	57	43	37	39	176	246
August AC	0	0	0	0	0	
September SE	14	0	3	1	8	
September GP	24	20	0	1	45	53
September AC	0	0	0	0	0	
October SE	0	0	0	0	0	
October GP	0	4	6	4	14	14
October AC	0	0	0	0	0	
Nov. SE	0	0	0	0	0	
Nov. GP	0	0	0	2	2	2
Nov. AC	0	0	0	0	0	

Key:
SE = Stream Edge
GP = Ground Pool
AC = Artificial containers
H.T = Habitat Total
GT = Grand Total(GT. 497)
MT = Monthly Total

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TABLE II. INTERACTIONS BETWEEN MONTHS AND SITES ANOPHELES GAMBIAE

Month Site	June	July	Aug.	Sept.	Oct.	Nov.	Total	Means
GSE	0	50	70	18	0	0	138	5.75
G.R.W	34	88	176	45	14	2	359	14.96
A.C	0	0	0	0	0	0	0	0
Totals	34	138	246	63	14	2	497	
Means	2.833	11.5	20.5	5.25	1.67	0.17		

LSD 6.42
P< 0.05

Key: GSE = Grassy Stream Edge
GRW = Ground Rain Water
AC = Artificial Containers

TABLE III. TABLE 3 LABORATORY REARING RECORD ANOPHELES GAMBIAE LAVAL INSTARS

Months	L_1	L_2	L_3	L_4	L_5	No. Dying	
June	4	10	9	11	33	1	
July	60	45	33	0	138	0	
August	20	146	38	42	246	2	
September 13	25	20	5	63	0		
October	3	5	4	2	14	0	
November 0	0	0	2	2	0		

IV. DISCUSSION

The majority of studies carried out on mosquitoes are in relation to the diseases they transmit, *Anopheles gambiae* being one of the most efficient vectors of malaria in the world. Laboratory colonization of *Anopheles gambiae* S.L. was unsuccessful. This behavior is well documented [16,17and 18].The successful breeding of the larvae under the laboratory condition provided evidence of their high survival and adaptive powers. Salivary gland dissections proved very difficult at the beginning due largely to inexperience and the very sensitive and delicate steps involved in the dissection and preparations of the chromosome squashes. The erratic overflowing of the breeding sites due to sudden and rapid flushes of rains made most breeding sites unproductive for the larvae. During the heavy rains the larval population were usually flushed away.

A 2 x 2 factorial design was used to investigate interaction between the seasons (months) and the sites (habitats) and an analysis of variance revealed a highly significant interaction between seasons and sites at (P<0. 05) level of significance. A least significance difference test (LSD) showed that the ground rain water habitat was significantly different from the other two habitats (stream edge and artificial container) with the highest number of larval collections. This agrees with other reports on *Anopheles gambiae* having a preference for breeding in open sunlit water with or without vegetation [16, 19, 17].There were several limitations that militated against the successful and detailed study intended for the purpose of this research work. Some of the limiting factors [20] are as follows:

(i) The fact that the insectary is only partially functional. This made laboratory colonization of the species very difficult; as the weather elements could not be manipulated to our advantage although study site weather elements were taken (appendix 3). (ii) Lack of a good photomicrograph system for immediate snap shots and successful processing and production of acceptable photomicrographs, (iii) Lack of Normaski microscopy. Structures in the polytene nucleus such as the nucleous and the Balbiani rings may not be readily apparent in preparations, which have been fixed, stained, or dehydrated. The use of phase contrast or Normarski interference microscope for visualization of micro morphological features of the polytene chromosome is advocated [21]. (iv)Incessant power supply, made working with both the dissecting and light microscope very frustrating. Although the supervisor provided a generator, the freezers and fridges with the fixed specimens had insufficient hours of power supply for the right temperature for such specimens. This greatly altered the chromosomes being prepared. Apart from the above reasons for chromosomal alterations, another source of variation in chromosome preparations [12] is the result of mechanical stresses of the squashing operation. This usually led to various degrees of stretching, which in turn may lead to some difficulty in using the maps. The mosquito *Anopheles gambiae* vector of malaria

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is regarded as undergoing incipient chromosomal speciation. This recent process of speciation appears mostly Centered in West Africa and possibly related to the late regression of the forest belt[14,13,10] .Man is said to represent an important factor in environmental changes and heterogeneities and as such acts as a 'powerful' evolutional force in the speciation. The knowledge of the genetical factors involved in the evolution of mosquito adaptation to man and his environment has more than theoretical interest: It has practical significant for planning of control strategies[22].The importance of studying the *Anopheles gambiae* is still relevant today. Polytene chromosomes provide the distinction of the sibling species and thus aid the medical entomologist in understanding vectoral capacity, the control and eradication of these disease vectors. Other tissues such as the malphigian tubules, ovarian nurse cells, in the adults require further investigation and the salivary gland techniques for the larvae need to be reviewed. Successful preparation of the chromosomes of the *Anopheles gambiae* larvae has provided me with the impetus to carry out further genetic investigations in more modern and well-equipped laboratories. These objectives could not be realized within the context of a short study such as this, but may only be realized in an adequately funded well-equipped laboratory and in a longer time span research work.

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APPENDIX 1

TABLE IV. MONTHLY LAVAL COLLECTIONS AT DIFFERENT HABITAT DURING THE PERIOD OF STUDY

Season sampling	Grassing stream	Ground (rain)	Artificial in position	
	Occasion	Edge	pools	containers
June	3 - 06 - 97	0 0 0 0	2 0 4 2	*
	16 - 06 - 97	0 0 0 0	0 2 1 2	*
	24 - 06 - 97	0 0 0 0	3 2 1 0	*
	30 - 06 - 97	0 0 0 0	5 2 6 2	
July	2 - 07 -97	3 4 5 5	10 8 4 2	*
	9 - 07 - 97	3 4 1 1	7 6 4 5	*
	21 - 07 - 97	0 2 2 4	8 10 4 2	*
	24 - 07 - 97	4 5 4 3	10 4 2 2	*
August	3 - 08 - 97	4 1 0 6	16 181310	*
	7 - 08 - 97	6 5 3 2	21 10 8 4	*
	13 - 08 -97	10 8 6 1	10 12 9 6	*
	23 - 08 - 97	0 6 8 4	9 13 10 7	*
September	3 - 09 - 97	4 2 6 2	8 5 7 4	*
	9 - 09 - 97	0 00 0	4 8 6 2	*
	17 - 09 - 97	0 1 1 1	0 0 0 0	*
	29 - 09 - 97	0 0 0 1	0 0 1 0	*
October	1 - 10 - 97	0 0 0 0	0 0 0 0	*
	6 - 10 - 97	0 0 0 0	2 1 1 0	*
	16 - 10 - 97	0 0 0 0	0 4 0 2	*
	26 -10 - 97	0 0 0 0	0 2 2 0	*
November	1 - 10 - 97	0 0 0 0	0 0 0 0	*
	7 - 10 - 97	0 0 0 0	0 0 0 0	*
	8 - 10 - 97	0 0 0 0	0 0 0 0	*
	10 - 10 - 97	0 0 0 0	0 1 1 0	*

* No larval were found in artificial containers

o No water in habitat.

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APPENDIX 2

TABLE V. TOTAL LARVAL COLLECTIONS FROM VARIOUS HABITATS DURING THE MONTHS OF STUDY ANOPHELES GAMBIAE TOTALS FOR EACH OCCASION

	Season sampling	Grassing Stream	Ground (rain)	Artificial
	Occassion	Edge	pools	containers
June	3 - 06 - 97	0	8	0
	16 -06 - 97	0	5	0
	24 - 06 - 97	0	6	0
	30 - 06 - 97	0	15	0
July	2 - 07 - 97	17	24	0
	9 - 07 - 97	9	22	0
	21 - 07 - 97	8	24	0
	24 - 07 - 97	16	18	0
August	3 - 08 - 97	11	57	0
	9 - 09 - 97	16	43	0
	17 - 09 - 97	25	37	0
	29 - 09 - 97	18	39	0
September	3 - 09 - 97	14	24	0
	9 - 09 - 97	0	20	0
	17 - 09 -97	3	0	0
	29 - 09 -97	1	1	0
October	1 - 10 - 97	x	0	0
	6 - 10 - 97	x	4	0
	16 - 10 - 97	x	6	0
	26 - 10 - 97	x	4	0
November	1 - 10 - 97	x	0	0
	7 - 10 - 97	x	0	0
	8 - 10 - 97	x	0	0
	10 - 10 - 97	x	2	0

Key X = habitat Dry
O = No larvae found

APPENDIX 3

TABLE VI. MONTHLY DISTRIBUTION OF WEATHER ELEMENTS, AIR TEMPERATURE, RELATIVE HUMIDITY AND RAINFALL DURING THE PERIOD UNDER STUDY

MONTHS	TEMP		R.H.	RAINFALL
	MX	MN		
June	30	20	71	188
July	28	20	77	208
August	28	19	77	253
Sept	28	19	67	115
Oct	29	19	65	91
Nov	29	16	35	44
Dec	29	15	24	0
Jan	29	14	22	0
Feb	32	18	18	0
March	-	-	-	-
April	-	-	-	-
May	-	-	-	-

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