Full Length Research Article
Advancements in Life Sciences – International Quarterly Journal of Biological Sciences

ARTICLE INFO

Date Received: 09/05/2023;
Date Revised: 20/07/2023;
Date Published Online: 30/09/2023;

Authors' Affiliation:
1 Alzaiem Alazhari University - Sudan
2. Faculty of Public Health and Informatics, Ummmm Al-Qura University - KSA
3. Kassala University - Sudan

***Corresponding Author:**
Hatim Matooq Badri
Email: hmbadri@uqu.edu.sa

How to Cite:
Nasir NS, Ali FF, Elamin MO, Alshehri AM, Natto HA (2023). A comparative study between X-chromosome mapping of Sudanese and Egyptian *Anopheles pharoensis* theobald (Diptera: Culicidae) strains. Adv. Life Sci. 10(3): 447-451.

Keywords:
Anopheles pharoensis; X-chromosome; Malaria; Karyotypes; Egypt and Sudan

Open Access

A comparative study between X-chromosome mapping of Sudanese and Egyptian *Anopheles pharoensis* theobald (Diptera: Culicidae) strains

Najlaa Siddig Nasir[1], Fatima Fadul Ali[1], Mohamed Osman Elamin[2], Ali. M. Alshehri[2], Hatim. A. Natto[2], Hatim M. Badri[2]*, Wahaj A. Khan, Ahmed A. Osman[3], Luay Alkazmi[2]

Abstract

Background: Current literature lacks information regarding *Anopheles pharoensis* malaria transmission patterns. *Anopheles pharoensis* succeeded in transmitting Malaria in Egypt. However, it was unsuccessful in doing the same in Sudan. From here arises these important questions: Why does it transmit malaria in Egypt but not in the Sudan or other Countries? Is it a Sibling species or a Sub-species? This investigation aimed to answer these questions by studying the genetics of *Anopheles pharoensis* collected from Egypt and Sudan.

Methods: A comparative study was conducted in Egypt and Sudan to determine the population genetic structure of *Anopheles pharoensis* species based on chromosomal inversion of karyotypes. Fourth-stage larvae of *Anopheles pharoensis* were collected from Egypt (Faiyoum government) and from Sudan (Khartoum, Gezira and Sennar states).

Result: Significant levels of differentiation were observed among the species studied.

Conclusion: Investigations suggest that *Anopheles pharoensis* species collected from Egypt can transmit malaria due to the presence of an inversion in the X-chromosome. The lack of the aforementioned inversion probably prevented the transmission of malaria by *Anopheles pharoensis* in Sudan. The results above provide insight into malaria transmission patterns by *Anopheles pharoensis*. However, more needs to be done and hence it is recommended to undergo further research on genetics and morphological studies using molecular biological tool son *Anopheles pharoensis* in Sudan.

Introduction

Approximately there are around 500 Anopheles species worldwide, many of which are sibling species that can only be identified by using genetic techniques. Of these species, 30 to 40 vectors of the protozoan "Plasmodium" cause malaria [1]. Previous statistics indicate that there are around 228 million cases of Malaria across the globe[2]. The female Anopheles is the only species of mosquitoes known to transmit malaria. Female prefers to feed on people and animals such as cattle. Males live for about one week while females can survive for up to one month. Malaria transmission occurs by different Anopheles species depending on climatic factors such as temperature, humidity and rainfall [3]. Numerous studies have reported the importance of temperature, low temperatures are believed to be affecting larval development and causing higher mortality rates. Increased ambient temperature will increase human biting frequency and increase parasitic sporogonic development thus increasing disease transmission. Increased rain and temperatures have a positive effect on Anophelinae breeding and on developmental rates [4].

In Egypt, previous studies have indicated that *Anopheles pharoensis* thrives in clean shallow, stagnant water which is thick with growth of vegetation shades especially in rice fields, sewage wells drains and canals) [5]. Results revealed *Anopheles pharoensis* to be one of five predominant anopheles species in Faiyoum, Aswan New Valley [6]. *Anopheles pharoensis* in modern Egypt was found widely distributed all over the country, especially in Al- Faiyoum governate, Nile delta, Cairo and Aswan governate indicating the re-emergence of malaria in Modern Egypt [7]. *Anopheles pharoensis* was proven the most important vector of Malaria in Egypt, especially in the Delta [6]. Due to the significant impact of malaria on populations, this study aimed to compare *Anopheles pharoensis* species collected From Egypt and Sudan.

Anopheles pharoensis in Sudan:

Known to be found across the Blue Nile and White Nile during rainy seasons. On the main Nile, it is present at Kadaro (and once at Zedab). Other Northern localities are Abu Kidada; WadiKaja; Keilak; AlAmira; Rahad; Hawata and Faras. *Anopheles pharoensis* is the most common Anophelinae in Jabal Awlia and Sennar reservoir [9,10]. *Anopheles pharoensis* was found limited in Eastern and Southern Sudan as observed in a study [8].

This research aimed to answer the Question: why *Anopheles pharoensis* was not proven a vector of malaria in Sudan?

The cytogenetic identification of Anopheles species permitted studies on the distribution of cryptic species. Vector capacity has been found to be closely related to a fixed inversion in the X-chromosome. Polymorphism can be used to examine evolutionary correlations across species [11]. The various members of different groups of cryptic species complexes within Anophelinae may be examined for inversion differences within species via polytene chromosomes of ovarian nurse cells. Cytogenetic techniques were used in this study in order to detect any mutations.

X-chromosome inversions and their relationship with disease transmission and vectorial capacity: There is evidence that some polymorphic inversion types detected in X-chromosomes differ in both behavioral and ecological characteristics which are relevant to disease transmission and its control [12]. So, inversions may affect the Vectorial efficiency of *Anopheles pharoensis* changing it from a non-vector in Sudan to a vector of malaria in Egypt as was revealed and observed by this research.

Methods

This is an Experimental analytical study conducted between Egypt and Sudan.

Study area:

Egypt: 500 samples were collected in the fourth larval stage. Samples were obtained from Egypt: Faiyoum Governate (Sinnuris Centre). Sinnuris is located between latitudes 29°15 and 30° 15 and longitudinal 30°0 and 30 °30.boundries: North-guaran Lake South-Western desert West Western desert. East-River Nile. It comprises an area of 1,778 km2 with an elevation of 30m from sea level.

Sudan: 500 samples were also collected in the fourth larval stage. Samples were collected from Gezira state (WadElshafie village), Sennar state and Khartoum state (Soba). Khartoum is located between 15 °26 and 15 °45 north and longitudes 32 °25 and 32° 40 east at an altitude of 405.6m above sea level.

Sample Collection:

500 samples were collected from Egypt and stored in a thermometer to be brought to Sudan for morphological identification. Also, 500 samples were collected from Sudan and bred in dishes containing normal water and placed in a room with an air conditioner. Larvae were fed with crushed bread (boxomat) for 3 days until changing to pupa.

Collection of Larvae:

Samples were collected via the dipping method according to O'Malley, (1989) was used [13]. The dipping technique is called Shallow skim. A WHO standard dipper was used to investigate the presence

and/or absence of larval stages of *Anopheles pharoensis* species. Larvae were then collected by a Bilge pump or a Baster. They were emptied into a white enamel pan from which the larvae were examined and moved to a laboratory to be properly stored in Carnoy's fixative and kept in a freezer pending the beginning of the experimental work.

Methodology:

a- Techniques for karyotyping:

Karyotyping was investigated using Polytene chromosomes.

1/Polytene Chromosomes:

Polytene chromosomes were obtained by the squash method.

Larval salivary glands were dissected and squashed using orecien stain method.

2/Cytotaxonomy and polytene chromosome map:

A reference system of chromosome mapping adopted by Sidky and Riad (1976) was used in this study[14].

b- Preparation of Orecien stain:

5 gm of orecien powder was added to 125 ml of acetic acid. The solution was shaken for 2-3 min at room temp. 125 ml of lactic acid was added to the solution and stirred for 2 minutes then it was filtered and stored in the laboratory.

c- Preparation of Carnoy's fixative:

Carnoy's fixative was prepared by adding 300 ml of ethanol to 150 ml of chloroform and 50ml of acetic acid [15].

d- Larval Salivary Polytene Chromosome Preparation:

Larval polytene chromosomes were prepared following Boakye et. al (1993)[16]. Fourth-stage larvae were removed from carnoy's fixative and placed in a petri dish. Full-sized larvae were chosen. Each larva was dissected and its salivary glands were removed and converted to a clean slide. A drop of 60% acetic acid was put on the slide. The salivary glands were covered with a clean cover slip and squashed smoothly with the thumb. A drop of Orcein stain was added to the edge of the coverslip and the slide was placed on a bench to dry. Four hundred slides were prepared from An. Pharoensis species. The chromosomes were viewed under a LeitzDiaux 20 phase-contrast microscope with a camera attached to the microscope.

Results

Mitosis in *Anopheles pharoensis*:

a-Polytene chromosomes:

Figure(1): shows a larval salivary gland polytene chromosome of Sudanese strains of *Anopheles pharoensis*. Polytene chromosomes have a characteristic clear banding pattern. No breakpoints and fragmentation were observed to occur in the Sudanese strain. The centromere is fan-shaped.

Figure (2): Clear bands appear in the Egyptian strain with two break points and fragmentation in the X -X-chromosome. The centromere is also fan-shaped.

Figure 1: Salivary Polytene chromosome of *Sudanese Anopheles pharoensis.*

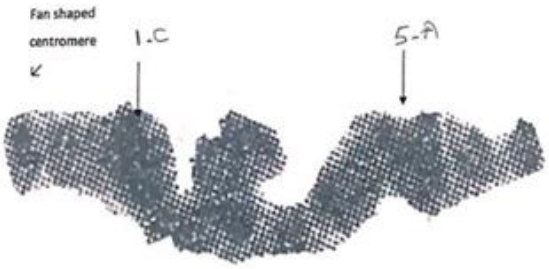

Figure 2: Salivary Polytene chromosome of Egyptian *Anopheles pharoensis* (Arrows show inversions).

b-X-chromosome Maps:

X-Chromosome Map of Sudanese strains:

Figure (3): Showing clear bands. No inversion was observed in the Sudanese stains of *Anopheles pharoensis*.

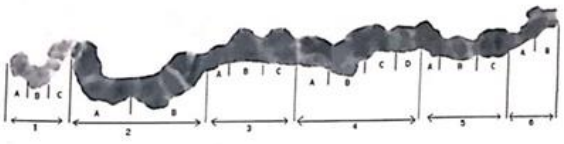

Figure 3: Salivary Polytene X-Chromosome map of Sudanese *Anopheles pharoensis* (No inversions detected).

X-Chromosome Map of Egyptian strains:

Figure (4): Showing clear bands with inversions at junctions 1-C and 5-A on the X-Chromosome of Egyptian strains of *Anopheles pharoensis*. Inversions are indicated by arrows. This Map was drawn by Sidky and Riad (1976). [14]

X-Chromosome Map of South Africa (Zululand sample):

Figure (5): is a chromosome map adopted by Miles et al., [17]. This map is a comparative Polytene Chromosome map between the Sudanese and Egyptian Chromosome maps. Inversions also appear at junctions 1-C and 5-A similar to inversions of the Egyptian strain of *Anopheles pharoensis*.

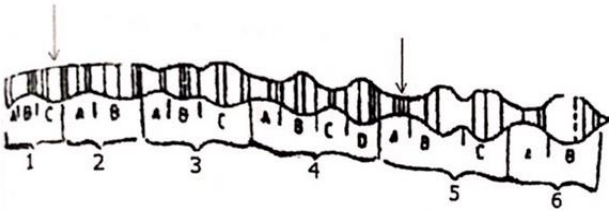

Figure 4: Salivary Polytene X-Chromosome map of Egyptian *Anopheles pharoensis* (Map drawn by Sidky and Riad, (1976)) (Inversions are indicated by arrows at junctions 1-C and 5-A).

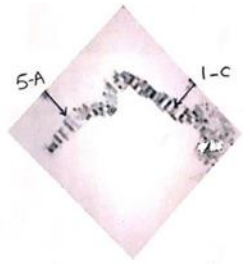

Figure 5: Salivary Polytene X-Chromosome map of South African (Zululand) *Anopheles pharoensis* (Miles et al., [17]) (Inversions are indicated by arrows at junctions 1-C and 5-A).

Discussion

Anopheles pharoensis as a malaria vector is well established in Egypt [7] but there is a lack of certainty in the south Saharan Deseret's of Africa even though a number of sporozoite-positive specimens were previously reported. Such conflicting views on the role of *Anopheles pharoensis* as a vector might be attributed to the existence of different sibling species deducted from Polytene chromosomes studied on materials from different African regions. In Egypt, *Anopheles pharoensis* vector populations are known by chromosomal Xa inversion [18].

The aim of this investigation was to study the genetics of *Anopheles pharoensis* in the Sudan and Egypt in-order to detect the presence of inversions in the X`-Chromosomes of Sudanese samples (the investigation also aimed to study the relation of this inversion with vectors in Sudan and Egypt).

The existence and apparent fixation of two different X-Chromosome arrangements in geographically separated populations of the taxon *Anopheles pharoensis* in nature represents either geographic variation within a single species, or at least two genetic species within the taxon [17].

A photo map of larval salivary gland Chromosomes of Anopheles annulipes (Cellia was presented by Booth et al., [19]. The standard Chromosome of Anopheles annulipes had one major puff with two prominent broken bands across it. The centromere end had a large puff. This agrees with the findings of this research where a large puff was observed at the centromeric end, also there are two broken bands, which indicate that an inversion in the X-Chromosome of the *Anopheles pharoensis* occurred in the Egyptian strain. The X-chromosome had also one major puff. In this research, Sudanese *Anopheles pharoensis* strains had no broken bands but had a large puff at the centromeric end and a major puff in the X-chromosome.

A photo map of the polytene chromosome of *Anopheles pharoensis* from Zululand was presented by Miles et al. (1983) inversions also appeared at junctions

1-C and 5-A [17], which agrees with the findings of this research similar to inversions observed in the polytene chromosome of the Egyptian strain of *Anopheles pharoensis*.

The Polytene Chromosome technique was used in this research to compare the X-chromosomes of the two strains (Egypt and Sudan) of *Anopheles pharoensis* and to give reasons why Sudanese strains cannot transmit Plasmodium parasites while Egyptian *Anopheles pharoensis* strains succeeded in transmitting the malaria parasite. Probably the absence of the inversion detected in the Sudanese strains of *Anopheles pharoensis* prevents Sudanese strains from acting as vectors of Malaria, while the presence of the inversion in the X-chromosome of the Egyptian strain increases its possibility of transmission of Malaria.

In Egypt, in general, and in Faiyoum Governate, in particular, *Anopheles pharoensis* is an important Malaria vector. In the Sudan, *Anopheles pharoensis* is not known to be causing malaria transmission anywhere in Sudan. The aim of this study was to answer the question of why *Anopheles pharoensis* is a major vector in Egypt and not a vector in the Sudan?

There were inversions detected in the X-chromosome of Egyptian strains at junctions 1-C and 5-A. From this finding one comes to the fact that *Anopheles pharoensis* in Egypt is capable of transmitting Plasmodium parasites due to the presence of inversions in the X-Chromosome while *Anopheles pharoensis* in the Sudan cannot transmit the Malaria parasite due to the absence of the inversions in the X-Chromosome.

It appears that *Anopheles pharoensis* is a Sudanese species that migrated to Egypt and on its migration it experienced different inversions in its X-chromosome when it reached Egypt. This changed its behavioural factors shifting it from a non–vector in Sudan to a vector of Malaria when reaching Egypt. Though further research is needed from the findings of this research, *Anopheles pharoensis* is probably a Sibling species.

Author Contributions

Najlaa Siddig Nasir: Conceptualization, writing-original draft, supervision, methodology, data curation, writing-Review, and editing.

Fatima Fadul Ali: Conceptualization, writing-original draft.

Fatima M. Mohamed: Conceptualization, writing-original draft, supervision, methodology, data curation.
Mohamed Osman Elamin: writing-original draft, methodology, writing-Review, and editing.
Ali M. Alshehri: writing-Review, and editing.
Hatim M. Badri: writing-Review, and editing.
Wahaj.A. Khan: writing-Review, and editing.
Hatim A. Natto: writing-Review, and editing.
Mashael Alfaifi: writing-Review, and editing.
Ahmed A. Osman: writing-original draft, methodology, writing-Review, and editing.

Competing Interest

The authors declare that there is no conflict of interest.

References

1. Escobar D, Ascencio K, Ortiz A, Palma A, Fontecha G. Distribution and phylogenetic diversity of Anopheles species in malaria endemic areas of Honduras in an elimination setting. Parasites & vectors, (2020); 13(1): 333.
2. World Health Organization. Global Malaria Programme. A framework for malaria elimination. 2017.
3. CDC-Centers for Disease Control, Prevention. CDC - Malaria - Malaria Worldwide - Impact of Malaria. 2009 [accessed 2023 Jul 23]. https://www.cdc.gov/malaria/malaria_worldwide/impact.html.
4. Yaw, A. Afrane, Andrew K. Githeko. and Guiyan Yan. The ecology of Anopheles mosquitoes under climatic change: Case studies from the effects of environmental changes in East Africa highlands. Annals of the New York Academy of Science, (2012); 1249: 204-210.
5. Tarek Y, Elsheikh, Kotb M, Hammad, Walaa Moselhi. Mosquito species diversity and abundance in relation to Riceland agroecosystem and filarial infection in Kafr Elseikh Governate. The Egyptian journal of hospital Medicine, (2010); 38(1): 100-114.
6. Wassim M. Secondary structure and sequence of its2-rDNA of the Egyptian malaria vector *Anopheles pharoensis* (Theobald). Journal of Egyptian society of parasitology, (2014); 44(1): 197-204.
7. Kenawy M. Review of Anopheles Mosquitoes and Malaria in Ancient and Modern Egypt, Journal of Mosquito Research, (2015); 5(4):1-8
8. Makhawi M. Identification of Anopheles species of the funestus group and their role in malaria transmission in Sudan. University of Khartoum. Journal of Applied and Industrial Sciences, (2015); 3(2): 58-62
9. Lewis J. Observations on the distribution and Taxonomy of Culicidae (Diptera)in the Sudan. Transactions of the Royal Entomological Society of London. (1945); 95.
10. Elsafi H, Haridi M. Field trial of the insect growth regulator, Dimilin for control of *Anopheles pharoensis* in Gezira Sudan. Journal of the American Mosquito Control Association, (1986); 2(3): 374.
11. Kitzmiller JB. Genetics, cytogenetics, and evolution of mosquitoes. Advances in genetics. (1976); 18:315–433.
12. Coluzzi M. Heterogeneities of the malaria vectorial system in tropical Africa and their significance in malaria epidemiology and control. Bulletin of the World Health Organization. (1984); 62 Suppl:107–113.
13. Rutgers New Jersey Agricultural Experiment Station -Center for vector control). Guidelines for Larval surveillance. 76th annual meeting. (2022); 44-55.
14. Sidky H, Riad Z. A chromosomal map of the salivary gland chromosomes of *Anopheles pharoensis*. Journal of the Egyptian society of parasitology, (1976); 6:153.
15. French W, Baker L, Kitzmiller J. Preparation of Mosquito Chromosomes. Mosquito News, (1962); 22(4):377-383.
16. Boakye D, Post R, Mosha F, Surtees, Baker R. Cytotaxonomic revision of the Simulium sanctipauli subcomplex (Diptera: Simulidae) in Guinea and the adjacent Countries including descriptions of two new species. Bulletin of Entomological Research, (1993); 83:171-186.
17. Miles S, Green C, Hunt R. Genetic observations on the taxon Anopheles (cellia) pharoensis Theobald (Diptera: Culicidae). Journal of Tropical Medicine and Hygiene. (1983); 86:153-157.
18. Carrarra G, Petrata V, Niang M, Coluzzi M. *Anopheles pharoensis* and transmission of Plasmodium falciparum in the Senegal river Delta West Africa. Medical and Veterinary Entomology, (1990); 4:421-424.
19. Rory D, Abee, Julie A. Christiansen, Anthony J.C Ornel. A detailed larval salivary gland polytene chromosome photomap for Culex quinquefasciatus (Diptera: Culicidae) from Johannesburg, South Africa. Journal of Medical Entomology, (2007); 44(2):229-237.