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Mitotic metaphase karyotype of the mosquito *Anopheles arabiensis* Patton (Diptera: Culicidae) from Kassala State, eastern Sudan

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Abstract. The mosquito *Anopheles arabiensis* Patton is the most important malaria vector in Sudan. The study was conducted for the first time to describe numerically the karyotype of *An. arabiensis* from Kassala State, eastern Sudan. Adults *An. arabiensis* were caught from human dwellings during the rainy season of 2022. We examined for the first time the utility of brain ganglia tissues of adult mosquitoes for mitotic chromosomal preparations using Giemsa stain - spreading technique. High-quality chromosomal preparations were examined and photographed. Chromosome measurements were carried out using computer software and analyzed statistically using SPSS® software. The diploid mitotic chromosome complement of *An. arabiensis* consists of three pairs of chromosomes, two pairs of metacentric autosomes (chromosome II and chromosome III) and one acrocentric dot-shaped pair, sex chromosome, which is homomorphic in females (XX) and heteromorphic in males (XY). Chromosome II was described as the longest (2.61±0.07) of the complement and constitute 44.39% of the total length (5.88 μm) of the haploid chromosomes set, while chromosome I (X=1.39 ±0.04; Y=1.04±0.04) as the shortest chromosome. Chromosome X appears in the males significantly larger than chromosome Y (P = 0.00). Chromosome III has an intermediate length (1.88±0.06) compared with the other chromosomes. Comparison of the average lengths of the three chromosome pairs by ANOVA test revealed highly statistical significant differences between them (P < 0.00). The study establishes a strong cytogentic data, which can contribute to accurate identification of the mosquito *An. arabiensis* and to planning human malaria vector control programs in Kassala State, eastern Sudan.

Keywords: *Anopheles arabiensis*, karyotype, brain ganglia, mitotic chromosomes, chromosome measurements, Sudan, Kassala.

1. INTRODUCTION

Of the nine recognized sibling species of the *Anopheles gambiae* complex (Barron et al 2019), *Anopheles arabiensis* Patton (Diptera: Culicidae) is one of the most significant malaria vectors globally (WHO 2018). It is considered the most efficient malaria vector in Kassala State, eastern Sudan and it has

been reported from Kassala town by (Hamza et al. 2014; Mustafa et al. 2021).

Chromosomes are the critical carrier of genetic information in eukaryotic cell nuclei, and their characteristics are typically stable within species (Vimala et al 2021). The karyotype is a fundamental characteristic of the chromosome complement (Marinho et al. 2016; Dehury et al 2021) and it represents the phenotype of the chromosomes including chromosome number, size, shape and position of the centromere (Tjong et al. 2012; Astuti et al. 2017). Cytogenetics is usually based on the examination of the fixed mitotic chromosomes during the analysis of metaphase of cell cycle, in which the DNA is folded up and chromatin is strongly condensed (Tüzün and Yükse 2009). In karyotype (ideogram) construction, the chromosomes are arrange on the basis of homologous chromosome pairs and sort out by chromosome size and centromere position from the longest to the shortest (Tjong et al. 2012) and can either be developed in haploid or diploid organism's cells. An ideogram construction following chromosome measurements is a versatile tool for cytogenetic studies (Kirov et al. 2017).

In the past chromosome measurements were carried out using classical karyological measurement methods (e.g. Anil et al 1970; Robert et al. 1986). Recently with advance in computer science, different computer software were developed for image processing and can be used for chromosome measurements from microphotographs (e.g. Image J, Rasband and Eliceiri 2012). In addition, others software beside chromosome measurements can be used in karyotype analysis (e.g. KaryoType, Altınordu et al. 2016). These software allow efficient, precise and rapid chromosome measurements.

Chromosomes cytologic information can be used for many purposes; such as, to study cytotaxonomy, phylogenetic relationships, karyotypic evolution (Felip et al. 2009; Guerra 2012), chromosomal aberrations and cellular function (Zhao et al. 2013) and chromosomal structural variation (Marinho et al. 2016; Dehury 2021). Karyotype analysis can serve as an additional tool in the species level identification of the species, which have morphological similarity and require additional identification methods. Using morphological, molecular, and karyotypic data, more precise species identification can be performed (Alekseeva et al. 2020).

The chromosome complement of *Anopheles* mosquitoes consists of three pairs of chromosomes (Baimai et al. 1996), two pairs of generally metacentric autosomal chromosomes of unequal size and one pair of heteromorphic sex chromosomes (XX in females and XY in males) (White 1980). Many workers carried out cytogenetic studies of *An. arabiensis* e.g: Coluzzi and Sabatini

(1967) described the karyotype of *An. arabiensis* using larval mitotic and polytene chromosomes. Coosemans et al. (1989) investigated the frequencies of inversion polymorphisms in polytene autosomal chromosomes of *An. arabiensis.* Ayala et al. (2017) investigated the role of chromosome inversion polymorphisms in environmental adaptation from a macro-ecological perspective. Sharma et al. (2020) analyzed the metaphase chromosomes in *An. arabiensis* using fluorescence in situ hybridization techniques.

The objective of the present study is to provide for the first time a base line data of the karyotype of the mosquito *An. arabiensis* from Kassala State, eastern Sudan. We examined for the first time the utility of brain ganglia tissues of adult female and male *An. arabiensis* for description of mitotic karyotype based on chromosome measurements using computer software program. This type of data is important for improving the cytogenetic identification of this species and for planning human malaria vector control programs in Kassala State, eastern Sudan.

MATERIALS AND METHODS

Mosquitoes used in the study

Adult *Anopheles* mosquitoes were collected from Kassala town, eastern Sudan. The town is located between 15°: 28″ N and 36°: 24″ E. in semi-arid climate with rainfall of varying intensity and duration.

Indoor resting wild adult *An. arabiensis* mosquitoes were caught from human dwellings by hand capture using sucking tube, aspirator (WHO 1975) during the rainy season of 2022. The collected samples were fixed alive in the field and preserved in freshly prepared modified Carnoy's solution (3 absolute ethanol: 1 glacial acetic acid by volume). Then the collected samples were transported to the laboratory and kept at -20C° for prolong storage. The processing of the materials for this study was carried out at the Molecular Biology Laboratory of Tuberculosis and Endemic Diseases' Center of Kassala University, Kassala town, Sudan.

The collected specimens were identified morphologically using morphological identification keys described by Gillies and De-Mellion (1968) and Gillies and Coetzee (1987) with the aid of the dissecting microscope.

Preparation of mitotic chromosomes

Brain ganglia tissues of adult females and males of *An. arabiensis* were dissected out and used for mitotic

slide chromosome preparations, following the karyotyping spreading technique described by Barker (1970) using giemsa stain, with minor modifications. For mitotic karyotype analysis, 48 chromosomal slide preparations derived from 4 specimens females and 4 specimens males (6 slides per specimen) were studied.

Chromosomal slide preparations were viewed under 40 X objective of A. X. L- GERMANY EGLASS light compound microscope with DG CAM 1600 equipped digital camera. The microscopic images were projected into a *hp* intel core i 2 computer screen and preparations with well spread chromosomes were selected and photographed using the S eye software package.

Chromosome measurements and karyotype

Metaphase images with the best chromosomes spreading; fewest overlaps and sharpest were selected for mitotic karyotype description. For chromosome measurements, an image of 1mm, Erma- Tokxc, micrometer stage having a linear scale of 100 divisions was taken at the same magnification as that of the chromosome preparations. This was used as a scale to measure the lengths of individual chromosomes - with a clear centromere - and their arms (in micrometer, μm) from the chromosome preparations. Chromosome measurements were made from about 84 metaphase images using Image J computer software version16 for Windows (Rasband and Eliceiri 2012).

Measurements were taken from male chromosomal preparations as follows: long arm length of chromosome (L), short arm length of chromosome (S), centromere length of chromosome (C) and total chromosome length (TCL) = [L + S+ C]. Then parameters were calculated based on chromosome measurements, which include: arm ratio of chromosome (AR) = [L / S], centromeric index (CI) = [S / (L + S) × 100] (Eroğlu et al. 2017) and relative length (RL%) = [TCL / Total length of all the chromosomes in haploid genome size x100]. Arm ratio was used to classify chromosomes according to classical method of Levan et al. (1964).

Computer imaging system Photoshop version 7.0 was used for image editing. First, the images were processed minimally by adjusting brightness and contrast. Then, the karyotype (ideogram) was constructed by arranging the homologous chromosome pairs (by cutting and pasting) based on the averaged length, shape and centromere position. Chromosomes were named according to the classical nomenclature for chromosomal complement in the *An. gambiae* complex (Coluzzi and Sabatini 1967).

Statistical analysis of chromosome measurements:

The computer soft ware package SPSS* (Statistical package for Social Science) version 16.0 for windows was used for statistical aspects of the *An. arabiensis* chromosomes analysis. Descriptive statistics (means, standard error, maximum, and minimum) of all chromosome measurements were recorded. The mean lengths of all the autosomal and sex chromosomes were compared by analysis of variance (ANOVA). T-test was used to compare the mean lengths of the long and short arms of the same chromosome and the sex chromosomes.

<div align="center">RESULTS</div>

Karyotype

Cytological observations of adult females and males brain ganglia tissues of *An. arabiensis* demonstrate a diploid mitotic chromosome complement consisting of three pairs of chromosomes (2n= 6), two pairs of autosomes (chromosome II and chromosome III) and one pair of sex chromosomes (chromosome 1), which is homomorphic in females (XX) and heteromorphic in males XY (Figure 1).

Chromosome analysis

Chromosome measurements and parameters calculated were used to describe the karyotype of *An. arabiensis* numerically. The measurement data of all chromosomes are given in Table 1. Chromosome lengths range between (2.61±0.07) μm and (1.04±0.04) μm from the longest to the shortest. Comparison of the average lengths of the three chromosome pairs by ANOVA test revealed highly statistically significant differences between them (f = 137.11; df. = 3; P = 0.00), as explained in details by the result of Scheffe Post Hoc Test.

From the analysis of chromosome length measurements, chromosomes were identified according to their length: chromosome II can be describe as the longest (2.61±0.07) of the complement, while chromosome III has an intermediate length (1.88±0.06) compared with the other chromosomes. Staining of chromosomes II and III by giemsa stain revealed a primary strong constriction, the centromere, so this allowed the estimating of the arm ratio of these two chromosomes and classify them according to the standard classification method of Levan et al. (1964). The calculated arm ratio of chromosomes II and III were 1.45 and 1.06, respectively and the centromeric index of the two chromosomes were 40.79
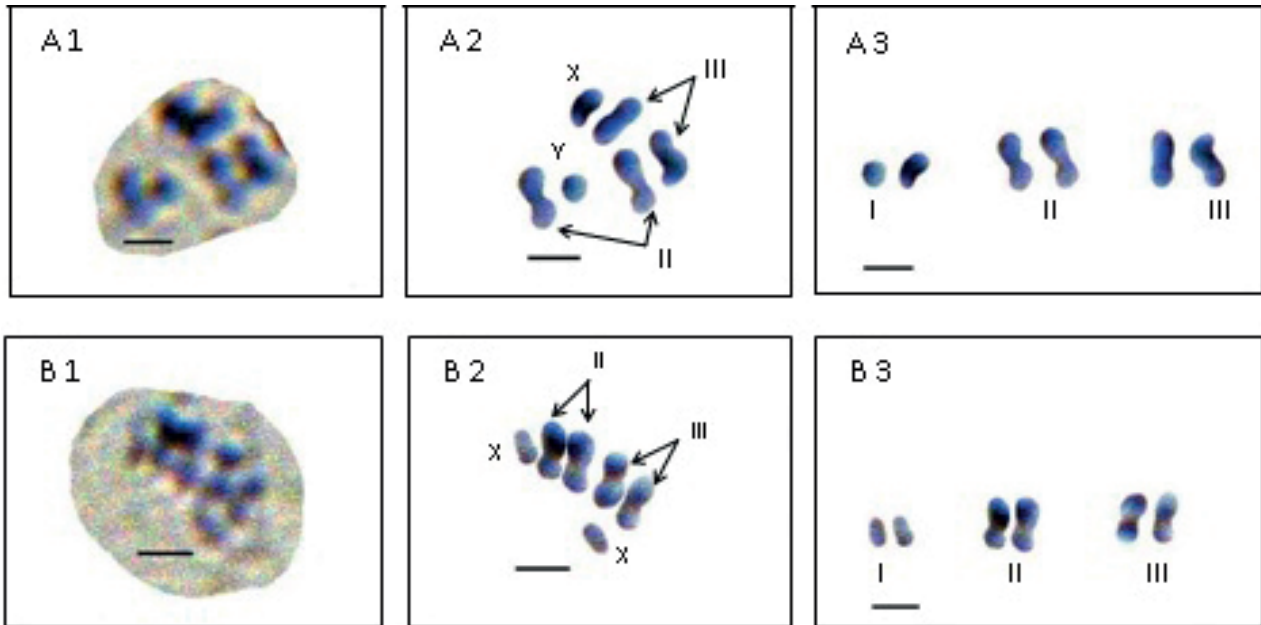
Figure 1. . Giemsa stained mitotic karyotype of adult female and male *Anopheles arabiensis* collected from Kassala town, eastern Sudan A: Male cell; A1- Plate of metaphase chromosomes. A2- Somatic pairing. A3- Karyotype B: Female cell; B1- Plate of metaphase chromosomes. B2 -Somatic pairing. B3- Karyotype Scale bar = 3 μm.

Table 1. Chromosome measurements of the mosquito *Anopheles arabiensis* from Kassala State, eastern Sudan.

Chromosome pair	No of chromosomes measured	Average length of long arm (L±SE) (μm)	Average length of short arm (S±SE) (μm)	Average length of centromere (C±SE) (μm)	Average total length (T±SE) (μm)	Arm ratio (r)	Centromeric index (SI) (%)	Relative length (%)	Chromosome type
X	15	-1,39 ±0.04 (1.17-1.58)	0	0	1,39 ±0.04 (1.17-1.58)	0	0	23.64	Telocentric
Y	19	1.04±0.04 (0.70-1.24)	0	0	1.04±0.04 (0.70-1.24)	0	0	17.69	Telocentric
2	29	1.35±0.04 (1.04-1.67)	0.93±0.04 (0.58-1.31)	0.33±0.02 (0.141-0.49)	2.61±0.07 (2.97-3.27)	1.45	40.79 Median	44.39	Metacentric
3	24	0.82±0.04 (048-1.13)	0.77±0.03 (0.55—1.10)	0.28±0.03 (0.12-046)	1.88±0.06 (1.38-2.52)	1.06	48.43 Median	31.97	Metacentric

Total length of the haploid genome: 5.88.
Number between two brackets represent the range of the measurement.
Chromosome type according to Levan et al. (1964).

and 48.43%, respectively, so the relative position of the centromere of the two autosomes meeting the parameters characteristic of metacentric chromosomes. However, the two chromosomes are metacentric, there was highly statistically significant difference (t = 8.00; df. = 56; P = 0.00) between the long arm and short arm of chromosome II, in contrast there was no statistically significant difference (t = 1.12; d.f. = 46; P > 0.05) between the long arm and short arm of chromosome III according to T- test analysis.

Chromosome I (X=1.39 ±0.04; Y=1.04±0.04) was described as the shortest chromosome, dot-shaped and with no obvious centromeric region, so it can be described as acrocentric. The X-chromosome appears in the males is larger than the Y-chromosome with highly statistically significant difference (t = 6.60; df. = 32; P = 0.00) between them.

The total haploid length (n, the two autosomes+ X chromosome) equal 5.88 μm, thus chromosome II constitutes 44.39% of the total length of the haploid chro-

mosomes set. Secondary constriction was not detected in chromosomal arms.

DISCUSSION

The karyotype information is important for understanding population differentiation and for the development of human malaria vector control programs (Rafael1 et al. 2005). In this study, the mosquito *An. arabiensis* has been cytologically investigated because of lack of information on karyotype of this important malaria vector in Kassala State, eastern Sudan.

The study demonstrates for the first time the utility of brain tissues of adult females and males *An. arabiensis* for mitotic chromosomes analysis using giemsa stain-spreading techniques. The protocol provides clear differential phases and visualized chromosomes in metaphase cell division. The techniques for chromosome preparation are always based on obtaining sources of dividing cells to produce high quality metaphase spreads with good chromosome definition (Felip et al. 2009). Most of the cytogenetic studies on *Anopheles* mosquitoes were performed on mitotic chromosomes from brain ganglia (Baimai et al. 1995; Salara, 1998; Rafael and Tadei 1998; Rafael et al. 2005, 2006) or leg and wing imaginal discs (Sharma et al. 2020) tissues of fourth instar larva and testis tissues of adult male (Salara 1998; Choochote 2011).

The computer software Image J allowed us to measure the length of chromosomes and their arms accurately as done by Bozek et al. (2012) and Sadílek et al. (2016). Here the description of the karyotype of *An. arabiensis* was updated numerically using chromosome measurements, so the chromosomes were identified and the characteristic features of each chromosome were described. The detailed study of *An. arabiensis* mitotic karyotype has confirmed a diploid number of six, agreeing with diploid numbers reported in other *Anopheles* species, e.g.: Brazilian *An. albitarsis* (Rafael et al. 2005, 2006), *An. darlingi* and *An. nuneztovari* (Rafael and Tadei 1998).

In the present study, chromosomes have been numbered according to the classical nomenclature for chromosomal complement in the *An. gambiae* complex (Coluzzi and Sabatini 1967) which was adopted by Sharma et al. (2020), in which the shortest chromosome is designated as chromosome I and the longest is II. In contrast, in other *Anopheles* mosquitoes, the chromosomes were numbered according to the nomenclature proposed by Rai (1963), in which the chromosomes were numbered in a descending order, i e the shortest chromosome is designated as chromosome I and the longest is III.

Our study revealed a karyotype consists of two pairs of metacentric autosomes (chromosome pair II and III) and acrocentric pair I, sex chromosome (X & Y). These findings differ with the findings of previous study of Coluzzi and Sabatini (1967) who described chromosome pair II in members of *An. gambiae* complex including *An. arabiensis* as submetacentric.

Secondary constriction that constitutes a satellite in chromosome arm was not detected in the study. Rafael and Tadei (1998) detected secondary constriction in chromosome II and chromosome III from different populations of the Brazilian *An. darlingi* and they stated that secondary constriction is an important aspect of chromosome morphology.

CONCLUSION:

The chromosomal measurements of the mosquito *An. arabiensis* from Kassala State, eastern Sudan were reported here for the first time. In the study, the mitotic chromosomes number, karyotype and ideogram of *An. arabiensis* were determined. The cytogenetic data of this study together with morphological characters could be used for accurate classification of *An. arabiensis* and other members of the *An. gambaie* complex.

AUTHORS' CONTRIBUTIONS

A. M. H. performed chromosome preparations and photographing, chromosome measurements, data analysis and wrote the draft manuscript. S. H. E. performed morphological identification of mosquitoes and participated in chromosome preparations and photographing. The two authors read and approved the final manuscript.

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