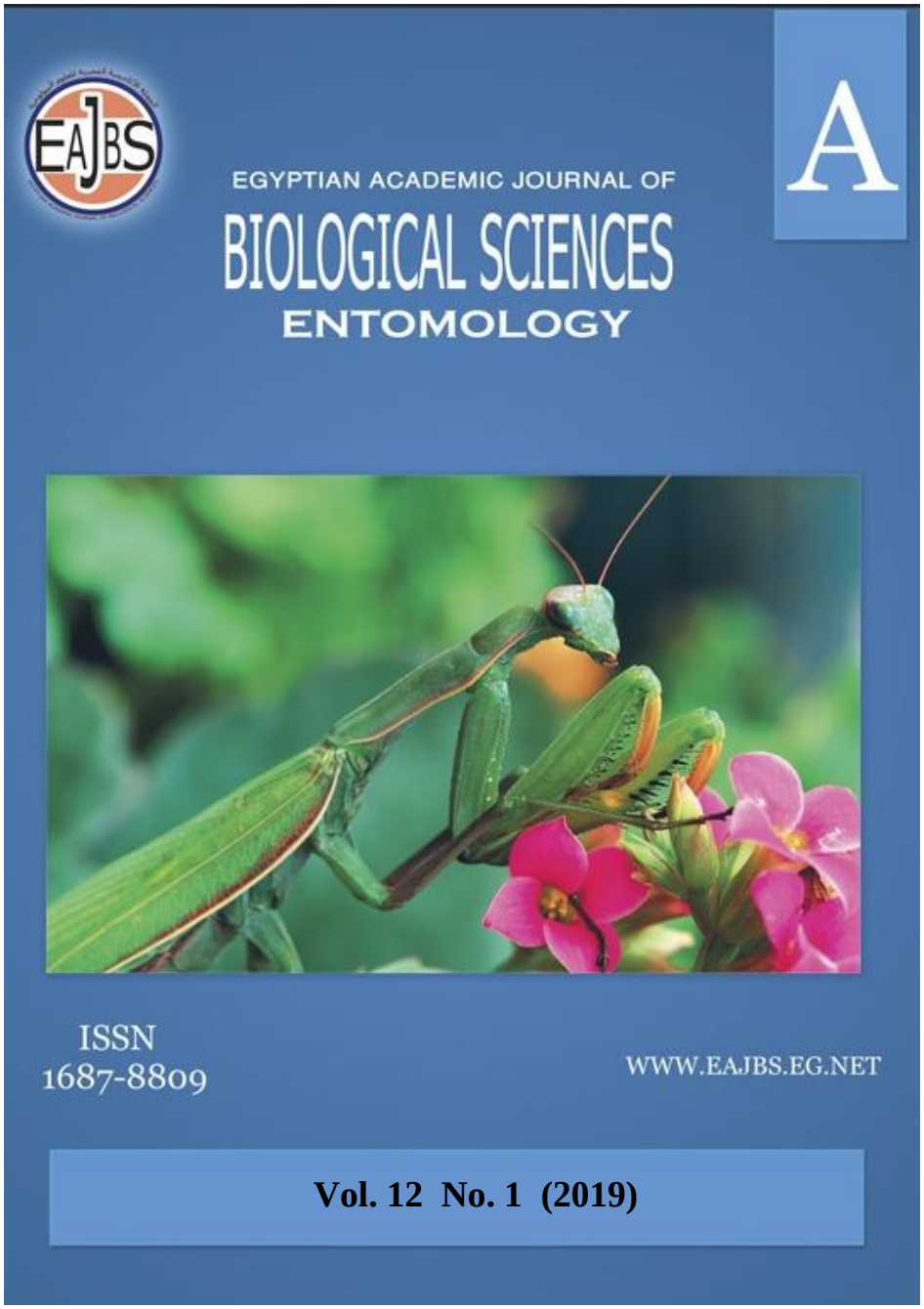

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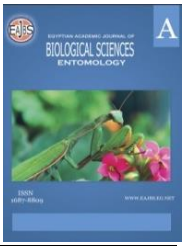

Cytogenetic and Meiotic Studies Reveal Conservatism in *Acrida turrita* (Linnaeus 1758) (Orthoptera: Acrididae) from Lagos, Nigeria

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ABSTRACT

The Acrididae exhibits a stable karyotypic uniformity or conservatism and are a typical specimen for cytological and meiotic investigations. Despite the diversity and cytotaxonomic value of this family, however there are only a few studies on their karyology. This paper is therefore aimed at describing the karyotype and meiotic behaviours of chromosomes of *Acrida turrita* from Nigeria, West Africa. Ten (10) male *A. turrita* grasshoppers were randomly collected from different locations in the University of Lagos community between May and June, 2018. Their chromosome smears were prepared using the lacto-propionic orcein squash technique. The prepared slides were viewed under 100X oil immersion objectives. The study revealed conserved 23 acrocentric chromosomes (2n = 23) in all 10 male specimens as reported by previous studies. We also found out that the 11 autosomes are made up of ten Large, three Medium and three Small chromosomes and the X chromosome is Medium in size. No chromosomal aberrations were observed in the meiotic stages as all chromosomes chiasmate. Mean chiasma frequency was found out to be 13±1.700. Total chromosome length ranges from 2.30±0.374 µm to 12.20±0.126 µm in chromosomes 1 and 11, respectively.

INTRODUCTION

Grasshoppers are a group of herbivorous insects in the order Orthoptera and suborder Caelifera (Ragge, 1965; Meinzingen, 1993). Globally, there are about 11,000 known species of grasshoppers distributed in six families and several genera. The genus *Acrida* is one of the most diverse genera in the order (Gupta and Chandra, 2018). *Acrida turrita* (Family Acrididae or Acridomorphoidea) is a short-horned grasshopper found in many parts of the world, often regarded as giant green slant face grasshopper. The males have a diploid chromosome number of 23, with an X0 constitution (Brown, 1972).

Several cytogenetic studies have shown that 23 uniform and conserved acrocentric chromosomes made up of 22 autosomes and an X chromosome are found in the grasshoppers of the family Acrididae and this conservation has been reported in Acrida (Adekoya and Williams, 2001; Seino and Akongnui, 2010) and other Orthoptera family (Souza and de Melo, 2007; Seino *et al*., 2008; Chadha and Mehta, 2011; Seino and Dongmo, 2013). The mitotic chromosomes' morphology of Acrida

has been reported to be more of acrocentric. However, due to not too apparent short arms of chromosomes in observed plates presenting as telocentrics, authors have described the behaviour as "acro-telocentric" (Adekoya, 2009; Seino and Akongnui, 2010). Also, findings have indicated that majority of the Acrididae species employs the X0-XX sex determination mechanism where 2n = 23 in males while it is 24 in females, except in the Neartic and Neotropical Acrididae, the Melanoplinae Acrididae, where there are fusions of the autosomes or the autosomes and sex chromosomes through a mechanism known as the Autosome Robertsonian fusion (Bidau and Marti, 2002; Bidau and Marti, 2004; Castillo *et al*., 2010).

Although there have been several studies on chromosome morphology and chiasma distribution in the Acrididae family (Burgov, 1999; Seino *et al*., 2008; Seino and Akongnui, 2010; Efe and Koca, 2016), there is still need for more information on cytogenetics of *Acrida turrita* from Lagos, Nigeria. This present study, therefore, is aimed at filling this void by providing detailed numerical and structural information on the chromosomes of *A. turrita* from Nigeria.

MATERIALS AND METHODS

1. Collection and Storage of Specimens:

A total of ten (10) adult males *A. turrita* were trapped from their natural habitat around the University of Lagos, Nigeria, Western Africa between May and June 2018 in a perforated plastic bottle. The grasshoppers were then taken to the genetics laboratory unit of the Department of Cell Biology and Genetics situated at the botanical and zoological garden, University of Lagos, where they were sacrificed with ethyl ether. The testes and testicular follicles were then harvested and preserved in saline solution (0.7 NaCl) and absolute alcohol and glacial acetic acid fixative in the ratio of 3:1, respectively at 4 ^{o}C until ready for use.

2. Chromosome Smears Preparation:

Chromosome smears were prepared for each sample from the preserved testes using the lacto-propionic orcein squash technique after Seino *et al*. (2012). To do this, about 4 – 5 testicular follicles were placed on a clean microscope glass slides and flooded with 45% acetic acid to swollen the tissue. Excess acid was blotted off with filter paper followed by the addition of 2% lacto-propionic orcein (2 drops) and the cells were subsequently macerated with the pointed end of a dissecting needle. This was left at room temperature on the laboratory bench for about 10 – 15 minutes to allow for deep penetration of the stain into the tissue. A clear size O covers slip was placed on the preparation. The cover glass was held in place while it was tapped gently with the flat wooden end of a dissecting needle to disperse the cells and force out the excess stain. The preparation was squashed further between folds of bibulous paper to absorb the excess stain. The preparations were preserved by sealing the edges of the cover glass with transparent (colourless) nail varnish.

3. Analysis of Chromosomes Smears and Chiasma Frequency Determination:

The prepared smears were viewed and the micrographs were taken under 100X oil immersion objective lens of a wild M20 microscope with MPS-55 photoautomat attachment. Five cells or slides were prepared for each specimen and were examined for chromosome number and morphology. The chromosomes were identified on the basis of their length (Stace, 1980) and their morphology was determined by examining the shapes of the chromosomes in meiotic anaphase I, anaphase II and metaphase II according to Williams and Ogunbiyi (1995) and Seino *et al*. (2008). Ocular and stage micrometers were used in determining the chromosomes' lengths.

Chiasma(ta) was examined at the diakinesis/diplotene stage from five prepared cells per sample and the average values were recorded. Other statistical parameters were performed using Statistical Package for Social Sciences (SPSS) version 23.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

The chromosomes of *Acrida turrita* obtained from the testis are mainly meiotic chromosomes. In anaphase I, the autosomes form 11 dyads and the sex chromosome remains single. This represents a chromosome constitution of 23. A chromosome number of 22 autosomes and 1 sex chromosome was observed in all five cells examined from the 10 specimens analyzed. The centromere position is also easily observed because the V-shaped double-stranded anaphase I chromosomes indicate acrocentric chromosomes (Plate 2). The acrocentric centromere is also clearly observable in metaphase II (Plate 3E).

Chromosome morphometric results as presented in Table 1 revealed that the chromosomes are made up of 5 pairs of large – chromosomes 1,2,3,4 and 5; 3 pairs of medium – chromosomes 6,7 and 8; and 3 pairs of small – chromosomes 9,10 and 11 (i.e. 5LL, 3MM, 3SS). Chromosomes that were adjudged to be large in the study ranged between 8.00 and 12.20 µm with an average length of 10.46 µm; the medium chromosomes i.e. chromosomes 6, 7, 8 and X chromosome measured 6.00, 5.60, 5.50 and 5.70 µm, respectively with an average length of 5.7 µm, while the short chromosomes ranged from 3.80 to 2.30 µm with an average length of 2.87 µm. Chromosome 1 which is the longest as a mean length of 12.20±0.126 µm while chromosome 1, the shortest, was 2.30±0.374 µm in length. The X chromosome falls in the medium length category with 5.70±0.300 µm long.

Table 1: Morphometrics of *A. turrita* chromosomes

Chromosome	Chromosome length (µm) Mean±SEM	Chromosome size	Chromosome morphology
1	12.20±0.126[a]	Large	Acrocentric
2	11.60±0.197[a]	Large	Acrocentric
3	11.10±0.321[a]	Large	Acrocentric
4	9.40±0.207[a]	Large	Acrocentric
5	8.00±0.316[a]	Large	Acrocentric
6	6.00±0.316[b]	Medium	Acrocentric
7	5.60±0.400[b]	Medium	Acrocentric
8	5.50±0.316[b]	Medium	Acrocentric
9	3.80±0.200[c]	Small	Acrocentric
10	2.50±0.500[c]	Small	Acrocentric
11	2.30±0.374[c]	Small	Acrocentric
X	5.70±0.300[b]	Medium	Acrocentric

a, b and c are statistically significant at $p < 0.005$

Chiasma frequency was evenly distributed in the specimens(Table 2). The frequency is highest in specimen 6 with a chiasma frequency of 16 and jointly lowest in specimens 2, 4, 5 and 10 with a frequency of 11. Mean chiasma frequency was found out to be 13±1.700. Bivalents that are either rod-shaped, ring-shaped or with up to three chiasmata were observed at diplotene stage (Plate 2D). The mean frequencies of rod-shaped, ring-shaped, 1-chiasma, 2-chiasma and 3-chiasma

bivalents were found to be 7.4±0.843, 2.6±0.843, 8.8±1.932, 2.1±0.876 and 1.0±0.816, respectively.

Table 2: Frequency of chiasma and bivalents distribution

Sample	Chiasma Frequency/ cell	Types and number of bivalents				
		Rod	Ring	1-chiasma	2-chiasmata	3-chiasmata
1	15	7	3	10	3	1
2	11	8	2	9	1	0
3	13	9	1	8	3	1
4	11	8	2	7	2	0
5	11	7	3	6	2	2
6	16	7	3	13	1	1
7	13	7	3	8	2	2
8	13	8	2	8	3	2
9	14	6	4	9	3	0
10	11	7	3	10	1	1
Total	130	74	26	88	21	10
Mean	13	7.4	2.6	8.8	2.1	1.0
Std Dev.	1.700	0.843	0.843	1.932	0.876	0.816

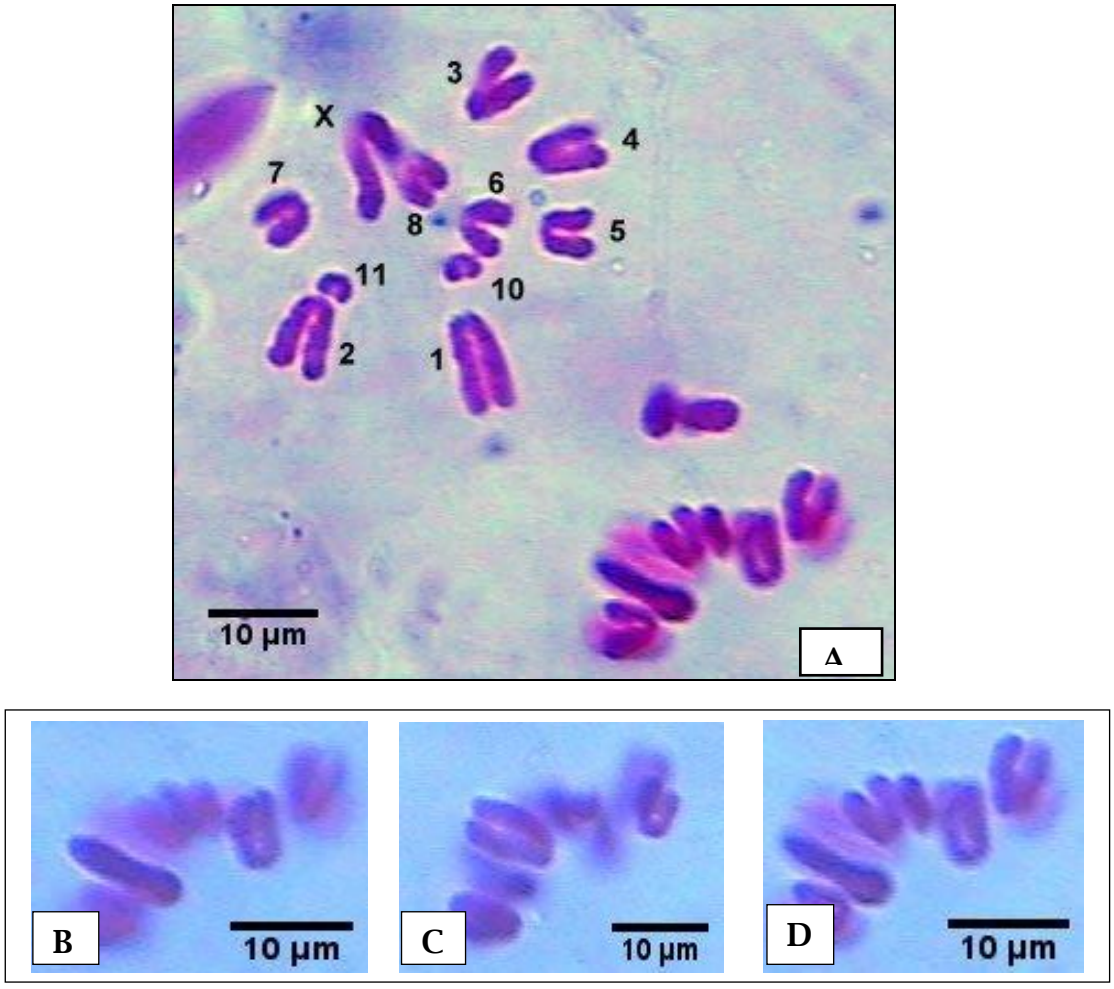

Plate 1: Karyotype of *A. turrita.*
Chromosomes are seen with 11 dyads and an X chromosome. B, C and D are different foci of the same groups of chromosomes showing chromosomes that were out of focus in micrograph A.

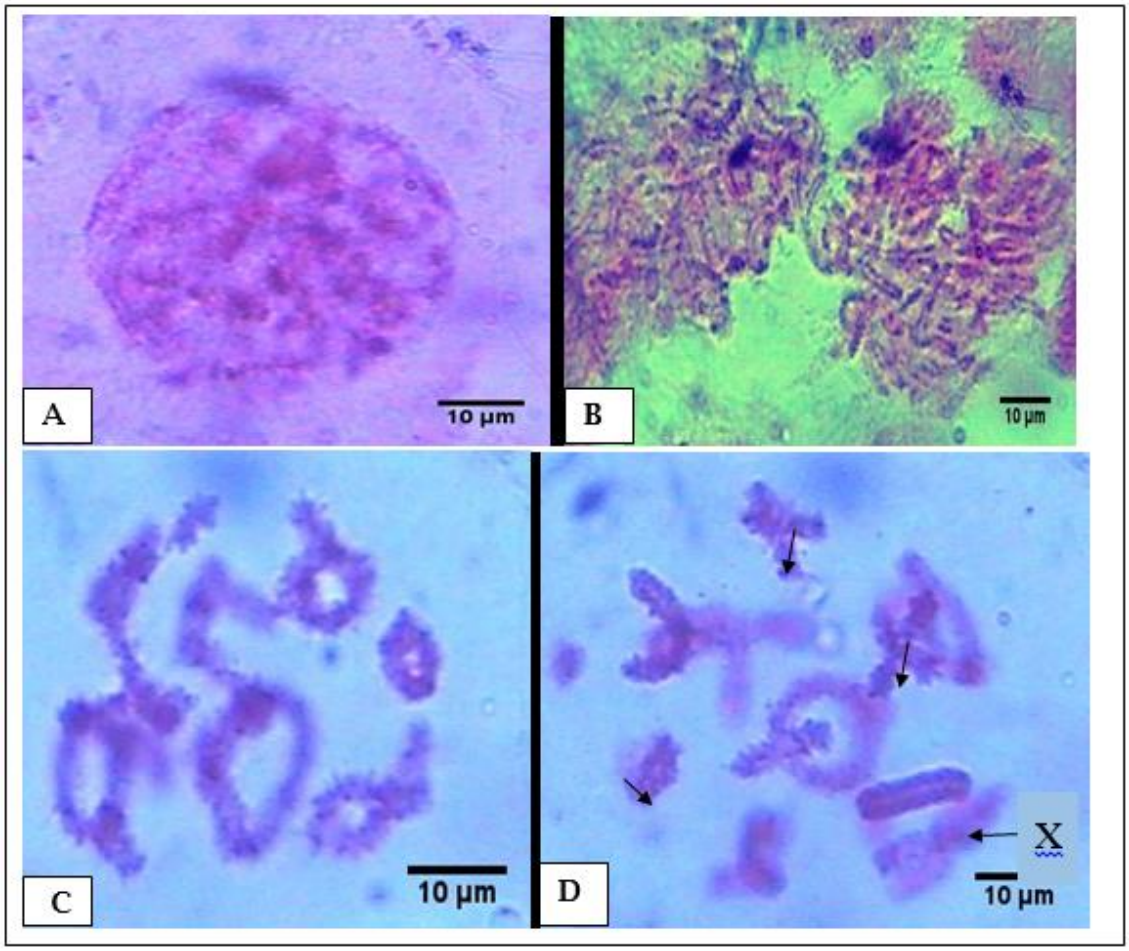

Plate 2: Chromosomes behaviour in meiosis: *A is leptotene with the chromatin threads still bunched up. B is the zygotene with the pair of strands appearing more distinct and less coiled. C and D are diplotene. Arrows in D indicate chiasmata. X is the sex chromosome showing positive heteropycnosis.*

From Plate 2, at leptotene (A), the chromosomes appear diffused and granular. At zygotene (B), the chromosomes appear more condensed and are double stranded with chromomeres along their lengths. At diplotene (C and D), bivalents were shorter and thicker than those in pachytene. The chromosomes are pulled apart and the homologues held together at chiasmata

From Plate 3C, micrographs A, B and C represent metaphase I where bivalents appear thicker and shorter. The two centromeres of each bivalent now lie on opposite sides of the equatorial plate. Also, the X chromosome (arrowed in A) appears more lightly stained than the autosomes. Micrograph D represents anaphase I which shows homologous chromosome migrating to the opposite poles, E is metaphase II showing each chromosome made up of two chromatids, F is anaphase II where sister chromatids are fully separated and move towards opposite poles. At telophase I (G and H), cleavage has occurred and two daughter cell are formed.

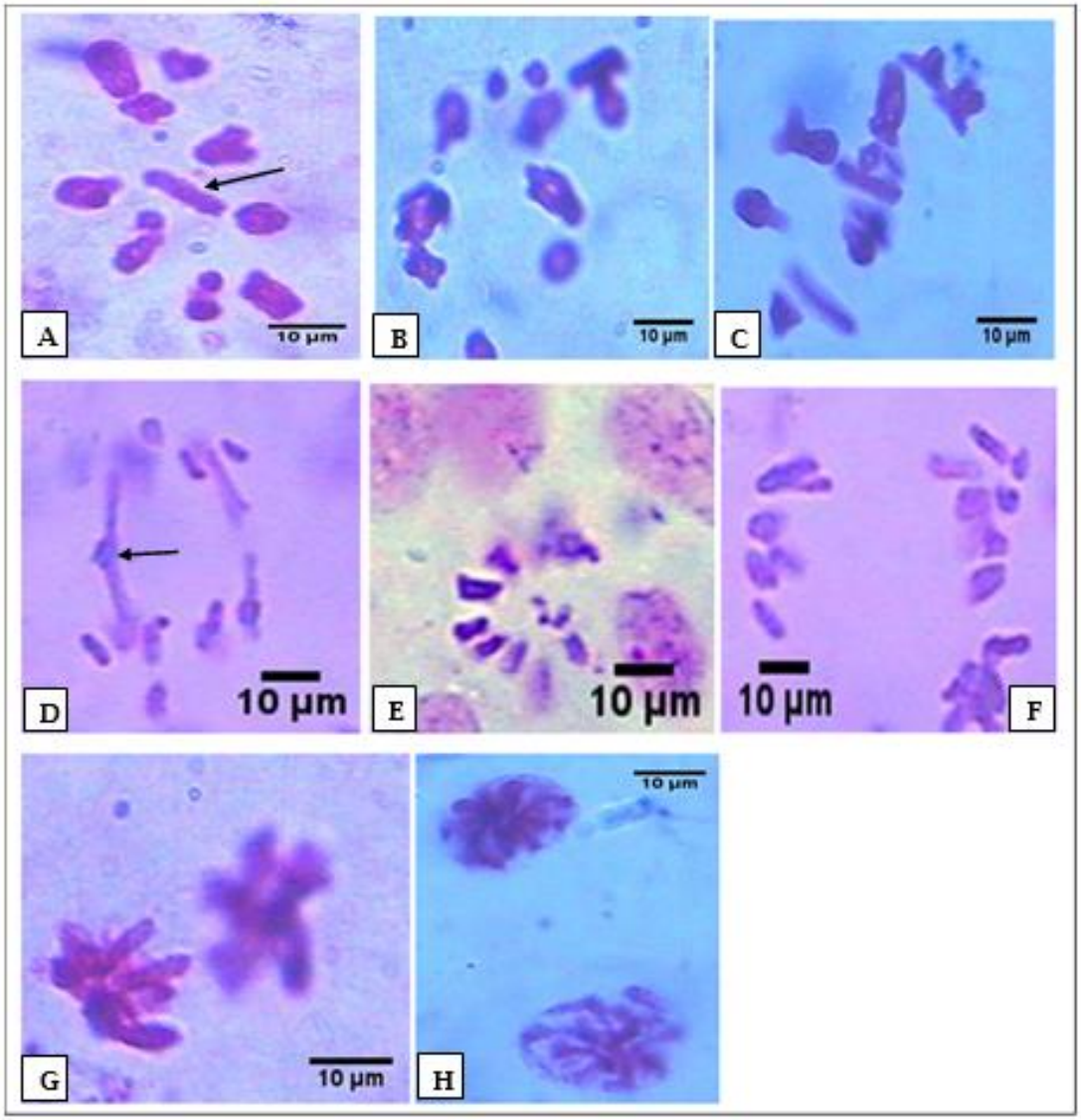

Plate 3*:* Further meiotic chromosomes: *A, B, C are metaphase I in polar view. The X-chromosome (arrowed in A) is 2-stranded. The metaphase stretch has changed from apparently ring-shaped bivalents into an α-shape. D is anaphase I with an arrow indicating a bridge. Entanglement of surface material between homologues causes elongation of some chromatids and* **G** *us connections between chromatids. E is metaphase II. F is anaphase II, the chromosomes are single-stranded. G and H are telophases I.* **H**

DISCUSSION

Chromosome numbers and morphologies are distinctive in organisms of the same species and they play major roles in the identification of species (Seino *et al*., 2012). Knowledge of karyotype analysis also helps in understanding evolutionary relationships and divergence that exists between organisms of the same species (Meera-Rao, 1990). It has however been shown that chromosome number and structure are not always constant in species. The short-horned grasshoppers (*Acrida turrita*) are unique in that they display a form of cytogenetic uniformity which is regarded as "karyotypic conservatism" (Aswathanarayana and Ashwath, 2006). The Acrididae family possesses a fixed number of chromosomes in which 2n can either

be 23 in males that exhibit X0 sex determination mechanism (2n♂ = 23), or 24 in females with XX mechanism (2n♀ = 24) (Efe and Koca, 2016).

The present study reveals that all 10 male specimens without exceptions have 23 chromosomes (i.e. 22A+X0) characteristic of the Acrididae family. All autosomes and sex chromosomes observed in the study are acrocentric. In Anaphase I, the chromosomes were seen to be V-shaped and consist of two sister chromatids. Repulsion between the sister chromatids confers the V shape characteristic of acrocentric chromosomes; while in Anaphase II (Plate 4F), the chromatids have fully separated and appeared I-shaped confirming that the chromosomes are acrocentric morphologically (Williams and Ogunbiyi, 1995; Seino *et al*., 2008), or acro-telocentric as suggested by Adekoya (2009) and Seino and Akongnui (2010).

Our results therefore, agree with the karyotypic conservatism phenomenon in the Acrididae family in terms of chromosome number (23 in X0 males) and structures (acrocentric or acro-telocentrics). This phenomenon has been attributed to several factors by different researchers. These factors include, but are not limited to habitat similarity and stability in climates as well as natural selection (Vij *et al*., 1980; Vosa, 2005).

Also, our finding is in conformity with previous studies on the karyotype of the Acrididae. Sharma and Gautam (2002) for example, reported similar results in their study of 11 species of grasshoppers from North-Western Himalayas. Results obtained from this study also corroborated that of Seino and Akongnui (2010) who reported 23 acrocentric chromosomes among Acrididae species from Limbe, South-Western Cameroon. Our results as well as these other findings from other countries therefore, prove that the short-horned grasshoppers demonstrate cytogenetic similarity in terms of chromosome number vis-à-vis their sex-determining mechanism despite different geographical locations. However, despite cytological uniformity and karyological conservatism expressed by the Acrididae, molecular studies and C-banding techniques have revealed certain structural re-arrangements such as deletion, insertion, transversion, reciprocal translocation and paracentric inversion that do not always translate to noticeable morphological changes in the family (Cabrero and Camacho, 1982).

Meiotic processes in all specimens were normal and there were no chromosomal aberrations observed in all meiotic stages observed in the study, which include anaphase I, metaphase I, telophase I, telophase I, anaphase II and metaphase II. The anaphase I with double-stranded V–shaped dyads (Plate 2D) indicates acrocentric chromosomes. The chromosomes were distinct and well separated at Anaphase I, an indication that there was no non–disjunction of chromosomes.

The number of chiasmata in the *A. turrita* varied from 1 to 3 with each bivalent having at least one chiasma. Also, the chiasma frequency per cell varied in different cells of the organisms tested with values ranging between 11 and 15, as shown in Table 2. Several authors had stated that Acrididae's eleven bivalents in the males show chiasma frequency ranging from 11.5 to 19.8 (White and Contreras, 1978; Grieco and Bidau, 1999). Chiasma frequency has been suggested to be under genetic influence. Its main function is a continuous release of genetic variability within and between species (Grieco and Bidau, 1999).

Conclusion

In conclusion, the chromosome number in the male *Acrida turrita* from Nigeria conforms with the conserved diploid number of 2n = 23 i.e. 22A+X0 that is found in the family Acrididae. There is also no meiotic chromosomal abnormality found in

any of the specimens observed in the study. It is believed, therefore, that the data presented in this study has helped enhance the knowledge on the conservation of chromosomes of the Acrididae from Nigeria.

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