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PRELIMINARY STUDIES OF CHROMOSOME MORPHOLOGY AND ABERRATIONS IN NATURAL POPULATION OF *ACRIDA TURRITA* (LINNAEUS), (ACRIDOMORPHOIDEA, ACRIDIDEA) IN EWEKORO, OGUN STATE, NIGERIA.

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ABSTRACT

Acrida turrita (Linnaeus) collected in the Lagos State University (LASU), Ojo campus and the fields around Ewekoro cement factory in Ogun State were investigated. The mitotic metaphase length, chiasma frequency heteropycnotic behaviour of x-chromosomes and structural aberrations of the grasshoppers were studied from the two sites. The species have diploid chromosome number of 2n=23 (B&) and the chromosomes were telocentric. The meiotic processes detected were mostly normal for LASU, various aberrations ranging from reduction in chromosome number, clumping and non-disjunction of chromosomes were recovered from Ewekoro. The mean chiasma frequency is 16.92. No significant difference was detected from the length of X-chromosomes from both sites. The X-chromosome however displayed the normal reversal type of heteropycnosis. The implication of aberration in morphology of chromosomes detected at Ewekoro site is discussed in relation to the mutagenic effects of cement dust on flora and fauna.

INTRODUCTION

The grasshopper Acrida turrita of the family Acridomorphoidea/Acrididea has a male diploid chromosome number of 23, with an XO constitution (Brown, 1972). The chromosomes were described as acrocentric (Ebiane, 1982). They are known to undergo normal mitotic and meiotic divisions for growth and production of gametes respectively. This grasshopper may be green or brown with a pink dorsal abdominal surface. The body and head are strongly elongated and stick like in appearance. The head bears two short ensiform antennae. The hind femura are strongly elongated. The karyotype which is the particular chromosome complement of each organism is used to characterize organisms. (Fincham, 1983). However karyotypes have been known to be affected by mutations in different organisms by various investigators.

Cement dust has been reported to affect flora and fauna both "in-vitro" and "in-vivo". (Iqbal et al, 1995; Madre and Klosieko, 1997; Adekoya and Williams, 2000; Osuna et al, 1996 and Makridis et al, 1996) cytogenetical effects of cement has been highlighted to decrease the mitotic index in Allium cepa (Ignacimuthu and Muraleytharam, 1994). Mutagenic effects of cement has been reported by Pandey et al, (1996)to decrease leaf chlorophyll content, plant biomass, plant protein, ash, fats and crude-fibre of wheat.

The cement has been known to increase the soil, pH, alkalinity and also lime content (sivakumar et al, 1995). The soil polluted with cement dust will loose it's nutrients and various heavy meal content was higher in surface soil samples than sub-surface. This led to the conclusion by Asubiojo et al (1991) that the vegetation near the vicinity of cement factories are contaminated with toxic materials.

This contamination which affects vegetation could in turn affect the organisms which feed on the plants in the vicinity. The effect or effects could build-up and lead to mutagenic damage. The effects could also be sudden and drastic, it could also have long term effects as a result of accumulation over time in the tissues of the organisms.

Data on direct effect (in-vitro) of cement dust on grasshoppers are scanty or non-existent. Moreover documented facts on the effect of cement dust on other organisms, could be used to evaluate the effects expected on grasshoppers more especially when grasshoppers being herbivorous feed on the grasses growing in the environment of the cement factory. The leaves which would contain deposits of cement dusts that consist of possible mutagenic materials such as pozzolan, granulated slag, hydrated lime, calcium carbonate, calcium oxide etc. It is with the above facts that this investigation was carried out to study the mitotic and meiotic division of Acrida turrita from Lagos state university fields as control and those from fields of Portland cement factory in Ewekoro, Ogun state.

MATERIALS AND METHOD

A total of fifty adult male grasshoppers were used for this study. Thirty of the grasshoppers were collected from the fields of the Lagos State University, Ojo campus and twenty were collected from the fields around Ewekoro cement factory in Ogun state between January and May 1999. The grasshoppers were killed with chloroform either on the day they were captured or on the next day. The grasshoppers were then dissected in 0.67% NaCI solution.

Five-ten fresh testicular follicles were placed on each glass slide, cut into three or four pieces and covered with a drop of 2% lactic – propionic orcein stain. After about 10-15 mins, a cover glass was placed on the material. The cover glass was held in place while it was tapped gently with 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 edges of the cover glass were sealed with nail varnish.

The length of the individual chromosomes in mitotic metaphase were measured from five cells in each individual. Measurements were carried out with the help of an ocular micrometer reading and the lengths were recorded in micrometers. Homologous chromosomes were determined on the basis of length. The frequency of chiasma occurrence at prophase I (Diplotene) of meiosis were recorded, heteropycnotic behaviour of X-chromosomes were determined by visual assessment. The numerical and structural aberrations of chromosomes were studied. Photomicrographs were made under the 100x oil immersion objective of a WILD M20 microscope with MPS55 photoautomat attachment.

RESULTS

The lengths of the mitotic metahase chromosomes in five cells in each of five individuals from LASU were measured. The homologous chromosomes were determined by matching the chromosomes on the basis of their lengths and consequently ranked as A (very long), B (long), C (medium) and D (short) Group A was made up of chromosome pairs ranked as 1 and 2 (1-4), they are the longest chromosome pairs in the karyotype with mean length of 10.56+ 0.62 micrometers. Group B, 3 and 4 pairs (5-8) have 7.99+ 0.68 micrometers. Group C, (5-9) pairs (9-19) consist of chromosome with length 5.28+0.18 micrometers. Group C was considered to include the X-chromosome since one of the chromosomes in the group lacked a homologous partner. The shortest group of chromosome are Group D, 10 and 11 pairs (20-23) has length 2.54+ 0.33 (Table 1)

Table 2 shows the mean lengths of spermatogonial mitotic chromosome measured from five individual of Ewekoro. Group A chromosomes have a mean of 10.12+0.62 micrometers and group B has 8.13+0.47, group C with 5.28+, 0.09 and 2.56+0.31 was recorded for those in group D. When the data on chromosome lengths from LASU and Ewekoro were compared using chi-square ($?^2$)- test, value of 0.015 at 3 degree of freedom was derived and this figure translates to $0.7 > P > 0.5$.

The number of chiasmata present in each of five cells from each of ten individuals were recorded for grasshoppers from LASU in Table 3 and those from Ewekoro were presented in Table 4. The mean frequency from LASU gives 16.80+1.49 while those from Ewekoro revealed 17.04+0.95. The test of significant difference gives ($?^2 = 0.017$, n=1, $0.7 > P > 0.5$). This translates to a no significant difference in the chiasma frequency recorded from the two sites.

X-chromosomes in all the slides investigated in this study stained darker than the autosomes (plates 1-4). In plates 1-3 the X- chromosome have two chromatids displayed. Plates 5 and 6 show the chromosomes at mitotic metaphase. It reveals the number of chromosomes in the cell, n=23 and the plates also present the sizes and telocentric nature of the chromosomes. These slides were used for the measurement and analysis of the length and ranking into groups. The slides show that the chromosome are rod-shaped and tapered at the end.

However, a couple of slides presents clumping of chromosomes (plate 7) and duplication of chromosomes (plate 8). These type of slides were mostly found from Ewekoro preparations. Other forms of aberrations were present in plates 9 and 10 where there were chiasma disruption and loss of chromosomes respectively. However normal chiasma has been presented in plates 11 and 12. Plate 13 shows late metaphase – II or early anaphase – II and plate 14 display normal anaphase showing the bridges of separation.

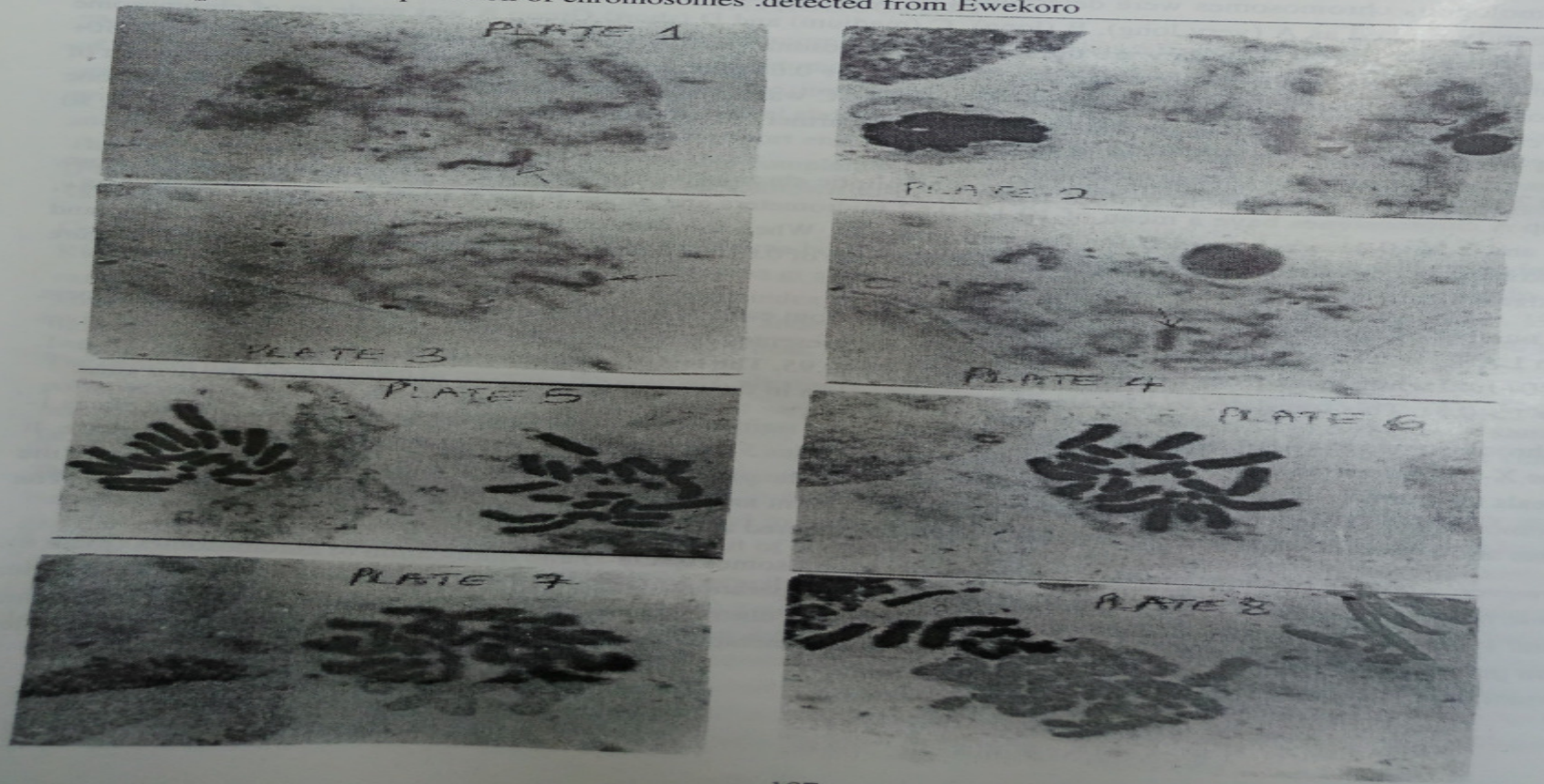
TABLE 1

Mean lengths (micrometers) of the spermatogonial mitotic metaphase chromosomes measured from five individuals in LASU.

INDIVIDUAL	A(1-4)	B (5-8)	C (9-19)	D (20 – 23)
I	10.94±011	8.08± 0.13	5.42± 0.08	2.82± 0.23
II	10.86± 0.19	8.36± 0.27	5.44± 0.36	2.76± 0.27
III	10.74± 0.11	8.48± 0.47	5.28± 0.31	2.33± 0.17
IV	10.82± 0.24	8.24± 0.59	4.98± 0.28	2.06± 0.15
V	9.46±0.11	6.80±0.22	5.30± 0.37	2.72±0.15
	10.56±0.62	7.99± 0.68	5.28±0.18	2.54 ± 0.33

LEGEND TO PLATES (1-8)

Plate 1 – Cell at Diplotene.X – chromosome (arrowed) show +ve heteropycnosis
Plate 2 - Cell at Diplotene.X – chromosome (arrowed) show +ve heteropycnosis
Plate 3 - Cell at Diplotene.X – chromosome (arrowed) show +ve heteropycnosis
Plate 4 - Cell at Diplotene.X – chromosome (arrowed) show +ve heteropycnosis
Plate 5 - Mitotic metaphase. n=23. Show the Telocentric nature
Plate 6 - Mitotic metaphase. Two strands of chromosome shown.
Plate 7 - Abnormality detected from Ewekoro clumping of chromosomes
Plate 8 - Duplication of chromosomes .detected from Ewekoro



LEGEND TO PLATES (9-14)

Plate 9 - Cell at Diakinesis, Chiasma distruption and loss of chromosomes

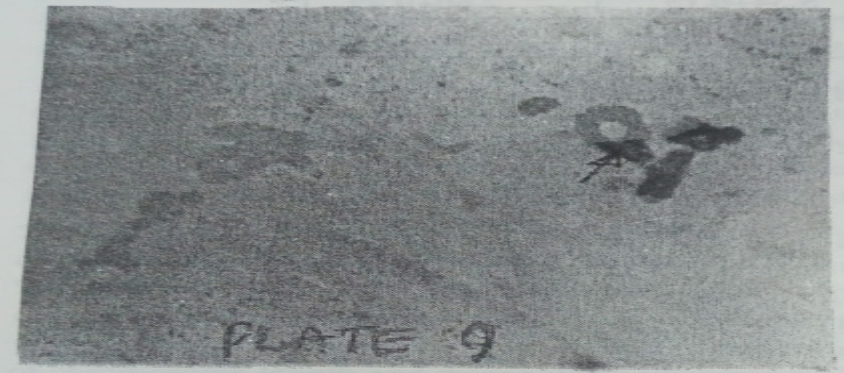
Plate 10 - Cell at pachytene, showing burst membrane and loss of chromosome

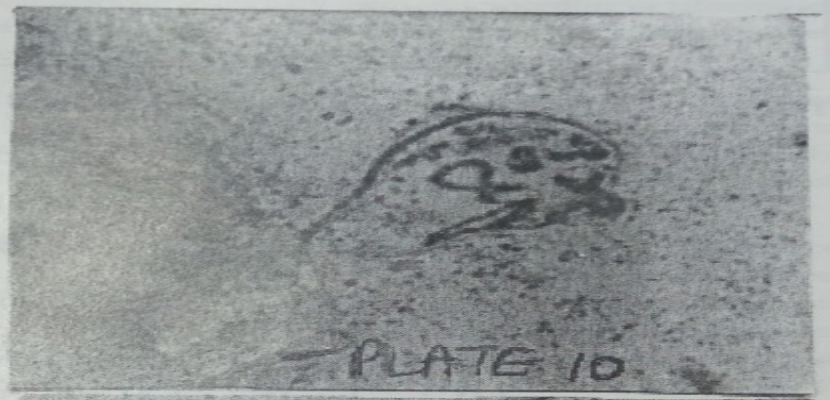
Plate11 - Cell at pachytene chiasma (arrowed)

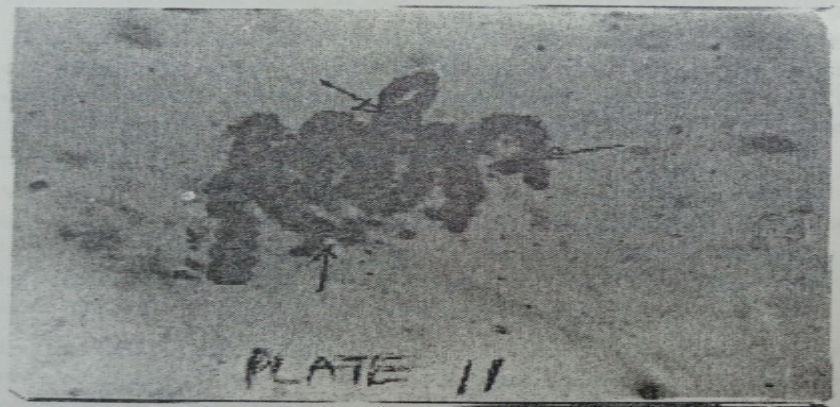
Plate 12 - Cell at pachytens chiasma (arrowed)

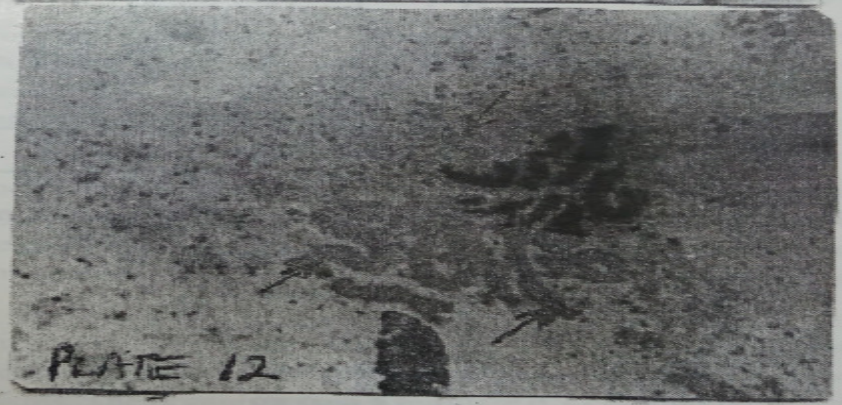
Plate 13 - Late metaphase II, showing chromatids without minute second arm.

Plate14 - Separation of chromosomes at anaphase. Bridges arrowed.









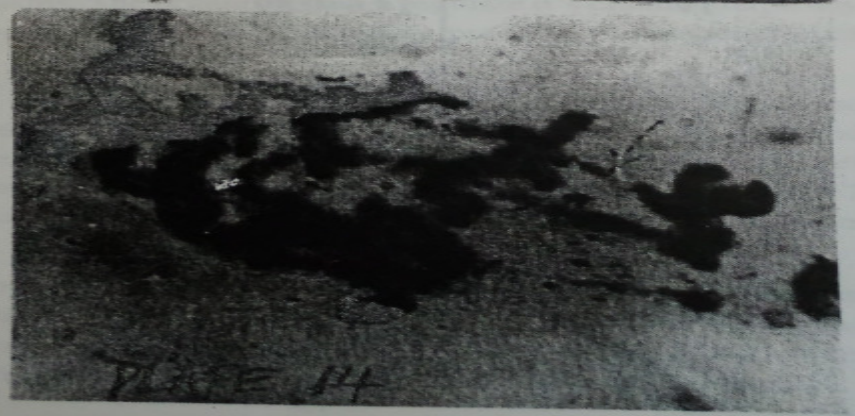


TABLE 2: Mean lengths (micrometers) of the spermatogonial mitotic metaphase chromosomes measured from five individuals in Ewekoro

INDIVIDUAL	A (1 – 4)	B (5 – 8)	C (9 – 19)	D (20 – 23)
I	10.74±0.11	8.24±0.59	5.40±0.08	2.84±0.27
II	10.34±0.59	8.13±0.13	5.30±0.45	2.04±0.10
III	9.60± 0.22	7.66±0.48	5.18±0.13	2.72±0.15
IV	10.80±0.24	8.72±0.19	5.20±0.16	2.60±0.34
V	9.44±0.09	8.80±0.20	5.34±0.15	2.60±0.49
?	10.12±0.09	8.31± 0.47	5.28±0.09	2.56± 0.31

TABLE 3: Chiasma frequency per cell in five cells in each of ten individual from LASU.

INDIVIDUAL	NUMBER/FREQ
I	15.8± 1.64
II	17.0 ± 1.22
III	17.2±3.11
IV	16.4 ± 2..07
V	13.6 ± 1.14
VI	18.2 ± 3.70
VII	16.2 ± 1.30
VIII	16.6 ± 2.51
1X	18.8 ± 1.92
X	18.2 +1.30
?	16.8+ 1.49.

TABLE 4: Chiasma frequency per cell in five cells in each of ten individual from Ewekoro

INDIVIDUAL	NUMBER/FREQ
I	18.2±0.84
II	16.8±2.17
III	17.0± 1.58
IV	14.6±0.89
V	17.6±1.14
VI	17.0±2.24
VII	17.2±1.92
VIII	17.2±2.05
IX	17.6±1.34
X	17.2±2.28
?	17.04±0.95

Furthermore, the species investigated from both sites studied were similar to one another in that the X- chromosomes were positively heteropycnotic (plate 1-4 and plate 12). These observations are common features of Acrididae and have been claimed to be characteristic to the Acridomophodiae (Seino, 1989) and in pyrgomorphoidae (Williams and Ogunbiyi, 1995).

The chromosomal aberrations detected and recorded in plates 7,8,9 and 10 which are characterized by clumping of chromosomes (plate 7), duplication of meiotic chromosomes (plate 8), loss of chromosomes and chiasma disruption (plate 9) and membrane rupture followed by loss of chromosomes (plate 10), Could be suggested to be as a result of cement dusts at Ewekoro. This is because the abnormalities were quite frequent from slides of Ewekoro where the grasshoppers feed on cement dusted leaves.

The ascertion that the cement dust might have been the cause of the abnormalities would be tenable but for the fact that aberrations were recorded from LASU slides too. The claim needs further investigation. Moreover, Olorode and Akingbohungbe (1975) and Lasebikan and Olorode (1977) has suggested that chromosome aberration are a regular feature of meiosis in Zonocerus variegatus studied by them. Although a repeat work done by Williams and Ogunbiyi in1995 disagreed sharply with the claims.

The similarities recorded between chromosomes from the two sites when length of chromosomes, chiama frequency and X-chromosome heteropycnotic behaviour were considered makes the abnormalities recorded not to be as a result of the cement dust. It is therefore suggested that before conclusion could be made on the effects of cement dust on morphology of chromosomes, more work of "in-vivo" as well as "in-vitro" effects should be studied at different concentrations of the cement dust.

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WILLIAMS, G.O AND OGUNBIYI, B.I.(1995). Chromosome morphology and meiosis in Zonocerus variegatus L. (Orthoptera,Pygomorphidae) Cytologia. 60: 111 - 116 PLATES (Legend)