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Karyotype and meiosis studies in *Oxycatantops spissus* (Walker) (Orthoptera: Acrididae)

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ABSTRACT

The standard karyotype of *Oxycatantops spissus* (Walker) (Orthoptera: Acrididae: Acridinae) from Limbe in the South West Province of Cameroon, comprises 2n = 23 acrocentric chromosomes in the male with the XO/XX sex determining mechanism [2n = 23 (22AA+XO)]. The karyotype structure is represented by five pairs of long (L1, L2, L3, L4, L5), three pairs of medium (M6, M7, M8) and three pairs of short (S9, S10, S11) autosomes. The acrocentric X chromosome is approximately equal in size to the short (S) autosomes. The meiotic process in this species was normal and chiasmate. Mean chiasma frequency in the wet season (16.36 ± 1.77) was significantly higher (p = 0.05) than in the dry season (15.00 ± 1.60). The differences probably lie in the fact that in the wet season many long bivalents had two or more chiasmata while in the dry season only a few long chromosomes had two or more chiasmata.

Keywords: Karyotype, Chiasma frequency, Dry & Wet season, Acrididae, Grasshopper, *Oxycatantops spissus.*

INTRODUCTION

Karyotype studies in the genus *Oxycatantops* of the subfamily Acridinae and Family Acrididae have for long been neglected due to the non-economic nature of this species and because karyotypes in the Acrididae have been shown to have a remarkable uniformity not known in any other Orthoptera family (White, 1973; Hewitt, 1979). Since one of the most important problems of comparative cytotaxonomy is the reconstruction of the basic (fundamental) chromosome number of a given group and consequently the elucidation of the evolutionary trends in diversification of those karyotypes, the necessity arose for the accumulation of cytotaxonomic information for the *Oxycatantops* species.

Oxycatantops spissus (Walker) belongs to the subfamily Acridinae of the family Acrididae. It is a medium sized grasshopper that is robust and characteristically dirty brown. The head of this grasshopper bears filiform antennae which are thick and slightly longer than the combined length of the head and pronotum. The tibia of the hind limbs has a white band which gives way to a black shade towards the tarsus. The forewings are wide and dirty brown while the hind wings are light brown in colour. *Oxycatantops spissus* is an African grasshopper, distributed along the seashores in the Gulf of Guinea (Dirsh, 1975; Jago, 1984; Mestre, 1984).

The absence of cytotaxonomic information on this species has stimulated this study. This study was designed to describe the karyotype (chromosome number, morphology and chromosome length), the meiotic process and seasonal variation in chiasma frequency in *O. spissus* collected from Limbe in the South West Province of Cameroon.

MATERIALS AND METHODS

Collection of specimen

Five of the ten adult male grasshoppers used for this study were collected in the dry season (January 2007) and the other five collected in the wet season (May 2007), on the premises of *Institut de Recherche Agricole pour le Développement* (IRAD) Batoke, in Limbe, South West Province of Cameroon. On capture, the insects were placed in bottle cages prepared by the method of Popov (1990). They were then taken to the laboratory, killed with chloroform fumes, dissected for the testes which were then fixed in 3:1 ethanol: acetic acid and stored in a refrigerator at 4 °C until used.

Preparation of chromosome smears

Chromosome smears were prepared by the Lacto-propionic Orcein squash technique (Seino, 1989; Seino et al., 2002). Two to three of the fixed testicular follicles were placed on a clean and siliconized microscope glass slide. They were first flooded with 45% acetic acid to allow the cells to become swollen. After blotting off the acid, the tissue was next flooded with one or two drops of lacto propionic Orcein stain and macerated using the sharp pointed end of a dissecting needle to allow the stain penetrate into the tissue. The preparations were then incubated at room temperature for between ten and fifteen minutes while making sure that the stain did not dry off. A cover slide was next placed on the tissue, held in place with the thumb and forefinger before gently tapping with the wooden end of a dissecting needle. This helped to disperse the cells and force out excess stain. The preparation was then wrapped in a filter paper and squashed between the thumb and the top of the laboratory table. The filter paper absorbed excess stain. The edges of the cover slide were sealed with colourless nail vanish to temporarily preserve the preparation.

Analysis of chromosome smears

The chromosome smears thus prepared were examined using the 40x objective of a Fisher laboratory microscope. Good smears were photographed using the 100x oil immersion objective of a Lietz photomicroscope.

The lengths of the chromosomes were determined by direct microscope measurements using ocular and stage micrometers. Five cells in mitotic Metaphase were examined from each of ten individuals. Chromosome pairs were identified on the basis of length (Stace, 1980), and chromosome morphology was determined by examining the shapes of chromosomes in the meiotic Anaphase–I, Metaphase–II and Anaphase-II (Williams and Ogunbiyi, 1995; Seino et al., 2002).

Chiasma frequency

Chiasmata were counted at Diplotene/ Diakinesis from five cells per individual. Five individuals were examined for each of the wet and dry seasons.

Statistical analysis of data

The data on relative length was subjected to the Duncan's Multiple Range Test, (DMRT) (Steel and Torrie, 1981) to separate the chromosomes into size groups of long, medium and short while data on chiasma frequency was subjected to variance analysis to determine significant differences between seasons.

RESULTS AND DISCUSSION

Chromosome number and morphology

The species revealed 23 rod-shaped chromosomes with sister chromatids separated gradually from a tapered end towards the other end. The two chromatids of each chromosome were not coiled around each other looking like C-mitotic chromosomes. Centromeres and short arms were not distinct in the chromosomes but centromeres were inferred to be in the tapered terminal regions where sister chromatids were in close contact (Fig. 1a). At this stage, the chromosomes in *O. spissus* were considered to be acrocentric or telocentric in morphology. The chromosomes were further examined in some meiotic stages to ascertain morphology. All anaphase–I chromosomes (Fig. 3) were made up of two chromatids held together at one end by the centromere. Distal to the centromere there was repulsion between sister chromatids (which were single stranded) conferring a V-shape appearance on the chromosome. This configuration is what is expected as a result of the repulsion between sister chromatids

characteristic of Anaphase–I acrocentric chromosomes. Metacentric chromosomes in Anaphase–I would appear as four armed V-shaped structures while acrocentric and telocentric chromosomes in Anaphase–I would have two arms with the centromere at the point of their convergence (John and Lewis, 1973, 1975; White, 1973; Williams and Ogunbiyi, 1995). Minute short chromosome (SA) arms were visible in some of the long chromosomes in Anaphase–I (Fig. 3) confirming that the chromosomes in this species were actually acrocentric in morphology. The chromosomes in Anaphase–II (Fig. 5) were clearly single stranded and appeared I–shaped. They were therefore not folded, further confirming that the chromosomes in *O. spissus* were acrocentric in morphology.

Cytological studies of known grasshopper species in the family Acrididae (Orthoptera) have revealed a characteristic karyotype of 2N= 23 (22A+X0) acrocentric chromosomes in male individuals. This standard karyotype has been variously found in Acrididae that include, the genus *Acrotylus* (Camacho and Cabrero, 1983), *Meianoplus senguinipes* (Zhan et al., 1984), *Podisma pedestris*, (John and Hewitt, 1970; Westerman and Hewitt, 1985), *Acrida turita*, *Paracenema luculenta* and *Morphacris fasciata* (Seino, 1989), the genus *Podisma* Bertold (Bugrov et al., 1994; Bugrov and Segreev, 1997), *Eyprepocnemis plorans* (Charp.) (Burgrov et al., 1999), *Podisma sapporensis* Shir (Burgrov, 1995; Burgrov et al., 2000, 2001), and Oedipoda *schochi schochi* and *Acrotylus insbricus* (Turkoglu and Koca, 2002). It is confirmed in this study that male individuals of *O. spissus* show the standard Acrididae karyotype of 23 acrocentric chromosomes.

Chromosome lengths

The results of morphometric measurements of metaphase chromosomes are shown in Table 1. These include mean and relative chromosome lengths.

Mean total chromosome length was 84.0 ± 0.61µm. The longest chromosome was 12.3 ± 0.07µm and the shortest chromosome was 2.6 ± 0.00µm. The chromosomes in this species occurred in three distinct sized groups of five long, three medium and three short (5L + 3M + 3S). The X chromosome was found to be among the short chromosomes (Fig. 1b). This is consistent with the chromosomes of other Acrididae (Shaw, 1976; Bugrov and Sergeev, 1997; Bugrov and Warchalowska-Sliva 1997; Bugrov et al., 1999; Turkoglo and Koca, 2002; Warchalowska-Sliva et al., 2002). For example the chromosomes in *E. plorans* (Charp.) occur in groups of 2L + 6M + 3S. The acrocentric X chromosome is about the same size as the M autosome (Bugrov et al., 1999). In *P. sapporensis* the chromosomes also occur in groups of 2L + 6M + 3S. The acrocentric X chromosome is approximately equal to the M4 autosome (Bugrov et al., 2001). It therefore follows that the karyotype of *O. spissus* here described is comparable to the characteristic Acrididae karyotype, which is made up of three distinct size groups of long, medium and short.

The standard karyotype of *O. spissus* from Limbe in Cameroon can therefore be described to consist of 2n = 23 acrocentric chromosomes in the male with the XO/XX sex determining mechanism. The karyotype structure is represented by five pairs of long (designated L1, L2, L3, L4, and L5), three pairs of medium (M6, M7, and M8) and three pairs of short (S9, S10, and S11) autosomes. The acrocentric X chromosome is similar in size to the short (S) autosomes.

Meiotic process and chiasma frequency in the wet and dry seasons

Meiosis in this species was normal and chiasmate. Prophase I and its substages, Metaphase I, Anaphase I, Metaphase II and Anaphase II stages were recorded. At Diplotene and Diakinesis bivalents with one, two or three chiasmata were present (Fig. 4 & 5).

Chiasma frequency was determined from Diplotene/Diakinesis of Prophase-I. Five cells from five individuals collected during each of the wet and dry seasons were examined. The data shown in Table 2 was therefore obtained from twenty-five cells. As expected, chiasma frequencies per cell were never below 11 or above 20. This is because chiasma frequency in a species with 11 bivalents will always fall between 11 and 23 (John and Lewis, 1973; White, 1973). In the dry season, the modal chiasma frequency was 14 and the mean chiasma frequency per cell was 15.00 ± 1.60. The modal chiasma

frequency in the wet season was 16 and the mean chiasma frequency per cell was 16.36 ± 1.77. Analysis of variance of the mean chiasma frequencies between seasons showed a significant difference. Mean chiasma frequency per cell was significantly higher (P=0.05) in the wet than dry seasons. The differences were probably because there were many more long bivalents with two or more chiasmata in the wet season than in the dry season. Seino (1989) reported a similar trend in the Acrididae species *Coryphosima stenoptera producta* (Walker) and *Chirista compta* (Walker).

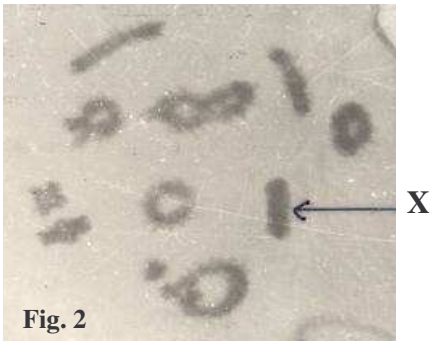
Table 1: Chromosomes lengths in *O. spissus.*

Chromosome	Chromosome length (µm) Mean ± SE	Relative length (% of 2N set)	Chromosome morphology
1	12.3 ± 0.07	14.64 ± 0.12[a]	Acrocentric
2	11.7 ± 0.91	13.93 ± 1.49[a]	Acrocentric
3	11.7 ± 0.91	13.92 ± 1.49[a]	Acrocentric
4	8.8 ± 0.02	10.47 ± 0.03[a]	Acrocentric
5	8.5 ± 0.50	10.12 ± .0.82[a]	Acrocentric
6	7.2 ± 0.90	8.57 ± 1.48[b]	Acrocentric
7	6.4 ± 1.70	7.62 ± 2.79[b]	Acrocentric
8	6.4 ± 1.70	7.62 ± 2.79[b]	Acrocentric
9	3.2 ± 0.00	3.81 ± 0.00[c]	Acrocentric
10	2.6 ± 0.00	3.09 ± 0.00[c]	Acrocentric
11	2.6 ± 0.00	3.09 ± 0.00[c]	Acrocentric
X	2.6 ± 0.00	3.09 ± 0.00[c]	Acrocentric
TOTAL	84.0 ± 0.61	-	-

Means followed by the same letters are not significantly different at 5% level of significance using the DMRT.

Table 2: Mean chiasma frequencies per cell in *O. spissus* during the wet and dry seasons.

Individual	Season	
	Wet	Dry
1	15.6 0 ± 1.52	15.00 ± 1.58
2	15.60 ± 1.67	14.60 ± 1.82
3	16.40 ± 1.14	14.60 ± 1.34
4	18.6 0 ± 3.68	15.00 ± 1.23
5	16.2 0 ± 0.84	15.8 0 ± 2.05
Mean	16.36 ± 1.77	15.00 ± 1.60



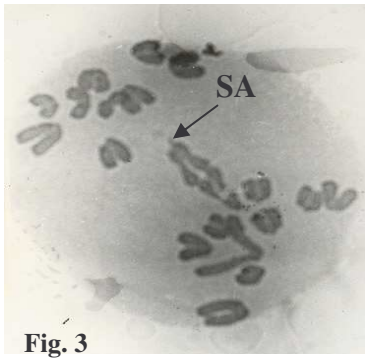


Fig. 2: Diplotene in *O. spissus*. The X- chromosome arrowed; Chiasma frequency = 15.
Fig. 3: Anaphase I in *O. spissus*. Chromosomes are V-shaped; SA = Short chromosome arm.

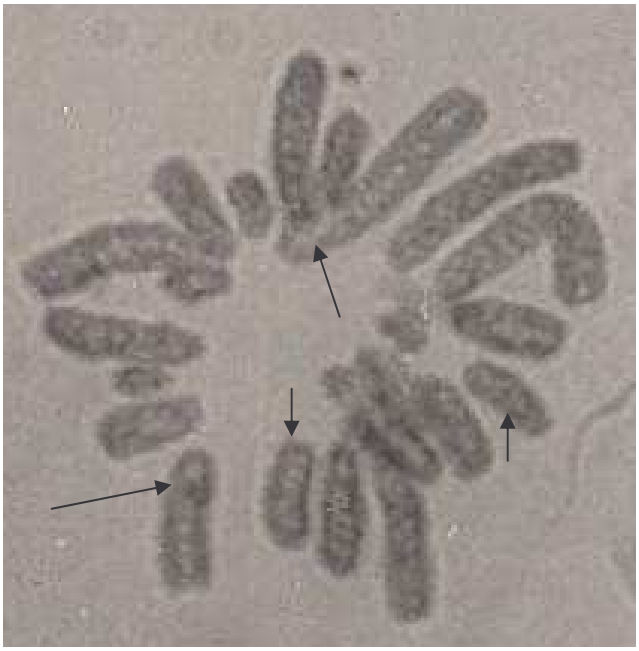


Fig. 1a: Mitotic Metaphase chromosomes in *O. spissus.* Sister chromosomes are not coiled around each other looking like c-mitotic chromosomes (short arrows). The centromeres are near terminal (long arrows). Short arms are not distinct.

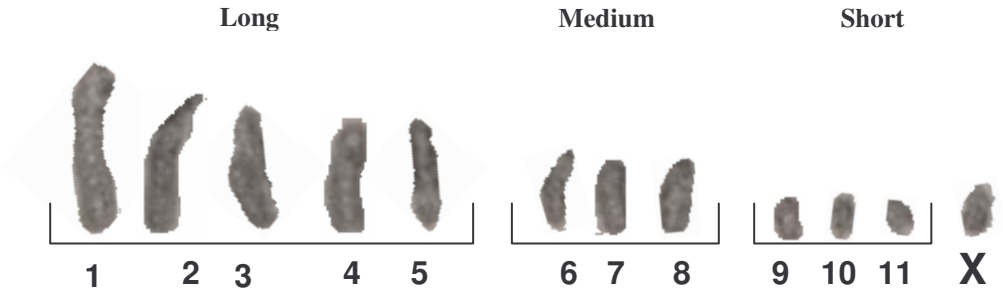


Fig. 1b: Karyotype of *O. spissus*

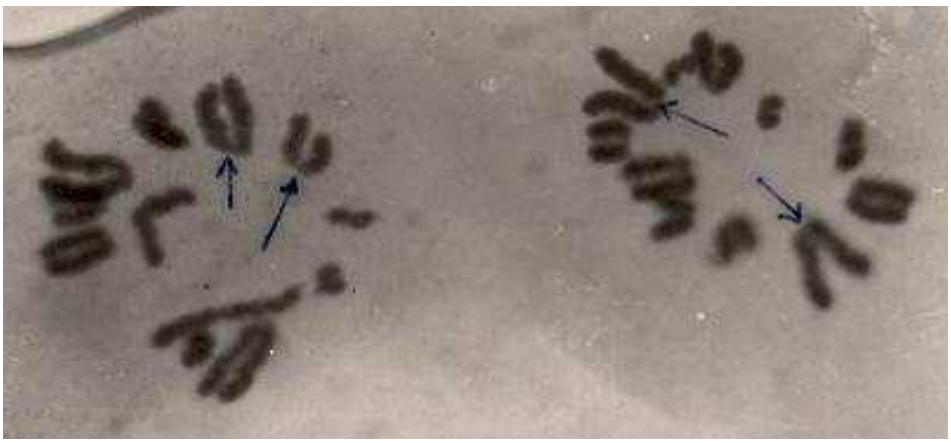


Fig. 4: Metaphase II in *O. spissus*. Chromosomes are V-shaped; Centromeres (arrowed) are toward points of convergence of chromosome arms.

Fig. 5: Anaphase II in *O. spissus*. Chromosomes are I-shaped and therefore typically acrocentric.

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