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Meiotic study of *Acrida turrita* (Linnaeus 1758), *Paracinema luculenta* Karsch 1896 and *Morphacris fasciata* (Thunberg 1815) (Orthoptera: Acrididae)

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ABSTRACT

This article is a first record on the karyotypic features of *Acrida turrita, Paracenema luculenta,* and *Morphacris fasciata* (Orthoptera: Acrididae). The lacto-propionic orcein squash technique was used to prepare chromosome smears from the testes of specimens collected in May 2009 from the premises of the Institute for Rural Development (IRAD) Batoke, Limbe, in the South West Region of Cameroon. Analysis of these preparations revealed that the three species have the basic Acrididae complement consisting of 23 acrocentric chromosomes [2n♂= 23(22A+X0)]. The autosomes could be divided into 3 size groups: long, medium and short. The number of chromosomes in the different size groups varied among the species. *A. turrita* had 2 long, 7 medium and 2 short pairs of chromosomes while *P. luculenta* and *M. fasciata* each had 6 long, 2 medium and 3 short chromosome pairs. The mean chromosome lengths in the species were 71.80 ± 0.661µm, 83.70 ± 0.39µm, and 67.10 ± 0.31µm for *A. turrita*, *P. luculenta* and *M. fasciata* respectively. The X chromosome was medium in *A. turrita* and *M. fasciata* and long in *P. luculenta*. The chromosomes in these species were acro/telocentric in morphology.

KEY WORDS: *A. turrita, P. luculenta, M. fasciata,* chromosome number, length morphology.

INTRODUCTION

Acrida turrita, Paracinema luculenta and *Morphacris fasciata* are short horn grasshoppers that belong to the family Acrididae. This large grasshopper family Acrididae is widely distributed all over the world. *Acrida turrita (Acridinae)* and *Morphacris fasciata (Oedipodinae)* span throughout Africa while *Paracinema luculenta (Oedipodinae)* is restricted to West Africa and parts of Central Africa (Dirsh, 1975; Mestre, 1988). Till date, there are no known records of chromosome investigations of these species.

Cytological studies of the known grasshopper species in the family Acrididae (Orthoptera) have revealed that over 90% of them have a characteristic karyotype of [2n ♂= 23(22A+X0)] acrocentric chromosomes (Burgrov et al., 1997; Seino, 1989; Turkoglu and Koca, 2002, Seino et al., 2007, 2008; Souza and De Melo, 2007). This remarkable uniformity is not known in any other Orthoptera family. Most species of Acrididae have 2n = 23/24(males / females) acrocentric chromosomes with the XX – XO sex chromosome mechanism and this karyotype is considered the most primitive in the family. But within the Acridoidea, most of the

families follow this tendency. A remarkable exception is Neotropical Melanoplines (Melanoplinae : Acrididae) due to the incorporation of neo-sex chromosomes by the establishment of X-Autosome Robertsonian fusions and the presence in natural populations of Autosome - Autosome Robertsonian polymorphism . In Neartic and Neotropical regions fixed centric fusions between autosomes or sex chromosomes and autosomes are numerous (Bidau and Marti, 2002, 2004; Castillo et al., 2010). Turkoglu and Koca (2002) have reported the presence of centric fusions in *Oedipoda schochi schochi* that have resulted in the reduction of chromosome number and the presence of metacentric chromosomes. Other exceptions are the result of presence of supernumerary or B- chromosomes (Burgrov et al., 1999; Camacho et al., 1997; Bugrov et al., 2003, 2004; Palestis et al., 2004).

This paper presents detailed information on the number, morphology and relative lengths of the hitherto unknown chromosomes in *A. turrita*, *P. luculenta* and *M. fasciata.* The data here presented are fundamental to increase the knowledge on the evolution and diversification of the Acrididae group of Orthoptera.

MATERIALS AND METHODS

Collection of specimens

Thirty adult males (ten for each species) from natural populations of *Acrida turrita, Paracinema luculenta* and *Morphacris fasciata* were collected in May 2009 on the premises of the Institute for Rural Development (IRAD) Batoke, Limbe, in the South West Province of Cameroon. On collection, the insects were immediately killed in ethyl ether and dissected for the testes in insect saline (0.67% NaCl) solution. The connective tissue surrounding the testicular follicles was torn off before the latter were stored in fixative (3:1 absolute alcohol – glacial acetic acid). At the Animal Ecology Laboratory of the Department of Animal Biology of the University of Dschang, Cameroon, the testes were stored in a refrigerator (at 4 °C) until used.

Preparation of chromosome smears

Chromosome preparations were made from testes using the lacto-propionic orcein squash technique described by Seino (1989) and Seino et al. (2002). Two to three testicular follicles were placed on a clean siliconized microscope glass slide and flooded with one or two drops of lacto-propionic orcein stain. The testes were next macerated using the sharp pointed end of a dissecting needle. This permitted the stain to penetrate into the tissue. A cover slip was then placed over the tissue, held in place with the thumb and forefinger before gently tapping with the wooden blunt end of a dissecting needle. The tapping forced out excess stain and helped to disperse the cells. The preparation was next wrapped in filter paper and squashed between the thumb and top of the laboratory table. The filter paper absorbed the excess stain. The chromosome preparations were preliminarily examined under the microscope. The edges of cover slip were sealed using colourless nail varnish in order to temporarily preserve good chromosome smears.

Analysis of chromosome smears

The slides were examined using the 100X oil immersion objective of the laboratory Fisher microscope. Photographs were taken with the Leitz photomicroscope using high contrast films and enlarged.

The lengths of the chromosomes were determined by direct microscope measurements using ocular and stage micrometers. Ten cells were considered from each of ten individuals per species. Individual chromosome pairs were identified on the basis of length (Stace, 1980) and chromosome morphology was determined by examining the shapes of chromosomes in meiotic anaphase – I, metaphase–II and anaphase-II (Williams and Ogunbiyi, 1995; Seino et al., 2007, 2008).

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.

RESULTS AND DISCUSSION

Chromosome numbers

The thirty male individuals of the three species analysed were each found to have 23 chromosomes per cell (Figures 1-3). These included 22 autosomes and the X or sex chromosome. They therefore exhibited the characteristic Acrididae karyotype of 2n♂= 23(22A+X0) and the basic Orthoptera sex determining mechanism of XX – XO (Bridle et al., 2002; Bugrov et al., 2001, 2002; Souza and De Melo, 2007; Seino et al., 2007, 2008).

Chromosome length and morphology

The results of morphometric measurements of metaphase chromosomes are shown in Tables 1–3. These include mean chromosome lengths, relative chromosome lengths and chromosome morphology.

Chromosome lengths

Total chromosome lengths were highest for *P. luculenta* (83.70 ± 0.39 µm) and lowest for *M. fasciata* (67.10 ± 0.31 µm). The longest chromosome (10.10 ± 0.43 µm) and the shortest chromosome (2.40 ± 0.40 µm) were found in *A. turrita*.

The chromosomes in the three species examined occurred in 3 - size groups of long, medium and short. The number of chromosomes in each size group varied among the species (Table 4). The number of long chromosomes was higher in *P. luculenta* and *M. fasciata* than in *A. turrita*. The number of medium sized chromosomes was higher in *A. turrita* than in the other two species. The number of short chromosomes was higher in *P. luculenta* and *M. fasciata* than in *A. turrita*. The X chromosome was long in *P. luculenta* and medium in *A. turrita* and *M. fasciata*. Chromosomes in the karyotypes of Orthoptera grasshoppers characteristically occur in three size groups of long, medium and short (Shaw, 1976; Bugrov and Sergeev, 1997; Seino et al., 2007, 2008). However, the number of chromosomes per size group has not been shown to be characteristic of the family, subfamily, genus or species as was the case in the present study.

Chromosome morphology

Short arms were not distinct in the metaphase chromosomes examined (Figures 1–3). Therefore, the chromosomes in the species were definitely not metacentric or submetacentric in morphology. Examination of anaphase –I (Figure 4) and metaphase –II (Figure 5) in the meiotic process of these species showed that the chromosomes were V-shaped and made up of two visible chromatids. Chromosomes in anaphase –II were I-shaped (Figure 6).

Table 1: *Acrida turrita* chromosomes morphometric measurements.

Chromosome	Chromosome length (µm) Mean ± SE	Relative length (% of 2N set)	Chromosome morphology
1	10.10 ± 0.43	14.06 ± 0.24[a]	Acrocentric
2	10.10 ± 1.33	14.06 ± 0.24[a]	Acrocentric
3	9.40 ± 1.70	13.10 ± 0.18[a]	Acrocentric
4	9.40 ± 1.70	13.10 ± 0.18[a]	Acrocentric
5	5.00 ± 0.58	6.96 ± 0.08[b]	Acrocentric
6	5.00 ± 0.58	6.96 ± 0.08[b]	Acrocentric
7	5.00 ± 0.58	6.96 ± 0.08[b]	Acrocentric
8	5.00 ± 0.58	6.96 ± 0.08[b]	Acrocentric
9	5.00 ± 0.58	6.96 ± 0.08[b]	Acrocentric
10	2.40 ± 0.40	3.34 ± 0.00[c]	Acrocentric
11	2.40 ± 0.40	3.34 ± 0.00[c]	Acrocentric
X	5.00 ± 0.08	6.96 ± 0.08[b]	Acrocentric
TOTAL	**71.80 ± 0.66**	-	-

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

Table 2: *Paracinema luculenta* chromosome morphometric measurements.

Chromosome	Chromosome length (µm) Mean ± SE	Relative length (% of 2N set)	Chromosome morphology
1	9.90 ± 0.28	11.83 ± 0.52[a]	Acrocentric
2	9.30 ± 0.63	11.11 ± 0.25[a]	Acrocentric
3	9.00 ± 0.50	10.75 ± 0.18[a]	Acrocentric
4	9.00 ± 0.50	10.75 ± 0.17[a]	Acrocentric
5	8.70 ± 0.16	10.39 ± 0.30[a]	Acrocentric
6	8.60 ± 0.19	10.39 ± 0.21[a]	Acrocentric
7	6.00 ± 0.59	7.17 ± 0.02[b]	Acrocentric
8	6.00 ± 0.92	7.17 ± 0.00[b]	Acrocentric
9	3.20 ± 0.27	3.82 ± 0.00[c]	Acrocentric
10	3.10 ± 0.26	3.70 ± 0.00[c]	Acrocentric
11	3.10 ± 0.26	3.70 ± 0.00[c]	Acrocentric
X	7.70 ± 0.59	9.20 ± 0.14[a]	Acrocentric
TOTAL	**83.70 ± 0.39**	**-**	**-**

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

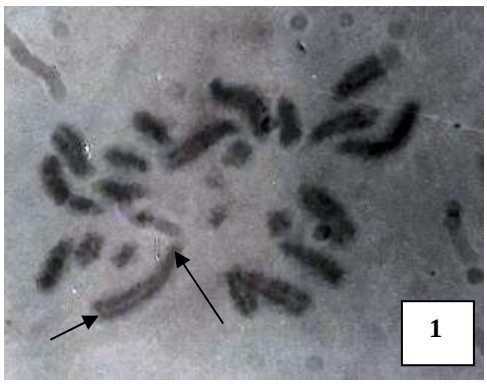
Table 3: *Morphacris fasciata* chromosomes morphometric measurements.

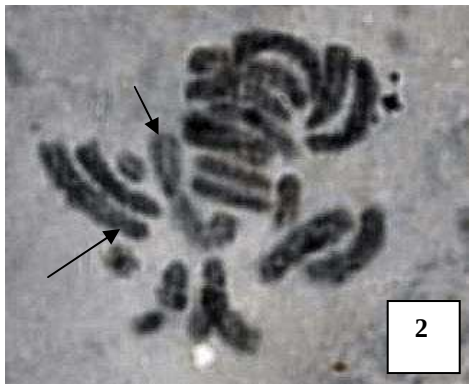
Chromosome	Chromosome length (µm) Mean ± SE	Relative length (% of 2N set)	Chromosome morphology
1	7.90 ± 0.39	11.77 ± 0.07[a]	Acrocentric
2	7.40 ± 0.85	11.03 ± 0.05[a]	Acrocentric
3	7.00 ± 0.44	10.43 ± 0.21[a]	Acrocentric
4	7.00 ± 0.57	10.43 ± 0.25[a]	Acrocentric
5	6.90 ± 0.55	10.28 ± 0.19[a]	Acrocentric
6	6.90 ± 0.55	10.28 ± 0.19[a]	Acrocentric
7	5.20 ± 0.24	7.70 ± 0.00[b]	Acrocentric
8	5.20 ± 0.24	7.70 ± 0.00[b]	Acrocentric
9	2.70 ± 0.00	4.02 ± 0.00[c]	Acrocentric
10	2.70 ± 0.00	4.02 ± 0.00[c]	Acrocentric
11	2.70 ± 0.00	4.02 ± 0.00[c]	Acrocentric
X	5.50 ± 0.24	8.20 ± 0.00[b]	Acrocentric
TOTAL	**67.10 ± 0.31**	**-**	**-**

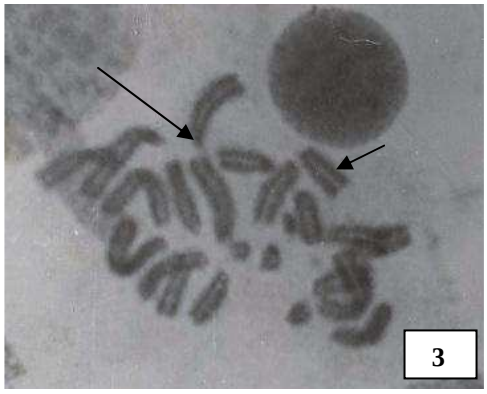
Means followed by the same letters are not significantly different at 5% level of significance using the DMRT.

Table 4: Distribution of chromosomes by size group in the species studied.

Species	N° of Long chromosomes pairs	N° of medium chromosomes pairs	N° of short chromosomes pairs
A. turrita	4	5 & X	2
P. luculenta	6 & X	2	3
M. fasciata	6	2 & X	3







Figures 1 – 3: Acrida. *turrita* (1), *Paracinema luculenta* (2) and *Morphacris fasciata* (3) spermatogonial metaphase. Sister chromatids are not coiled around each other looking like C- mitotic chromosomes (long arrows). The centeromeres are near terminal (short arrows) with no evident short arms.

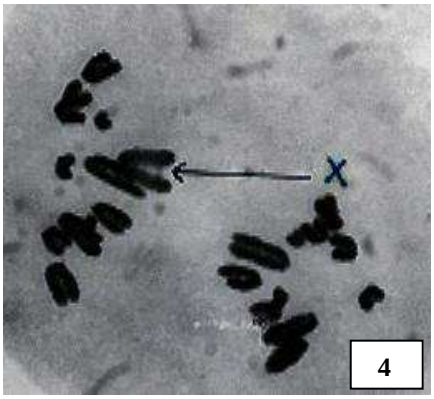


Figure 4: Anaphase –I in *A. turrita*. Dyads are made up of two chromatids held together towards one end by centeromeres. The V-shape indicates acro/telocentric chromosomes.

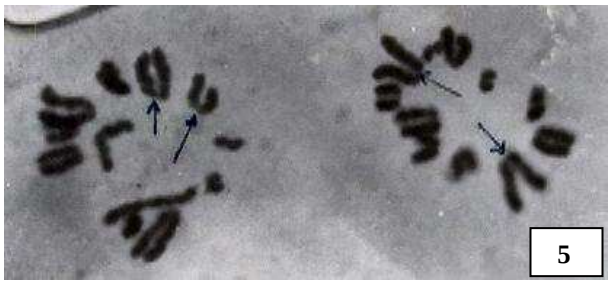


Figure 5: Metaphase –II in *P. luculenta* (Polar view). Dyads with two arms and are V-shaped indicating acro/telocentric chromosomes.

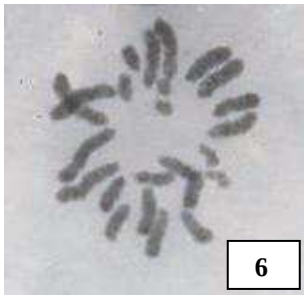


Figure 6: Anaphase -II in *M. fasciata* (Polar view). All chromosomes are one stranded confirming that they are acro/telocentric chromosomes.

These indicated that the chromosomes in these three species were acrocentric or telocentric. This is therefore a confirmation that the standard chromosomes in the Acrididae are characteristically acro/ telocentric in morphology (White, 1973; Shaw; 1976; Hewitt, 1979; Seino, 1989; Williams and Ogunbiyi, 1995; Bugrov and Sergeev, 1997; Turkoglu and Koca, 2002; Seino et al., 2007, 2008).

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