International Journal of Applied Bioresearch

A study on the karyotype of *Spathosternum pygmaeum* Karsch 1893 (Orthoptera: Acrididae: Spathosterninae) from Cameroon

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Published: 15 April, 2012; Vol. No.6:1-7; Online: www.bioresjournal.com/documents/ijab0018

Chromosome analyses were performed in *Spathosternum pygmaeum* Karsh (1893) using standard staining procedures. Chromosomes with detailed information on number, length, relative length, meiosis and chiasma formation are discussed. *S. pygmaeum* presented a diploid number of 2n = 23(XO) in males and the karyotype was made up of exclusively acrocentric chromosomes. The chromosomes occurred in size groups of long (Chromosome pairs 1, 2, 3, & 4), medium (chromosome pairs 5, 6, 7, 8, & 9) and short (Chromosome pairs 10 & 11). The X-chromosome was acrocentric and short. Meiosis in the species was normal and chiasmate. Analyses of Diplotene / Diakenesis sub-stages of Prophase-1 revealed a mean chiasma frequency of 16.5 ± 0.99 per cell. Bivalents with 1, 2 and 3 chiasmata were recorded. Bivalents with 1 chiasma contributed least to cell chiasma frequency while bivalents with 3 chiasmata contributed most to cell chiasma frequency.

Chromosome /Cytology

The subfamily Spathosterninae Rehn, 1957 belongs to the order Orthoptera, Sub –order Calefera and family Acrididae. Spathosterninae Rehn, 1957 is the smallest sub-family within the Acrididae. It is made up of 5 genera and 9 known species amongst which is *Spathosternum pygmaeum* (Mestre, 1988, Mestre and Chiffaud, 2006). The Spathosterninae are widely distributed in West and Central Africa and are well known in South Asia and Australia (Dirsh, 1975; Mestre, 1988; Seino, 1989; Mestre and Chiffaud, 2006; OSF, 2011).

Spathosternum pygmaeum is a small grasshopper with a light brown dorsal surface. The head bears two filiform antennae. The femur and tibia of the hind limbs are pale brown with a black band occurring where they meet. The fore-wings are brown, with black dots interspersed along the edges. The species is terrestrial and herbivorous (Seino, 1989; OSF, 2011).

This article is IJAB direct Email Submission.
Freely available on online through the IJAB open access www.bioresjournal.com.

Received: March, 14, 2012
Accepted: March,30,2012

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This article contains supporting information online at www.bioresjournal.com/documents/ ijab0018

Information regarding the genomic structure of the short-horned grasshoppers of the family Acrididae has been largely on the sub-family Acridinae probably because most members of this sub-family are amongst the worst pests grasshoppers ever known to man. The Acrididae are characterised by a conservative chromosome number of 2n= 23 acrocentric chromosomes in male individuals. Meiosis in most members of the family is normal and chiasmate. Information concerning the genomic structure of members of the sub-family Spathosterninae (Acrididae) is rare because they are not pest of agricultural importance. Available literature revealed that only *S. prasiniferum* (from Asia and India) has had its diploid number described to comprise 2n = 23, XO in males and 2n= 24, XX in females (Yao, 2006; Chadha and Mehta, 2011). The karyotype comprised of exclusively acrocentric chromosomes and the sex determining mechanism is XO.

There is therefore need to cytogenetically characterise *S. pygmaeum* since the information obtained should accumulate data for the family Acrididae and could be useful in their cytotaxonomy and evolution. The present study describes the mitotic and meiotic chromosomes in adult males of *Spathosternum pygmaeum* and establishes the karyotype and meiotic process in the species.

Material and Methods

Biological material

Adult grasshoppers used for this study were collected on the campus of the University of Dschang in May 2010. They were identified by comparing them to specimens found in the collections of one of the authors (Seino R.A.).

Cytogenetic study

The grasshoppers were not treated with colchicine in order to avoid possible distortion of the meiotic process (Tepperberg *et. al.*, 1997). Chromosome smears were made from testes using the lacto-propionic-orcein squash technique described by Seino *et. al.* (2007 & 2008). Two to three testicular follicles were placed on a clean siliconised microscope glass slide and flooded with one or two drops of 2% 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 blunt wooden 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 smears so prepared were preliminarily examined under the microscope.

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. After sufficient photographs were taken and the films developed and checked, the slides were discarded. Photographs of mitotic metaphases were scanned and processed using the Microsoft Office Picture Manager. They were next cut out, paired up according to length before arranging them into karyotypes.
The lengths of the chromosomes were determined by direct microscope measurements using ocular and stage micrometers. Five cells were considered from each of the ten individuals used. 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 stages (Williams and Ogunbiyi, 1995; Seino *et. al.*, 2002).

Statistical analysis

The data on relative chromosome lengths 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

Chromosome number and morphology

Though the grasshoppers were not treated with colchicine, individual chromosomes could be easily identified, counted and measured in a polar view of mitotic metaphase. Mitotic metaphase smears of *S. pygmaeum* reveled 23 rod-shaped chromosomes indicating a karyotype of 2n=23 X0 in the male. This was confirmed by the presence of 11 bivalents and one univalent in Diplotene of Prophase-I and Metaphase-I smears (Figs. 2 & 3). The mitotic Metaphase chromosomes appeared to be in C -

metaphase since there was no relational coiling between sister-chromatids. The sister chromatids separated gradually from a tapered end towards the other end. The two chromatids of each chromosome were therefore not coiled around each other looking like C- mitotic chromosomes. Centromeres were not distinct in these chromosomes smears but were inferred to be in the tapered terminal regions where sister chromatids were in close contact. Short chromosome arms were not also clearly visible in the mitotic smears. All the chromosomes in Anaphase-I were V-shaped and had two arms. Each arm was single stranded (Fig. 4). In Metaphase-II, the chromosomes were V-shaped and had two arms each, each of which was single stranded. In Anaphase-II, the chromosomes were I-Shaped and single stranded (Fig. 5). By the standards of Williams and Ogunbiyi (1995) and Seino *et. al*. (2008), the chromosomes in *Spathosternum pygmaeum* could be considered to be acrocentric in morphology.

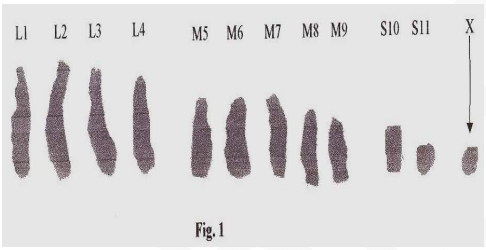

Fig. 1: Karyotype of *S. pygmaeum*

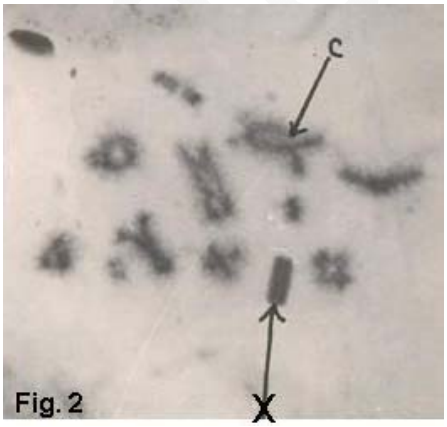

Fig.2: Diplotene in *S.* pygmaeum. The X-chromosome is positively heteropycnotic and C represents the position of a chiasma.

Chromosome length

Total chromosome length for a diploid set in this species was 162.7+ 0.91µm. The individual chromosomes were not of equal lengths. Table 1 revealed that the lengths of individual chromosomes ranged from 12.3µm to 1.7µm. The chromosomes could therefore be separated into distinct size groups of long, medium and short. In this species were found 4 long chromosomes (L1, L2, L3, and L4), 5 medium chromosomes (M5, M6, M7, M8, and M9) and 2 short chromosomes (S10 and S11), and the X-chromosome was also short (Fig. 1). Long chromosomes had mean lengths ranging from 8.4+0.53µm to 12.3+0.03µm. Medium chromosomes had mean lengths ranging from 5.7+1.41µm to 7.7+0.78µm while short chromosomes had mean lengths ranging from 1.7+00µm to 2.7+0.0µm. The X-chromosome had a mean length of 1.7+0.0µm.

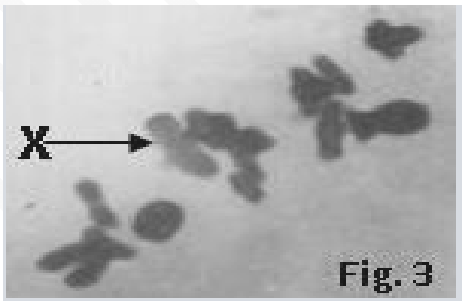

Fig 3: Metaphase-I in *S.* Pygmaeum (side view). The X-chromosome is negatively heteropycnotic

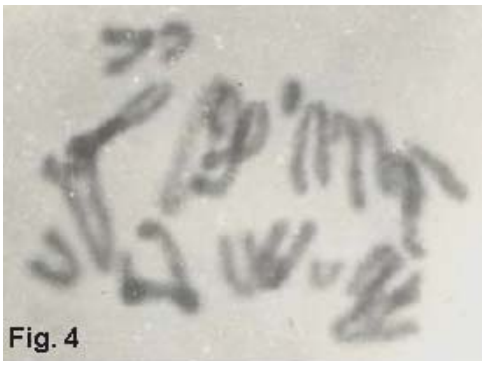

Fig. 4: Anaphase- I in *S. pygmaeum*. Dyads have two chromatids held together at one end by centromeres. The V-shape indicates acrocentric morphology.

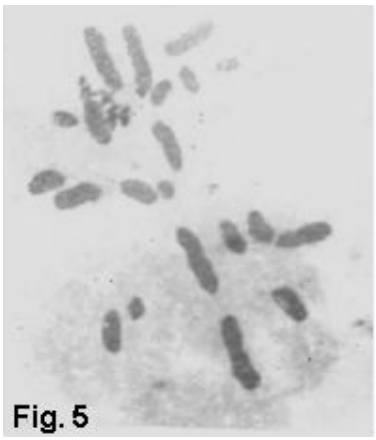

Fig.5: Anaphase-II in *S. pygmaeum*. Chromosomes are I-shaped and therefore typically acrocentric in morphology

Meiotic process

The following meiotic stages and sub stages were recorded in *S. pygmaeum* during this study: Prophase-I (Zygotene, Pachytene, Diplotene, & Diakinesis), Metaphase-I, Anaphase-I, Metaphase-II and Anaphase-II. Chiasmata were visible in late Prophase-I and Metaphase-I. Anaphase- I and II separations were normal with no precocious movements of any chromosome. These observations indicated that meiosis in *Spathosternum pygmaeum* was normal and chiasmate.

Heteropycnosis of the X-chromosome

It was also observed that the X - chromosome stained darker than the autosomes in Prophase-I (Fig.2) and was therefore positively heteropycnotic, while in Metaphase-I (Fig. 3) and Anaphase-I (Fig. 4), the X chromosome stained lighter than the autosomes indicating that it was negatively heteropycnotic. By the standard of Seino (1989), the X chromosome in *Spathosternum pygmaeum* could therefore be said to exhibit the reversal type of heteropycnosis.

Chiasma frequency

Chiasma frequency per cell varied from 13 to 18. The modal chiasma frequency per cell was 17 while the mean chiasma frequency per cell was 16.5+0.99. Bivalents with 1, 2 or 3 chiasmata were recorded and they contributed differently to cell chiasma frequency. Bivalents with one chiasma contributed most (51.2%) to cell chiasma frequency while bivalents with three chiasmata contributed least (11.0%) to cell chiasma frequency (Table 2). Also, long, medium and short bivalents with three chiasmata contributed differently to cell chiasma frequency. As expected, long bivalent contributed most (60.2%) while short bivalents contributed least (13.3%) to cell chiasma frequency (Table 3).

Chiasma frequency in *Spathosternum pygmaeum* was also observed to vary with season. In the dry season, chiasma frequency per cell ranged from 14 to 18 with a modal frequency of 16 while in the wet season chiasma frequency per cell ranged from 13 to 18 with a modal frequency of 16. Mean chiasma in the individuals investigated are shown in Table 4. Individual means ranged from 15.4 ± 1.4 to 17.4 ± 0.55 in the dry season and 16.2 ± 0.58 to 17.0 ± 0.71 in the wet season. Mean chiasma frequency per cell was found to be slightly higher in the wet (16.56±) than in the dry season (16.52±). However, Student's t-test analysis of the data revealed that mean chiasma frequencies were not significantly different (P=0.05) in the dry and wet seasons.

Discussion

The results revealed that *Spathosternum pygmaeum* had a karyotype of 23 acrocentric chromosomes in male individuals and the sex determining mechanism was XO. The karyotype consisted of four long (L1, L2, L3, L4) five medium (M5, M6, M7, M8, M9) and two short (S10, S11) chromosomes. The X chromosome was short. The karyotype of 2n=23 acrocentric chromosomes in male individuals of S. pygmaeum is in agreement with that previously described for the family Acrididae. This standard karyotype has been variously reported for Acrididae such as *Acrotylus* (Camacho & Cabrero, 1983), *Melanopus senguinipes (*Zhan *et. al.*, 1984), *Podisma pedestris* (Westerman & Hewitt, 1985), the genus *Podisma* (Bugrov *et. al.*, 1994), *Eyprepocnemis plorans* Charp (Bugrov *et. al.*, 1999), *Podisma Sapporensis* Shir (Bugrov *et. al.*, 2001), *Oedipoda schochi schochi* and *Acrotylus insbricus* (Turkoglu & Koca, 2002), *Oxycatantops spissus* Walker (Seino *et. al.*, 2008), *Stethophyma grossum*, (Viera *et. al.*, 2010) *Acrida turrita, A. exaltata,*

Phaeoba infumata & P. antennata (Chadha & Mehta, 2011), *Orthoscapheus rufipes* and *Eujivarus fusiformis* (Rocha *et. al.*, 2011) . This contention is further substantiated by the chromosome occurring in the size groups of long, medium and short, which is consistent with the chromosomes of several Acrididae already studied (Shaw, 1976: Bugrov & Segreev, 1997; Bugrov & Warchalowska-Sliwa, 1997; Bugrov *et. al.*, 1999; Turkoglu and Koca, 2002; Warchalowska-Sliwa *et. al.*, 2002; Seino *et. al.*, 2008). Therefore the standard karyotype of *Spathosternum pygmaeum* from Dschang in Cameroon can be said to consist of 2n=23 acrocentric chromosomes in the male with the XX-XO sex mechanism. The X - chromosome is similar in size to the short (S) autosomes.

Table -1: Relative chromosomes lengths in *S. pygmaeum*

Chromosome	Chromosome length (µm) mean + SE	Relative length (% of 2N set)	Chromosome morphology
1.	12.3 ± 0.03	15.47[a]	Acrocentric
2.	11.9 ± 0.08	14.97[a]	Acrocentric
3.	9.0 ± 0.21	11.32[a]	Acrocentric
4.	8.4 ± 0.53	10.57[a]	Acrocentric
5.	7.7 ± 0.78	09.69[b]	Acrocentric
6.	7.7 ± 0.78	09.69[b]	Acrocentric
7.	6.5 ± 1.27	08.18[b]	Acrocentric
8.	5.9 ± 1.58	07.42[b]	Acrocentric
9.	5.7 ± 1.41	07.17[b]	Acrocentric
10.	2.7 ± 0.00	03.39[c]	Acrocentric
11.	1.7 ± 0.00	02.14[c]	Acrocentric
Total	79. 5 ± 0.91	100.00	-
X	1.7 ± 0.00		Acrocentric

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

Table - 2: Percentage contribution of bivalents with 1, 2, & 3 chiasmata to the mean cell chiasma frequency in *S. pygmaeum.*

	Bivalents with one chiasma	Bivalents with two chiasmata	Bivalents with three chiasmata
No of chiasmata per cell	8.4	6.2	1.8
% contribution to chiasma frequency	51.2	37.8	11.0
Mean cell Chiasma frequency	16.4		

Table -3: Percentage contribution of long, medium & short bivalents to mean cell chiasma frequency in *S. pygmaeum.*

	Long Bivalents	Medium Bivalents	Short Bivalents
No of chiasmata per cell	10	4.0	2.2
% contribution to chiasma frequency	60.2	24.1	13.3
X		16.4	

Table -4: Mean cell chiasma frequency in *S. pygmaeum* during the wet and dry seasons.

Individual	Season	
	Dry	Wet
1.	15.40 ± 1.14	16.60 ± 0.89
2.	16.80 ± 1.58	17.00 ± 0.71
3.	16.60 ± 0.81	16.80 ± 0.45
4.	17.4 0 ± 0.55	16.20 ± 1.79
5.	16.52 ± 0.89	16.20 ± 0.56
6.	16.4 0± 0.81	16.56 ± 0.89
7.	16.20 ± 0.58	17.00 ± 1.23
8.	17.40 ± 0.55	17.00 ± 0.84
9.	16.00 ± 0.31	16.60 ± 1.14
10.	16.20 ± 0.58	16.80 ± 1.36
Mean	16.49 ± 1.14	16.67 ± 0.89

As concerns the meiotic process in the species, the following facts are noted from the results: Chiasmata were formed in prophase-I of meiosis in *Spathosternum pygmaeum*. In Diplotene and Diakinesis, bivalents with one, two or three chiasmata were recorded. Chiasma frequencies were never below 11 or above 20 which could be judged as normal, since White (1973) observed that chiasma frequency in Orthoptera species with 11 bivalents will always fall between 11 and 23. Therefore meiosis in *Spathosternum pygmaeum* can be judged to be normal and chiasmate and typical of the Orthoptera insects. Long bivalents formed two or more chiasmata while short bivalents formed invariably one chiasma. Chiasma formation was directly proportional to lengths of bivalents. This confirms the assertion of Henderson (1963) and Seino (1989) that chiasma formation is positively correlated to chromosome length. Bivalents with more than two chiasmata contributed most to cell chiasma frequency. Chiasma frequency was not significantly different in the dry and wet seasons. Similar trends have been reported in some Acrididae notably *Acrida turrita* Linnaeus and *Paracinema luculenta* Karsh (Seino, 1989; Seino & Akongnui, 2010). The X-chromosome stained darker than the autosomes in prophase-I and stained lighter than the autosomes in metaphase-I. Under the conditions of the present study, the meiotic process in *Spathosternum pygmaeum* was normal and chiasmate.

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