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Karyotype analysis and meiosis in *Coryphosima stenoptera producta* (Walker) and *Chirista compta* (Walker) (Orthoptera: Acrididae: Acridinae) from Cameroon

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Abstract

This article describes the hitherto unknown karyotypes of the short-horned grasshoppers C*oryphosima stenoptera producta* (Walker) and C*hirista compta* (Walker) that belong to the family Acrididae and subfamily Acridinae of the order Orthoptera. Chromosomes smears prepared by the lactic-propionic-orcein squash technique and chromosome analysis performed in the two species revealed a conserved karyotype of 2N= 23, XO in males composed exclusively of acrocentric chromosomes. The chromosomes occurred in size groups of long (L), medium (M) and short (S). In *C. stenoptera producta* were 2LL + 6MM + 3SS chromosomes whereas in *C. compta* were 4LL + 4MM + 3SS chromosomes. The X chromosome was medium in size in both species with mean lengths of 5.60µm ± 0.56 in *C. s. producta* and 7.3 µm ± 0.52 in *C. compta*. The mean chiasma frequency was found to be 12.20± 0.77 and 16.20 ± 0.72 in *C. stenoptera producta* and C. *compta* respectively. The significantly lower chiasma frequency found in *C. stenoptera producta* could have been due to the consistent absence of bivalents with 3 or more chiasmata in this population. Bivalents with 1 chiasma contributed most to cell chiasma frequency in *C. stenoptera producta*, while bivalents with 2 chiasmata contributed most to cell chiasma frequency in *C. compta*. This article aims to offer some basal data for the cytotaxonomy of the Orthoptera.

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Introduction

The Orthoptera grasshoppers *Coryphosima stenoptera producta* (walker) and *Chirista compta* (walker) are African grasshoppers that belong to the subfamily Acridinae in the family Acrididae. The Acrididae constitute one of the largest families within the Superfamily Acridoidea, forming a cosmopolitan group and one of the richest in species number within the Orthoptera. The Acrididae comprise eleven subfamilies and over 218 described species of West African distribution. The Acridinae is a highly diverse subfamily that comprises over 38 described species belonging to 20 genera spread widely in West and Central Africa (Mestre and Chiffaud (2006).

Several representatives of the subfamily Acridinae are cytologically characterized to date. The karyotype (chromosome number and morphology) in the family Acrididae are highly conserved predominantly presenting a diploid complement of 2N= 23, XO and 2N= 24, XX acrocentric chromosomes in males and females respectively (White, 1973; Mesa *et al.,* 1982; Seino, 1989; Bugrov, 1996; Bugrov *et al.*, 2002; Bridle *et al.*, 2002; Turkoglu and Koca, 2002; Rocha *et al.*, 2004; Souza & de Melo, 2007, Seino *et al.,* 2007 & 2008). This chromosome number is considered to have come from that of 2N=19 (Pyrgomorphidae and Pamphagidae), the additional chromosomes being acquired in the course of evolution (Hewitt, 1979). Meiosis in the Acrididae is normal, chiasmate and chiasma frequency always fall between 11 and 23 since they usually have 11 bivalents (White, 1973, Seino, 1989)

Cytogenetic studies on the genera *Coryphosima* and *Chirista* are rare and this paper presents a pioneer description of the karyotypes of *C. stenoptera producta* and *C. compta*. This paper also offers some basal data to the cytotaxonomy of the Orthoptera. It describes the karyotypes (chromosome numbers, morphology), chromosome lengths, chiasma formation and discusses the relationship between chromosome length and chiasma frequency in *C. stenoptera producta* and *C. compta*.

Materials and methods

Five adult individuals of each species used in this study were collected on the Campus of the University of Dschang in the West Region of Cameroon in May 2012. On capture, the insects were immediately killed with chloroform fumes and dissected in insect saline (0.68% NaCl) for the testes. The testes were then placed (fixed) in 3:1 ethanol: acetic acid fixative and stored in the refrigerator at 4°C until used.

Chromosome preparation

Chromosome smears were prepared using the Lactic-Propionic-Orcein squash technique (Seino *et al.,* 2010). Two to three testicular follicles were placed on a clean microscope glass slide. They were first flooded with 45% acetic acid for five minutes. This made the cells to swell. After blotting off the acid, the tissue was next flooded with one or two drops of lactic-Propionic-Orcein stain and macerated using the sharp pointed end of a dissecting needle. This permitted the stain to penetrate into the tissue. The preparations were then incubated at room temperature 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 enabled the cells to disperse 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.

Microscopical examination and photography

The chromosome smears thus prepared were examined using the Fisher laboratory microscope. Slides were initially scanned under a 10X objective and nuclei of interest further examined under a high power objective 40X.

Chromosome morphology was determined by examining the shapes of chromosome in meiotic Anaphase-I, Metaphase-II and Anaphase-II and then classified as per the criteria of Williams and Ogunbiyi, (1995) and Seino *et al.,* (2012). The number of chiasmata in five cells per individual was scored from cells at Diplotene / Diakinesis for five individuals of each species.

Table 1. Chromosome length, Relative Chromosome Length (RCL), chromosome size groups in *C. s. Product.*

	Chromosome pair											
	1	2	3	4	5	6	7	8	9	10	11	X
Chromosome length	10.70 ± 0.40	9.80 ± 0.67	6.00 ± 1.60	5.90 ± 0.52	5.80 ± 0.53	5.80 ± 0.53	5.70 ± 0.57	5.60 ± 0.56	2.90 ± 0.00	2.70 ± 0.00	2.70 ± 0.00	5.60 ± 0.56
RCL	16.82	15.41	9.43	9.28	9.12	9.12	8.96	8.81	4.56	4.25	4.25	8.09
Size groups	Large				Medium					Small		Medium
Mean chromosome length	10.25				5.80					2.77		-

RCL is calculated as a percent of the haploid chromosome set.

Table 2. Chromosome length, Relative Chromosome Length (RCL), chromosome size groups in *C. Compta*.

	Chromosome pair											
	1	2	3	4	5	6	7	8	9	10	11	X
Chromosome length	12.20 ± 0.00	11.90 ± 0.37	11.90 ± 0.37	11.90 ± 0.37	7.80 ± 0.34	7.50 ± 0.35	7.30 ± 0.52	6.80 ± 0.74	2.80 ± 0.11	2.70 ± 0.11	2.70 ± 0.00	7.3 ± 0.52
RCL	14.26	13.92	13.92	13.92	9.12	8.77	8.50	7.95	3.28	3.16	3.16	7.87
Size groups	Large					Medium				Small		Medium
Mean chromosome length	11.98					7.35				2.73		-

RCL is calculated as a percent of the haploid chromosome set.

Photographs were taken with the Lietz photomicroscope using the oil immersion lens, 100X and immersion oil was applied to the preparation. After sufficient photographs were taken and the film 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 into karyotypes.

Measurements and calculations

The lengths of the chromosomes were measured directly from the microscope using ocular and stage micrometer. Five cells were considered from each of ten individuals examined. Individual chromosome pairs were identified on the basis of length (Cody and Jeffrey, 2006).

Relative chromosome length (RCL) is the length of each chromosome expressed as a percentage of the total haploid autosome length in the nucleus (Paris Conference, 1971). This was calculated by adding the lengths of all the autosomes in one nucleus together, then dividing by 2 because they are paired, to obtain the total haploid length. Then each chromosome length is divided by the total haploid length and multiplied by 100 to gain a percentage result.

Table 3. Mean chiasma frequency per cell in five individuals each of *C. stenoptera producta* and *C. compta*. Five cells were scored for each individual studied;

Species	Chromosome pair						
	1	2	3	4	5	Total	Mean
C .s. producta	12.20 ± 1.09	13.00 ± 1.0	12.40 ± 0.55	11.80 ± 1.30	11.60 ± 0.55	61	12.20 ± 0.77
C .compta	16.40 ± 0.89	17.20 ± 0.84	15.80 ± 0.84	15.40 ± 0.55	16.20 ± 0.45	81	16.20 ± 0.72

Table 4. Percentage contribution of long, medium and short chromosomes to mean cell chiasma frequency in *C. stenoptera producta* and *C. compta*; X= mean number of chiasmata calculated from twenty-five cells.

Species	Mean chiasma frequency	Long Bivalents		Medium Bivalent		Shorts	
		X	%	X	%	X	%
C. s. producta	12.20 ± 1.09	2.37	19.40	8.02	65.70	1.81	14.90
C .compta	16.40 ± 0.89	9.850	60.80	3.03	18.70	3.32	20.50

The RCL were also subjected to the Duncan's Multiple Range test, (DMRT) (Clewer and Scarisbrick, 2001) so as to separate the chromosomes into size groups of long, medium and short, a characteristic of Orthoptera species. No attempt was made to determine minor chromosomal variations between individuals.

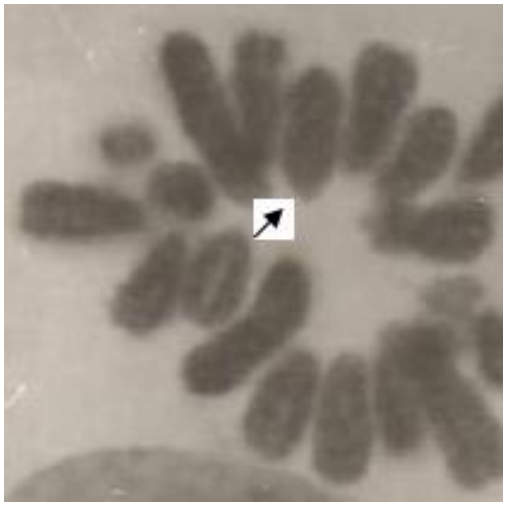

Fig. 1. Mitotic chromosmes in *C. Stenoptera producta*. Sister chromatids are coiled around each other looking like C-mitotic chromosomes. Centromeres are near terminal (arrow).

Mean chiasma frequencies were determined for the data obtained and these means were next subjected to the Student's t – test, the purpose of which was to determine if chiasma frequencies were different between the two species.

Results

Mitotic chromosomes of the two species are shown in Figs 1 and 2. Meiotic preparations are shown in Figs 3. Actual and Relative Chromosome Lengths (RCL) are given in Table 1 & 2, while chiasma frequencies in Tables 3 & 4.

Chromosome number and morphology

The analysed individuals revealed that both species studied have a chromosome number of 2n=23 (22A+X) (FN=32) and the basic Orthoptera sex determining mechanism, XX/XO.

In each of the two species the chromosomes were rod-shaped and the sister chromatids separated gradually from a tapered end towards the other end. Centromeres and short arms were not distinct in these mitotic chromosomes but the centromeres were inferred to be in the tapered terminal regions where sister chromatids were in close contact (Fig. 1 & 2); hence the chromosomes were acrocentric in morphology. Examination of Anaphase I chromosomes (Fig. 3c) revealed that chromosomes were V-shaped and some of the long chromosomes revealed minute short arms. This confirmed that the

chromosomes in these species were acrocentric and not telocentric in morphology. To further confirm that the chromosomes were acrocentric in morphology, Anaphase II chromosomes (Fig. 3d) were single stranded and appeared I-shaped.

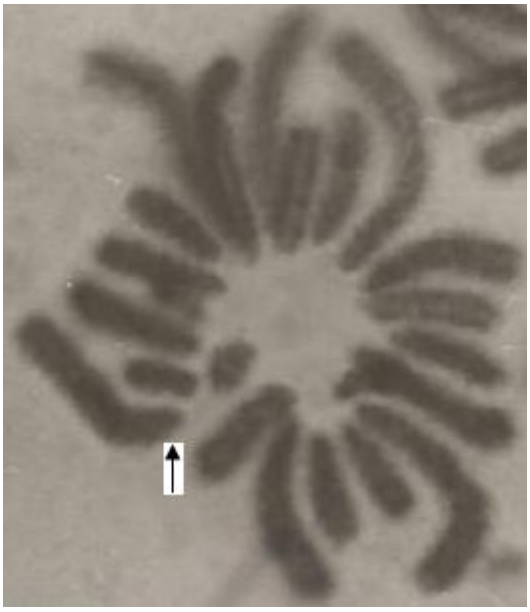

Fig. 2. Mitotic chromosmes in *C. compta*. Sister chromatids are coiled around each other looking like C-mitotic chromosomes. Centromeres are near terminal (arrow).

Chromosome length and Relative Chromosome Length (RCL)

The lengths of chromosomes and RCL obtained for *C. s. producta* are shown in Table 1. In this species, the lengths of the chromosomes ranged from 10.70 µm to 2.70 µm with a total haploid length of 63.60 µm. The RCLs (Table 1) were used to construct the graphs in Fig. 4 and the graph revealed the chromosomes to occur in size groups of large, medium and small. The length of the 2 long pairs of chromosomes 1& 2 ranged from 10.70µm ± 0.4 to 9.80µm ± 0.67, the 6 pairs of medium chromosomes 3 to 8 ranged from 6.00µm ± 1.6 to 5.60µm ± 0.56 , while the mean length of the 3 pairs of short chromosomes 9 to 11 ranged from 2.90µm ± 0.0 to 2.70µm ± 0.0.The mean chromosome length per size group was 10.25 µm for the large chromosomes, 5.80 µm for the medium chromosomes and 2.77 µm for the small chromosomes. DMRT revealed the large chromosomes to be significantly longer (P<0.05) than medium chromosomes and the medium chromosomes to be significantly longer (P<0.05) than the small chromosomes. This DMRT analysis therefore confirmed that the autosomes in *C. s. producta* occurred in size groups of 2 long, 6 medium and 3 short chromosomes (2LL + 6MM + 3SS) (Fig,5). The X-chromosome was medium in size with a mean length of 5.60µm ± 0.56.

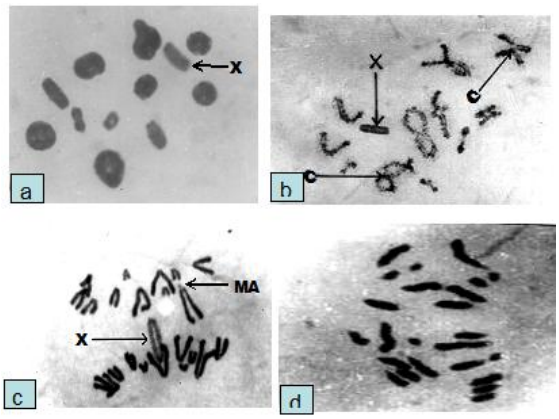

Fig. 3. Different meiotic stages in *C. stenoptera producta* and *C. compta*.

a) Diplotene in *C. stenoptera producta*. Bivalents with three chiasmata absent

b) Diplotene in *C. compta*. Bivalent with three chiasmata present (C= chiasma)

c) Anaphase – 1 in *C. compta*. Chromosomes V-shaped with minute chromosome arms present. (MA= minute arm)

d) Anaphase – 2 in C. *stenoptera producta*. Chromosomes single stranded, I-shaped.

In *C. compta* the length of the chromosomes and RCL obtained are shown in Table 2. In this species, chromosome length ranged from 12.2 to 2.7 µm with a total haploid length of 85.50 µm. The RCLs (Table 2) were used to construct the graphs in Fig. 6 and the graph revealed the chromosomes to occur in size groups of large, medium and small. The length of the 4 long pairs of chromosomes ranged from 12.20µm ± 0.4 to 11.90µm ± 0.67, the 4 pairs of medium chromosomes 5 to 8 ranged from 7.80µm ± 1.6 to 6.80µm ± 0.56 , while the mean length of the 3 pairs of short chromosomes 9 to 11 ranged from 2.80µm ± 0.0 to 2.70µm ± 0.0. The

mean chromosome length per size group was 11.98 µm for the large chromosomes, 7.35 µm for the medium chromosomes and 2.73 µm for the small chromosomes. DMRT revealed the large chromosomes to be significantly longer ($P<0.05$) than medium chromosomes and the medium chromosomes to be significantly longer ($P<0.05$) than the small chromosomes. This DMRT analysis therefore confirmed that the autosomes in *C. compta* occurred in size groups of 4 long, 4 medium and 3 short chromosomes (4LL + 4MM + 3SS) (Fig, 7). The X-chromosome was medium in size with a mean length of 7.3 µm ± 0.52.

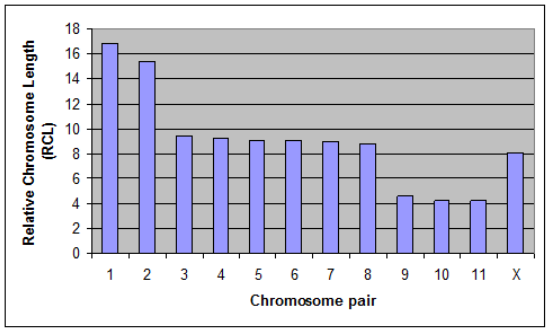

Fig. 4. Relative chromosome length by chromosome pair in *C. stenoptera producta* X= X or sex chromosome.

Chiasma frequency distribution

The mean chiasma frequencies for the two species studied are presented in Table 3. Chiasma frequency ranged from 13.00 ± 1.0 to 11.60 ± 0.55 in *C. stenoptera producta and 17.20* ± 0.84 to 15.40 ± 0.55 in *C. compta*. Chiasma frequency was therefore generally lower in *C. stenoptera producta* than in *C. compta*. This was reflected in the significantly lower ($P< 0.05$) mean chiasma frequency of 12.20 ± 0.77 in *C. stenoptera producta*. In *C. compta* a significantly higher ($P< 0.05$) mean chiasma frequency of 16.20 ± 0.72 was recorded.

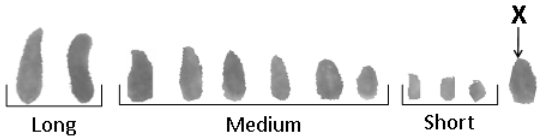

Fig. 5. Karyotype of *C. stenoptera producta*.

Table 4 shows the percentage contribution of the long, medium and short bivalents to mean cell chiasma frequency. In *C. stenoptera producta* medium size bivalents contributed most (65.70%) to mean cell chiasma frequency, while the short bivalents contributed least (14.90%). On the other hand, in *C. compta* the long bivalents contributed most (60.80%) to mean cell chiasma frequency, while the medium bivalents contributed least (18.70%).

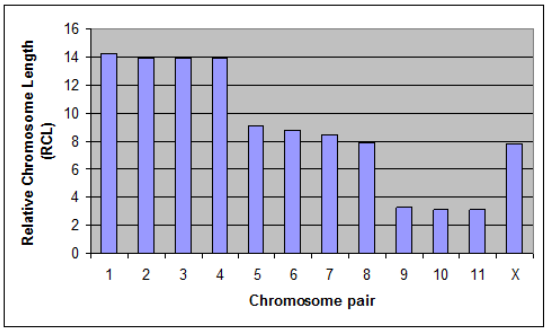

Fig. 6. Relative chromosome length by chromosome pair in *C. Compta* X= X or sex chromosome.

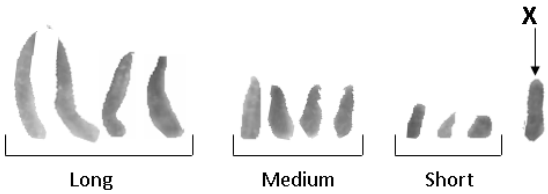

Fig. 7. Karyotype of *C. compta*.

The distribution of the number of chiasmata per bivalent shown in Table 5 revealed that bivalents with 3 chiasmata were nonexistent in *C. stenoptera producta* (Fig.3a). In this species, bivalents with only one chiasma contributed most (80.3%) to mean cell chiasma frequency. Contrarily, bivalents with 3 chiasmata were present in the *C. compta* population (Fig.3b) and contributed 11.10% of the mean cell chiasma frequency. In *C compta*, bivalents with 2 chiasmata contributed most (51.90%) to mean cell chiasma frequency.

Discussion

In West Africa, the genus *Coryphosima* (Walker) has three species, while only *Chirista compta*

(Walker) is known in the genus *Chirista* (Mestre *et al.,* 2006). *C. stenoptera producta* and *C. compta* here studied are known in Nigeria and Cameroon (Dirsh, 1975). These species which have previously been collected on the University of Lagos Campus (Seino, 1989) are morphologically distinct.

In this article, the chromosome complements of C. *stenoptera producta* and C. *compta* are described for the first time. Both species have 2N=23 acrocentric chromosomes in male individuals. Most species of the family Acrididae have 2N=23 (XO) chromosomes in males and all the chromosomes are acrocentric (White, 1973; Bugrov *et al.*, 2002; Bridle *et al.*, 2002; Turkoglu and Koca, 2002; Sharma and Gautam, 2002; Rocha *et al.*, 2004; Seetharama *et al.,* 2004, Souza & de Melo, 2007; Chadha and Mehta, 2011). This suggests that the 2N=23 acrocentric chromosomes in the male is the model karyotype for this family. It therefore follows that *C. stenoptera* and *C. compta* here described have the model Acrididae karyotype. So the short horn grasshoppers of different regions are showing cytogenetic uniformity regarding chromosome number and sex determining mechanism.

The chromosomes of both *C. stenoptera producta* and *C. compta* occur in size groups of long, medium and short. In *C. stenoptera producta* were found 2LL, 6MM and 3SS chromosomes while in *C. compta* were found 4LL, 4MM and 3SS chromosomes. This kind of arrangement in which chromosomes of a complement occur in three size groups of long, medium and short has been severally reported in Orthoptera grasshoppers (Burgrov and Warchalowska – Silva, 1997; Bugrov *et al.,* 1999; Turkoglu and Koca, 2002; Warchalowska-Silva *et al.,* 2002; Ren *et al.*, 2008, Seino *et al.,* 2012). However, the number of chromosomes per size group varies with the species as was the case with *C. stenoptera producta and* C. *compta* described in this study.

Chiasma frequency shows a wide variation both within and between different species of the Orthoptera. Its dependence on the genotype is well known (Verma and Agarwal, 2005). In the Acrididae with 11 bivalents, chiasma frequency per cell is often between 11.50 and 19.80 (White, 1973; Seino *et al.,* 2010). During this study, chiasma frequency in *C. stenoptera producta* and *C. compta* was never below 11 or above 18. The mean chiasma frequencies obtained were 12.20 ± 0.77 and 16.20 ± 0.72 in *C. stenoptera producta* and *C. compta* respectively. Mean chiasma frequency was significantly higher ($P < 0.05$) for *C. compta* than *C. stenoptera producta* and this difference can be attributed to the presence of 4 long bivalents in *C. compta* as compared to only 2 long bivalents present in *C. stenoptera producta*. Also, bivalents with 3 chiasmata were present in the population of *C. compta* but absent in the population of *C. stenoptera producta* here studied. Since a positive correlation between chromosome length and chiasma formation has been reported in Acrididae grasshoppers (Seino, 1989), these results could further explain why long bivalents contributed significantly higher to cell chiasma frequency in *C. compta* (60.80%) than in *C. stenoptera producta* (19.40%). The results of this study confirmed an earlier observation (Seino, 1989) of the consistent absence of bivalents with 3 chiasmata in *C. stenoptera producta*.

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