Rec. zool. Surv. India : **106**(Part 4) : 25-32, 2006

CHROMOSOME EVOLUTION IN *TRISTRIA PULVINATA* (UVAROV) (ORTHOPTERA : ACRIDIDAE : CATANTOPINAE)

ASHOK K. SINGH

Cytotaxonomy Research Laboratory, Zoological Survey of India, M-Block, Kolkata-700053, India

Family ACRIDIDAE

Subfamily TROPIDOPOLINAE

Species ***Tristria pulvinata*** (Uvarov)

Locality of collection : Golf club, Kolkata.

INTRODUCTION

Karyological features of a species are of considerable importance in studying the origin of chromosomal changes and trend of selection within and between populations. Acridids and Pyrgomorphids have long been recognized as chromosomally conservative groups. Majority of the species have 19, XO males; 20, XX females (Pyrgomorphidae) and 23, XO males; 24, XX females (Acrididae) with all the members of the chromosome complement to be *acrocentrics*. A number of deviations from this uniformity have however been recorded (White 1973; Hewitt 1979). In *Tristria pulvinata,* some of the autosomes have been observed to have been eliminated resulting in monosomic and nullisomic conditions. Individuals with nullisomic chromosome complement (2n = 21) have been found to be in greater frequency in the populations. This we observed to be somewhat unique, because the parental complement of this species comprises 23 acrocentric elements, and no other mechanism which reduces the 2n or FN could be recorded in this species. In all the cytotypes published, 2n and FN are equal with XO and XX sex-mechanism in the present series of investigation. The 21, 22 or 23 chromosomes scored for different karyotypes of the individuals was studied with the conventional C- and Hoechst 33258 banding technique.

MATERIAL AND METHODS

One hundred and forty males and two females comprised the material for the present series of investigation. The insects were collected from Golf club, Kolkata. The females are very similar to males except that they are little larger.

For metaphase arrest, individuals were injected with colchicine at a concentration of 0.05% and with an amount of 0.03 to 0.04 ml in each case. Thereafter they were kept alive for at least 4 hrs., in insect cages. Chromosomal preparations were made from the testes and hepatic caecae cells. The tissues were dissected out and cleaned in 0.67% sodium chloride solution. The testes were pretreated in 0.9% sodium citrate solution (hypotonic treatment) for 45 minutes and hepatic caecae for 1 hr. in the same solution, diced and fixed in freshly prepared methanol-acetic acid (3 : 1) with three changes of 15 minutes. In the field the tissues were fixed to small tubes, brought to laboratory and stored at 4°C. For preparation of the slides the fixed tissues were transferred to 50% acetic acid till it became soft. The materials were then teased and squashed in one or two drops of 50% acetic acid. The slides were stored in vapours of 50% acetic acid overnight in cold. Next morning the slides were brought at room temperature and immersed in a solution of 3 : 1 methanol-acetic acid for an hour. The cover slips were removed with the sharp edge of a blade, while immersed in the medium and dried at room temperature in a dust free chamber. Staining was done in 2% Giemsa prepared in phosphate buffer (pH 6.8). After differentiating in phosphate buffer (pH 6.8), slides were air-dried, cleaned in xylene and mounted in DPX. Only well spread and well stained mitotic and meiotic stages were photomicrographed with a Leitz Ortholux microscope. For photographs black and white film viz., NP 22 (120 ASA) were used. The negatives were printed on sterling SW glossy 3 and glossy 4 papers of Allied Photographics India Ltd. Fine grain film and paper developers of Agfa-Gevaert (A901 and A902) and Kodak (D76 and D163) were used in developing negatives and also for positive prints.

C-banding was carried-out following the method by Sumner (1972) with some minor modifications. The air-dried slides were treated with 0.2 N Hydrochloric acid for 30-60 minutes at room temperature, rinsed in distilled water and dried. The slides were then dipped in a freshly prepared 5% aqueous solution of Barium Hydroxide octahydrate kept at 50°C for 1-10 minutes; the timing depended on the age of the slides; the slides produced sharper bands with comparatively longer treatment. After a thorough rinsing with several changes in distilled water, the slides were incubated for an hour at 60°C in 2 X SSC (0.3 M sodium chloride and 0.03 M tri-sodium citrate at p^H 7), rinsed in distilled water and were then dried. The slides were then stained for 30 to 90 minutes in Giemsa (2.5 ml of stock solution added to 50 ml of buffer at p^H 6.8), rinsed briefly in distilled water, blotted, allowed to dry thoroughly, soaked in Xylene and mounted in DPX.

Fluorescence banding with HOECHST 33258 In this method, which was first introduced by Hilwig and Gropp (1972) and which is now popularly called Hoechst 33258, for staining the heterochromatic regions of mouse chromosomes, we slightly modified the technique by soaking the

air dried slides first in McIlvain's buffer at pH 5.4 for 10 min, and then staining them in freshly prepared Hoechst solution (0.5 μg/ml – 1 μg/ml) in the same buffer (at pH 5.4) for 15-20 minutes at room temperature. The slides were then rinsed in the same buffer and mounted either in same buffer or in a glycerol buffer mixture. The preparations were examined in a Fluorescence microscope or were stored in cold. For fluorescence a Leitz Ortholux photomicroscope was used.

Chromosome morphology and nomenclature were made according to the system of Levan *et al.* (1964). In order to compare the chromosome pairs, histograms were constructed from the relative length. Every individual of this species were tagged with a code number and details of the locality were carefully recorded in our Cytotaxonomy Lab.

OBSERVATIONS

Karyological Details :

Diploid Number, Chromosome Morphology and Sex Chromosome Constitution :

The diploid number of the chromosomes were 21 in males and 22 in females. All the chromosomes were acrocentrics (Figs. 1-13). The males were XO and the X chromosome was distinct by its negative heteropycnocity within the spermatogonial metaphase stages. In later spermatogonial stages it attained an intensity comparable to the autosomes. On an average the X was the 2nd largest element of the chromosome complement, comprising 15.06% of the relative length.

Karyotype : Thirty five conventionally stained karyotypes were analysed from 11 individuals, twenty five C-banded karyotypes of 6 individuals and fifteen Hoechst 33258 stained karyotypes of 5 individuals were analysed. Hoechst stained 8 meiotic karyotypes of 2 individuals were studied for meiotic progression and chiasma localization. In this species a very interesting variation in chromosome numbers was recorded in the male individuals, which was a variation in the diploid number from 21 to 23 in the gonads of the same individuals. The insects were collected from Golf club, a locality in the Southern part of the metropolis. It may be mentioned that this population was a community of potentially closely breeding individuals. In this population with same variation in the diploid number of chromosomes, it could be seen that in individuals with 21 and 23 chromosomes, the smallest chromosomes were the 9th 10th and 11th pairs (Figs. 1- 9). On the other hand an individual with 22 chromosomes, one of the 8th pair of chromosomes was found to be missing. And in individual with 21 chromosome, the smallest pair (the 11th) was found to be missing.

Relative length (RL) in percent : The table below (No. 1) represents the chromosome wise distribution of the relative length of the normal karyotypes of the population.

1	2	3	4	5	6	7	8	9	10	X
15.92	13.65	11.48	9.63	8.30	7.49	6.84	5.86	3.19	2.59	15.06

Supernumerary chromosomes : Two very minute (RL : 2.12%) acrocentric chromosomes of similar size were present in 17% of the individuals of the population (Figs. 1, 2 and 5, 6). On arranging the karyotypes they were found to be the smallest pair of chromosomes. These elements were of similar staining intensity as that of the other two smallest pair of chromosomes (the 9^{th} and the 10^{th} pair). Out of 24 individuals of the population investigated, these supernumeraries were present in 7 spermatogonial plates of only 4 individuals.

Idiogram : As mentioned earlier, we observed that in the idiogram, the last 2 pairs of chromosomes were of considerably smaller size. The X was the 2^{nd} largest chromosome and the size difference was large among pairs 1^{st}, 2^{nd}, 3^{rd} and 4^{th} chromosome. The medium-sized chromosome pairs 5^{th} to 8^{th} did not show much variation in length (Fig. 13).

C-Banding : Of the 25 C-band karyotypes studied, no variation could be recorded. All the chromosomes of the complement exhibited centromeric band. The last pair was distinct comparatively with its more prominent C-band. The X chromosome had the additional feature of having an interstitial band close to the centromere. The feature can be taken as maker element for the X (Figs. 7 and 8).

H-33258 Banding : The chromosomes exhibited a very bright fluorescing centromeric region. But the X chromosome was not that distinct as it was in C-band preparations as the interstitial heterochromatic band did not fluoresce well. Also with the technique the last pair of chromosomes showed a dull centromeric region.

Chiasma Distribution : The table below (Table 2) gives an account of the distribution of chiasmata in each bivalent. Each bivalent has been arbitrarily divided into three equal parts as proximal (P) interstitial (I) and distal (D).

Bivalents	1	2	3	4	5	6	7	8	9	10
Chiasma	P, D-3	P, D-2	P-1	P-5	P, D-1	P-5	P-3	P-4		I-2
Location and Number	I, D-5	I, D-6	P, D-2 I-3 I-D-2	P, D-2 D-1	I-1 I, D-1 D-5	I-1 I, D-1 D-1	I-3 D-2	P, D-1 I-1 D-2	I-1 D-7	D-6
Total	P = 3 I = 5 D = 8	P = 2 I = 6 D = 8	P = 3 I = 5 D = 4	P = 7 P = 1 D = 3	P = 1 I = 2 D = 7	P = 5 I = 2 D = 2	P = 3 I = 3 D = 2	P = 5 I = 1 D = 2	I = 1 D = 7	I = 2 D = 6
	16	16	12	10	10	9	8	9	8	8 = 106

A total of 106 chiasmata were scored in 80 bivalents, showing 29 proximal (P), 28 interstitial (I) and 49 distal (D) type.

The 3^{rd}, 5^{th} and 8^{th} bivalents at diplotene showed terminalized chiasmata (arrow; Fig. 12). Such configurations were also found in other meiocytes at later stages of meiosis of this individual.

DISCUSSION

A very interesting variation in chromosome numbers could be recorded in this species, both between and within the individuals involving the 8th and 11^{th} pairs of chromosomes. Within them, the last pair (11^{th}) was of considerably smaller size, sharing only 2.12% (relative length) of the complement. Both the elements of this pair were missing in the majority of the karyotypes resulting to a reduced chromosome number (21). Whereas when both the elements of the 8^{th} pair, were present it contributed alone 5.86% (relative length) of the 21 chromosome complement. Sometimes however it retains one of the supernumerary, resulting in a chromosome count of 20 in some karyotypes. Both the pairs of the species *T. pulvinata* have been designated as supernumeraries, as they did not show Mendelian segregation. We also noted that the 8^{th} pair was associated with a chiasma in the meiocytes.

If the minute 11^{th} pair is not considered as supernumerary, then a condition with 23 acrocentric chromosomes appears to be the normal karyotype of all the Acridids which is acknowledged to be the parental number from which a decrease or increase have been proposed to have been derived (White 1973, Hewitt 1979). However, in this species, intraindividual polymorphism of a "transient" type is observed that evolved towards elimination of a small pair (11^{th}) with a consequent fixation of a lower chromosome number in their parental karyotype. Occurrence of any orthodox mechanism *viz.*, "Robertsonian" type of translocations (Robertson, 1916) is not convincing since acrocentric nature of all the chromosomes in the complement was evident. Also a fusion followed by pericentric inversion (to restore acrocentric condition) is opposed by the observation of small increase in the relative lengths of the 21 chromosome groups. This is expected in case of a loss or elimination of a small chromosome pair alone.

Loss of a normal chromosome reflects a decrease in the recombination potential in the species. The recombination potential of the 11^{th} pair, due to its minute size might have been very low. Consequently, a lack of its pairing and lagging of unpaired homologues, led to the production of gametes with deficient genomes. A high frequency of spontaneous non-disjunctions along with a high degree of inbreeding increased the possibilities for a rapid spread of various chromosome numbers in the population. This type of chromosome polymorphism, approaching with the extinction of a pair of chromosome, which has lost its property of Mendelian segregation and turned to be a supernumerary, is quite unusual in the Acridoids.

The behaviour of the 8^{th} pair as supernumerary was not so pronounced. Both the homologues of this pair were mostly represented in the karyotypes and found associated with a chiasma. This pair was nowhere distinct as supernumerary in conventional staining (Figs 1-6). In C-banding, centromeric region of all the chromosomes in the complement were C-band positive (Figs. 7 and 8). In one karyotype, one chromosome at 8^{th} position was found to be more stained (Fig. 8). In a Hoechst 33258 stained karyotype, the 8^{th} pair was found comparatively brighter (Fig. 9), while in other cells (Figs. 10-12) all the chromosomes were uniformly stained. One reason for this might be that there was no uniform condensation of this pair or like all the other members of a complement at a given

stage of the mitotic cycle. This pair was also did not agree with the concept of it to be a megameric element. This is because a large part of a megameric chromosome is characteristically heterochromatic in male meiotic prophase and for this reason, it is frequently associated with similarly condensed X-chromosome (Hewitt, 1979). The etiology underlying heterochromatic parts of a megameric chromosome is that they are duplications of some part of the basic genome that have been heterochromatinised (Hewitt, 1979). In the present case (i) by the Barium hydroxide saline Giemsa technique which provides the most consistent method for identifying constitutive heterochromatin, this pair was not found to be C-band positive, (ii) biosynthetically inactivated or switched off euchromatic elements are not depicted by this C-banding procedure. For example, the facultative heterochromatinized X-chromosome of grasshoppers in male meiosis do not give positive C-banding, (iii) regarding 'H' fluorescence in grasshoppers, it has been proposed that "H-fluorescence is modulated by chromosome condensation brought about by differential ratios of DNA/protein at different chromosome regions and at different stages" (Das *et al.* 1979). With this procedure no bivalent had displayed prominently its condensed nature, as is seen for the condensed X chromosome in diplotene (Fig. 12).

We know that there is no direct relationship between a heterogeneity identified by one staining technique and those identified by others. But the Sumner's method employed here has been ~~yet enough in~~ revealing recognizing the chromosomes which were otherwise indistinguishable in the conventional staining method. This technique constantly revealed a marker element as an interstitial C-band on the X-chromosome. In the present case 'C' and Hoechst procedures were kept uniform throughout. The 8^{th} pair is therefore not supported as a megameric pair in this species.

The cytological features which have favoured the proposal that this pair is supernumerary are as follows :

(i) At meiosis, homologues had paired with one another and formed chiasma. They had not paired with members of the normal chromosomes of the complement i.e., not homologous with any other member of the normal complement.

(ii) This pair had never revealed any secondary constrictions, therefore did not have the probability of having a nucleolus organizer.

(iii) Non-disjunction at anaphase of spermatogonial mitosis ~~duplicated~~ its non-Mendelian type of inheritance.

(iv) This pair had revealed constitutive heterochromatin (centromeric C-band) which presumably contains no Mendelian genes and is not transcribed (Therman, 1980). However our proposal regarding the absence of any major genes in the euchromatic region of this pair can not be explained only on the above findings alone. But to explain our finding we refer Jones and Rees (1982) who on the genetic material of B-chromosomes have concluded that "the organization of the genetic material within B chromosomes is distinctive in that the B-chromosomes at metaphase of mitosis are more densely coiled than the A chromosomes, with the result that the DNA density per unit volume is greater."

SUMMARY

Karyological features of *Tristria pulvinata* (Uvarov) have been investigated. In the present series of investigation the grasshoppers belonged to a community of close breeding individuals from Golf club, a locality in the Southern part of" Kolkata. Karyotypes with 21, 22 and 23 chromosomes were observed within and between individuals of this species. A deviation from 23 (parental complement) to 21 and 22 chromosomal types have been attributed to loss of 2 and 1 normal elements respectively. Individuals with 21 chromosomes were considerably more in number in the population. The idiogram of this cytotype depicted that the last two pairs had a small gap and first 4 pairs had ♂ wider gaps ~~within~~ On the C-banding ~~them~~ distinct prominent centromeric band revealed 1st pair. On Hoechst staining, all the chromosomes showed brighter fluorescing centromeric region but proximal C- band of the X did not fluoresce prominently. In meiotic study, chiasma was not restricted to any particular region of the bivalents.

ACKNOWLEDGEMENTS

I am indebted to Dr. J.R.B. Alfred, Director, Zoological Survey of India for providing facilities and for his continued interest and encouragement in the work. I thank Prof. J.S. Yadav, Kurukshetra University for promptly providing me all his reprints. I also thank Prof. B.N. Singh of Banaras Hindu University and Prof. A.K. Duttagupta of Calcutta University for their hospitality during consultation of literature in their laboratories. I am thankful to Dr. Ch. Satyanarayana of Z.S.I. For helping me in shaping the manuscript in electronic form and to Dr. M.S. Shishodia for identifying all these grasshoppers.

REFERENCES

Das B.C., Raman R. and Sharma, T. 1979. Chromosome condensation and Hoechst 33258 fluorescence in meiotic chromosomes of the grasshopper *Spathosternum prasiniferum* (Walker). *Chromosoma*, **70** : 251-258.

Hewitt, G.M. 1979. Animal Cytogenetics, Vol. **3,** Insecta 1. Orthoptera. Gebrüder Borntraeger Berlin Stuttgart : 170.

Hilwig, I. and Gropp, A. 1972. Staining of constitutive heterochromatin in mammalian chromosomes with a new fluorochrome. *Exptl. Cell Res.*, **75** : 122-126.

Jones, R.N. and Rees, H. 1982. B Chromosomes. Academic press, London : 266.

Robertson, W.R.B. 1916. Chromosome studies. I. Taxonomic relationships shown in the chromosomes of Tettigidae and Acrididae. V-shaped chromosomes and their significance in Acrididae, Locustidae and Gryllidae : chromosomes and variation. *J. Morph.* **27** : 179-331.

Sumner, A.T. 1972. A simple technique for demonstrating centromeric heterochromatin. *Exptl. Cell Res.*, **75** : 304-306.

Therman, E. 1980. Human Chromosomes : Structure, Behaviour, Effects. Springer-Verlag, Berlin : 235.

White, M.J.D. 1973. Animal Cytology and Evolution. Cambridge University Press, London : 961.