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MALE MEIOSIS IN STRUCTURALLY HETEROZYGOUS AND HOMOZYGOUS TRERON, *PHOENICOPTERA PHOENICOPTERA* (LATHAM) (COLUMBIFORMES: AVES) FROM JHARKHAND

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ABSTRACT
Treron phoenicoptera phoenicoptera (Latham) is polymorphic for a pericentric inversion involving chromosome pair 2 in Jharkhand population. Analysis of meiotic stages was based on the study of ten male birds collected from Chotanagpur and Singhbhum areas of Jharkhand. Three distinct karyomorphs were observed for chromosome 2. Chromosome 2 has two to four chiasmata. In order to ascertain whether inversion heterozygotes leads to a significant reduction in chiasma frequency, the number of chiasmata were counted in bivalent 2 from fifty cells of each karyomorph. The observed and expected frequencies of bivalent 2 with less than three chiasmata and with three or more than three chiasmata have been calculated. A chi-square test was performed. The chi- square values are significant which indicate that there has been a decrease in chiasma frequency in the inversion heterozygotes.

INTRODUCTION

One of the most important causes of evolution in organism is their store of genetic variability. Such variability when present as a structural chromosomal change modifies the karyotype. If the original and modified chromosomes are both retained in the population the result is chromosomal polymorphism. Such polymorphism may be due to a variety of changes such as inversions, translocations, deletions or variability in heterochromatin.

Inversion polymorphisms are particularly frequent in natural in natural populations of *Drosophila* and other dipterans, where they have been studied extensively owing to the presence of giant polytene chromosome which facilitates their detection (Dobzhansky, 1970; White, 1973). Descriptions of chromosomal polymorphisms in birds, however, are rare. Thorneycroft, (1966, 1975) analysis of pericentric inversion polymorphism in white-throated sparrow *Zonotrichia albicollis* and Shields (1973, 1976) reports on chromosomal polymorphism common to several species of *Juncos* are both excellent critical accounts of chromosomal polymorphism within these taxa.

The chromosomal polymorphism in yellow-legged green pigeon *Treron phoenicoptera phoenicoptera* (Latham) was first reported by Ansari and Kaul (1979) in the population from Allahabad (Uttar Pradesh). The diploid complement comprises of 74 chromosomes with seven pairs of macro chromosomes (including the sex chromosomes) and thirty pairs of microchromosomes. The Allahabad population was found to be polymorphic for chromosome pairs 1 and 2. Thus, chromosome pair 1 was present in either as a metacentric (1^{m}) or a subtelocentric (1^{st}) condition. Similarly chromosome 2 was also present as either a subtelocentric (2^{st}) or a metacentric (2^{m}). The polymorphism was explained on the basis of two independent pericentric inversions involving chromosome pairs 1 and 2. Further from the same area (Allahabad population) inversion polymorphism in this species was recorded in a sample of fourteen birds (Roy *et al.,* 1988; Roy, 1990). Similar polymorphism was also recorded in Jharkhand (Gupta, 1993), Odisha (Gupta, 1993) and Kurushetra (Haryana). Thus, this polymorphism appeared to be a regular feature of *T. phoenicoptera* populations at least from Northern India.

Meiotic studies in birds have been carried out by several workers (Thorneycroft, 1966; Shields, 1973). Meiotic studies in *Treron phoenicoptera* are based on the study of germ cells from ten adult males from the Chotanagpur and Singhbhum populations of Jharkhand. Jharkhand populations being homomorphic for chromosome 1 ($1^{m}1^{m}$), there were no difference in chromosome 1 among these individuals. Thus, only three different karyomorphs, 2^{st} /2^{st}, 2^{st} /2 ^{m} and 2^{m} /2^{m} were observed.

The present communication reports the meiotic analysis of adult male birds and the condition of macrobivalent 2 in the three different karyomorphs encountered in the study.

MATERIALS AND METHODS

In the present paper, ten adult male individuals of common

green pigeon *Treron phoenicoptera phoenicoptera* (Latham) belonging to the family Columbidae of the order columbiformes were utilized for the analysis of meiotic chromosomes. The birds were sampled from Chotanagpur and Singhbhum areas of Jharkhand.

Preparation of meiotic chromosomes

Method of chromosome preparations from testicular tissue from the adult male birds is as follows

Squash preparation

The testicular tissue was taken from the adult bird during breeding season (March - June) and *in vivo* pretreatment of colchicine was avoided. The testes were excised from the anaesthesized bird and placed in a hypotonic solution (0.9 % tri-sodium citrate) at room temperature for 30 minutes. The tissue mass was macerated with fine needles for perfect penetration of hypotonic solution. Squash preparations were made by taking small bits of testicular material fixed in aceto-alcohlol for 30 minutes and then stained with 3% aceto-orcein.

Flame - dried preparation

For this the suspension was made homogenous with the help of pasture pipette by gentle flushing. The cell suspension was incubated at 37ºC for 15-20 minutes, the supernatant was discarded and freshly prepared fixative (1:3, acetic acid: methanol) was added drop by drop. Chromosome preparations were made on slides which were dipped overnight in 50% methanol at 4ºC. A small amount of homogenous suspension was taken in the Pasteur pipette and dropped to the chilled slide. The slides were immediately flame-dried. The chromosome preparations were stained for 15 minutes in 4% Giemsa diluted in Sorensen's buffer (pH 6.8).

RESULTS

Meiotic studies are based on the study of germ cells from ten adult males from Chotanagpur and Singhbhum populations of Jharkhand. The observations for male spermatogonial mitosis and meiosis are described first in homozygous individuals (2^{st} 2^{st}), followed by heterozygous individuals (2^{st} 2^{m}) and finally in inversion homozygous individuals (2^{m} 2^{m}) for chromosome 2. Both of these populations being homomorphic for chromosome 1 (1^{m} 1^{m}), there were no differences in chromosome 1 among these individuals.

Standard karyomorph ($1^{m}1^{m}$/ $2^{st}2^{st}$)

Like in somatic metaphases, in spermatogonial metaphases also seven pairs of macro chromosomes 1, 2, 3, 4 (ZZ), 5, 6 and 7 could be identified (Fig. 1). Both the homologs of chromosome pair 1 are metacentric while those of chromosome pair 2 are subtelocentric. The larger micro chromosomes appear as discrete stained bodies whereas some of the smaller micro chromosomes are lightly stained and remain indistinct at this stage. However, in a few well spread metaphases, thirty pairs of micro chromosomes could be counted giving a diploid count of seventy four chromosomes while other metaphases showed hypo modal values.

In the primary spermatocyte division cycle, the chromosomes after leptotene (Fig, 1b) emerge at pachytene as completely paired bivalents which are uniformly stained (Fig. 1c). Due to

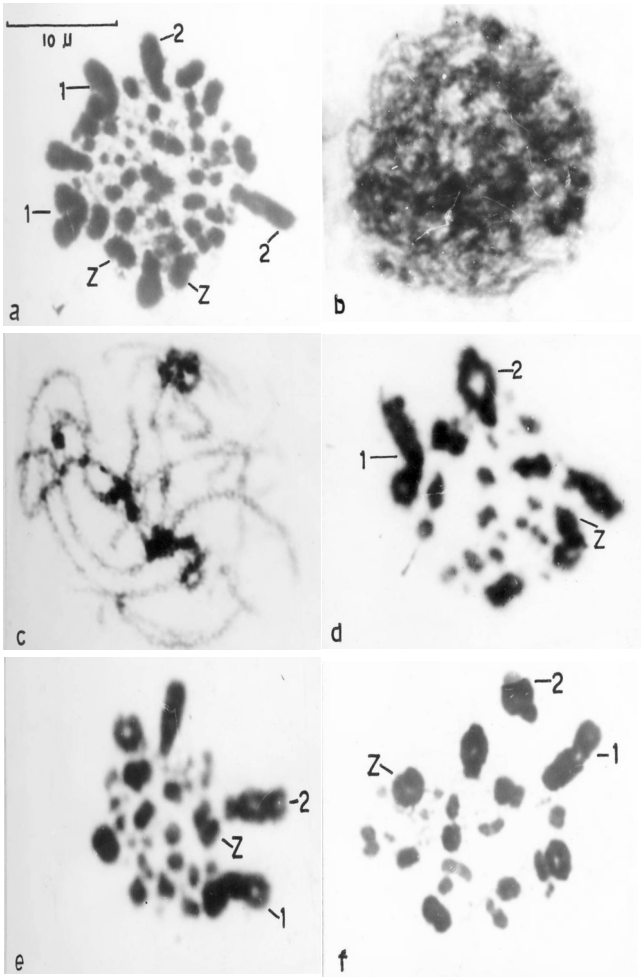

Figure 1: Meiotic stages in standard karyotype ($1^{m}1^{m}/2^{st}2^{st}$)
(a) spermatogonial metaphase; (b) leptotene; (c) pachytene; (d-f) diplotene/ diakinesis

the large number of micro bivalents at this stage, it becomes difficult to recognize the seven macro bivalents individually as well as to ascertain the total number of bivalents. The bivalents gradually condense and at diplotene / diakinesis all the seven macro bivalents corresponding to the chromosome pairs 1, 2, 3, 4 (ZZ), 5, 6 and 7 become distinct (Figs. 1d to f). The number of micro bivalents however, is not very clear as they usually tend to aggregate in centre of the nuclei. The position of the chiasma in the micro bivalents could not be deciphered. Therefore, the analysis of chiasma frequency was restricted only to macro bivalents in this as well as in other two karyomorphs. The chiasma count is a correlation between the length of a chromosome and the number of chiasmata present.

The macro bivalent 1 which is the largest element being 7.45 $\pm$ 0.08μ long has 4 to5 chiasmata (Table 1). Macro bivalent 2 which is 5.80$\pm$0.04μ long has 3 to 4 chiasmata. The macro bivalent 3 is 4.59 $\pm$0.03μ long and has 3 chiasmata. The macro bivalent 4 is smaller than micro bivalent 3 (3.42 $\pm$0.03 μ long) and shows 2 chiasmata. The macro bivalent 5 is 3.04 $\pm$ 0.03μ long with 2 chiasmata. Macro bivalents 6 and 7 are often indistinguishable and are normally about 2.75$\pm$0.70 and 2.45 $\pm$0.10μ long, respectively. Both are characterized

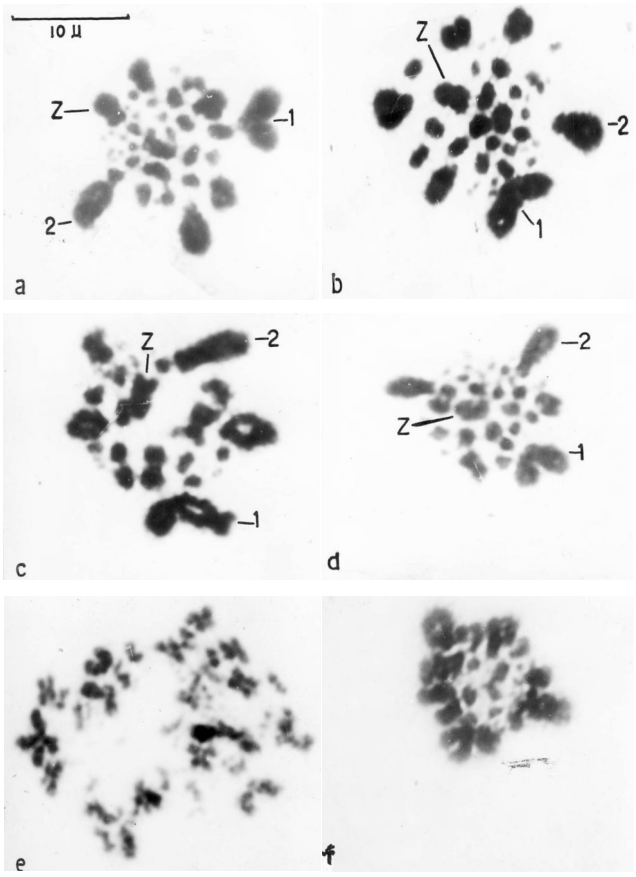

Figure 2: Meiotic stages in standard karyotype ($1^m1^m/2^{st}2^{st}$)
(a-c) diplotene / diakinesis;(d) metaphase II; (e) anaphase I, (f) metaphase II.

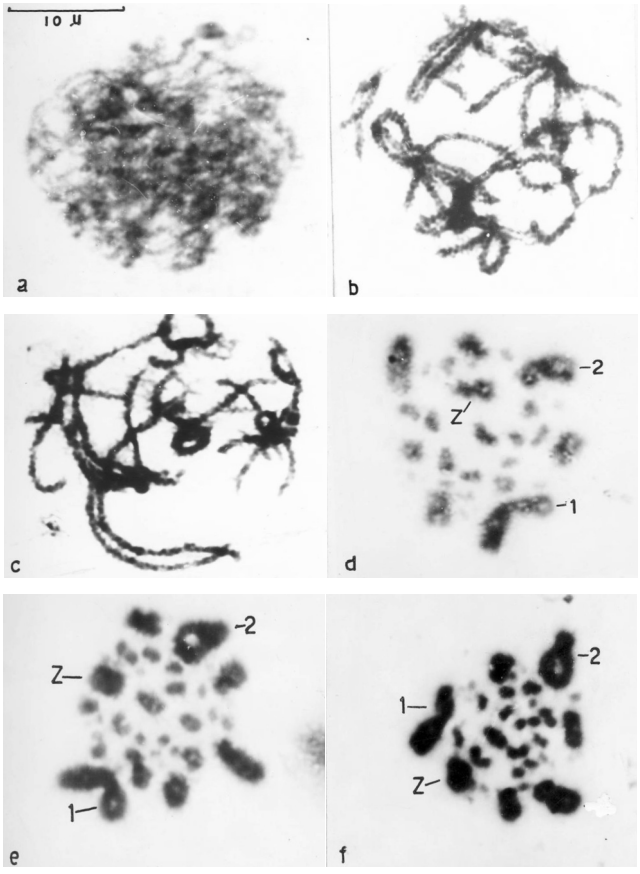

Figure 3: Meiotic stages in heterozygous karyotype ($1^m1^m/2^{st}2^m$)
(a) leptotene; (b-c) pachytene; (d-e) diplotene / diakinesis; (f) diakinesis /metaphase I.

by the presence of single chiasma representing a cross like configuration in most of the diplotene stages (Figs. 2 a, b). The chiasma count is a correlation between the length of a chromosome and the number of chiasmata present.

Table 1: Standard karyomorph ($1^m1^m/2^{st}2^{st}$)

Macro bivalents	Length (μ)	No. of chiasma/chiasmata
1	7.45 ± 0.08	5-4
2	5.80 ± 0.04	4-3
3	4.59 ± 0.03	3
4(ZZ)	3.42 ± 0.03	2
5	3.04 ± 0.03	2
6	2.75 ± 0.70	1
7	2.54 ± 0.01	1

At diakinesis, the bivalents become more condensed and chiasmata in all the bivalents are more or less terminalized (Fig. 2c). At metaphase I all the bivalents appear as compact bodies. The micro bivalents generally tend to aggregate at the centre while the macro bivalents are arranged at the periphery (Fig 2d). At anaphase I, the homologs separate and migrate to the opposite poles (Fig. 2e). Both the homologs of pair 1 and 2 reveal their centromeric position at median and subtelocentric region, respectively (Fig. 2e).

During second meiotic division, the cells were observed at metaphase II where chromosomes appear as condensed bodies (Fig. 2f).

Heterozygous karyomorph ($1^m1^m/2^{st}2^m$)

Table 2: Heterozygous karyomorph ($1^m1^m/2^{st}2^m$)

Macro bivalents	Length (μ)	No. of chiasmata
1	7.88 ± 0.13	4
2	6.05 ± 1.0	3
3	4.92 ± 0.07	3
4(ZZ)	3.75 ± 0.04	1-2
5	3.54 ± 0.04	1-2
6	3.04 ±0.04	2
7	2.63 ± 0.04	2

In the heterozygous karyomorph, the chromosomes primary spermatocyte division appears as bivalents at the pachytene stage (Fig. 3b). No pachytene loop was observed under light microscope with conventional Giemsa staining (Fig. 3c). The micro bivalents usually form aggregates, which are similar in appearance to the macro bivalent making it difficult to discriminate all the bivalents individually.

The bivalents gradually condense to emerge at diplotene/ diakinesis where seven macro bivalents are regularly observed (Fig. 3d). The large micro bivalents appear as dots (Fig. 3e). In good preparations, thirty seven bivalents could easily be counted at diakinesis (Fig. 3e). The largest macro bivalent is $7.88 \pm 0.13\mu$ long and has 4 chiasmata (Table 2). Macro bivalent 2 is $6.05 \pm 1.0\mu$ long and has 3 chiasmata. Macro bivalent 3 is slightly smaller ($4.92 \pm 0.07\mu$ long) and also has 3 chiasmata. The macro bivalents 4 and 5 are $3.75 \pm 0.04\mu$ and $3.54 \pm 0.04\mu$ long, respectively, and show 1 or 2 chiasmata at

their terminal ends. The macro bivalents 6 and 7 mostly appear as rings characterized by 2 chiasmata. Their absolute lengths are $3.04 \pm 0.04\mu$ and $2.63 \pm 0.04\mu$, respectively.

At diakinesis /metaphase I, all the seven macro bivalents could be easily identified (Figs. 4a and b). The micro bivalents, in general, tend to aggregate in the centre surrounded by the macro bivalents (Fig. 3f).

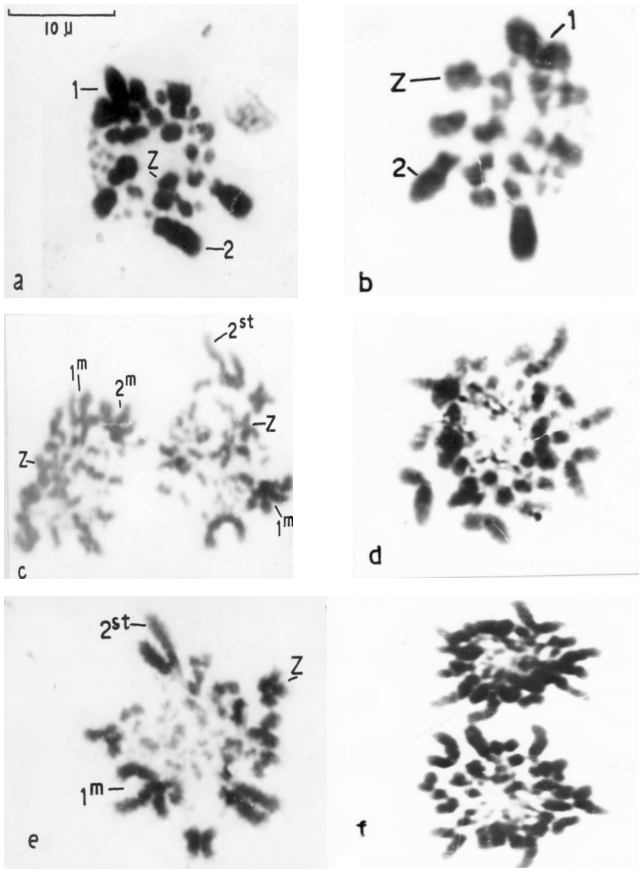

Figure 4: Meiotic stages in heterozygous karyotype ($1^m1^m/2^{st}2^m$)
(a-b)metaphase I; (c) anaphase I; (d-e) metaphase II; (f)anaphase II.

At anaphase I when migration of homologs to opposite poles has taken place, at one pole one observes the second chromosome in 2^m condition while at the other it is in 2^{st} condition (Fig. 4c).

In second spermatocytic division cycle, the micro chromosomes show a tendency to aggregate at the centre of the ring of macro chromosomes (Figs. 4d and e). In second spermatocyte metaphases, the chromosomes are less condensed. However, in good metaphase II (Fig. 3e) 2^{st} condition of chromosome 2 is visible. At anaphase II the chromosomes migrate to opposite poles (Fig. 4f).

Inversion homozygous karyomorph ($1^m1^m/2^m2^m$)

In the early primary spermatocyte, the chromatin threads are conspicuous and several dark staining masses also appear (Fig. 5a). The leptotene chromosomes usually appear as long slender lying entangled in the compact mass.

At zygotene/pachytene, the paired bivalents are visibly thicker than the leptotene threads (Fig. 5b). At this stage, some of the micro bivalents exhibit definite length and appear similar in

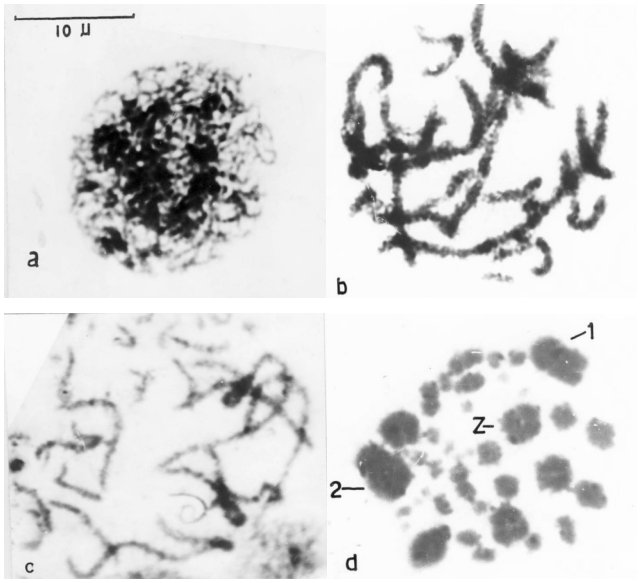

Figure 5: Meiotic stages in inversion homozygotes ($1^m1^m/2^m2^m$)
(a) Leptotene; (b-c) zygotene/pachytene; (d)diplotene / diakinesis

structure to the macro bivalents (Fig. 5c).

During diplotene / diakinesis, the bivalents gradually condense and the macro bivalents can be distinguished from the micro bivalents because of their larger size (Fig. 5d). The mi-

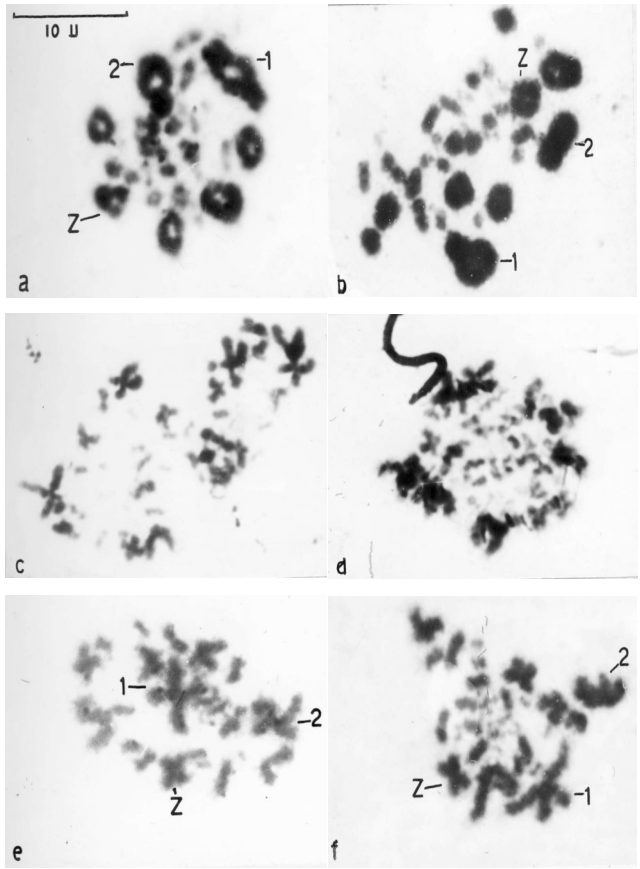

Figure 6: Meiotic stages in inversion homozygote ($1^m1^m/2^m2^m$)
(a) diplotene/ diakinesis; (b) metaphase I; (c) anaphase I; (d) prophase II; (e-f) metaphase II.

cro bivalents are, however, not very distinct and the general tendency of the micro chromosomes to aggregate in the centre is also prevalent in this karyomorph (Fig. 6a).

Table 3: Inversion homozygous karyomorph ($1^m1^m/2^m2^m$)

Macrobivalents	Length (μ)	No. of chiasmata
1	5.63 ± 0.2	4
2	4.84 ± 015	3
3	4.09 ± 0.15	2-3
4(ZZ)	3.11 ± 0.04	1-2
5	2.75 ± 0.05	1-2
6	2.50 ± 0.03	1-2
7	2.38 ± 0.04	1-2

Thirty seven bivalents could easily be counted in well spread preparations (Fig. 6b). Macro bivalent 1 is the largest in the complement (5.36±0.2μ) and reveals 4 chiasmata (Table 3). Macro bivalent 2 is 4.84 ± 0.17μ long and has 3 chiasmata. Macro bivalent 3 is 4.09 ± 0.15μ long with 2 to3 chiasmata. Macro bivalents 4 and 5 are 3.11 ± 0.04 and 2.75 ±0.05μ long respectively and show 1 or 2 chiasmata. Macro bivalent 6 is 2.50 ± 0.30μ long and has 1 or 2 chiasmata. Macro bivalent 7 is 2.38 ± 0.04μ long and reveals 1 or 2 chiasmata.

At metaphase I, the chiasmata are fully terminalized and the bivalents appear as compact bodies (Fig. 6b). As in other karyomorphs, the macro chromosomes are peripherally located with micro bivalents lying in the centre.

At anaphase I the homologs migrate to opposite poles. The centromeric position of both the homologs of macro bivalent 2 appears as median (Fig. 6c).

The chromosomes emerge out of prophase II at second spermatocytic division cycle as discrete bodies with aggregation of macro chromosomes (Fig. 6d). The chromosomes gradually condense at metaphase II (Figs. 6e and f).

DISCUSSION

The most outstanding and masterly studies on inversion polymorphism based on the study of meiocytes in natural populations of several species of grasshoppers have been conducted by White and coworkers (White, 1973, 1978). An analysis of meiotic chromosomes to a very large sample of white-throated sparrow *Zonotrichia albicollis* suggests that

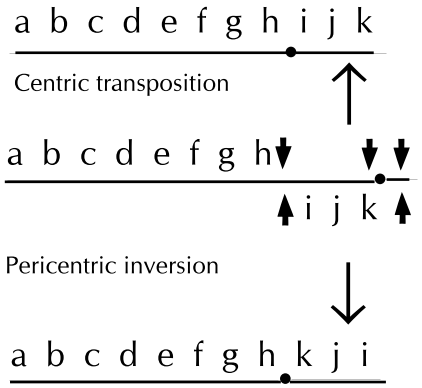

Figure 7: Diagrammatic representation of the possible mode of centric shift resulting from chromosome rearrangements

pericentric inversions disrupts the chromosome pairing in heterozygous condition (Shields, 1976). The changes which led to the establishment of polymorphism in the population of this bird have been explained on the basis of pericentric inversion of a chromosomal segment so far may alternatively be explained by a centric transposition. The difference between the two modes is explained in Fig. 7.

It is clear that both pericentric inversion and centric transposition results in a shift of the position of centromere , the former is a two break rearrangement and leads to the formation of a reverse loop at pachytene, the latter is a three break arrangement in which there should be straight pairing at pachytene. Meiotic analysis also supports the contention that the centric shift in the dimorphic pairs is because of pericentric inversion and not due to centric transposition. Pairing need not be disrupted in a transposition heterozygote and hence no reduction in chiasma frequency expected. Crossing over between the centromeres in a transposition heterozygote should give rise to a dicentric bridge and an acentric fragment at the first anaphase.

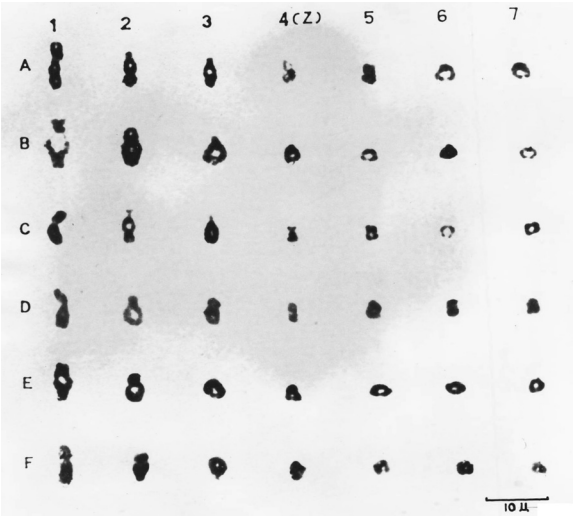

Figure 8: Partial karyotype of diplotene / diakinesis of *T. p. phoenicoptera* A and B – standard karyotype 1^m1^m / $2^{st}2^{st}$); C and D – heterozygous karytype (1^m 1^m / 2 st 2^{st}); E and F - inversion homozygous karyotype (1m 1^m / 2^m 2^m)

Male meiosis was studied in homozygous and heterozygous birds from Chotanagpur and Singhbhum areas of Jharkhand. Partial karyotypes of the seven macro bivalents at diplotene / diakinesis in the three karyomorphs are represented in Fig. 8. At pachytene no reverse loop was observed in heterozygous birds. Chromosome 2 has two to four chiasmata. Bivalent 2 from fifty cells of each karyomorph (Table 4).

In order to ascertain whether inversion herterozygosity leads to a significant reduction in chiasma frequency the number of chiasmata were counted in bivalent 2 from fifty cells of each karyomorph.

The observed and expected frequencies of bivalent 2 with less than three chiasmata and with three or more chiasmata have been calculated and a chi-square performed. The data is presented in Table 5. The chi-square value is significant in both the cases and indicates that there has been a decrease in chiasma frequency in the inversion heterozygote. The decrease

Table 4: Chiasma count in the bivalents $2^{st}2^{st}$,$2^{st}2^{m}$ and $2^{m}2^{m}$

Karyomorphs	Number of chiasmata			
	2	3	4	Total
2^{st} 2^{st}	8	30	12	50
2^{st} 2^{m}	19	26	5	50
2^{m} 2^{m}	10	24	16	50
Total	37	80	33	150

Table 5: Chiasma frequencies of $2^{st}2^{st}$, $2^{st}2^{m}$ and $2^{m}2^{m}$ bivalents

Chromosome morph	Chiasma class			
	< 3		≥3	
	Observed	Expected	Observed	Expected
2^{st} 2^{st}	8	13.50	42	36.5
2^{st} 2^{m}	19	13.50	31	36.5

$$x\frac{2}{1} = 6.14, p<0.01$$

Chromosome morph	Chiasma class			
	< 3		≥3	
	Observed	Expected	Observed	Expected
2^{m} 2^{m}	10	14.5	40	35.5
2^{st} 2^{m}	19	14.5	31	35.5

$$x\frac{2}{1} = 3.94, p<0.01$$

in chiasma frequency may be taken as indicative of the fact that the inversion is pericentric. This is supported by the observed lack of dicentric bridges and acentric fragments at anaphase I. However, a final verdict regarding pericentric inversion/ centric transposition must wait till a study of the synaptonemal complex has been carried out.

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