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C-BANDING EVIDENCE FOR PERICENTRIC INVERSION POLYMORPHISM IN COMMON GREEN PIGEON *TRERON PHOENICOPTERA PHOENICOPTERA* (LATHAM)

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ABSTRACT
Forty eight birds of common green pigeon *Treron phoenicoptera phoenicoptera* (Latham) collected from different geographical areas like Allahabad (U.P), Chotanagpur and Singhbhum Divisions (Jharkhand) and Ganjam district (Odisha) have been subjected to C-banding analysis. The bird was found to be polymorphic for chromosome pairs 1 and 2. The polymorphism was explained on the basis of two independent pericentric inversions taking place in these chromosome pairs. With two chromosomes involved in inversions, ideally there should be nine types of karyomorphs. In the present investigation five different karyomorphs were encountered. All the karyomorphs were subjected to C-banding analysis. All the macrochromosomes have distinct C-bands at the centromere and three band sizes large, medium and small have been categorized purely on visual basis. Intercalary or terminal C- bands have not been observed in any of the macrochromosomes. No variant forms of C-bands have been recorded in the individuals examined during the present study. However, the shift in the position of C- band in the chromosome pairs 1 and 2 confirms that an inversion is responsible for the heterozygosity.

INTRODUCTION

The presence of chromosomal polymorphism in the populations of *Treron phoenicoptera* from Allahabad was initially identified in our laboratory by Ansari and Kaul (1979b). The report was based on chromosomal examination of twelve individuals from Allahabad population. Later from the same area, Roy *et al.,* (1988b), reported presence of the same polymorphism in a sample consisting of eight male and six female individuals. During the present study the sample showed same polymorphism. Two forms of autosomes 1, *i.e.,* 1^m and 1^{st} and also of autosomes 2, *i.e.,* 2^{st} and 2^m exist in the population and have been explained on the basis of pericentric inversions taking place in these chromosomes. A sample of eighteen individuals from Allahabad, nine individuals from Singhbhum division, thirteen individuals from Chotanagpur division (Jharkhand) and a single female from Berhampur showed same polymorphism for chromosome pairs 1 and 2. After specific staining of constitutive heterochromatin, the inversion has also been found associated with C-band polymorphism for the same pairs of chromosomes. This paper presents analysis of C-banding studies in the different karyomorphs population wise.

MATERIALS AND METHODS

In the present study, forty eight individuals of common or yellow-legged green pigeon *Treron phoenicoptera phoenicoptera* (Latham), belonging to the family Columbidae of the order Columbiformes were utilized for the analysis of mitotic and C-banding kayotypes. The birds were sampled from different geographical localities which have been grouped as three distinct populations (Fig. 1a). Population A consists of birds from Allahabad. Populations B and C consist of birds from Chotanagpur Division and Singhbhum Division of Jharkhand, respectively. Besides these individuals a single female bird from Berhampur (Ganjam district), of Coastal Orissa has also been karyotyped. The details of localities, number of males and females and the period of collection are given in Table 1.

Preparation of mitotic chromosomes

The mitotic chromosome preparations have been made from the bone marrow cells following the flame drying technique of Rothfels and Siminovitch (1958) with some modifications. Adult birds were administered intraperitoneal injection of 0.5% colchicine (U.S.P. Ph. - Intern., Italy) solution at a dose of 0.5 mg /100g of body weight. Birds were sacrificed forty minutes after injection. The bone marrow was extracted from femur and tibio-tarsus in hypotonic solution (0.56% KCl), prewarmed at 37ºC. The cell suspension was homogenized by repeated flushing out of the marrow with the help of a Pasteur pipette and kept at 37ºC for twenty minutes. Incubation was followed by centrifugation of the suspension at 1000 rpm for 5 minutes. The supernatant was discarded and the pellet was fixed with freshly prepared fixative (1 part glacial acetic acid: 3 part methanol), which was added slowly with a Pasteur pipette to get a homogenous suspension. The material

Table 1: Localities, number of males and females and the period of collection

Population and locality	No. of individuals studied Males	Females	Total	Distance from allahabad (in kms)	Period of collection
Population A Allahabad	8	10	18	--	1988-90
Population B (East from Allahabad) (Chotanagpur Division)					
Ranchi	1	3	4	420	1989-90
Giridih	0	1	1	480	1989-90
Hazaribag	1	1	2	400	1989-90
Daltonganj	7	6	13	280	1989-90
Population C (East from Allahabad) (Singhbhum Division)					
Chaibasa	2	3	5	508	1990-91
Berhampur (South from Allahabad) (Coastal Odisha)	0	1	1	740	1990-91

was then kept for thirty minutes at room temperature followed by centrifugation at 1000 rpm for 5 minutes. After centrifugation the supernatant was decanted and fresh fixative were given at intervals of 5 minutes. After the final change material was shaken vigorously to obtain a cloudy suspension.

Chromosome preparations were made on slides which were dipped overnight in 50% methanol at 4ºC. However, for air dried preparations dry and clean slides were used. 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 (Merck) diluted in Sorensen's buffer (pH 6.8). The excess stain was removed by rinsing the slide in distilled water. Staining was controlled by examining the slides from time to time under microscope. After staining the slides was washed in double distilled water (DDW), air dried and mounted in DPX.

C-banding method

The constitutive heterochromatin staining method of Sumner (1972) was followed with minor modifications. Four to six days aged slides were treated with 0.2 N HCl for one hour at room temperature followed by thorough washing in DDW. The slides were then treated with saturated solution of Ba $(OH)_2$, filtered before use, at 50ºC for 1-2 minutes in water bath. The chromosome preparations were rinsed in 0.2 N HCl and washed thoroughly in DDW. Thereafter, the slides were incubated in 2x SSC (0.3 M NaCl, 0.03 M Na citrate) at 60ºC for one hour, washed thoroughly with DDW, dried and stained in 2.5% Giemsa diluted with Sorensen's buffer at pH 6.8. The staining intensity of chromosome s was controlled by examining the slides from time to time. After staining the slides were washed in DDW, air dried and mounted in DPX.

Analyses and schematic representation of C- bands

The C-banding patterns are based on observations of ten metaphase plates of each karyomorph. The banding pattern appears to be quite variable from one plate to another due to condensation of chromosomes. Thus, the idiograms showing schematic presentation of C-bands are based on the chromosomes showing minimal condensation which reveal optimum number of bands the idiograms depicting C- bands have been constructed using the relative length (L^R) and centromeric indices (I^C) data. The representations of banding patterns on the idiograms are based on visual and photographic examination.

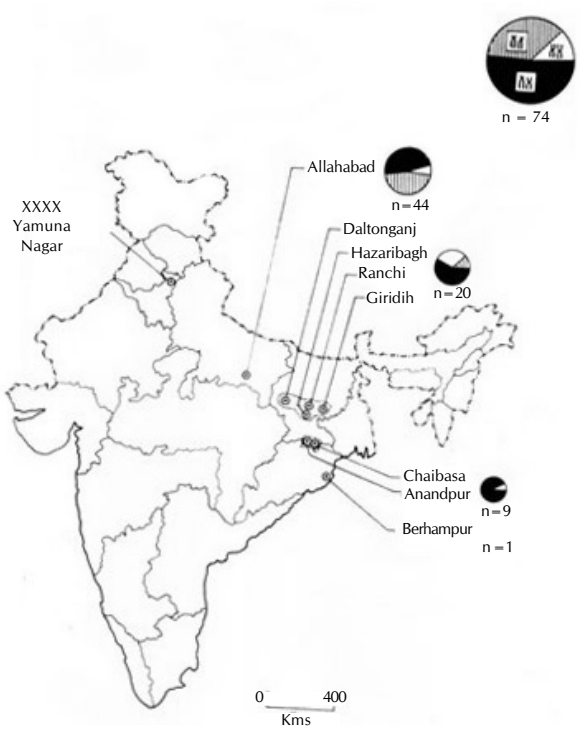

Figure 1a: The sites from where *T.p.phoenicoptera* has been karyotyped and found polymorphic for chromosome 2. Frequency of birds with the three sample size is represented by dimension of circles: the white, black and striped areas show frequency of birds with 2^m2^m, $2^{st}2^m$ and $2^{st}2^{st}$, respecively

RESULTS

In the present investigation, mitotic chromosomal analysis has been carried out on common or yellow - legged green pigeon, *Treron phoenicoptera phoenicoptera* (Latham) drawn from three populations from U.P and Jharkhand and a single female from Berhampur, Odisha (for details see Table 1). The basic chromosome number in all the individuals examined showed a prominent peak at 74 and has been taken as the diploid chromosome number of this species. During the present study the sample showed same polymorphism initially identified by Ansari and Kaul (1979b) from Allahabad population. Two forms of autosomes 1, *i.e.,* 1^m and 1^{st} and also of autosome 2, *i.e.,* 2^{st} and 2^m exist in the population and have been explained

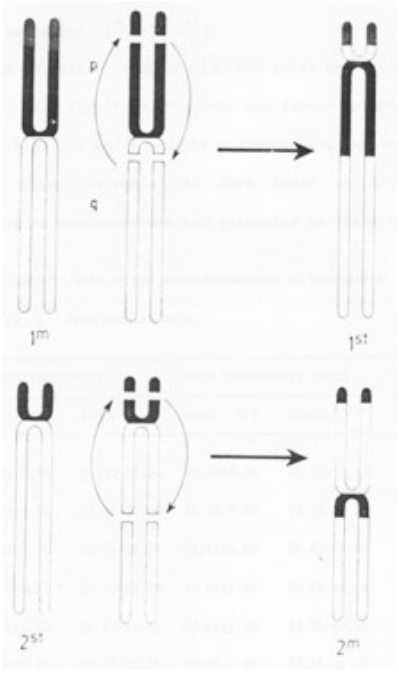

Figure 1b:Schematic representation of the proposed mechanism of pericentric inversion. Gaps indicate chromosome breakage and reunion points

on the basis of pericentric inversions taking place in these chromosomes. The scheme of events giving rise to the polymorphism is given in Fig. 1(b).

As a result of these karyotypic changes, a total number of nine different karyomorphs (not including the sex chromosomes) are expected to be present in the population.

Somatic karyotype

In the present study nineteen males and twenty five females constituted the samples from three different geographical areas. In these samples five karyomorphs were encountered. The most frequently observed doubly homozygous karyomorphs, *i.e.*, $1^m1^m/2^{st}2^{st}$ has been designated as the standard karyotype. The details of karyotype are described first followed by the details of other karyotype.The basic karyotype consists of seven pairs of macrochromosomes (including the sex chromosomes) which can be easily distinguished from the remaining thirty pairs of micro chromosomes. The macrochromosomes have been divided into two size groups. Group A includes three pairs of large autosomes 1, 2 and 3. Pair 1 is the largest chromosome with median centromere and it has been designated as 1^m. Chromosome 2 has its centromere in the sub terminal region and is designated as 2^{st}.Chromosome 3 is the third largest macrochromosomes with its centromere at subterminal region.

Group B consists of four pairs of medium sized macrochromosomes designated as pairs 4, 5, 6 and 7. The Z (pair 4) chromosome belongs to this group and has centromere at median position. The W chromosome of the females is the smallest submetacentric macrochromosome. In female karyotype, W chromosome has always been placed next to Z chromosome. Chromosome 5 is a metacentric chromosome. Chromosome pairs 6 and7 are in decreasing order. The remaining thirty pairs of chromosomes are categorized as microchromosomes.The centromeric position of the micro chromosomes cannot be fully ascertained though some of the large micro chromosomes appear to have their centromere at the terminal point.

C-banding Karyotype

Good C- bands were generated in air-dried preparations of birds with karyomorphs $1^m1^m/2^{st}2^{st}$ (Figs. 3a, b), $1^m1^m/2^{st}2^m$ (Figs. 4a to d) , $1^m1^m/2^m2^m$ (Figs. 5a, b) , 1^m1^{st} / $2^{st}2^{st}$ and $1^{st}1^{st}$ / $2^{st}2^m$ (Figs. 6a to d) from population A; Karyomorphs $1^m1^m/2^{st}2^m$ and $1^m1^m/2^m2^m$ (Figs.7a to d) from population B; karyomorphs $1^m1^m/2^{st}2^{st}$ and 1^m1^m/ $2^{st}2^m$ (Figs. 8a to d) from population C and the karyomorph $1^m1^m/2^{st}2^m$ (Figs. 9a, b) from Berhampur, Odisha.

C-bands were studied in a large number of metaphase plates of each karyomorph. An idiogram of C-banded macrochromosomes based on these observations is represented in Fig. 2.

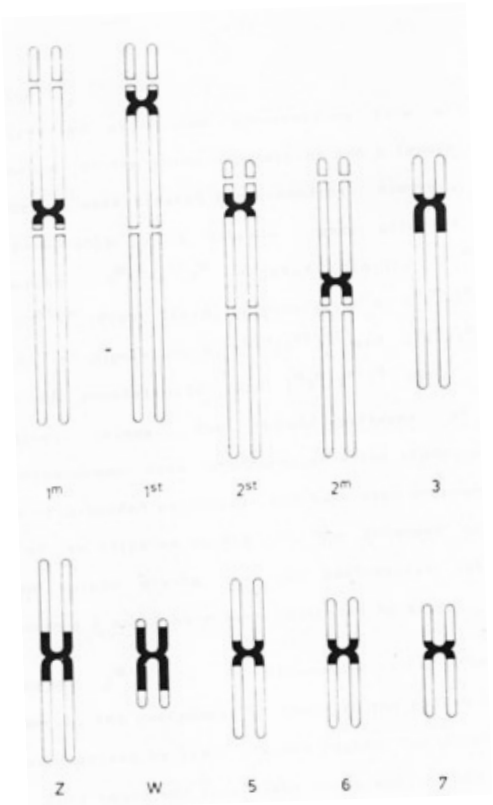

Figure 2: Idiogram representing C-bands of seven macrochromosomes of heterozygous complement

Table 2: The position and size of C-band in the macrochromosomes

Macrochromosomes	C-bands
1^m	medium
1^{st}	medium
2^{st}	medium, extending on the p arm
2^m	medium, extending on the q arm
3	large, bipartite, more towards q arm
Z	large
W	Very large, covering almost both the arms
5	medium
6	medium
7	Small

All the macrochromosomes have distinct C-bands at the centromere and three band sizes large, medium and small have been categorized purely on visual basis (Table 2).
C- bands in the microchromosomes are not very clear and therefore analysis is restricted to the macrochromosomes only.

Intercalary or terminal C- bands have not been observed in any of the macrochromosomes. No variant forms of C-bands have been recorded in the individuals examined during the present study.

Chromosome 1: Chromosome 1 possesses pericentromeric C- band of medium size (Fig. 2). In individuals heterozygous for chromosome 1(1^m1^{st}), both the homologs have similar medium sized C-bands (Figs. 6)

Chromsome 2: Chromosome 2 has a band size comparable to that of chromosome 1. In subtelocentric chromosome 2, the C-band extends more on the short arm (Figs.3-9) while its partner in heterozygous karyotype ($2^{st}/2^m$) reveals homologous medium sized c-bands extending in the long arm (Figs.4,6, 7, 8,9). Thus in inversion heterozygotes for pair 2, the C-band can be clearly seen shifted together with the centromere.

Chromosome 3: The C-band in the chromosome 3 is the largest and extends well into the short arm where it is bipartite.

Sex chromosomes: The Z chromosome has clear centromeric band of large size. The W chromosome is largely C- band

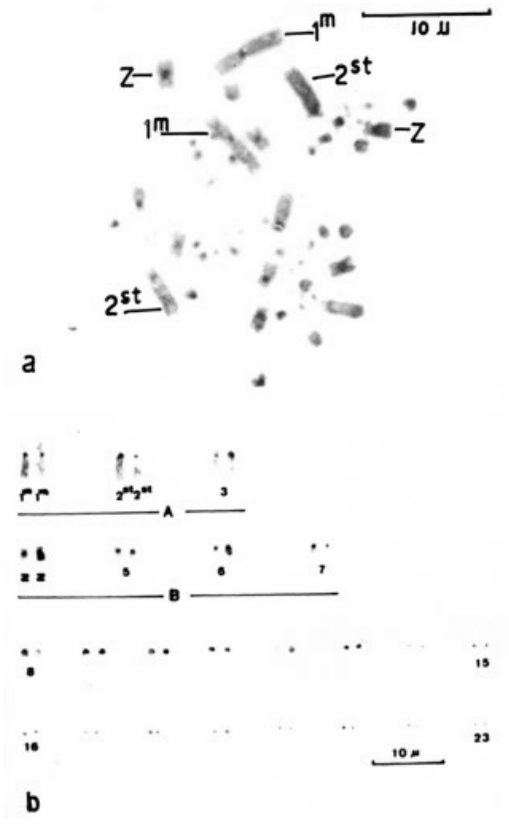

Figure 3: C-banded $1^m1^m2^{st}2^{st}$ karyotype from population A. (a) male metaphase (b) Partial male karyotype karyotypes in figs. 3 to 9 include seven pairs of macrochromosomes and sixteen pairs of microchromosomes only

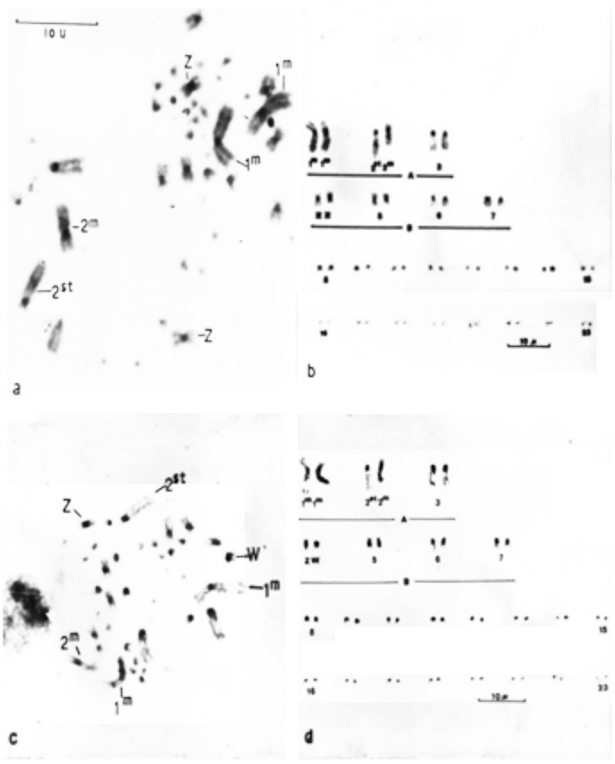

Figure 4: C-banded karyotype of $1^m1^m2^{st}2^m$ individuals from population A. (a) Male metaphase; (b) Male karyotype; (c) Female metaphase; (d) female karyotype

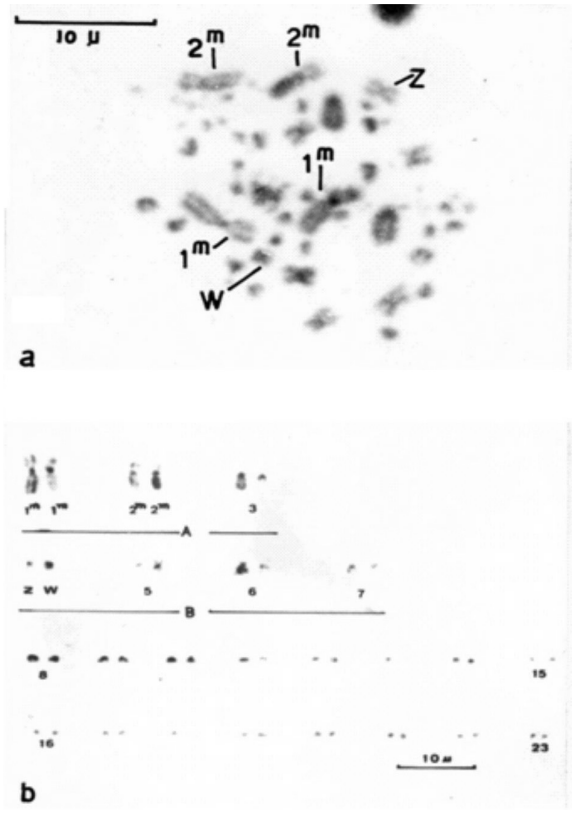

Figure 5: C-banded karyotype of $1^m1^m2^m2^m$ individuals from population A. (a) Female metaphase; (b) female karyotype

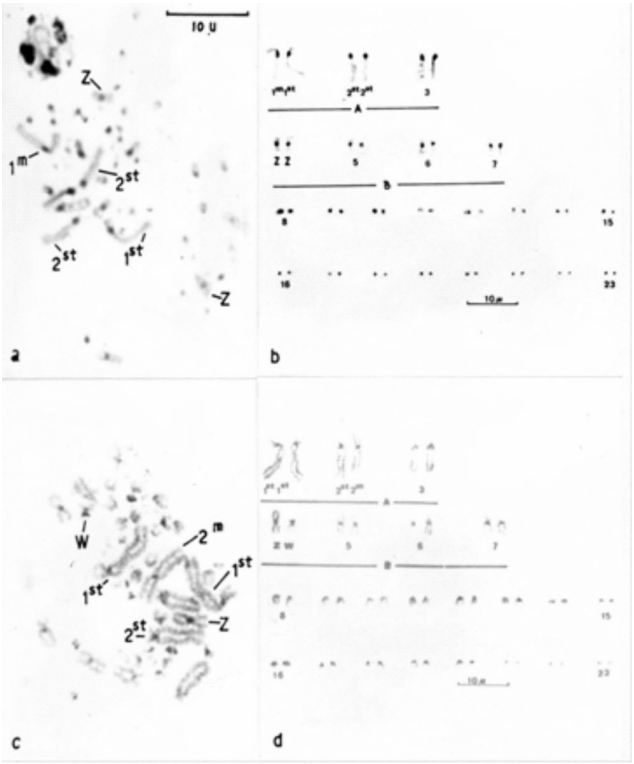

Figure 6: C-banded karyotype of $1^m1^{st}/2^{st}2^{st}$ (a and b) individuals from population A. (a) Male metaphase; (b) Male karyotype; (c) Female metaphase; (d) female karyotype

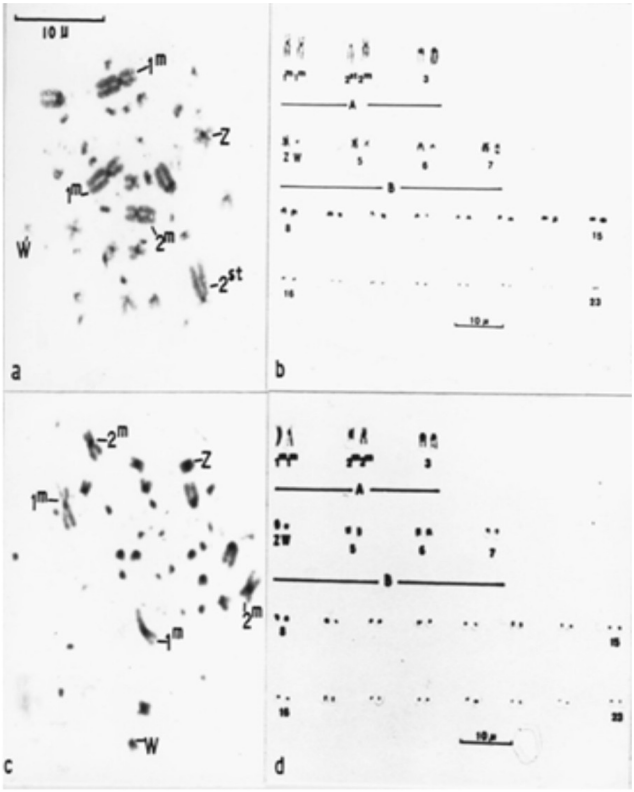

Figure 7: C-banded karyotype of $1^m1^m/2^{st}2^m$ (a and b) and $1^m1^m/2^m2^m$ (c and d) individuals from population B. (a) Male metaphase; (b) Male karyotype; (c) Female metaphase; (d) female karyotype

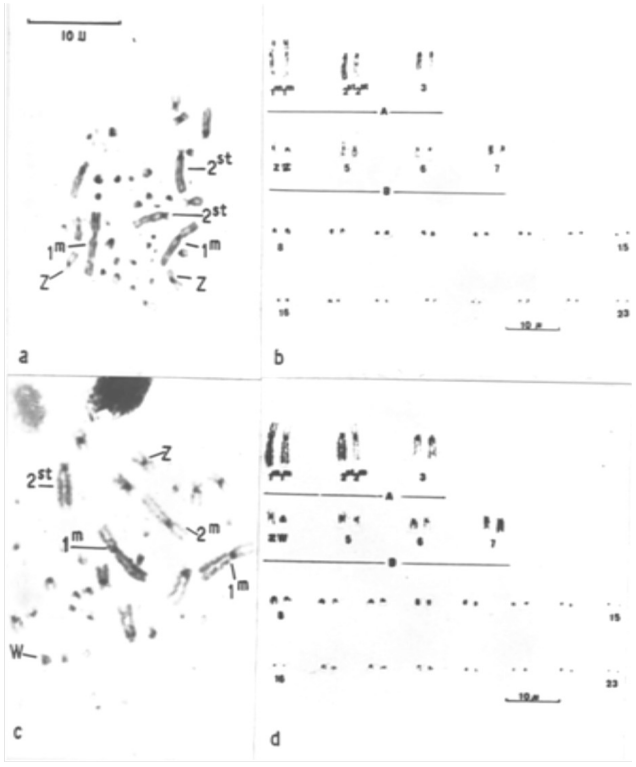

Figure 8: C-banded karyotype of $1^m1^m/2^{st}2^{st}$ (a and b) and $1^m1^m/2^{st}2^m$ (c and d) individuals from population C. (a) Male metaphase; (b) Male karyotype; (c) Female metaphase; (d) female karyotype

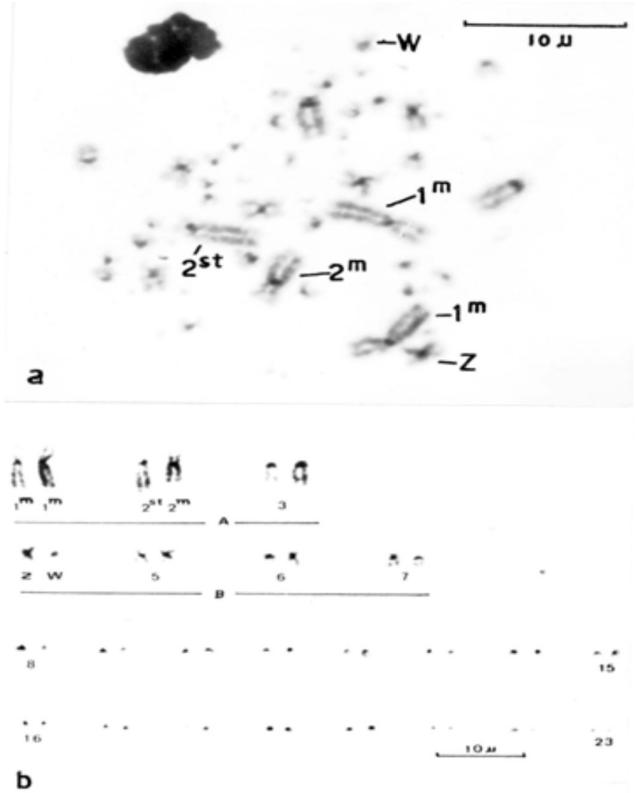

Figure 9: C-banded karyotype of $1^m1^m/2^{st}2^m$ individuals from Berhampur, Odisha. (a) Female metaphase; (b) female karyotype

positive throughout its length except very small regions at the telomeric ends of short and long arms.

Chromosomes 5, 6 and 7: chromosomes 5 and 6 possess medium sized centromeric C-band while chromosome 7 shows centromeric C-band of small size.

DISCUSSION

There were several reports of C-band polymorphism in birds. The C-banding studies in an Australian population of *C.chloris* by Christidis (1986c) showed inversion polymorphism for chromosome pair 1. In basic karyotype, a distinct centromeric C-band is present in the subtelocentric chromosome 1 but the same characteristic band is lacking in the corresponding submetacentric morph. Besides, the Z chromosome also possesses C-band polymorphism. Both the Z chromosomes of the male show a large telomeric C-band which was absent in the females. Christidis (1986b) studied chromosomal constitution of thirteen species of the genus *Lonchura* by conventional Giemsa staining and C-banding. In nine individuals of *L. punctulata* chromosome pairs 6, 7 and 8 were recognized to be dimorphic with respect to independent pericentric inversions. In addition C-band polymorphism was also present in autosome pairs 1, 3 and 5 and in these cases heterozygosity was associated with the presence or absence of centric C-band.C-banded karyotype revealed that the acrocentric form of chromosome 6 lacked centromeric heterochromatin. The submetacentric Z lacked the terminal C-block present in the metacentric Z chromosome. Banding analysis *Poephila acuticauda* by (Chritidis, 1986a) revealed a very interesting case of Z chromosome polymorphism which depends on two unrelated factors *i.e.,* additional C-band and position of the centromere. In *P.cincta* a centric dimorphism was identified involving originally acrocentric chromosome 7. In heterozygous condition acrocentric chromosome 7 becomes telocentric. C-band polymorphism for the presence/ absence of an additional interstitial band in chromosome 2 has also been found. Another case of Z polymorphism on the basis of C-band pattern was identified in *P.bichenovii* (Christidis, 1986a). Three distinct karyomorphs were observed with varied distribution of terminal blocks of heterochromatin. The three different karyomorphs of Z chromosomes are due to the presence of large telomeric block on the short arm or small telomeric block on long arm or a third type possessing both types of terminal blocks. *Aegintha temporalis* also shows C-band polymorphism resulting from the presence of an interstitial C-band on the long arm of chromosome pairs 2 as well as on the Z chromosome. However, male and female individuals differ in the position of interstitial C-band in the Z chromosome. While in the males it is situated proximally, in the females the band is located on the distal end of the long arm (Christidis, 1986a). C-band heteromorphism in the short arm of chromosome 4 was recorded in *Aratinga cactorum* (De Lucca, 1948b). Similar C-banding patterns were observed in several cells from 182 Japanese quail embryos to detect presence of stable variants (Sena *et al.,* 1991). Distinct variants of chromosome 4 and the Z chromosome were observed. In the Z chromosome a C-band at the terminal region of the short arm was markedly in some embryos. Likewise, the short arm of chromosome 4 was much more prominent in one or both of the homologues in some embryos.

In the present study comparisons of C-bands between birds with different karyomorphs do not provide much information on the nature of inversions, owing to the fact that no intercalary or terminal C-bands are found in any microchromosome. In chromosome 1 the centromeric C-band is so symmetrical that the change from 1^{m} to 1^{st} condition cannot be decided on the basis of C-bands. However, the position of centromere, median or subtelocentric, becomes very clear after C-banding.

In chromosome 2, the centromeric C-band extends well into the short arm while in 2^{m} condition the long arm displays the C-band extends into it. This finding further provides evidence that there has been a pericentric inversion in chromosome 2.

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