Rec. zool. Surv. India : **101** (Part 3–4) : 147-157, 2003

PHYLOGENETIC SIGNIFICANCE OF ROBERTSONIAN FUSIONS IN *CHORTHIPPUS INDUS* UVAROV (ORTHOPTERA : ACRIDIDAE : GOMPHOCERINAE)

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INTRODUCTION

The grasshopper species *Chorthippus indus* Uvarov, has been investigated for the first time. Subfamily Gomphocerinae is an assemblage of large number of genera. *Chorthippus* comprises of six sub-genera of which sub-genus *Chorthippus* is an enormous one with not less than 65 species (Jago 1971). So far 23 species under this genus are known chromosomally, each having 2n ♂ = 17 (XO), instead of 23 acrocentrics. Their three meta/submetacentric pairs had originated by Robertsonian fusions. Several species under different genera have also been reported for their Robertsonian fusions. Had these species obtained their three fusions independently? or they form a natural phylogenetic group pleading their origin monophyletic (i.e., fusions truly homologous). The findings on the submetacentric chromosomes of *C. indus* carries unique phylogenetic significance. This species had obtained its fusions independently, so can not be placed with so called natural phylogenetic group. The present investigations also include morphometric analysis of the published karyotype of several species which support polyphyletism in the Gomphocerinae.

MATERIAL AND METHODS

Six males collected from Saproon and Renuka lake in Himachal Pradesh were injected with 0.03 to 0.04 ml of 0.05% colchicine prior to dissection to arrest metaphases. After 4–6 hours of injection, the testes and hepatic caecae were dissected-out and cleaned in 0.67% solution of insect saline. The tissues were then transferred for a hypotonic treatment in a solution of 0.9% sodium citrate for 45–60 minutes then were fixed for a minimum of 40 minutes. In the field, tissues were transferred to small tubes and brought to laboratory and stored preferably at 4°c in refrigerators. The slides were prepared by the air-dry technique. The fixed tissues were transferred to 50% acetic acid till it became soft. Squashed this material in a few drops of 50% acetic acid, and stored in vapours of 50% acetic acid in refrigerator for overnight. Next morning the slides were brought at room temperature and immersed in 1 : 3 acetic acid methanol mixture for an hour. The coverslips were removed with the sharp edge of a blade in the immersed condition and dried at room temperature in a dust proof chamber. After a preliminary scanning of the unstained slides, the

selected ones were stained in 2-3% solution of Giemsa [E. Merck (India) Pvt. Ltd.] in phosphate buffer at pH 6.8 to 6.9. Staining was continued till appropriate contrast was obtained; they were then rinsed in distilled water (two changes) and rapidly air dried under lamp. Selected slides were soaked in xylene and mounted in DPX.

The cells with good chromosome spreads were photomicrographed with the help of Leitz Ortholux microscope. Black and white negative film viz., NP22, 120 ASA (35 mm) were mostly used. The negatives so obtained were printed on photosensitive Agfa bromide papers of normal and hard grades. Fine grain film and paper developers of Agfa-Gevaert (A901 and A902) and Kodak (D76 and D163) were used in developing negatives and positive prints.

The diploid number (2n) was determined by basic or most predominant number observed. The cut-out of individual chromosomes which appeared similar in morphology and staining intensity were paired to construct karyotypes. Morphometric measurements of each chromosome was taken from several karyotypes. Their mean values were used calculating the relative length of the chromosome in percent of the total haploid length. The measurements were used in constructing idiogram i.e. a diagrammatic karyotype of the species. The nomenclature of Levan *et al.* (1964) has been followed. The individually numbered specimens of the species with locality details are preserved and maintained in the Cytotaxonomy Research Laboratory, Zoological Survey of India, Kolkata.

OBSERVATIONS

KARYOLOGICAL DETAILS :

Diploid Number, Chromosome Morphology and Sex Chromosome Constitution

17 chromosomes were present in the males. This consisted of 6 metacentric and 10 acrocentric autosomes and 1 acrocentric X chromosome. Smaller arms of the metacentrics were larger than the last four chromosomes. The 1st, 2nd and 3rd largest metacentric elements were the fusion products of the 2nd and 4th, 1st and 8th and 3rd and 7th acrocentric chromosomes of the parental complement (Pl. refer table I & RL in percent). The X was the 4th largest in the complement with its centromeric region negatively heteropycnotic at late prophae I and metaphase I stages (Figs. A-D arrow).

Karyotype :

Study of 66 cells—2 zygotene, 50 diakinesis/metaphase I and metaphase plates of 5 males confirmed the morphological feature of the complement. Chiasma localization of the bivalents was also studied.

Gaps/Constrictions :

Gaps/constrictions were not present. Centromeric region of the biarmed elements appeared as uncoiled constrictions at diakinesis (Figs. A, B & D arrow).

Relative length (RL) in percent :

1	2	3	4	5	6	7	8	9
22.52	20.65	16.60	8.53 (X)	8.20	6.82	5.29	5.12	4.27

Idiogram :

The X or the 4th largest in the complement, was half the size of the 3rd pair.

Chiasma Distribution :

For study of chiasma distribution, each bivalent was arbitrarily divided into 3 equal segments as proximal (P), interstitial (I) and distal (D). The chiasmata were randomly distributed in all the bivalents except the last one which appeared to have distally located chiasma.

Bivalents	1	2	3	4	5	6	7	8
Chiasma	2I, 1D 1	I, D 2	2D 1	P, D 1	D 3	D 3	D 3	D 4
	2I, 2D 1	I, 2D 1	2I 1	I 2	I 1	P.D 1	I 1	
Location	3I 2	2I 1	2I, 2D 1	D 1				
& number			D, I 1					
Total	I = 10	I = 5	I = 5	P = 1	I = 1	P = 1	I = 1	
	D = 3	D = 4	D = 5	I = 2	D = 3	D = 4	D = 3	D =4
				D = 2				
	13	9	10	5	4	5	4	4

The 54 chiasmata scored for 32 bivalents were 2(P), 24(I) and 28(D). Small arm of the submetacentric pairs was found to form chiasma in every cell (Figs. A-D).

DISCUSSION

Jago (1971) arranged species under genus *Chorthippus* into six subgenera, and of which subgenus *Chorthippus* was an enormous one with not less than 65 species. The large complex of *Chorthippus* is distributed in all zoogeographical regions except Australia (Dirsh 1975). Bhowmik (1985) recorded 3 species from India and adjoining regions viz., *Chorthippus* (Chorthippus) *almoranus* Uvarov (from Almora in Uttar Pradesh), *C.* (Glyptobothrus) *hammarstroemi* (Miram) (India (H.P.), Siberia and China) and *C.* (Chorthippus) *indus* Uvarov (from Kashmir; Simla (H.P.); Kumaon Hills (U.P.) and N.W.F. Province, Pakistan). *C. hammarstroemi* had 2n = 21 in males (20 + X) (Kiknadze and Vysotskaya 1970). *Chorthippus schmidti* from Russia had 2n = 23, all acrocentrics in males (Bugrov 1996). Karyologically twenty three *Chorthippus* species known so far had 2n = 17 ♂ with 6 meta/

Table-1. Chromosome measurements of species under subfamily Gomphocerinae.

Species & References	Chromosome number n : 2n									Constituents of metacentrics		
	1	2	3	4	5	6	7	8	9			
Chorthippus biguttulus	5	9	8							1	2	3
Santos J.L. and Giraldez	8.5	7.2	7.4	10.6(X)	7.8	7.5	6.9	6.2	4.2	(1 + 5)	(2 + 9)	(4 + 8)
R. (1978) (C-band)	13.5	11.9	10.3									
	1	2	4	3	6	7	10	11	12			
Chorthippus brunneus	6	5	8							1	2	3
John, B. and Hewitt,	14.1	14.1	11.0	15.3(X)	12.9	10.6	7.7	6.3	5	(1 + 6)	(2 + 5)	(3 + 8)
G. M. (1966)	21.3	19.5	16.2									
	1	2	3	4	7	9	10	11	12			
Chorthippus brunneus	8.8 6.5	6.6 7.5	4.8 5.2		7.2 : 6.9	6.5 : 5.5	5.2 : 4.5	4 : 3.8	3.5 : 2.6			
Jones G. H., Stamford	16 14.3	10.6 10.2	10.1 7.5									
W. K. and Perry P. E.	5	6	9							1	2	3
(1975) (C-band)	7.65	7.05	5	7.9(X)	7.05	6	4.85	3.9	3.05	(1 + 5)	(2 + 6)	(3 + 9)
	15.15	10.4	8.8									
	1	2	3	4	7	8	10	11	12			
Chorthippus brunneus	9.8 9.7	8.3 10	9 10		9.4 : 8.2	8.2 : 7.8	7.7 : 6.6	5.8 : 4.3	3.7 : 3.7			
John B. and Hewitt	15.5 15	13.2 13.1	11.9 10.7									
G. M. (1963)	5	7	6							1	2	3
(C-band)	9.75	9.15	9.5	10.3(X)	8.8	8	7.15	5.05	3.7	(1 + 5)	(2 + 7)	(3 + 6)
	15.25	13.15	11.3									
	1	2	3	4	8	9	10	11	12			
Chorthippus brunneus	8.7 9.4	10.5 9.1	8.5 8.7		10.5 : 9.4	8 : 7.7	7.3 : 7	5 : 3.2	3.2 : 3			
John B., Lewis K. R. and	15.1 15.0	14.5 13.7	11 10.5									
Henderson S. A. (1960)	7	6	8							1	2	3
	9.05	9.8	8.6	11.4(X)	9.95	7.85	7.15	4.1	3.1	(1 + 7)	(2 + 6)	(4 + 8)
	15.05	14.1	10.75									
	1	2	4	3	5	9	10	11	12			
	8	4	7							2	1	3
Chorthippus indus	4.3/8	5.8/7.5	4.7/6.6	5.3(X)	5.2	4.2	3.5	3.5	2.1	(1 + 8)	(2 + 4)	(3 + 7)
Present observation	1	2	3	5	6	9	10	11	12			

Table-1. (*Contd.*).

Species & References	Chromosome number n : 2n									Constituents of metacentrics		
	1	2	3	4	5	6	7	8	9			
Chorthippus jucundus	6	9	7							1	2	3
John B. (1973)	13.2	11.0	12.2	18.7(X)	15	11.4	8.0	6.0	5.0	(1 + 6)	(2 + 9)	(4 + 7)
	21.6	20.7	18.0									
	1	2	4	3	5	8	10	11	12			
Chorthippus parallelus	7	5	10							1	2	3
John B. and Hewitt	11.5	13.1	9.3	13.1(X)	12.2	10.9	10	7.2	4.12	(1 + 7)	(2 + 5)	(3 + 10)
G. M. (1966)	19.5	17.3	14.3									
	1	2	3	4	6	8	9	11	12			
Chorthippus parallelus	8	9.2	8.6	9.5	7.6	7.8		10 : 8.5	8.2 : 8	6.6 : 6.1	4.7 : 4.5	3.3 : 3
John B. and Hewitt	14.9	14.7	14.4	13.9	12.5	11.3						
G. M. (1966)	7	6	9							1	2	3
	8.6	9.05	7.7	11.2(X)	9.25	8.1	6.35	4.6	3.15	(1 + 7)	(2 + 6)	(3 + 9)
	14.8	14.15	11.9									
	1	2	3	4	5	8	10	11	12			
Chorthippus parallelus	9.2	7.6	8.6	8	6.6	7.5		8.2 : 8.6	7.7 : 6.9	6.5 : 6.4	5 : 4.4	3.8 : 2.2
Hewitt G.M. and	15.3	15.1	14.5	13.7	11.4	10.5						
John B. (1968)	6	7	9									
	8.4	8.3	7.05	12.3(X)	8.4	7.3	6.45	4.7	3	1	2	3
	15.2	14.1	10.95							(1 + 6)	(2 + 7)	(4 + 9)
	1	2	4	3	5	8	10	11	12			
Euchorthippus pulvinatus	7	6	9							1	2	3
Arana P. Santos J.L. and	6	6.1	5.0	7.6(X)	7.0	5.5	5.0	2.8	2.0	(1 + 7)	(2 + 6)	(3 + 9)
Giraldez R. (1980)	11	10.2	9.2							The last two pairs are quite distinct		
(C-band)	1	2	3	4	5	8	10	11		as short pairs in *E. pulvinatus*		
Euchorthippus pulvinatus	11.6	11.3	11.2	10.7	10.5	9	17 : 16(X)	14.6 :	12.5 :	11.4 :	5.5 :	5.4 :
Santos J.L. and Giraldez	20.5	20.4	18.8	18	16.4	15.8		14.1	12.1	11.1	5.5	5.3
R. (1982) (C-band)	7		9		10							
	11.45		10.95		9.75		16.5(X)	14.35	12.3	11.25	5.5	5.35
	20.45		18.4		16.1							
	1		2		4		3	5	6	8	11	12

Constituents of metacentrics		
1	2	3
(1 + 7)	(2 + 9)	(4 + 10)
The last two pairs are quite distinct as short pairs in *E. pulvinatus*		

Table-1. (*Contd.*).

Species & References	Chromosome number n : 2n									Constituents of metacentrics		
	1	2	3	4	5	6	7	8	9			
Gomphocerus sibiricus	6	4	8							1	2	3
Gosalvez J. and Lopez-	3.5	3.7	3.3	3.5(X)	3.3	2.3	2.2	1.6	1.6	(1 + 6)	(2 + 4)	(3 + 8)
Fernandez C. (1981)	6.1	5.3	5.2									
	1	2	3	5	7	9	10	11	12			
Myrmeleotettix maculatus	8	5	7							1	2	3
John B. and Hewitt G.M.	11.4	13.5	11.5	16.7(X)	13.0	10.1	8.1	5.1	4.5	(1 + 8)	(2 + 5)	(4 + 7)
(1965)	21	18.2	15.7									
	1	2	4	3	6	9	10	11	12			
Myrmeleotettix maculatus	7	5	8							1	2	3
John, B. and Hewitt,	12.5	14.2	10.6	16.5(X)	13.2	10.5	7.4	5.6	4.5	(1 + 7)	(2 + 5)	(3 + 8)
G. M. (1966)	23.5	19.5	16.5									
	1	2	3	4	6	9	10	11	12			
Myrmeleotettix maculatus	10	9.5	8.2	7.5	6.9	7.2		9 : 8.2	6.9 : 6.6	4.8 : 4.7	4 : 3.6	3.5 : 3.1
Gallagher A. Hewitt G. M.	14	13.8	13.6	13.1	10.5	10.4						
and Gibson I. (1973)	5	7	8							1	2	3
	9.75	7.85	7.05	10(X)	8.6	6.75	4.75	3.8	3.3	(1 + 5)	(2 + 7)	(3 + 8)
	13.9	13.35	10.45									
	1	2	3	4	6	9	10	11	12			
Omocestus viridulus	6	5	8							1	2	3
John B. and Hewitt	14	14.6	11.6	15.6(X)	12.5	10.6	9.8	5	4.4	(1 + 6)	(2 + 5)	(3 + 8)
G.M. (1966)	24	20.7	17.2									
	1	2	3	4	7	9	10	11	12			
Omocestus viridulus	5	6	8							1	2	3
Santos J.L. and Fox D. P.	7.12	6.3	5.7	7.4(X)	6.3	5.6	4.5	2.5	2.5	(1 + 5)	(2 + 6)	(3 + 8)
(1988) (N-band)	10.7	10	8									
	1	2	3	4	7	9	10	11	12			

submetacentric chromosomes. The species being *C. albomarginatus* (Santos and Fox 1988), *C. apicalis* (Santos and Fox 1988), *C. apricarius* (del Cerro and Santos 1995; Santos and Fox 1988), *C. biguttulus* Santos and Giraldez 1978 and Santos and Fox 1988), *C. binotatus* (Santos and Fox 1988), *C. brunneus* (Camacho et al. 1984; Hewitt 1964; 1965; John and Hewitt 1963, 1966b; John et al., 1960; Jones et al. 1975; Lewis and John 1963; Santos and Fox 1988; Southern 1967), *C. dorsatus* (Santos and Fox 1988), *C. hammarstroemi* (Kiknadze and Visotskaya 1970), *C. jucundus* (Cano and Santos 1990; John 1973; Santos and Fox 1988), *C. longicornis* (Coleman 1947), *C. parallelus* (Camacho et al. 1984; Cano and Santos 1990; Fox et al. 1974; Hewitt 1964, 1965; Hewitt and John 1968, 1970; John and Hewitt 1966a, 1966b, 1968, 1969; Santos and Fox 1988; Westerman 1969, 1970) and *C. vagans* (Cano and Santos 1990; del Cerro and Santos 1995 : Santos and Fox 1988). Bugrov (1996) reported another 11 species of *Chorthippus* of the Russian sub-continent with 17 chromosomes viz., *C. angulatus, C. dichrous, C. fallax, C. montanus, C. intermedius, C. jacobsoni, C. loratus, C. macrocerus, C. ferganensis, C. saxatilis* and *C. vicinus.* Hewitt (1979) reported Robertsonian fusion in another 12 genera of Gomphocerinae viz., *Chloealtis, Chrysochraon, Euchorthippus, Euthystira, Gomphocerus, Mongolotettix, Myrmeleotettix, Napaia, Omocestus, Podismopsis, Stauroderus* and *Stenobothrus.* He had suggested their monophyletic origin, and thus had formed a natural phylogenetic group.

It was found worth while in tracing the validity of this natural phylogenetic grouping, because cytogenetic studies carried out mostly on individual species had not been summarized in this regard. And, phylogenetic grouping on morphological features by Jago (1971) had no correlation with this natural phylogenetic group suggested by Hewitt (1979). In the present study, published metaphase complements of 8 species of 5 different genera were measured. The species were *C. biguttulus, C. brunneus, C. jucundus, C. parallelus, C. indus, Euchorthippus pulvinatus, Myrmeleotettix maculatus, Omocestus viridulus* and *Gomphocerus sibiricus.* All the arms were measured and numbered serially according to their length (Table 1) which indicated their position in the parental complements (i.e. 2n ♂ = 23). The 1st, 2nd and 3rd largest chromosomes of parental complement always fused with other elements but they retained their positions in the complements after fusions. In *C. indus* (present observation) the 2nd largest parental chromosome after undergoing fusion with the 4th chromosome has become the biggest chromosome in the complement. In *Euchorthippus pulvinatus* (Santos and Giraldez 1982, collected from Spain), 2nd and 4th chromosomes had fused with 9th and 10th respectively. This feature was not noticeable in any of the species measured for the size of the chromosomes involved in Robertsonian fusions. *Chorthippus hammarstromi* with 2n ♂ = 21 (20 + X) with 1 fusion and *Chloealtis abdominalis* 2n ♂ = 19 (18 + X) with two fusions (Hewitt 1979) were also suggestive of their separate fusion events. Bugrov (1996) has reported 2n ♂ = 19 with 2 submetacentric pairs in *Erepippus mistschenkoi* and *E. sobolevi* belonging to Gomphocerini. It appears that the biarmed chromosomes in the 6 species, belonging to 4 different genera viz., *C. indus, C. hammarstroemi, E. pulvinatus, C. abdominalis, E. mistschenkoi* and *E. sobolevi,* did not originate monophyletically. Multivalents were not formed in any meiotic cell of *C. indus* studied, which apparently indicated that fusions had not shared any monobrachial homology.

SUMMARY

Karyological investigations carried-out on male individuals of an Acridid species viz., *Chorthippus indus* Uvarov (Subfamily : Gomphocerini) showed 2n ♂ = 17 (XO), comprising three submetacentric paris. These pairs were found to be the fusion products of 1st (2 + 4), 2nd (1 + 8) and 3rd (3 + 7) of parental complement i.e. its 23 chromosome karyotype, as the result of Robertsonian translocation. The X showed understained centromeric region, designated as nucleolus organiser. Submetacentric bivalents revealed non-localized chiasmata pattern. On comparative study of published metaphase complements of several species of *Chorthippus* and of other Gomphocerine genera, it was found that *C. indus* of Himachal Pradesh (India) had obtained its three fusions independently (i.e. not monophyletic).

ACKNOWLEDGEMENTS

The author is thankful to Dr. J. R. B. Alfred, Director, Zoological Survey of India, for providing facilities and encouragement in the work. Dr. M. S. Shishodia of Zoological Survey of India has obliged me by identifying the grasshoppers. Help rendered by Mr. R. Banerjee in typing the manuscript is also thankfully acknowledged.

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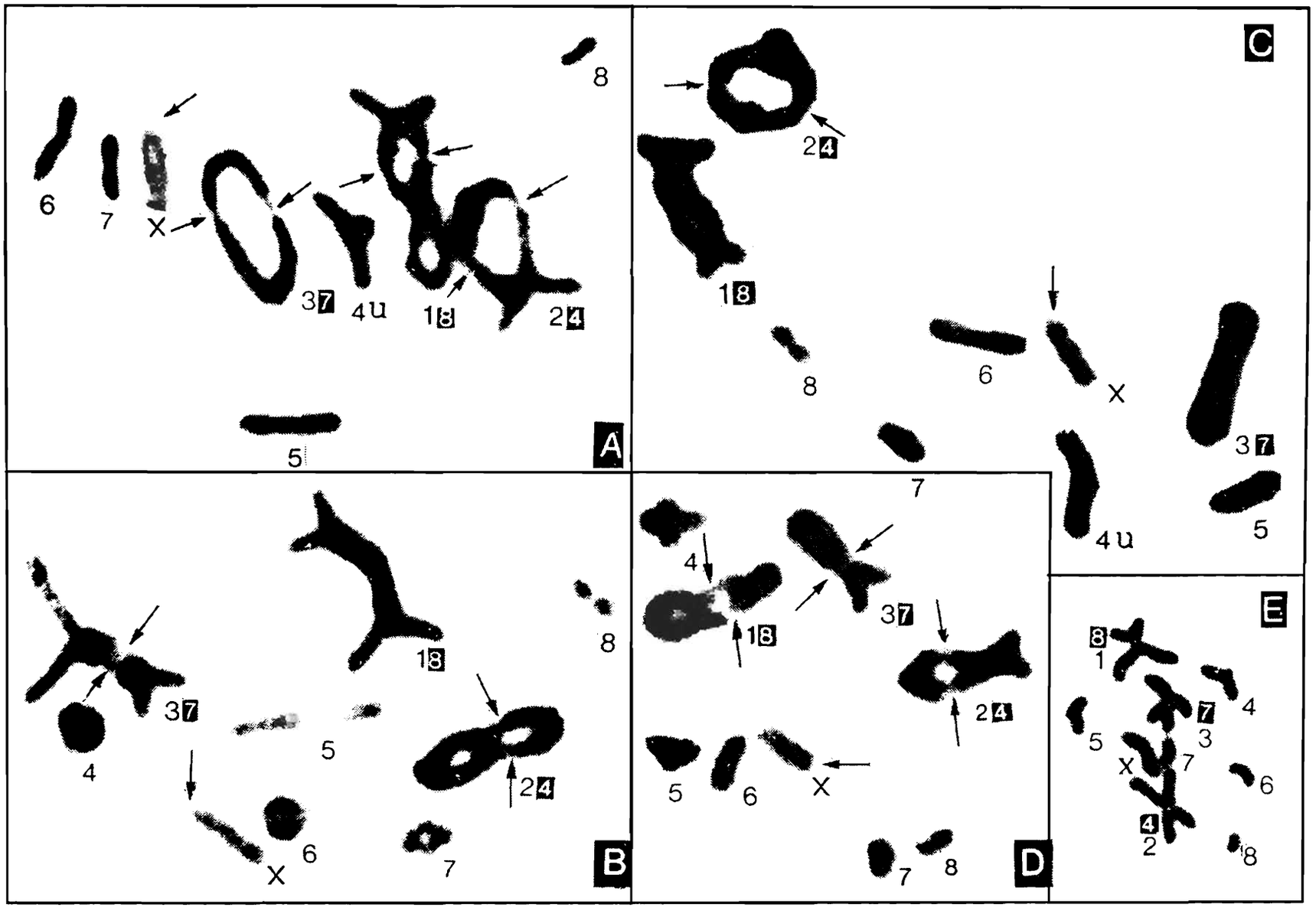

Figs. A-D. Late prophase I and metaphase I stages. Arrrows on the submetacentric bivalents indicate relatively uncoiled centromeric constrictions. In figs. A and C the 4th bivalent appears to be unequal. Arrow on the X indicates understained centromeric region, considered to be the NOR in the complement. Also observe considerable variation in the meiotic configurations of the bivalents. Fig. E : A metaphade II stage showing three submetacentric chromosomes. They have been found to be the fusion products of 1st (2 + 4), 2nd (1 + 8) and 3rd (3 + 7) of the parental complement i.e. 23 acrocentrics. Each arm has been numbered according to length.