

# Karyotypic Features of Eyprepocnemidinae Grasshoppers from Russia and Central Asia with Reference to the B Chromosomes in *Eyprepocnemis plorans* (Charp.)

Alexander BUGROV, Elżbieta WARCHAŁOWSKA-ŚLIWA and Ludmila VYSOTSKAYA

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Karyotypes and C-banding patterns of grasshoppers, members of the subfamily Eyprepocnemidinae *Thisoicetrinus pterostichus* (F.-W.); *Heteracris adspersa* (Redt.), *Eyprepocnemis unicolor* Tarb., *Eyprepocnemis plorans* (Charp.), and *Shirakiacris shirakii* (I. Bol.) are described. Chromosome complements of these species are composed of 23 acrocentric chromosomes in the male. The sex-determining mechanism is X0♂/XX♀. The B chromosomes were observed in the Northern Caucasus population of *E. plorans*. There was no evidence for any significant effect of 2Bs on either mean chiasma frequency or between cell-variance. There was a significant increase in the mean chiasma frequency for specimens with 1B.

Key words: Grasshoppers, Eyprepocnemidinae, karyotype, C-banding, B-chromosome, meiosis.

Alexander BUGROV, Institute of Systematics and Ecology of Animals, Siberian Branch of Russian Academy of Sciences, 630091 Novosibirsk, and Novosibirsk State University, 630090 Novosibirsk, Russia.

E-mail: bugrov@fen.nsu.ru

Elżbieta WARCHAŁOWSKA-ŚLIWA, Department of Experimental Zoology, Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, 31-016 Kraków, Poland

E-mail: warchalowska@isez.pan.krakow.pl

Ludmila VYSOTSKAYA, Novosibirsk State University, 630090 Novosibirsk, Russia

E-mail: vysot@fen.nsu.ru.

More than a hundred species of grasshopper, members of the subfamily Eyprepocnemidinae, are widely distributed in the tropical and subtropical areas of the Palearctic (UVAROV 1966). The karyotypes of only nine species and subspecies from Southern Europe (CAMACHO *et al.* 1980; CANO & SANTOS 1989), Africa (JOHN & LEWIS 1965), India (MANNA 1954; CHATERJEE *et al.* 1971; MITTAL & SONI 1977), and Japan (INOUE 1985) have been studied. This group is of interest in view of the unusually high diversity of the B chromosomes in the *E. plorans* karyotype (for a broader consideration, see LOPEZ-LEON *et al.* 1993). The B chromosomes were observed also in the *Heteracris littoralis* (CAMACHO *et al.* 1980; CANO & SANTOS 1988); the karyotypes of these two species have been, so far, studied in detail. The present report is the first to describe the hitherto

unknown karyotypes and C-banding patterns in five species of the Eyprepocnemidinae grasshoppers from the Caucasus and maritime areas of Russia and Central Asia.

## Material and Methods

The species of the grasshoppers Eyprepocnemidinae are listed in Table 1. Five or six males of each species were injected with 0.1% colchicine for 1.5-2.0 hours. The testes were excised and fixed in a 3 ethanol : 1 glacial acetic acid mixture. The fixed material was washed and kept in 70% ethanol. Air-dried preparations were made by squashing tissues in 45% acetic acid and freezing them on dry ice. The C-banding examination was

Table 1

List of grasshopper species and collection localities (No – number of individuals)

Species	No	Collection locality
<i>Thisiocetrinus pterostichus</i> (F.-W.)	9	North Caucasus (Daghestan), near Sulak settlement, August 1987
<i>Heteracris adspersa</i> (Redt.)	11	North Caucasus (Daghestan), near Sulak settlement, August 1987
<i>Eyprepocnemis unicolor</i> Tarb.	9	Tajikistan, near Kurgan-Tube town, July 1983
<i>Eyprepocnemis plorans</i> (Charp.)	9	North Caucasus (Daghestan), near Buinaksk settlement, August 1987
<i>Shirakiacris shirakii</i> (I. Bol.)	12	Maritime territory (Primorskij kray), near Slavyanka settlement, August 1982

carried out according to SUMNER *et al.* (1972) with minor modification.

Chiasma frequency was scored from the cells at diplotene/diakinesis I from all five species.

## Results

### The basic chromosome complement

The karyotype of all the species consists of  $2n\sigma=23$  chromosomes. The acrocentric autosomes can be divided into three size groups: 2 long (L), 6 medium (M), and 3 short (S) pairs (2L+6M+3S). The acrocentric X chromosome is about the same size as the M.

### C-heterochromatin variation

C-banding variation is not wide among the examined grasshoppers *E. plorans* (Table 2). The centromeric C-band patterns were observed in all the chromosomes of each species. The centromeric C-bands alone were revealed in the *Sh. shirakii* and *E. unicolor*. In *Sh. shirakii* the C-bands in all the chromosomes are very small and about the same size (Fig. 1a, b). The chromosomes of *E. unicolor* showed, as a rule, size variations for the C-bands. The C-blocks in the autosomes of the M<sub>3</sub> pair and the X chromosome are larger than in the other chromosomes (Fig. 2a, b).

Several chromosome pairs in *Th. pterostichus*, *H. adspersa* and *E. plorans* have paracentromeric C-bands near the true centromeric C-blocks. In *Th. pterostichus* the C-blocks are localized in the L<sub>1</sub> and L<sub>2</sub> autosome pairs and in the X chromosome (Fig. 3a, b). The paracentromeric C-blocks were revealed in the S<sub>9</sub> pair of the *H. adspersa* and *E. plorans* (Figs 4, 5a, b).

The telomeric C-bands were found only in the L<sub>2</sub> chromosome pairs of *E. plorans* (Fig. 5a, b; Table 2).

Table 2  
C-heterochromatin variation in five grasshopper species

Species	C-band localization, number of chromosome pairs		
	Centromeric	Paracentromeric	Telomeric
<i>Thisiocetrinus pterostichus</i> (F.-W.)	1 - 11, X	L <sub>1</sub> , L <sub>2</sub> , X	–
<i>Heteracris adspersa</i> (Redt.)	1 - 11, X	S <sub>9</sub>	–
<i>Eyprepocnemis unicolor</i> Tarb.	1 - 11, X	–	–
<i>E. plorans</i> (Charp.)	1 - 11, X	S <sub>9</sub>	L <sub>2</sub>
<i>Shirakiacris shirakii</i> (I. Bol.)	1 - 11, X	–	–

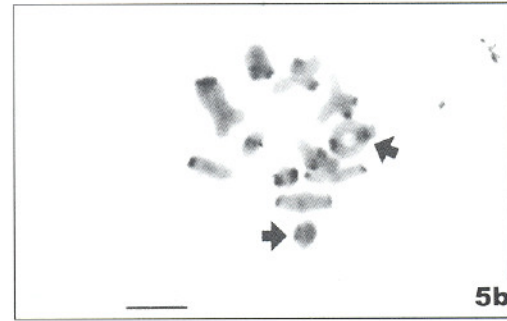
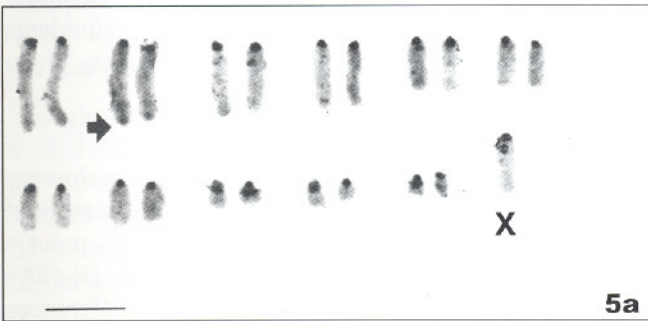
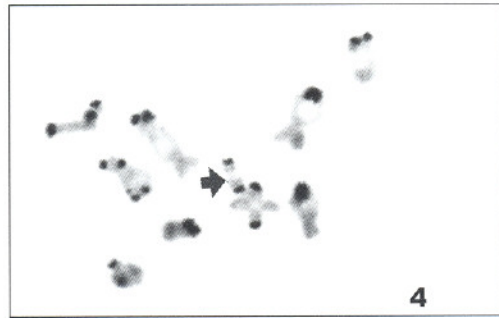
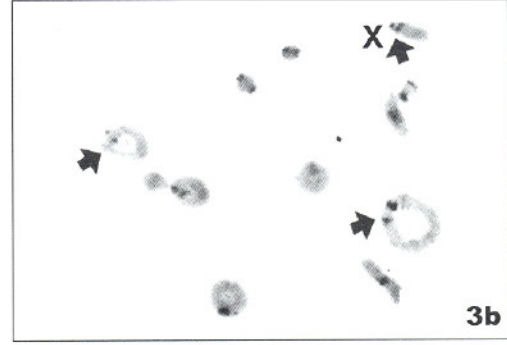
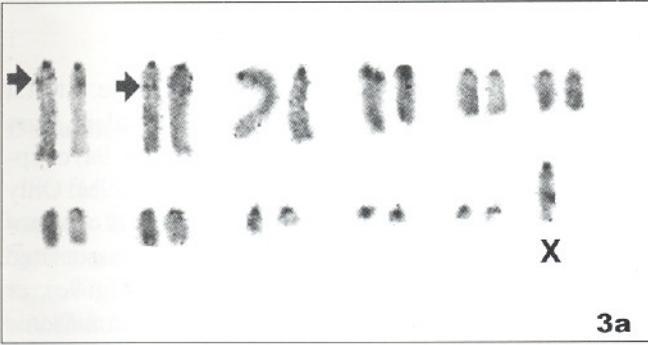
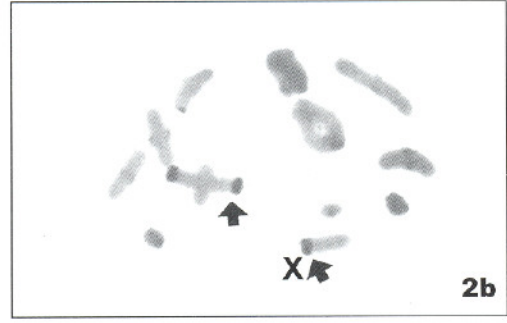
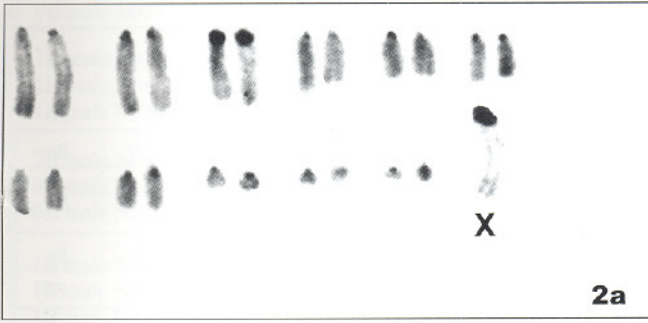
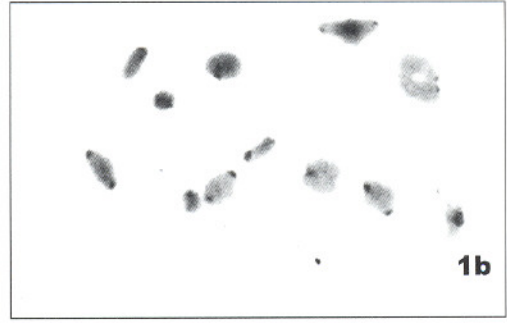
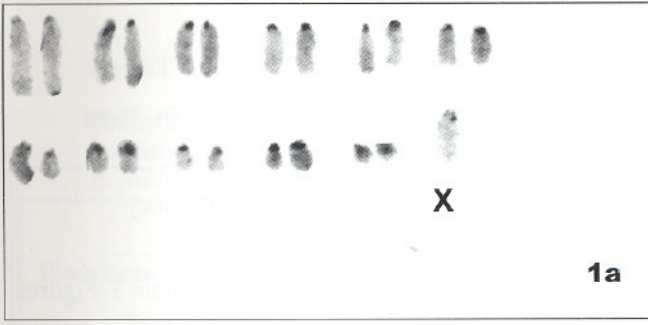
Chiasma frequency and B chromosome meiotic behaviour

The long bivalents usually form 2-3 chiasmata, 1-2 medium and a single short, at first prophase of male meiosis. The mean chiasma frequency for each species is given in Table 3 (the data are for B0 specimens of *E. plorans*).

Table 3

Mean chiasma frequency ( $\bar{x}$ ,  $\pm$ SD) in five species (No – number of cells)

Species	No	$\bar{x}$	$\pm$ SE
<i>Th. pterostichus</i>	61	15.90	0.12
<i>H. adspersa</i>	61	15.89	0.12
<i>E. unicolor</i>	60	13.88	0.10
<i>E. plorans</i>	60	14.30	0.13
<i>Sh. shirakii</i>	62	14.56	0.12



Figs 1-5. Fig. 1a,b. *Shirakiacris shirakii*. (a) Mitotic metaphase and (b) metaphase I. Fig. 2a,b. *Eyprepocnemis unicolor*. (a) Mitotic metaphase and (b) diakinesis; arrows indicate large C-bloks in  $M_3$  and X. Fig. 3a,b. *Thisoicetrinus pterostichus*. (a) Mitotic metaphase and (b) metaphase I; arrows indicate paracentromeric C-bands in  $L_1$ ,  $L_2$  and X. Fig. 4. *Heteracris adpersa*. Diakinesis with paracentromeric C-block in  $S_0$ . Fig. 5a,b. *Eyprepocnemis plorans*. (a) Mitotic metaphase and (b) diakinesis; arrows indicate telomeric C-band in  $L_2$  and paracentromeric C-block in  $S_0$ . Bars equal 10  $\mu$ m.

Fisher's variance ratio (F) (SNEDECOR & COCHRAN, 1956) was applied to test the significance of the difference in mean chiasma frequency between the species (Table 4).

Table 4

Analysis of differences in mean chiasma frequency between species (F\* – calculated variance ratio; No – number of cells)

Species	No	F*	Theoretical points of F	
			5%	1%
<i>Th. pterostichus</i> <i>H. adspersa</i>	61 61	6.487	3.92	6.84
<i>E. unicolor</i> <i>E. plorans</i>	60 60	19.409	3.92	6.84
<i>E. unicolor</i> <i>Sh. shirakii</i>	60 62	2.33	3.92	6.84
<i>Sh. shirakii</i> <i>E. plorans</i>	62 60	0.017	3.92	6.84

Fisher's test criterion demonstrated significant differences in mean chiasma frequency between the five species, which exceeded the random variations in chiasma number among the cells (F\* = 62.32 at the 5% points of F = 2.24). The contribution of each species to the differences between them varied. Paired comparisons of the species showed that *Sh. shirakii* and *P. plorans* differ least in the chiasma number (F\* = 0.017), and likewise *E. unicolor* and *Sh. shirakii* (F\* = 2.33 at 5% point = 3.92). The other compared species differed significantly in chiasma number.

#### B chromosomes of *E. plorans*

Four specimens have B chromosomes. A single B was present in 3 males (Fig. 6 a) and two B chromosomes (2B) in one male (Fig. 6 b). At prophase I of male meiosis, the B chromosomes appeared positively heteropycnotic (Fig. 7). At meiosis the 1B chromosome had the appearance of a univalent. At first prophase of meiosis, the B univalent in many cells was seen to be associated with the X chromosome or less frequently with the C-positive centric blocks of a small or medium-sized bivalent (Table 5).

Table 5

B-univalent associations in three males of *P. plorans*

Association type	Association frequency (%)			Figures
	male 1 (per 119 cells)	male 2 (per 136 cells)	male 3 (per 114 cells)	
Free B-univalent	37.8	26.8	36.8	8a
B-X <sub>centromere</sub>	21.0	26.8	26.3	8b
B-X <sub>telomere</sub>	12.6	15.9	14.9	8c
B at the side of the X	22.6	15.9	14.9	8d
B-S-bivalent	2.7	10.8	6.3	8e
B-M-bivalent	3.3	2.3	3.5	8f

Two Bs were identified in a single male. At first prophase of meiosis, the Bs, like the bivalent, were associated with each other (64.8%) (Fig. 9a) or appeared as two univalents (33.3%) (Fig. 9b). Only 1.9% of the examined cells lacked the B chromosomes. The B-bivalent was, as a rule, associated with the centromeric region, (38.3%) (Fig. 9c), or with the telomeric region of the X chromosome (22.2%) (Fig. 9d). In 1.2% of cells the B-univalents were associated with both the centromeric and telomeric regions of the X chromosome (Fig. 9e). The B/X chromosome associated temporarily, but the associations frequently persisted throughout first anaphase and, as a result, spermatocytes had either 2Bs or none (Fig. 10a, b).

The chiasmata were scored to ascertain whether or not the B chromosomes had any effect on recombination level in the *E. plorans* population. The results of analysis of *E. plorans* individuals with and without the B chromosomes are given in Table 6.

There were no significant differences in chiasma number between individuals with 2Bs and without the B chromosomes (F\* = 11.55 at the 5% point = 3.11) nor between the individuals with 1B (F\* = 0.75 at the 5% point = 3.11), while the differences in the chiasma number between the two groups were highly significant (F\* = 32.8 at 5% level = 3.89).

Table 6

Group comparisons in chiasma number between 0B, 1B and 2B males of *E. plorans*

B-variants	No	$\bar{x} \pm SE$	F*	Theoretical points of F	
				5%	1%
0B male No1	30	14.40 0.18	0.6	4.0	7.08
0B male No2	30	14.20 0.18			
1B male No3	30	15.20 0.17	0.175	3.11	4.88
1B male No4	30	15.10 0.17			
1B male No5	30	14.63 0.18			
2B male No6	50	14.02 0.14	1.51	3.09	4.82
0B male No1	30	14.40 0.18			
0B male No2	30	14.20 0.18			
1B male No3	30	15.20 0.17	12.44	2.68	3.94
1B male No4	30	15.10 0.17			
1B male No5	30	14.63 0.18			
2B male No6	50	14.02 0.14			

## Discussion

So far, the karyotype of nine species of grasshoppers from the subfamily Eyprepocnemidinae: *Eyprepocnemis plorans plorans* (Charp.), *Heteracris littoralis* (Ramb.) (CAMACHO *et al.* 1980; CANO & SANTOS 1988); *Eyprepocnemis plorans meridionalis*, *E. plorans ornatipes* (JOHN & LEWIS 1965); *E. roseus* Uv., *E. alacris* (Serv.), *Thisoicetrus pulcher* (Boliv.), *Heteracris illustris* Wlk. (MANNA 1954; CHATERJEE *et al.* 1971; MITTAL & SONI 1977), and *Shirakiacris shirakii* (I. Bol) (INOUE 1985) have been described. All the species have typical acridoid 23 acrocentric chromosomes karyotypes (the sex determining mechanism is X0/XX). The five species described at present (*Thisoicetrinus pterostichus* (F-W.), *Heteracris adspersa* (Redt.), *Eyprepocnemis unicolor* Tarb., *E. plorans* (Charp.), and *Shirakiacris shirakii* (I. Boll) are characterized by the same structural karyotype conservatism.

Similar to some groups of grasshoppers (SANTOS *et al.* 1983; CABRERO & CAMACHO 1986; GOSALVEZ *et al.* 1997), interspecific comparison of C-banding patterns in Eyprepocnemidinae reveal the existence of differences between species of either the same or different genera, which emphasize the dynamic nature of heterochromatin.

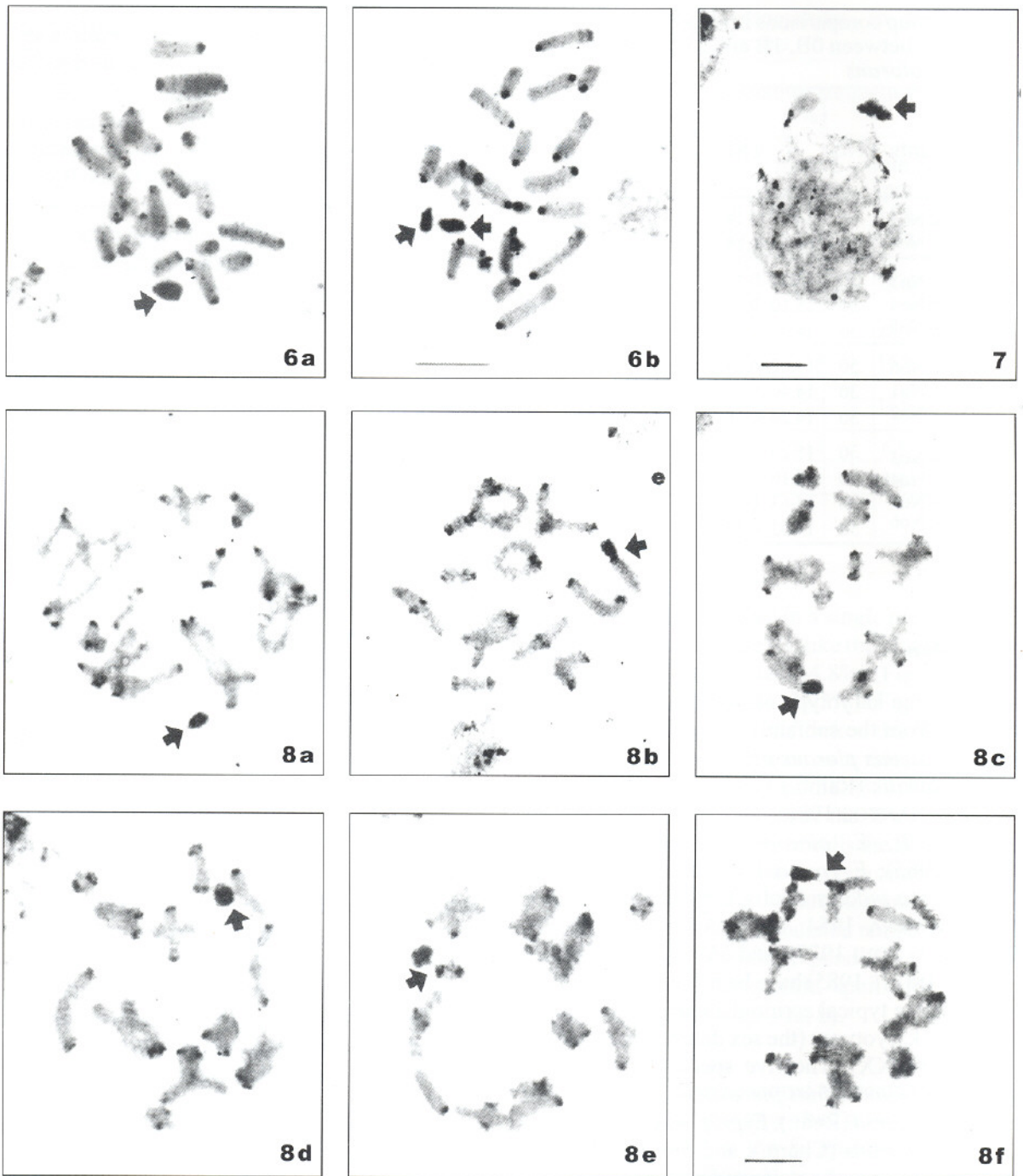
Among the subfamily Eyprepocnemidinae the presence of B chromosomes (supernumeraries) has been described in the karyotypes of *Heteracris*

*littoralis* and *E. plorans* (CAMACHO *et al.* 1980; CANO & SANTOS 1988).

In Europe *E. plorans* subspecies *plorans*, a species originally from Africa, shows a wide distribution, existing around the Mediterranean coasts of Spain, Italy, Greece, North Africa, Caucasus, and Turkmenistan (DIRSH 1958). *E. plorans* exhibits extremely widespread polymorphism for B chromosomes in natural Spanish populations, which seems to be one of the most complex systems hitherto reported, given that about 40 different B chromosome variation have been distinguished by C-banding (HENRIQUEZ-GIL & ARANA 1990; LOPEZ-LEON *et al.* 1993). B chromosome polymorphism is not limited to the Iberian Peninsula but also reaches Africa. The fact that B chromosomes from Morocco are made up of the same two DNA sequences (ribosomal DNA and a 180-bp repeat) as Spanish B chromosomes (LOPEZ-LEON *et al.* unpublished) suggests that the B chromosome polymorphism is relatively old (CABRERO *et al.* 1997). Indeed, in several individuals of *E. plorans* from North Caucasus a single morphological type of B chromosomes occurred but the number of specimens is too small for the study of the diversions of these supernumerary chromosomes in the eastern distribution areas of this species. However, the presence of individuals with B chromosomes in the population from Caucasus, besides Spanish populations, shows a very wide geographical distribution of these supernumerary chromosomes existing in *E. plorans*.

Irrespective of whether one B or two Bs are present in the karyotype, the B(s) remain(s) associated with the X to the end of anaphase I, and this promoted the accumulation of accessory chromosomes in the gametes with the X chromosome, at least in the male here examined. This strongly reminds the B chromosome accumulation mechanism in the Spanish populations of *E. plorans* (HENRIQUES-GIL *et al.* 1984; 1989) and *Heteracris littoralis* (CANO & SANTOS 1989).

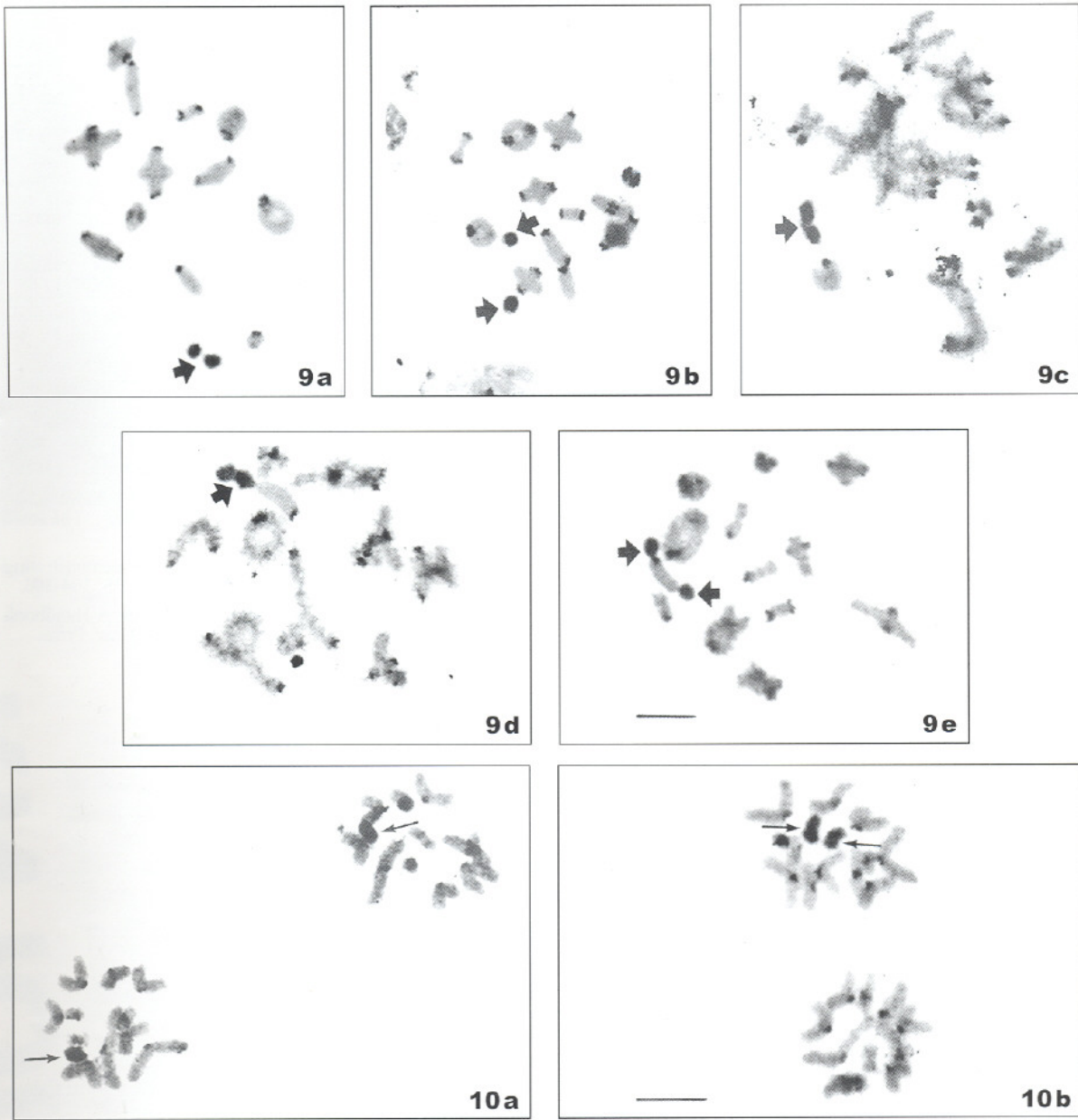
Chiasma frequency shows a wide variation both between and within different species and its dependence on the genotype is well known. In the Acrididae, the effect of B chromosomes on chiasma frequency has been studied by several workers. The effect of the B chromosomes on chiasma number and localization is a debatable question. An increase in the mean chiasma frequency has been found in *Myrmeleotettix maculatus* (JOHN & HEWITT 1965; HEWITT & JOHN 1970), *Eyprepocnemis plorans* from south of the Iberian Peninsula (CAMACHO *et al.* 1980), *Amblytropidia australis*,



Figs 6-8. *Eyprepocnemis plorans*. Fig. 6a,b. Mitotic metaphase; arrows indicate (a) single B and (b) two B chromosomes. Fig. 7. Early prophase I with B (arrow). Fig. 8a-f. Diakinesis with one B associated with X or small/medium bivalent (arrows). See Table 5. Bars equal 10  $\mu$ m.

and *Dichroplus elongatus* possessing one or two Bs (REMIS 1989; LOREY *et al.* 1991), whereas in other cases the absence of effect has been observed (JOHN & FREEMAN 1975). The present data demonstrated that chiasma number in individuals carrying a single B was significantly increased, while

it remained unaltered in carriers of two Bs. No increase in the chiasma frequency has been consistently observed in individuals with an uneven number of the B chromosomes. Why may an uneven B number occasionally affect chiasma number? What mechanism may underlie the ef-



Figs 9 & 10. *Eyprepocnemis plorans*. Fig. 9a-e. Diakinesis or metaphase I with two B (a) associated with each other, (b) two univalents, (c) associated with centromeric region, (d) with telomeric region, and (e) univalents associated with both centromeric and telomeric region with X. Fig. 10a, b. Anaphase; arrows indicate B chromosomes. Bars equal 10  $\mu$ m.

fect of the B chromosome on recombination exchange level in the basic chromosome set? These questions remain open.

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