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B-Chromosome Polymorphism and Interchromosomal Chiasma Interference in *Eyprepocnemis plorans* (Acrididae; Orthoptera)

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Abstract. Using the C-banding technique, the morphology and meiotic behaviour of four different types of B-chromosomes present in several populations of *Eyprepocnemis plorans* have been studied. The possible relationship between these four types is discussed. The analysis of chiasma frequency in A and B-chromosomes suggests the existence of interchromosomal interference and demonstrates that the chiasma frequency of B-s depends on the genetic background of the individual carrying them.

Introduction

Supernumerary or B-chromosomes are of fairly frequent occurrence in grasshoppers and can show variation both in respect of morphology and meiotic behaviour (Jones, 1975). Within species variation in the structure of B-chromosomes has been reported in *Myrmeleotettix maculatus* (John and Hewitt, 1965) and especially in *Chortoicetes terminifera* (Webb, 1976; Webb and Neuhaus, 1979). Sin some cases, as in *Melanoplus femur-rubrum* (Stephens and Bregman, 1972), these variations appear to result in differences in the meiotic behaviour of the supernumeraries.

In the present paper, a polymorphic system of B-chromosomes in *Eyprepocnemis plorans* has been studied using the C-banding technique which allows an accurate analysis of the different types of B-chromosomes, their relationships and their meiotic behaviour.

Materials and Methods

Adult males of *Eyprepocnemis plorans* were collected in three spanish populations all of which carry relatively high frequencies of B-chromosomes: Daimuz (Valencia), 1979 and 1980; Gandía (Valencia), 1978 and 1979 and Jávea (Alicante), 1979. B-chromosome behaviour was also analysed in the male offspring of 15 fertilised females collected at Daimuz (1979).

Testes of adult males were fixed without previous treatment in acetic:alcohol 1:3. Squash preparations of the fixed material were stained following the Giemsa C-banding technique described previously (Santos and Giraldez, 1978).

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Results

The basic karyotype of *E. plorans* is composed of 23 telocentric chromosomes (22+X) in the males and 24 (22+2X) in the females (John and Lewis, 1965). The C-banding technique revealed the presence of centromeric heterochromatin in all chromosomes but with quantitative variation in several pairs (Fig. 3c–e).

B-Chromosomes Morphology and Frequency

Figure 1 shows the four types of supernumerary chromosome found (B_1, B_2, B_3 and B_4). All of them are mitotically stable, telo- or subtelocentric in character and their C-banding pattern is clearly different from that of any of the A-chromosomes.

The B_1 type is similar in size to M8 but it differs from the latter in that it has two C-bands near the centromere. The banding pattern of B_2 is similar to that of B_1 but it is smaller in size, having a smaller euchromatic distal region. B_3 is the smallest supernumerary, it has nearly the same length as autosome S11 but has an interstitial C-band. Finally, B_4 has aproximately the same size as M5 and is characterised by two very prominent interstitial C-bands.

Table 1 shows the frequency of each type of B in the populations analysed. The B_1 type was the most frequent and was the only type present in the Jávea population.

Although the frequencies of the different karyotypic classes in laboratory populations are not significantly different from their population of origin (Daimuz collections, 1979 and 1980; $\chi^2=1.54$, d.f.$=2$, n.s.) it should be pointed out that the six individuals with more than two B-chromosomes appeared only in the laboratory population, and that these were obtained from the offspring of four different females. This suggests that laboratory conditions favour the development of individuals with higher numbers of supernumerary chromosomes and excludes the possibility that sampling error might explain the observed differences.

B-Chromosome Meiotic Behaviour

During prophase I all types of B-chromosomes appeared positively heteropycnotic. Chromosomes B_1, B_2 and B_3 maintain this condition throughout the entire meiosis. Chromosome B_4, however, appears isopycnotic at metaphase I and subsequent stages.

All types of B's show a tendency to be associated at prophase I either with the X-chromosome or with the other B-chromosomes present. These associations invariably lapse by metaphase I.

1. B_1. The number of B_1-chromosomes present can vary from one to four. When only one B_1 was present, it segregated reductionally in 95.7% of the anaphase I cells, moving at random with respect to the X-chromosome. In the remaining 4.3% of the cases it segregated equationally.

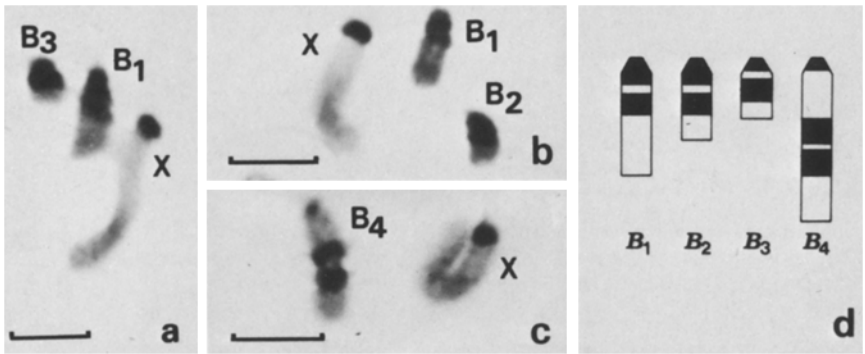

Fig. 1. The four types of B-chromosomes. **a** and **c** pachytene. **b** diplotene. **d** a diagram illustrating the differences between the four B types. Bar represents 5 μm

Table 1. Frequency of the four types of B-chromosomes in the different populations analyzed

Population	0B	$1B_1$	$1B_2$	$1B_4$	$2B_1$	$1B_1+1B_2$	$1B_1+1B_3$	$3B_1$	$4B_1$	Total
Gandía (78)	8	3	–	–	1	1	–	–	–	13
Gandía (79)	17	29	2	–	4	1	1	–	–	54
Jávea	40	20	–	–	2	–	–	–	–	62
Daimuz (79)	33	29	–	1	11	1	–	–	–	75
Daimuz (80)	32	35	1	–	6	1	–	–	–	75
Laboratory	83	63	1	–	16	3	–	5	1	172

Table 2. Pairing behaviour at diplotene and metaphase I of the different B-chromosome types

B-Chromosomes configuration	$1B_1+1B_2$	$1B_1+1B_3$	$2B_1$	$3B_1$	$4B_1$
All univalents	863 (85.2%)	200 (100.0%)	684 (43.5%)	229 (57.3%)	291 (26.0%)
One bivalent[a]	150 (14.8%)	0 (0.0%)	889 (56.5%)	165 (41.3%)	489 (43.7%)
One trivalent[b]	–	–	–	6 (1.5%)	39 (3.5%)
Two bivalents	–	–	–	–	289 (25.8%)
One quadrivalent	–	–	–	–	12 (1.1%)
B-Chiasmata per cell	0.148	0.000	0.565	0.443	1.054
B-Chiasmata per B-chromosome	0.074	0.000	0.283	0.148	0.264
Number of individuals	6	1	16	4	1

[a] In the individuals with 3 and 4 B's one and two univalents repectively were also present.
[b] In the individual with 4 B's one univalent was also present.

When two B_1-chromosomes were present they formed a monochiasmate bivalent with a mean frequency of 56.5% (Table 2). There is, however, some variation in this frequency between individuals and this will be described later. The chiasma formed by two B_1-chromosomes can be located either distally (13.4%) or proximally between the two C-bands (86.6%, see Fig. 2a, b). In order to investigate the independence of anaphase I behaviour of the univalents

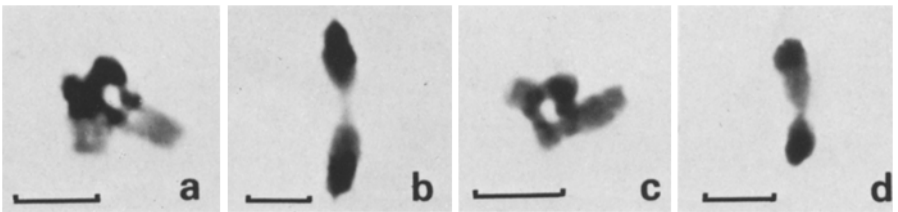

Fig. 2. Chiasma formation between B-chromosomes. **a** B_1-B_1, proximal chiasma (early diplotene). **b** B_1-B_1, distal chiasma (metaphase I). **c** B_1-B_2, proximal chiasma (diplotene). **d** B_1-B_2, distal chiasma (metaphase I). Bar represents 5 μm

Table 3. Frequencies of Anaphase I poles and Metaphase II cells with different B-chromosome constitution in ten $2B_1$ individuals, compared with expected values under the hypothesis of independence. (Probabilities of B_1-B_1 bivalents at metaphase I, b=0.416; two B_1 univalents, u=0.584; reductional division of one B_1, r=0.957; and equational division e=0.043)

0B	1B chromosome	2B chromosomes	$1B_1$ chromatid	$1B_1$ chromosome + $1B_1$ chromatid	$2B_1$ separated chromatids	Total AI poles and MII cells
Observed						
46	217	30	10	8	2	322
Expected						
43.15 ($1.4\times r^2u$)	219.93 ($b+{}^1/_2r^2u$)	43.15 ($1/4\times r^2u$)	7.73 (eru)	7.73 (eru)	0.32 (e^2u)	

$\chi^2_3=0.883$ n.s. Only half of the total Anaphase I poles and Metaphase II cells have been considered independent observations

appearing at metaphase I, the different constitutions of anaphase I and metaphase II cells were analysed in ten individuals carrying $2B_1$. Table 3 shows the frequencies expected in case of independence as a function of the probabilities of bivalent (b) and univalent (u) appearance at metaphase I in these individuals, and the probabilities of equational (e) and reductional (r) division of B_1 univalents in individuals carrying $1B_1$. The differences with the observed values were not significant.

In the individuals with three or four B_1-chromosomes, multivalent associations arose (Fig. 3c, e). Table 2 shows the frequency of the different types of configurations (univalents, bivalents, trivalents and quadrivalents) in individuals with two, three and four B_1-chromosomes. It is worth mentioning that after multivalent pairing the opportunity for two chiasmata in one chromosome arises, which was never realised after bivalent pairing.

2. B_2. The frequency of this chromosome is quite low in all populations analysed and only two types of individuals with B_2 were found, namely $1B_2$ or $1B_1+1B_2$ (Table 1). When only a single chromosome of this type is present its meiotic behaviour is similar to that of B_1, dividing reductionally in 94.8% of the anaphase I cells. In individuals having $1B_1$ and $1B_2$ these two chromosomes form

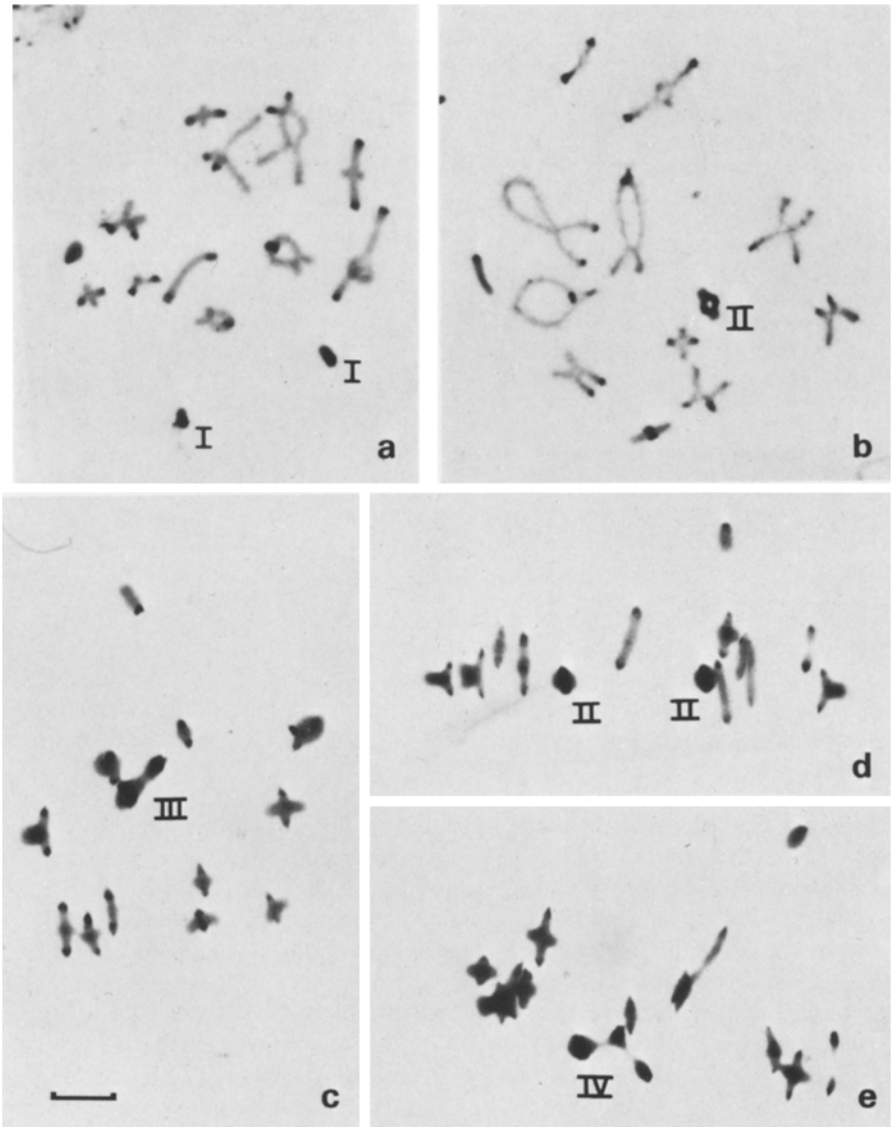

Fig. 3. Diplotene and metaphase I cells of individuals with different numbers of B-chromosomes. **a** diplotene cell having two B_1 univalents (*I*). **b** diplotene cell showing one B_1-B_1 bivalent (*II*) with a proximal chiasma. **c** metaphase I cell with a trivalent (*III*) formed by three B_1 chromosomes. **d** metaphase I cell with two B_1-B_1 bivalents (*II*). **e** metaphase I cell showing a quadrivalent (*IV*) formed by four B_1 chromosomes. *Arrows* identify A bivalents formed by two chromosomes showing quantitative differences in centromeric C-bands. Bar represents 10 μm

a monochiasmate bivalent with a mean frequency of 14.81% (Table 2). This chiasma can be distally (19.3%) or proximally (80,7%) located (Fig. 2c, d). In these individuals, the different constitutions of anaphase I and metaphase II cells depend on the probabilities of bivalents with a proximal (b_p) or distal (b_d) chiasma, the probability of univalence (u) and the probabilities of equational

Table 4. Frequencies of Anaphase I poles and Metaphase II cells with different B-chromosome constitution in two $1B_1+1B_2$ individuals, compared with the expected values under the hypothesis of independence. (Probabilities of bivalent with proximal chiasma $b_p=0.040$; bivalent with distal chiasma, $b_d=0.010$; two B univalents, $u=0.950$; reductional division of B_1, $r_1=0.957$; equational division of B_1, $e_1=0.043$; reductional division of B_2, $r_2=0.948$; and equational division of B_2, $e_2=0.052$)

	Observed	Expected (Probability)
0B	35	38.79 ($1/4\ r_1r_2u$)
$1B_1$ chromosome	44	39.64 ($^1/_2b_d+1/4\ r_1r_2u$)
$1B_2$ chromosome	41	39.64 ($^1/_2b_d+1/4\ r_1r_2u$)
2B chromosomes	27	38.79 ($1/4\ r_1r_2u$)
1 Recombinant B-chromosome	10	7.29 (b_p)
$1B_1$ chromatid	4	3.49 ($^1/_2e_1r_2u$)
$1B_1$ chromatid and $1B_2$ chromosome	6	3.49 ($^1/_2e_1r_2u$)
$1B_2$ chromatid	4	4.25 ($^1/_2r_1e_2u$)
$1B_2$ chromatid and $1B_1$ chromosome	9	4.25 ($^1/_2r_1e_2u$)
$1B_1$ and $1B_2$ chromatids	0	0.38 (e_1e_2u)
Total	180	

$\chi^2_4=4.326$ n.s. Only half of the total Anaphase I poles and Metaphase II cells have been considered independent observations

and reductional anaphase I division of B_1 (e_1, r_1) and B_2 (e_2, r_2) univalents. The frequencies of the different anaphase I poles and metaphase II cells under the hypothesis of independence are given in Table 4. The differences with the observed values were not significant.

3. B_3. This type of B-chromosome appeared in only one individual which also had a B_1-chromosome (Table 1). These two chromosomes were invariably in a univalent state during pro and metaphase I (Table 2). Unlike the B_1, the B_3 type divided in an equational manner at anaphase I, (Fig. 4a) in 85% of the cases. In the remaining cells (dividing reductionally) it segregated at random with regard to the X-chromosome.

In agreement with the anaphase I observations four different types of metaphase II cells were observed with respect to the constitution of the B_3-supernumerary: cells with OB_3, cells with $1B_3$-chromosome, cells with $2B_3$ chromatids and cells with $1B_3$ chromatid (Fig. 5b, c and d). Three different types of anaphase II cells with respect to the B_3-chromosome behaviour were found: cells with $1B_3$-chromosome dividing normally and cells with one or two chromatids lagging at the equatorial plate (Fig. 4e). Table 5 shows the frequency of the different types of anaphase I, meta and anaphase II cells in this individual. Chromosomes B_1 and B_3 behaved independently.

The existence of metaphase II cells with one or two separated B_3 chromatids and of anaphase II cells with one or two lagging B_3 chromatids can be the result of equational division of the B_3-chromosome at anaphase I. Second division cells with two separated B_3 chromatids would indicate that, in some cases, after the initiation of equational anaphase I division, both chromatids move to the same pole.

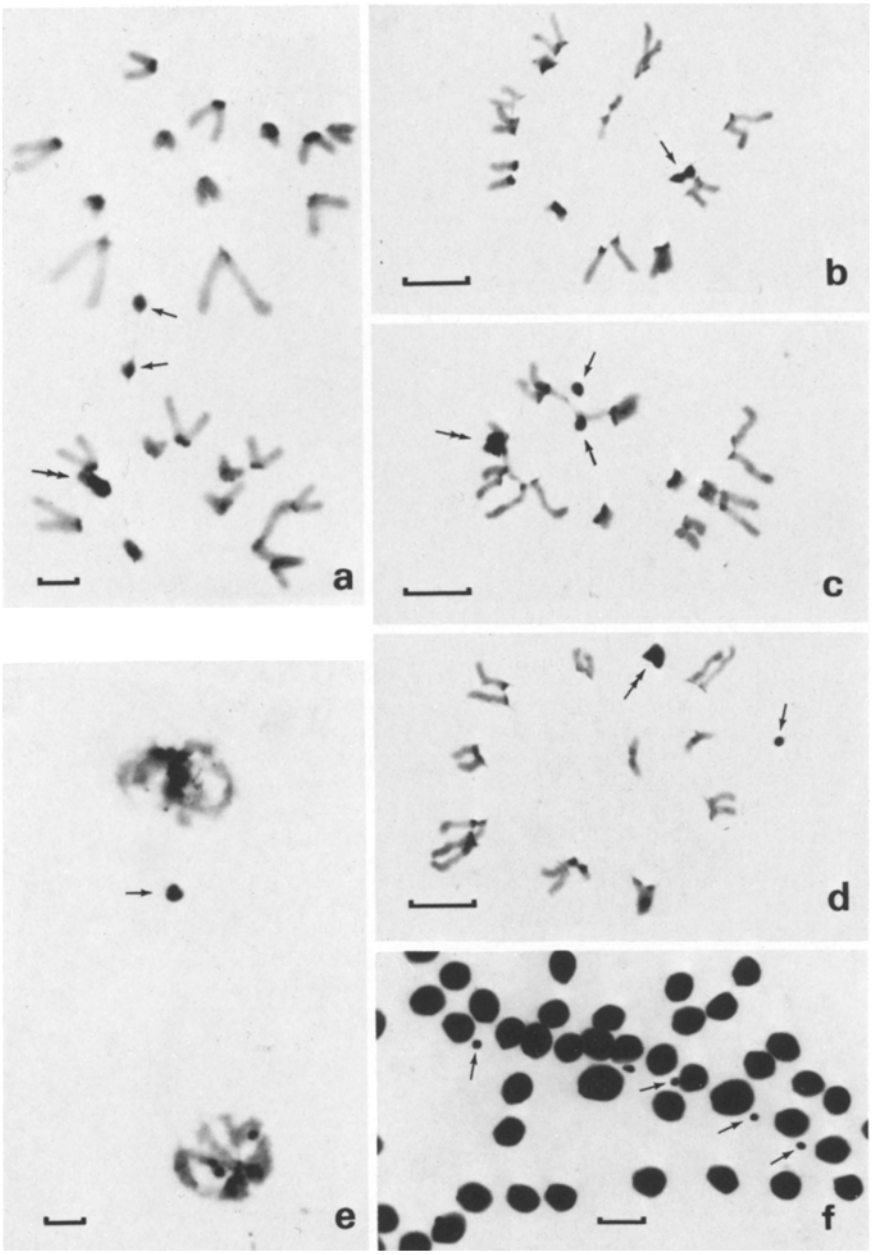

Fig. 4. **a** Anaphase I cell with one B_3-chromosome dividing equationally and one B_1-chromosome dividing reductionally. **b**, **c** and **d** Metaphase II cells: $1B_3$ chromosome (**b**), $1B_1$ chromosome and $2B_3$ chromatids (**c**) and $1B_1$ chromosome and $1B_3$ chromatid (**d**). **e** Telophase II cell showing one B_3 chromatid lagging. **f** Spermatids showing micronuclei in the individual with $1B_1+1B_3$. B_1 and B_3 chromosome types are pointed by a *double* and a *single headed arrow* respectively. Bar represents 10 μm

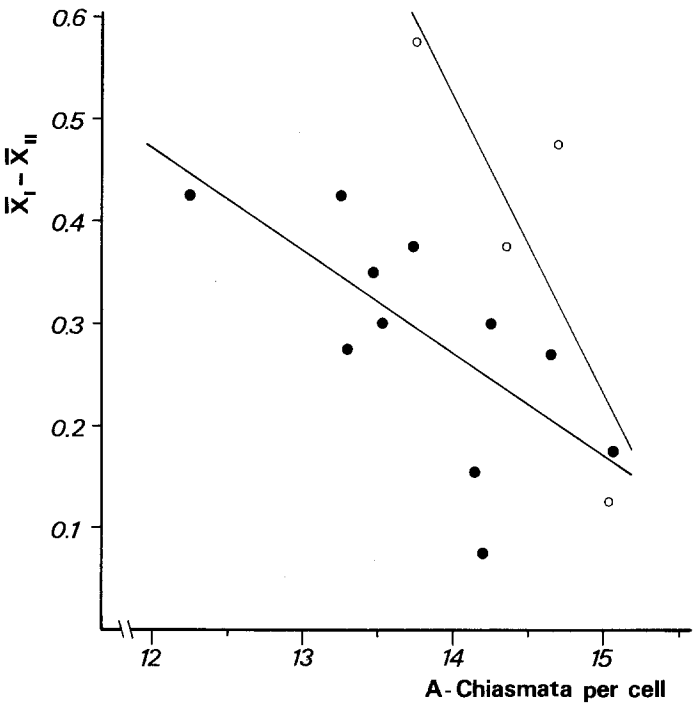

Fig. 5. The means chiasmata per cell formed by the A-chromosomes plotted against the decrease produced by B-chiasma formation, $X_I - X_{II}$: the difference between mean chiasma frequency of autosomes in cells with two B_1 univalents and that of the cells with one B_1 bivalent in individuals with two B_1-chromosomes (●) ($b = -0.10$, $t = 3.33$, $0.01 > p$) and in individuals with three B_1 chromosomes (○) ($b = -0.29$, $t = 2.07$, $0.2 > p > 0.1$)

Table 5. Comparison between anaphase I, metaphase II and anaphase II behaviour of B_1 and B_3 in the individual having these two B's (the behaviour of the two chromosomes was independent)

Type of B	Anaphase I (51 cells)		Metaphase II (328 cells)				Anaphase II (55 cells)			
	Reductional division	Equational division	0B	One B chromatid	Two B chromatids	1B	0B	One lagging B chromatid	Two lagging B chromatids	One B chromosome dividing
B_1	51	0	156	11	0	161	29	0	0	26
B_3	8	43	58	219	22	29	10	38	1	6

The low frequency (15%) of micronuclei observed in the spermatids (Fig. 4f) indicated that the anaphase II laggards are incorporated in the daughter nuclei with a high frequency, or that such cells degenerated.

4. B_4. This chromosome shows a regular meiotic behaviour. Equational divisions have not been observed during anaphase I (84 cells) nor have single chromatids

Table 6. Relation between chiasma frequencies in A and B chromosomes at diplotene in different cells in the 4B individual

B-Xta.	A-Xta. per cell	Number of cells
0 (4I)	13.18 ± 0.11	102
1 (1II+2I)	13.07 ± 0.08	180
2 (2II or 1III+1I)	12.86 ± 0.08	127
3 (1IV)	12.20 ± 0.49	5
$t_{0-1}=0.81$	$t_{0-2}=2.34$[a]	$t_{0-3}=2.17$[a]
$t_{1-2}=1.79$	$t_{1-3}=1.95$	$t_{2-3}=1.48$

[a] $P<0.05$

in metaphase II (112 cells). Segregation with respect to the X-chromosome was also at random.

Chiasma Relationships Between A and B-chromosomes

The frequency of B_1–B_1 and B_1–B_2 bivalents varies in different individuals. Moreover, the chiasma frequency of the B-chromosomes and that of the autosomes showed a positive between-individual correlation ($b=0.28$; $t=4.13$; $0.005>p$ for B_1–B_1 and $b=0.10$; $t=2.44$; $0.1>p>0.05$ for B_1–B_2). A similar tendency was also observed in the individuals with $3B_1$. This trend, however, was not statistically significant because of the low number of individuals with $3B_1$. These results suggest that chiasma formation in A and B-chromosomes is influenced by the same factor or factors (Jones and Rees, 1967).

If the autosome chiasma frequency is compared in cells having one B_1 bivalent versus cells with two B_1 univalents in a series of individuals with $2B_1$ chromosomes then in all cases, the mean chiasma frequency of autosomes is consistently higher in the latter than in the former (Totals: 13.90 ± 0.06 in cells with two B_1 univalents, 13.63 ± 0.06 in cells having a B_1 bivalent; $t=3.26$, $0.01>p$). A similar picture merges when $3B_1$ univalent cells are compared with cells with one B bivalent and one B univalent in four $3B_1$ individuals (Totals: 14.57 ± 0.10 in cells with $3B_1$ univalent, 14.16 ± 0.10 in cells with one bivalent and one univalent; $t=3.10$, $0.01>p$).

Table 6 shows the numbers of chiasmata formed by autosomes in cells where B-chromosomes form 0, 1, 2 and 3 chiasmata in one $4B_1$ individual available for analysis. Here too there is a significant reduction in the number of chiasmata present in the A-chromosomes as the number of chiasmata in the B-chromosomes rises.

Fig. 5 is a plot of the mean A chiasma frequency per cell in the different $2B_1$ and $3B_1$ individuals against the decrease produced by B-chiasma formation (i.e. the difference between mean chiasma frequency of A-chromosomes in cells with two or three B_1 univalents and that of the cells with one B_1 bivalent). This indicates that the lower the A-chiasma frequency, the higher the degree of interchromosomal interference.

Discussion

Relationship Between the Various B Types

As in other species of grasshoppers studied (King and John, 1980), the types of supernumerary chromosomes of *E. plorans* described in this work have C-banding patterns which are quite distinct from those of the A-chromosomes.

From the meiotic behaviour of individuals with $1B_1+1B_2$, a clear homology between these two chromosomes can be concluded. B_1 and B_2 form a bivalent with a noticeably lower frequency than two B_1 chromosomes, but the relative frequencies of proximal and distal chiasmata are similar in both cases. The higher frequency of B_1 found would suggest that the B_2 type has been derived from the B_1 by an interstitial euchromatic deletion. However, Camacho et al. (1980) employing conventional staining techniques, described two types of supernumerary chromosomes (B and B′) in southern spanish populations of *Eyprepocnemis plorans*. Size and C-band pattern of the most frequent B-chromosome type in this material (Camacho, 1980) is identical to that observed by us in the B_2 type. If this means that their B is identical with our B_2, it is most frequent in southern Spain, whereas B_1 is more common in the eastern populations. Then, the possibility of B_1 being derived from B_2 by duplication can not be excluded. Both a deletion or a duplication would go some way to explaining the low pairing frequency observed between B_1 and B_2 types. B′ of Camacho et al. probably represents a fifth category of B-chromosome since it is much smaller than our B_3 type.

The B_3 chromosome could have originated either from B_1 or B_2 through a loss of both euchromatic and heterochromatic regions though there is no clear evidence for this. Nevertheless, it is probably of fairly recent origin since its meiotic behaviour leads to a low transmision rate. A comparable behaviour has been described by Parker (1976) for the B-chromosomes of *Hypochoeris maculata*. Here the B-chromatids lag at anaphase II but finally reach the poles since no micronuclei are observed in the later stages. The behaviour of B_3 is more irregular since some micronuclei have been observed in spermatids (Fig. 4f). The higher anaphase I equational segregation frequency of B_3 in comparison with the other supernumerary chromosomes present in *E. plorans* is unexplained.

The relationship of B_4 and other B types remains unclear since both the euchromatic and the heterochromatic regions of the B_4 are larger than those present in other B's. Unfortunately we have as yet been unable to study possible homology in terms of meiotic pairing.

Chiasma Relationships Between A and B-Chromosomes

The between-individual variation in chiasma frequency of B-chromosomes is positively correlated with that of the autosomes. A similar correlation was observed in rye by Jones and Rees (1967), who concluded that the same factors influence the variation in chiasma frequency in both A and B-chromosomes, and suggested that the differences in the A genotype could be responsible for the observed variation. In *Locusta migratoria* too it was demonstrate that varia-

tion in B-chromosome pairing was due to the genetic background of each individual (Lespinasse, 1973).

If chiasma formation in both A and B-chromosomes in controlled by the same processes, it would be possible for competition for chiasmata (Mather, 1939) to give rise to interchromosomal interference. Although Jones and Rees (1967) did not find evidence of chiasma interference between both types of chromosomes in rye it was observed by Darlington (1933) in the same species.

From the results obtained in this work, it can be concluded that there is a positive interchromosomal chiasma interference between A's and B's in *Eyprepocnemis plorans*. Moreover, the negative correlation shown in Fig. 5 supports Mather's hypothesis that interchromosomic interference is produced by a competition for chiasmata, chiasma competition being maximal when chiasmata are scarce and minimal in individuals having high chiasma frequency.

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