The B-chromosomes of *Locusta migratoria*
I. Detection of negative correlation between mean chiasma frequency and the rate of accumulation of the B's; a reanalysis of the available data about the transmission of these B-chromosomes

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Abstract

B-chromosomes were studied in two Spanish populations of *Locusta migratoria*. Both exhibit a high frequency of B-carrying individuals (75% and 94%). In both the B-chromosomes are mitotically unstable and they accumulate in the male germ line. The mean rate of accumulation is 28.5% in Baños de la Encina and 31.9% in Carboneras. There are no significant differences in mean cell chiasma frequency and between-cell variance of chiasmata between follicles with different number of B's within individual males. Likewise, there are no significant differences in mean cell chiasma frequency and between-cell variance between males with and without supernumeraries. However, a significant negative correlation exists between mean chiasma frequency and the rate of accumulation.

Introduction

Effects of B-chromosomes on chiasma formation have been detected in several plant and animal species (see Jones, 1975; Hewitt, 1979). In grasshoppers an increase in chiasma frequency due to the presence of supernumerary chromosomes has been reported in *Myrmeleotettix maculatus* (John & Hewitt, 1965), *Melanoplus differentialis* (Abdel-Hammed *et al.*, 1970), *Melanoplus femur-rubrum* (Stephens & Bregman, 1972), *Melanoplus frigidus* (Gosalvez *et al.*, 1980) and *Eyprepocnemis plorans* subsp. *plorans* (Camacho *et al.*, 1980). In all five species the B-chromosomes are mitotically stable elements. On the other hand, the influence of mitotically unstable B-chromosomes on chiasma formation has been studied in only two grasshopper species. In *Camnula pellucida* they have no influence on this endophenotypic character (Schroeter & Hewitt, 1974). Likewise Dearn (1974) did not observe any influence of unstable B-chromosomes on chiasma frequency in material of *Locusta migratoria* from both laboratory culture and field. However, he did not study this effect at an intraindivual level.

In this paper we describe a negative correlation between mean cell chiasma frequency and the rate of B-accumulation in *Locusta migratoria*.

Material and methods

Eight males from Baños de la Encina (BE) (Jaén, Spain) and fourteen males and three females from Carboneras (C) (Almería, Spain) were used for the present study. Additionally, four males from Granada (GR) and a six day old embryo from Torremolinos (Málaga, Spain) were studied.

Testes were fixed in 1:3 acetic alcohol and subsequently squashed in acetic orcein. Females were injected with 0.3 ml of 0.05% colchicine. Eight hours later the ovarioles were fixed and subsequently squashed in acetic orcein. The embryo was analyzed cytologically following the technique described by Webb *et al.* (1978).

To ascertain the mean number of B's in the testes

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50 follicles per male were analyzed individually in the majority of the B-containing males. From this mean number of B's the rate of accumulation was assessed by dividing its decimal fraction by its integral part. In males without supernumeraries from 6 to 10 follicles per male were studied cytologically. To analyze chiasma frequency ten diplotene cells were scored in each follicle wherever possible.

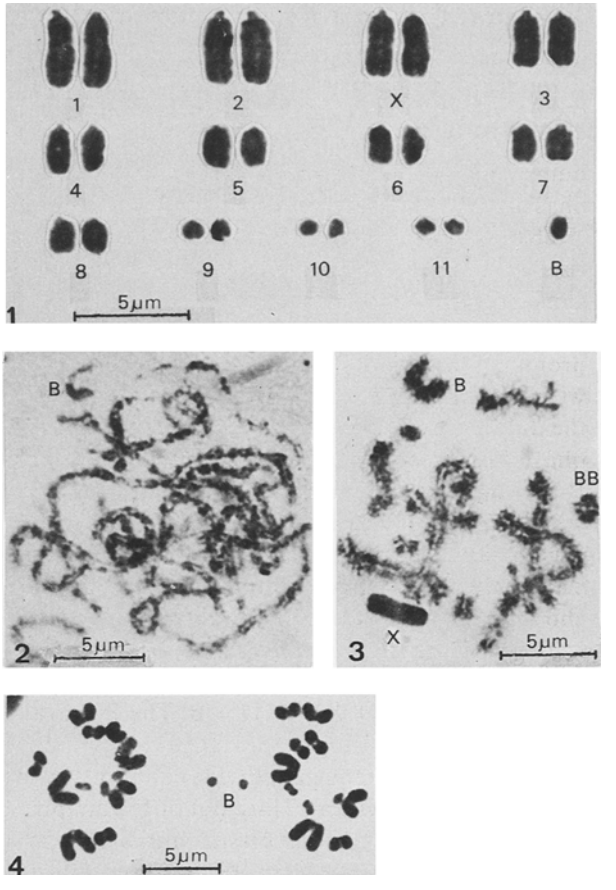
Statistical analyses were carried out in the Centro de Informática de la Universidad de Granada, by means of an Univac 1100 Multiprocessor System.

Results

The B-chromosomes

Six of eight males from Baños de la Encina and thirteen of fourteen males and the three females from Carboneras carried supernumerary chromosomes in addition to their standard chromosome complement (2n = 22 + XO/XX). The frequency of B-carriers was thus very high, namely 75% in Baños de la Encina and 94% in Carboneras.

These B-chromosomes are telocentric and slightly larger than the smallest autosomes (Fig. 1). Furthermore, B's are partially heterochromatic and at zygotene they show a proximal euchromatic segment and a positively heteropycnotic terminal segment (Fig. 2) which is consistent with C-banding behaviour (Figs. 5 and 6). The C-banding pattern of the B-chromosomes studied by us agrees with that of the B's from other Spanish populations (see Santos, 1980) and also that of the B's from Australian populations (B. John, personal communication).



Figs. 1-4. B-chromosomes of *Locusta migratoria*: (1) Karyotype of a female with 1 B. Note that the B-chromosome is slightly larger than the S-autosomes 9, 10 and 11; (2) Zygotene cell showing the pycnotic behaviour of the B-chromosomes. Note the positively heteropycnotic distal segment and a proximal euchromatic segment in the B; (3) Pachytene cell showing structure of a B-bivalent. Note that the chiasma is located in the negatively heteropycnotic segment; (4) Equational division of the B-univalents in anaphase I. Bar = 5 μm.

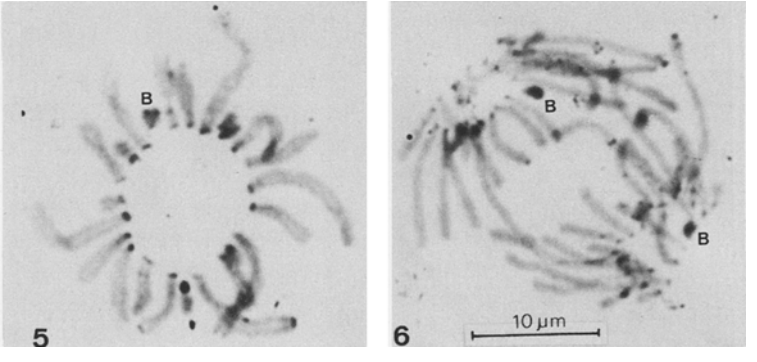


Fig. 5-6. C-banding pattern of the B-chromosome of a male embryo of *L. migratoria*. Neuroblast cells at metaphase (5) and anaphase (6) of mitosis. Note the presence of a distally located large block of C-heterochromatin in addition to a small procentric one. Bar = 10 μm.

Meiotic behaviour of the supernumeraries

Associations between the X-chromosome and the B-univalent during prophase-I and metaphase-I of meiosis are very rare and were present in only 4 out of a total of 942 diplotene, diakinesis and metaphase-I cells analyzed.

The overall pairing behaviour of B's is summarised in Table 1. Pairing between two B-chromosomes leads to the formation of a single chiasma in the proximal euchromatic segment (Fig. 3). The pronounced tendency to form bivalents in all cells with more than 1B is striking. The frequencies of the different configurations at diplotene and metaphase-I are very similar, which indicates that the B-bivalents persist as is expected of a chiasmate system. This contrasts with the situation found in *Eyprepocnemis plorans* subsp. *plorans* where associations of B's rarely persist to metaphase I (Camacho *et al.*, 1980).

The B-bivalents divide regularly in the first meiotic division since all anaphase-I cells observed with 2B consisted of 11 + X + B/11 + B. The B-univalents sometimes divide reductionally (in about 39% of cells), but they frequently divide equationally in the first meiotic division. Thus, out of 152 anaphase-I cells with 1B, 61.8% showed equational division of the B-univalent (Fig. 4). Moreover, 61% of the 41 anaphase-I cells with 3B examined showed equational division of the B-univalent. Despite their lagging, B-univalents dividing equationally move to the poles and integrate regularly into them, as indicated by the fact that only two microspermatids were found among 787 spermatids scored in follicles with 1B. Lagging of the B-univalents does not affect cytokinesis, since not a single macrospermatid was observed.

Accumulation of supernumeraries in male germ line

The fact that B's tend to accumulate in the male germ line of *L. migratoria* was first suggested by Nur (1969) and definitively demonstrated by Kayano (1971). In the Spanish populations examined in this report B-chromosomes also accumulate as a result of the mitotic instability they show in the male germ line. Table 2 lists the mean number of B's and their rate of accumulation in each male. The latter value could be reliably assessed only in those males with a mean number of B's less than 3. The mean rate of accumulation was 28.5% in Baños de la Encina and 31.9% in Carboneras. These frequencies are very similar to those found by Nur (1969) in Japanese populations: 27% in Misima and 32% in Hakozaki.

In the female germ line, B's are mitotically stable elements since all ovariole cells analyzed in each female carried the same number of supernumeraries. Two females had 1B each and one female had 2B's.

Effects of B-chromosomes on chiasma frequency

Table 3 gives analyses of variance for the mean chiasma frequency of individual follicles with different number of B's in each of ten males, four from BE and six from C. The data show that, within each male, the mean chiasma frequency is usually similar between follicles with different numbers of B's. However, in three males, BE-5, BE-6 and C-12, in which follicles with only 1B or 2B were present, the mean chiasma frequency was higher in the follicles with 1B, and in each case the difference was statistically significant.

Table 1. Meiotic pairing patterns of B-chromosomes in *Locusta migratoria*.

Number of B's	Stage	Configuration					Total cells	Contingency χ^2 test
		II	2 I					
2	Diplotene	538 (99.63%)	2 (0.37%)				540	$\chi^2_{(1)} = 2.46$
	Metaphase I	72 (97.30%)	2 (2.70%)				74	P:0.1 - 0.2
		III	II + I	3 I				
3	Diplotene	6 (5.08%)	92 (77.97%)	20 (16.95%)			118	$\chi^2_{(2)} = 0.06$
	Metaphase I	4 (5.56%)	55 (76.39%)	13 (18.05%)			72	P:0.95 - 0.98
		IV	III + I	2 II	II + 2 I	4 I		
4	Diplotene	0	0	29 (64.44%)	12 (26.67%)	4 (8.89%)	45	

Table 2. Mean number of B's, rate of accumulation, mean cell chiasma frequency and between-cell variance in 26 males of *L. migratoria* from Spain.

Population	Male no	Number of follicles analyzed	Number of diplotene cells analyzed	Mean number of B's	Rate of accumulation	Mean chiasma frequency	Between-cell variance	Mean log. variance
BE	1	50	82	1.14	14%	16.13	1.03	0.0128
	2	10	30	0	–	15.33	1.20	0.0792
	3	50	70	1.36	36%	15.17	1.97	0.2945
	4	50	195	1.29	29%	15.71	1.60	0.2041
	5	50	152	1.42	42%	16.00	1.54	0.1875
	6	50	154	1.10	10%	17.21	1.58	0.1987
	7	50	40	1.40	40%	14.08	0.74	-0.1308
	8	7	30	0	–	15.03	1.07	0.0294
C	1	18	53	4.41	?	15.11	1.41	0.1492
	2	50	112	3.79	?	15.21	1.14	0.0569
	3	50	75	1.30	30%	15.59	1.22	0.0864
	4	7	30	0	–	16.13	1.22	0.0864
	5	50	50	3.16	?	14.90	0.91	-0.0410
	6	50	33	2.58	29%	15.45	0.88	-0.0555
	7	50	82	1.44	44%	15.01	2.14	0.3304
	8	50	66	1.44	44%	15.42	1.94	0.2878
	9	50	54	3.76	?	16.56	1.08	0.0334
	10	50	75	1.84	84%	14.72	1.10	0.0414
	11	50	60	1.34	34%	16.12	1.39	0.1430
	12	50	310	1.03	3%	17.05	1.56	0.1931
	13	50	290	2.33	16.5%	14.64	1.07	0.0294
	14	25	150	2.05	2.5%	17.95	1.70	0.2304
GR	1	10	30	0	–	15.83	1.32	0.1206
	2	6	19	0	–	15.79	0.51	-0.2924
	3	10	30	0	–	16.10	2.30	0.3617
	4	10	30	0	–	15.40	1.57	0.1959

Table 3. Analyses of variance comparing within each male the mean cell chiasma frequency of follicles with different number of B's. In each male only those follicles in which chiasmata could be scored in ten diplotene cells are included. The number of follicles belonging to each karyotypic class is shown in brackets.

	Mean chiasma frequency of follicles with								Total follicles			
Male	0 B	1 B	2 B	3 B	4 B	5 B	6 B	7 B	analyzed	F	P	
BE-1	15.50 (1)	16.00 (4)	16.60 (1)	–	–	–	–	–	6	2.76	0.0720	N.S.
BE-4	15.85 (2)	15.45 (8)	15.60 (1)	15.93 (3)	–	–	–	–	14	1.49	0.2208	N.S.
BE-5	–	16.55 (4)	15.88 (5)	–	–	–	–	–	9	6.69	0.0113	*
BE-6	–	17.47 (7)	16.63 (4)	–	–	–	–	–	11	13.23	0.0004	***
C-1	–	–	–	14.70 (1)	15.20 (1)	14.60 (1)	–	15.65 (2)	5	2.73	0.0549	N.S.
C-2	–	15.30 (1)	15.40 (1)	15.05 (2)	15.40 (2)	15.50 (1)	14.90 (1)	14.60 (1)	9	0.59	0.7086	N.S.
C-3	15.70 (2)	15.75 (2)	15.60 (1)	15.00 (1)	–	–	–	–	6	1.16	0.3346	N.S.
C-12	–	17.10 (27)	16.68 (4)	–	–	–	–	–	31	3.84	0.0510	≅*
C-13	–	14.20 (1)	14.76 (18)	14.49 (9)	14.30 (1)	–	–	–	29	2.39	0.0692	N.S.
C-14	–	18.20 (2)	17.99 (9)	17.75 (4)	–	–	–	–	15	0.86	0.4262	N.S.

In three additional males (BE-1, BE-4 and C-3) follicles with and without B's were observed within the same individual. In BE-1 one follicle without B-chromosomes had a mean chiasma frequency of 15.50 ± 0.22 while in five follicles containing B's it was 16.12 ± 0.16, a difference which is statistically significant ($t = 2.28$, P:0.02 - 0.05). In BE-4 twelve follicles with, and two others without, B-chromosomes had a similar mean chiasma frequency: 15.85 ± 0.26 and 15.58 ± 0.11, respectively ($t = 0.96$, P:0.2 - 0.5). In the C-3 male the situation was similar to that observed in BE-4: $\bar{X}_{+B} = 15.53 \pm 0.18$ (4 follicles), $\bar{X}_{-B} = 15.70 \pm 0.23$ (2 follicles) ($t = 0.58$, P:0.5 -0.9). In BE-1 the rate of accumulation of the B (14%) was less than that of BE-4 and C-3 (29% and 30%, respectively).

Barlett's tests of homogeneity of the between-follicle variance of chiasmata showed that within each male there are no significant differences for this character among follicles with different numbers of B's.

To compare chiasma frequency at an inter-individual level we have used all the males scored (see Table 2). An analysis of variance for chiasma frequency in the eight BE-males showed significant differences between them ($F = 46.7$, df = 7 and 745, $P = 0$). Similar results were obtained from an analysis of variance for the fourteen C-males ($F = 98.6$, df = 13 and 1394, $P = 0$) and the pooled data from both populations ($F = 76.2$, df = 21 and 2139, $P = 0$). Thus there are statistically significant differences among males for mean chiasma frequency.

Because of the scarcity of males without supernumeraries (two from BE and one from the C population), the comparison of chiasma frequency between males with and without B-chromosomes was made after pooling the data from the two populations, adding those from four OB males from Granada (GR). Mean chiasma frequency was similar in males with (15.69 ± 0.224) and without (15.66 ± 0.157) B-chromosomes ($t = 0.1$, $P > 0.90$). Between-cell variance was also similar between males with and without B's when a t-test was applied to the log of variances ($t = 0.37$, P:0.70 - 0.90).

Accumulation and chiasma frequency

To investigate the origin of the heterogeneity for chiasma frequency observed among males (see the previous section) we have carried out a series of correlation analyses between mean cell chiasma frequency and between-cell variance versus the rate of accumulation and the mean number of B's (Table 4). These analyses demonstrate a significant negative correlation between mean chiasma frequency and the rate of accumulation ($r = -0.673$, $F = 10.74$, $P = 0.006$).

Discussion

After conventional orcein staining, the B-chromosomes of *L. migratoria* from Spanish populations are very similar to those found in natural popula-

Table 4. Correlation analyses. In the first two classes only males in which the rate of accumulation could be assessed have been used.

Population		Class			
		Mean chiasma frequency versus rate of accumulation	Between-cell variance versus rate of accumulation	Mean chiasma frequency versus mean number of B's	Between-cell variance versus mean number of B's
BE	r	-0.743	0.023	0.130	0.318
	F	4.92	0.01	0.10	0.67
	P	0.09	0.96	0.76	0.44
C	r	-0.682	-0.079	-0.245	-0.354
	F	6.09	0.04	0.76	1.72
	P	0.04*	0.84	0.40	0.21
Pooled	r	-0.673	-0.046	-0.108	-0.172
	F	10.74	0.03	0.24	0.61
	P	0.006**	0.87	0.63	0.44

tions from Japan (Itoh, 1934; Nur, 1969; Kayano, 1971), China (Hsiang, 1958) and Mali (Dearn, 1974), and also to those found in laboratory cultures initiated from collections made in the Sudan (Rees & Jamieson, 1954) and in Mali (Dearn, 1974; Lespinasse, 1973, 1977). In all these cases they are telocentric, slightly larger than the smaller autosomes, partially heterochromatic, and they show mitotic instability in the male germ line which leads to their accumulation. Lespinasse (1973, 1977, 1981) has claimed that accumulation of B's in this species also occurs by preferential segregation at female meiosis, and that there is an elimination mechanism operative during embryonic and larval development. We have, however, reanalyzed Lespinasse's data and, as we show in the next section, find no support for female accumulation.

Transmission of B-chromosomes of Locusta migratoria

To date, the only available information on the transmission and survival of B's in *Locusta* comes from controlled crosses performed by Lespinasse with material from two natural populations (1981) and a laboratory culture which originated from material collected in Mali of *L. m. migratorioides* (1973, 1977). From his studies Lespinasse concluded that there is an accumulation of B's during female meiosis. In our opinion his published data are not adequate to support this conclusion and his method of statistical analysis is open to criticism. In consequence we have re-analyzed his data.

In his 1973 paper Lespinasse reported that during mitosis B-chromosomes are very stable and at meiosis they are paired or not. When there are two B-chromosomes, in case of synapsis, the number of animals with two B's in the progeny is slightly lower than previous; in case of asynapsis, there is a higher lethality of individuals and oocytes with two B-chromosomes. We have re-examined his experimental results by pooling data from all identical crosses to obtain a larger number of progeny for statistical analysis (Tables 5 and 6). From his 1973 data we conclude that the proportions of progeny from crosses involving 1 B fit a 1:1 distribution. In crosses involving 2 B-chromosomes the proportions are, in general, those expected for a 1:2:1 distribution when each parent carries 1 B. When the two B's are carried by the male while the transmission to embryos is still independent (1:2:1) there is a differential survival to the adult stage which leads to an elimination of B's (mean number of B's = 0.80). This B-elimination involves a higher mortality of 2 B embryos and these crosses showed the lowest hatching frequency (63.65%) in all Lespinasse's crosses. When the female carries the two B's, the transmission to embryos is not independent and the mean number of B's in them (0.90) is lower than that expected (1.00) which actually indicates an elimination of supernumeraries in the 2 B females during meiosis. Thus, we conclude from Lespinasse's results that there is both a meiotic elimination of B's in 2 B females and an elimination of B's in the progeny of 2 B males by means of a higher mortality of 2 B embryos.

In 1977, Lespinasse calculated the frequency of embryos with 2 and 3 B's from crosses involving 2 and 3 B's, respectively, and compared it between crosses in which the two B's were carried respectively by the male and by the female. He concluded that in *L. m. migratorioides* there exists an accumulation mechanism of B's in the females (with two or more B's) which presumably results from preferential segregation of the B's in the female pronucleus, and also a B-chromosome elimination during embryonic and larval development by means of mitotic nondisjunction. In our re-analysis of these same experiments (Table 6) we find that in crosses involving 2 or 3 B-chromosomes there is neither B-accumulation nor B-elimination since in all cases the transmission of B's is independent (1:2:1 in 2 B crosses; 1:3:3:1 in 3 B crosses) and there is a similar survival of the different classes of embryos with different number of B's. In crosses involving 4 B's (♂2 B × ♀2 B), the transmission of the B's is not independent (1:4:6:4:1) and the mean number of B's in the embryos (2.15) is higher than that expected (2.00). However, the mean number of B's in the adults (1.76) is appreciably lower than that expected due to an elimination of embryos with 3 and 4 B's and a corresponding increase in the number of 2 B embryos. This B-elimination must occur during embryonic and larval development, as Lespinasse claims, since the hatching frequency of these crosses (78.96%) is similar to those of the majority of crosses.

Actually, it is difficult to determine whether the increased transmission of B's in crosses involving 4 B's is made through the male, the female or both.

Table 5. Results obtained from the experimental crosses performed by Lespinasse in *L. migratoria*. We have pooled the data from those similar crosses to obtain higher numbers of individuals among progeny and so increase the significance of the statistical tests.

Year of Lespinasse's experiments	Number of B's involved in the cross	Cross class	Code number of each cross class	No. of Lespinasse's crosses	Mean percent of hatching	Progeny									
						Embryos with					Adults with				
						0 B	1 B	2 B	3 B	4 B	0 B	1 B	2 B	3 B	4 B
1973	1	♂1 B × ♀0 B	1	4, 6, 9, 13, 20, 25	89.95	131	121	–	–	–	70	60	–	–	–
		♂0 B × ♀1 B	2	5, 12, 17, 19, 21, 22, 27, 30	90.64	192	175	–	–	–	85	81	–	–	–
	2	♂2 B × ♀0 B	3	3, 28	63.65	28	48	22	–	–	16	39	4	–	–
		♂0 B × ♀2 B	4	11, 24, 33	87.00	35	110	18	–	–	22	61	7	–	–
		♂1 B × ♀1 B	5	15, 18, 23, 26, 29, 31	82.55	93	213	84	–	–	49	97	30	–	–
1977	2	♂2 B × ♀0 B	6	62, 69, 70	82.83	12	36	12	–	–	6	9	5	–	–
		♂0 B × ♀2 B	7	50, 54, 71, 77	82.43	21	46	28	–	–	10	22	16	–	–
		♂1 B × ♀1 B	8	51, 53, 59, 66, 75, 81, 88	81.71	35	85	33	–	–	11	25	12	–	–
	3	♂1 B × ♀2 B	9	52, 55, 57, 58, 64	78.32	19	55	49	23	–	6	22	26	2	–
		♂2 B × ♀1 B	10	56, 67, 80 86, 90	79.30	15	48	44	11	–	5	23	28	4	–
	4	♂2 B × ♀2 B	11	(see Table 3 from Lespinasse's report)	78.96	23	83	253	121	43	9	37	98	6	6
1981	1	♂1 B × ♀0 B	12	E_4	83.70	26	30	–	–	–	12	10	–	–	–
		♂1 B × ♀0 B	13	P_5	79.42	10	10	–	–	–	–	–	–	–	–
		♂0 B × ♀1 B	14	E_7, E_8	81.41	66	55	–	–	–	13	11	–	–	–
		♂0 B × ♀1 B	15	P_{10}	88.40	9	11	–	–	–	7	8	–	–	–
	2	♂2 B × ♀0 B	16	E_5	78.39	15	42	13	–	–	6	20	8	–	–
		♂2 B × ♀0 B	17	P_2	71.88	6	9	5	–	–	4	11	5	–	–
		♂0 B × ♀2 B	18	P_1	82.57	4	9	7	–	–	9	19	12	–	–
		♂1 B × ♀1 B	19	E_1	81.85	6	9	5	–	–	4	7	3	–	–
		♂1 B × ♀1 B	20	P_3	75.03	5	10	5	–	–	–	–	–	–	–
	3	♂2 B × ♀1 B	21	P_7	81.80	2	9	9	3	–	1	5	12	2	–
		♂1 B × ♀2 B	22	P_4, P_9	67.55	7	26	42	15	–	4	10	20	2	–
	4	♂2 B × ♀2 B	23	P_8	81.56	1	4	13	8	4	2	2	16	0	0

Lespinasse (1977) found that supernumeraries are mitotically unstable in the ovarioles of females with 2, 3 or 4 B's, and also in the testes of males with 2, 3 or 4 B's, when these individuals are progeny from crosses involving 3 or 4 supernumeraries. It is possible that this instability could explain the increased transmission of B's in 4 B crosses.

In 1981 Lespinasse studied 7 natural populations of *L. migratoria* from different geographical origins and concluded again that B-chromosome transmission is similar for all the populations: the binomial transmission is modified by an accumulation mechanism with the preferential segregation of the B-chromosomes in the female pronucleus and by an elimination mechanism during embryonic and larval development. On re-examination of his experimental crosses (Table 6) we have again found that the transmission of the B's is binomial in all the cases. Furthermore, having in mind the accumulation mechanism which exists in the testes of the males of *L. migratoria* (Nur, 1969; Kayano, 1971; this report) males with whole numbers of supernumeraries (1, 2, 3 . . .) rarely exist in natural populations; rather they have a mean B number (e.g., 1.2,

Table 6. Our analysis of the results from Lespinasse's experiments expressed in Table 5. In some of the latter crosses in which there were low numbers of progeny, we have pooled the data from embryos and adults to obtain higher and more significant numbers.

Code number of each cross	Expected mean number of B's	Mean number of B's in the embryos	Mean number of B's in the adults	Transmission to embryos	Transmission to adults	Survival to adult stage: Contingency χ^2 test
1	0.50	0.48	0.46	$\chi^2_{(1)} = 0.40$ (1:1)	$\chi^2_{(1)} = 0.77$	$\chi^2_{(1)} = 0.12$
2	0.50	0.48	0.49	$\chi^2_{(1)} = 0.79$ (1:1)	$\chi^2_{(1)} = 0.05$	$\chi^2_{(1)} = 0.06$
3	1.00	0.94	0.80	$\chi^2_{(2)} = 0.78$ (1:2:1)	$\chi^2_{(2)} = 11.00$**	$\chi^2_{(2)} = 7.44$*
4	1.00	0.90	0.83	$\chi^2_{(2)} = 23.00$***	$\chi^2_{(2)} = 16.38$***	$\chi^2_{(2)} = 0.85$
5	1.00	0.98	0.89	$\chi^2_{(2)} = 3.74$ (1:2:1)	$\chi^2_{(2)} = 5.94$	$\chi^2_{(2)} = 1.99$
6	1.00	1.00	0.95	$\chi^2_{(2)} = 2.40$ (1:2:1)	$\chi^2_{(2)} = 0.30$	$\chi^2_{(2)} = 1.44$
7	1.00	1.07	1.13	$\chi^2_{(2)} = 1.13$ (1:2:1)	$\chi^2_{(2)} = 1.83$	$\chi^2_{(2)} = 0.22$
8	1.00	0.99	1.02	$\chi^2_{(2)} = 1.73$ (1:2:1)	$\chi^2_{(2)} = 0.12$	$\chi^2_{(2)} = 0.27$
9	1.50	1.52	1.43	$\chi^2_{(3)} = 1.87$ (1:3:3:1)	$\chi^2_{(3)} = 4.95$	$\chi^2_{(3)} = 6.86$
10	1.50	1.43	1.52	$\chi^2_{(3)} = 1.28$ (1:3:3:1)	$\chi^2_{(3)} = 3.82$	$\chi^2_{(3)} = 1.93$
11	2.00	2.15	1.76	$\chi^2_{(4)} = 40.8$***	$\chi^2_{(4)} = 56.2$***	$\chi^2_{(4)} = 36.6$***
12	0.50	0.54	0.45	$\chi^2_{(1)} = 0.29$ (1:1)	$\chi^2_{(1)} = 0.18$	$\chi^2_{(1)} = 0.15$
13	0.50	0.50	–	$\chi^2_{(1)} = 0.00$ (1:1)	–	–
14	0.50	0.45	0.46	$\chi^2_{(1)} = 1.00$ (1:1)	$\chi^2_{(1)} = 0.17$	$\chi^2_{(1)} = 0.001$
15	0.50	0.54		$\chi^2_{(1)} = 0.26$ (1:1)		$\chi^2_{(1)} = 0.01$
16	1.00	0.97	1.06	$\chi^2_{(2)} = 2.91$ (1:2:1)	$\chi^2_{(2)} = 1.29$	$\chi^2_{(2)} = 0.44$
17	1.00	1.00		$\chi^2_{(2)} = 0.00$ (1:2:1)		$\chi^2_{(2)} = 0.6$
18	1.00	1.10		$\chi^2_{(2)} = 1.47$ (1:2:1)		$\chi^2_{(2)} = 0.16$
19	1.00	0.94		$\chi^2_{(2)} = 0.35$ (1:2:1)		–
20	1.00	1.00		$\chi^2_{(2)} = 0.00$ (1:2:1)		–
21	1.50	1.65		$\chi^2_{(3)} = 2.83$ (1:3:3:1)		–
22	1.50	1.72	1.56	$\chi^2_{(3)} = 6.65$ (1:3:3:1)	$\chi^2_{(3)} = 5.48$	$\chi^2_{(3)} = 3.11$
23	2.00	2.33	1.70	$\chi^2_{(4)} = 4.76$ (1:4:6:4:1)	–	–

2.5, 3.6 . . .). Lespinasse (1981) did not mention this accumulation mechanism in the males he studied, but we believe it is an important factor to consider when analysing his experimental crosses.

In summary, we find that the only accumulation mechanism of B-chromosomes which has been demonstrated to date in *L. migratoria* is that which stems from their mitotic instability in the male germ line (see Kayano, 1971) and additionally, that there may be some elimination of B's when individuals carry high numbers of them.

Mitotic and meiotic behaviour of B-chromosomes

Since the B-chromosomes from Lespinasse's stock usually follow an independent transmission when a parent carries two B's, the B's cannot pair during meiosis. The B-chromosomes from the Spanish populations studied by us and those from the Japanese populations studied by Kayano (1971) show a great tendency to pair, forming bivalents when more than 1 B is present in meiocytes. Hence, the B's from natural populations seem to differ in pairing behaviour from those from the laboratory culture studied by Lespinasse. There is a further difference between these two classes of B's, namely their mitotic instability in the male germ line leading to their accumulation. While in all natural populations of *L. migratoria* so far analyzed the B's are mitotically unstable, in the laboratory stocks analyzed by Lespinasse they are apparently mitotically stable elements (Lespinasse, 1973). The only exception involved males with 2 or more B's produced from crosses involving up to 3 or 4 supernumeraries; here the B's were mitotically unstable (Lespinasse, 1977). These differences may be explained in two alternative ways: (1) The B's studied by Lespinasse are in some sense different from those from Spanish and Japanese natural populations and this determines their differential behaviour, or (2) Both classes of B's are structurally the same but culture conditions have influenced their mitotic and meiotic behaviour.

We think that the second possibility is most likely since Dearn (1974), who studied the B-chromosomes of *L. migratoria* from three different locali-

ties in Mali (where the initial collection of Lespinasse's stock was made) found the B's to be mitotically unstable. Certainly culture conditions can influence the chiasma phenotype since as Dearn points out the chiasma frequency in Mali populations is much higher than in the laboratory stocks derived from them.

Chiasma frequency and accumulation of B-chromosomes

From the chiasma-frequency analyses carried out in this report we conclude that, within males, both mean cell chiasma frequency and the between-cell variance of follicles with different number of B's are not usually significantly different. However, there are two exceptions: (1) In three males in which only follicles with 1 B or 2 B were found, mean chiasma frequency was significantly higher in those with 1 B, indicating what appears to be a negative dosage effect. No equivalent effect was found in males with more than two classes of follicles. (2) In the BE-1 male, follicles with B-chromosomes had a mean chiasma frequency significantly higher than those without B's, but this was not the case in two other males (BE-4 and C-3) where a comparison of +B and -B follicles was made. A possible relation may exist between mean cell chiasma frequency and the rate of accumulation of the B-chromosomes since in BE-1 the rate of accumulation was less than that of BE-4 and C-3.

At the inter-individual level, our results indicate that there are significant differences among males of *L. migratoria* for mean chiasma frequency. However, the B-carrying males have a similar mean chiasma frequency and between-cell variance to those without supernumeraries. So, our results agree with those obtained by Dearn (1974) in this species.

On the other hand, the existence of a significant negative correlation between the mean cell chiasma frequency and the rate of accumulation of the B implies that the influence which B-chromosomes have on mean chiasma frequency in males of *Locusta* depends on their degree of accumulation within the male. That is, males with a high rate of accumulation of B's in their germ line show a mean chiasma frequency similar to that of males without B's. On the other hand, males with a low degree of accumulation for the B's show an increased mean chiasma frequency which undoubtedly leads to an increase in the amount of genetic variation released by recombination. Whether it would have some adaptive signification will be tried to clear in future investigations.

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