

Random mating and absence of sexual selection for B chromosomes in two natural populations of the grasshopper *Eyrepocnemis plorans*

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The effects of B chromosomes on mating behaviour in natural populations of the grasshopper *Eyrepocnemis plorans* were investigated by collecting mating pairs in the field. The results have shown random mating and the absence of sexual selection related to the presence of Bs. Possible hypotheses explaining the maintenance of this polymorphism, despite the absence of drive and sexual selection, are discussed.

Keywords: B chromosomes, heterotic, mating pattern, mating success, neutral, parasitic.

Introduction

B chromosomes constitute one of the most frequent polymorphisms in natural populations of many plant and animal species. Their biological role, however, is not yet clear. They are mostly considered as genomic parasites (Östergren, 1945) because they are frequently harmful for the individuals carrying them (Müntzing, 1963; Nur, 1966, 1969; Puertas *et al.*, 1985) but maintain themselves at the expense of accumulation mechanisms (see Jones, 1985). Most B chromosome polymorphisms hitherto studied fit this pattern. However, there are at least two species where the maintenance of the B polymorphism may be based on more complex and until now unknown mechanisms: *Allium schoenoprasum* (Holmes & Bougourd, 1989) and *Eyrepocnemis plorans* (López-León *et al.*, 1992) in which the absence of any accumulation mechanism of B chromosomes open the possibility that they could be B chromosomes fitting the heterotic model suggested by White (1973), by which Bs could have some beneficial effect on individuals carrying them. Thus, it is necessary to investigate the possible effects of these B chromosome systems on various components of individual fitness.

The present paper analyses the possible effects of B chromosomes on mating behaviour in natural populations of the grasshopper *E. plorans*. The prolonged copulation period usually carried out by mating pairs in this species (about 24 h, M. D. López-León *et al.*, unpublished data) facilitated sampling of a high number of mating pairs in the field. The results showed random mating and absence of sexual selection in both sexes in relation to the presence of B chromosomes.

Materials and methods

Mating pairs of the grasshopper *Eyrepocnemis plorans* were collected in the field at Jete and Salobreña (Granada, Spain). The former population was sampled in 1986 and 1990, and the latter only in 1990. Males and females of each mating pair were stored in a vial, carried to the laboratory and fixed for cytological analysis. To test the possible existence of sexual selection, samples of non-mating males and females were simultaneously collected in each population.

Testes were fixed in 1:3 acetic ethanol without any pretreatment. Females, however, had to be injected with 0.05 per cent colchicine in insect saline solution for 6 h prior to fixation of ovarioles in 1:3 acetic ethanol. Squashes of testis follicles and ovarioles were C-banded by the technique described in Camacho *et al.* (1984).

Results

The analysis of the results was made in terms of the three main components of mating behaviour: differential male mating success, differential female mating success and pattern of mating (Santos *et al.*, 1986). Three samples were studied, J1986 (Jete in 1986), J1990 (Jete in 1990) and S1990 (Salobreña in 1990). Karyotypic classes with two or more B chromosomes were grouped in the class 2B⁺ for statistical analyses. Comparisons were made with the log-likelihood ratio test, or *G* statistic (Sokal & Rohlf, 1981).

J1986

Table 1 shows the karyotype frequencies observed in both mating and non-mating individuals from Jete collected in 1986. Mating and non-mating males did not show significant differences in karyotype frequencies ($G_2 = 0.01$, $P = 0.99$), and the same result was obtained for mating and non-mating females ($G_2 = 3.67$, $P = 0.16$). Consequently, there was no

differential male or female mating success related to the presence of the B chromosome. Table 2 shows the composition of the 71 mating pairs analysed. A *G*-test shows that mating occurs at random with respect to B chromosomes ($G_4 = 8.40$, $P = 0.08$).

J1990

Table 3 shows the karyotype frequencies in mating and non-mating individuals collected at Jete in 1990. The results are consistent with those observed 4 years before because there is no differential male ($G_2 = 2.26$, $P = 0.32$) or female ($G_2 = 0.07$, $P = 0.97$) mating success. Furthermore, the composition of the 72 mating pairs analysed shows that mating is at random in this population (Table 4) ($G_4 = 1.31$, $P = 0.86$).

S1990

Karyological frequencies of mating and non-mating individuals are shown in Table 5. *G*-tests show the absence of differential male ($G_2 = 4.30$, $P = 0.12$) or

Table 1 Karyotypic frequencies of mating and non-mating males and females collected at Jete in 1986

Sex	Type of individuals	Number of B chromosomes						Total	\bar{x}_B
		0B	1B	2B	3B	4B	5B		
Male	Mating	22	30	15	3	1	—	71	1.028
	Non-mating	26	34	20	2	—	—	82	0.976
	Total	48	64	35	5	1	—	153	1.000
Female	Mating	19	36	13	2	—	1	71	1.028
	Non-mating	22	49	9	—	—	—	80	0.838
	Total	41	85	22	2	—	1	151	0.927
Total		89	149	57	7	1	1	304	0.964

\bar{x}_B = mean number of B chromosomes.

Table 2 Frequency of mating pairs collected at Jete in 1986

Female	Male						Total	\bar{x}_B
	0B	1B	2B	3B	4B			
0B	3	8	7	1	—	19	1.316	
1B	11	15	7	2	1	36	1.083	
2B	6	6	1	—	—	13	0.563	
3B	2	—	—	—	—	2	0	
5B	—	1	—	—	—	1	1	
Total	22	30	19	3	1	71	1.028	
\bar{x}_B	1.318	1.067	0.632	0.667	1.000	1.028		

\bar{x}_B = mean number of B chromosomes.

Table 3 Karyotypic frequencies of mating and non-mating males and females collected at Jete in 1990

Sex	Type of individuals	Number of B chromosomes					\bar{x}_B
		0B	1B	2B	3B	Total	
Male	Mating	22	31	18	1	72	0.972
	Non-mating	58	62	25	3	148	0.818
	Total	80	93	43	4	220	0.868
Female	Mating	32	26	14	—	72	0.750
	Non-mating	24	21	8	2	55	0.782
	Total	56	47	22	2	127	0.764
Total		136	140	65	6	347	0.830

\bar{x}_B = mean number of B chromosomes.

Table 4 Frequency of mating pairs collected at Jete in 1990

Female	Male					\bar{x}_B
	0B	1B	2B	3B	Total	
0B	9	16	6	1	32	0.969
1B	8	10	8	—	26	1.000
2B	5	5	4	—	14	0.929
Total	22	31	18	1	72	0.972
\bar{x}_B	0.818	0.645	0.889	0	0.750	

\bar{x}_B = mean number of B chromosomes.

Table 5 Karyotypic frequency of mating and non-mating males and females collected at Salobreña in 1990

Sex	Type of individuals	Number of B chromosomes					\bar{x}_B
		0B	1B	2B	3B	Total	
Males	Mating	13	25	11	3	52	1.077
	Non-mating	58	66	21	4	149	0.805
	Total	71	91	32	7	201	0.876
Females	Mating	17	25	10	—	52	0.865
	Non-mating	15	11	2	1	29	0.621
	Total	32	36	12	1	81	0.778
Total		103	127	44	8	282	0.846

\bar{x}_B = mean number of B chromosomes.

female ($G_2 = 3.07$, $P = 0.22$) mating success with respect to the B chromosome. The frequencies of the different mating combinations (Table 6) are consistent with random mating ($G_4 = 4.48$, $P = 0.34$).

Table 6 Frequency of mating pairs collected at Salobreña in 1990

Female	Male					\bar{x}_B
	0B	1B	2B	3B	Total	
0B	3	10	3	1	17	1.118
1B	9	9	7	1	26	1.000
2B	1	6	1	1	9	1.222
Total	13	25	11	3	52	1.077
\bar{x}_B	0.846	0.840	0.818	1.000	0.846	

\bar{x}_B = mean number of B chromosomes.

Discussion

This is the first study of the effect of B chromosomes on mating behaviour performed in natural conditions. The results indicate random mating and an absence of sexual selection related to the presence of B chromosomes. In *E. plorans* there is paternity displacement based on a strong second male sperm precedence (M. D. López-León *et al.*, submitted) and the existence of sexual selection at the level of sperm competition cannot be ruled out because some karyotypes could be fitter than others in assuring paternity. This should be investigated.

The present results are consistent with the general idea of the dispensability of B chromosomes which could be the result of their heterochromatic nature. In fact, the only structural genes hitherto reported in B chromosomes are ribosomal RNA genes (see Jones & Rees, 1982; Green, 1990; López-León *et al.*, 1991), which are usually redundant with those of the normal genome. Unspecific transcriptional activity of B chromosomes has been detected in some cases (Green, 1988; Brockhouse *et al.*, 1989), but not in others (Sieger *et al.*, 1970; Fox *et al.*, 1974; Ishak *et al.*, 1991). The absence of effects of B chromosomes of *E. plorans* on mating behaviour could be a logical consequence of their genic inactivity. In fact, the B₂ type (the most frequent variant in Jete and Salobreña populations) possess a nucleolus organizer region (NOR) which is usually inactive due to methylation (López-León *et al.*, 1991). It is reasonable to think, therefore, that the effects of these B chromosomes are mainly derived from their physical presence rather than from genetic activity. Thus, it seems that the presence of B chromosomes in *E. plorans* has more influence at an endophenotypic level, i.e. on chiasma frequency (Camacho *et al.*, 1980) and NOR activity (Cabrero *et al.*, 1987), than at an exophenotypic level (Camacho *et al.*, 1980); the present results reinforce this argument.

Several hypotheses try to explain the maintenance of the widespread B chromosome polymorphism lacking drive existing in *E. plorans* (López-León *et al.*, 1992). Two antagonistic models have hitherto been proposed, the parasitic (Östergren, 1945) and the heterotic (White, 1973). A B chromosome system should be classified as heterotic or parasitic only after analysing all the fitness components in the most natural conditions possible, the transmission rate in controlled crosses and at populational level, as deduced from the study of gravid females and their offspring. The same B chromosome may be beneficial for a certain character but harmful for another. The absence of effects of B chromosomes on mating behaviour in *E. plorans* suggests that they may be neutral for this character. Given that they do not exhibit accumulation mechanisms and our current understanding of the mechanisms involved we can provisionally conclude that B chromosomes in this species are neither parasitic nor heterotic, they seem to be neutral.

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