The effect of B chromosomes on mating success of the grasshopper *Eyprepocnemis plorans*

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**Abstract**

The mating ability of *E. plorans* was tested in laboratory conditions in six experimental units composed of ten males and fifteen females during 31 days. When significant differences were found (three from the six cages, and in totals) they involved a decrease of matings involving males with B chromosomes. The same tendency seems to exist in females, but to a lesser extent, so that a significant effect is only detected when the totals are considered. Accessory chromosomes also delay, in both sexes, the occurrence of the first mating. No mating preferences depending on the number of Bs were detected.

**Introduction**

The existence of accessory or B chromosome systems has been in many cases explained under the 'parasitic' model, which claims that Bs are harmful but they are maintained by special mechanisms that increase their transmission to the next generations. However, an important number of unsolved questions about the B systems of many species still exist. An alternative point of view — the 'heterotic' model — claims that in some moments of the life cycle or under some circumstances, the individuals carrying a low number of B chromosomes may have a selective advantage over those showing the basic chromosome complement. Hence accessories could be maintained without a special mechanism of accumulation.

Most studies on the accumulation or loss of these chromosomes have traditionally focused on the germ line and particularly on gametogenesis (see the review of Jones & Rees, 1982). The relationship between Bs and fitness has been studied in some plants (see, for instance, Teoh & Jones, 1978; Rees & Hutchinson, 1974; and Puertas, Romera & Peña, 1985) and in animals like *Rattus fuscipes* (Thompson, 1984) or *Pseudococcus obscurus* (Nur, 1966a, 1966b, 1969).

In the grasshopper *Eyprepocnemis plorans* the B chromosome system shows a very high polymorphism for C chromosome structure (Henriques-Gil, Santos & Arana, 1985) and the distribution of different B types among natural populations indicates that newly arisen types replaced the previous ones (Henriques-Gil & Arana, 1990). The main question with this system is the cause for both the presence of Bs in this species and the substitution of one B type by another, since no accumulation mechanisms have been found in *E. plorans* (López-León et al., 1992a).

Because in animals mating is obviously a critical moment for any kind of genetic transmission, how it is affected by B chromosomes needs detailed analysis. López-León et al. (1992b) collected male–female pairs in natural populations of *E. plorans* and found no relationship with their B chromosome constitution. In this study we analyse the effect of B chromosomes on mating proficiency under controlled conditions.

**Materials and methods**

Fertilized females of *Eyprepocnemis plorans* subsp. *plorans* were collected from three Spanish natural pop-
ulations: Guadalhorce (GH), Fuengirola (FG) and Torrox (TX). Each one shows a different predominant type of B chromosome (B1, Bs-B6 and B2 respectively; see Henriques-Gil & Arana, 1990). Pods were obtained in the laboratory and the offspring were simultaneously reared in the conditions described by Henriques-Gil, Santos and Giraldez (1982) except that individuals of the same population were raised together in a common cage. The fifth instar hoppers were sexed and separated. All individuals selected for the experiment moulted in a four day interval.

For each of the three populations two different cages (FG1, FG2, GH1, GH2, TX1, and TX2) were established, with 10 males and 15 females per cage, introduced two weeks after the emergence of adults. One male (at FG1) and three females (one at GH2 and two at TX2) died during the first week of observation and were excluded from the analysis. The grasshoppers were selected from their main population culture by having a normal phenotype (a low number of individuals had some morphological abnormality due to a defective moult and so they were not included in the experiment). All the animals were marked with a numbered color flag on the femur of each of the jumping legs. The six experimental units were established at the same time and during each of the following 31 days, the observed couples were scored twice a day (morning and evening). True matings were considered those with more than 15 min duration. Because the total time of mating may last several hours, a ‘same pair’ mating registered in the morning and in the evening was considered a single mating.

Finally, after the period of observation, all the animals were weighed (excluding the third pair of legs since during the experiment, as in natural conditions, one of them was frequently lost). The ovarioles of females previously treated with 0.25% colchicine and male testes without previous treatment were fixed in acetic-ethanol 1 : 3. The preparations of the fixed material were stained with conventional C-banding. When one or more Bs were present, they corresponded in all cases to the predominant type of their respective geographic area.

Results

The matings scored in the six cages during the 31 days of observation are shown in Table 1. The data are given per day since the numbers obtained in the mornings and the evenings were very similar. It can be observed that the number of matings increases with time; this is probably the result of the achievement of complete maturity by the grasshoppers.

The total number of matings, however, differs significantly among populations, being higher for GH and lower for TX. The reasons for such differences remain unknown, but they must reflect some genetic difference since all the animals were obtained and cultured in the same laboratory conditions, developed simultaneously, and the experiment was carried out at the same time. The number of B chromosomes in the males or in the females (both given in Table 2) cannot be responsible for the different behaviour because no correlation was detected with the number of matings.

Matings do not seem to be established in a completely random manner. Indeed, despite the number of males being lower than that of females in a cage (10 and 15), there were a considerable number of males (11 from 59) that were never seen in a mating, while others were involved in a surprisingly high number of matings (like male 6 of FG2 which mated 19 times with 10 different females). The number of females was higher but, by contrast, non-mating females were scarcer (5 from 87); nevertheless, there are also extreme cases, like female 14 of GH1 which mated 20 times with 7 different males.

No significant correlation was detected between the number of matings and weights, though it is important to note that the animals employed were selected by having a completely ‘standard’ phenotype (see Materials and methods).

The role of B chromosomes

Matings per individual. Assuming that accessories have no effect on this variable, the number of matings involving an individual with one or more Bs (+B) should be proportional to the frequency of B carriers in a given cage. The $\chi^2$ comparisons with the observed data are given in Table 3.

In three of the six cages there was a significant increase in the matings involving non B (−B) males; the same phenomenon may be observed when the totals are considered. By contrast, the proportion +B/−B in the females showed no significant differences with the correspondent observed data; there is, however, a similar tendency towards a lower number of +B matings so that, when the totals are compared, the $\chi^2$ value is significant at a 0.05 level.

The mean number of matings per individual was compared by an analysis of variance for paired obser-