

## Brief report

# Supernumerary heterochromatin does not affect several morphological and physiological traits in the grasshopper *Eyprepocnemis plorans*

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Supernumerary heterochromatin in the form of autonomous B chromosomes or A-linked supernumerary chromosome segments (SCSs) constitutes the most frequent genomic polymorphism in grasshoppers (HEWITT 1979). Most B chromosomes are considered selfish genetic elements because they show drive mechanisms that enhance their maintenance in natural populations despite the negative effects they may produce on carriers (JONES 1985; BEUKEBOOM 1994). Even though SCSs have received less attention in the literature, it is now clear that many show drive (LÓPEZ-LEÓN et al. 1992a) and should also be considered selfish elements integrated in the standard genome. The maintenance of these polymorphisms depends mainly on a balance of positive (drive) and negative (harmful effects) factors that influence their frequency in natural populations. In order to reveal their evolutionary role it is necessary to investigate these two properties (drive and effects).

The grasshopper *Eyprepocnemis plorans* has both polymorphisms in natural populations from the Iberian Peninsula. The three most widespread B types (B<sub>1</sub>, B<sub>2</sub> and B<sub>5</sub>) lack drive (LÓPEZ-LEÓN et al. 1992b) and fitness effects (LÓPEZ-LEÓN et al. 1992c; CAMACHO et al. 1997a,b), so that they are expected to go extinct unless they are either replaced by a new selfish B variant (CAMACHO et al. 1997a,b) or they have unknown beneficial effects on fitness. The SCS located proximally on the smallest autosome shows a transmission ratio significantly lower than the Mendelian one through females possessing B chromosomes (LÓPEZ-LEÓN et al. 1991, 1994). Therefore, it is also expected to become extinct unless it is beneficial for carriers. Surprisingly, this polymorphism seems to show frequency stability over time in natural populations (LÓPEZ-LEÓN et al. 1995), suggesting the existence of some unknown factors influencing SCS frequency. Therefore, it is important to analyse the effects of both polymorphisms on as many different traits as possible. Here, we report the

results of such an analysis for several morphological and physiological traits. It has indicated the absence of any significant effects of the B<sub>2</sub> chromosome and the SCS on these characters.

## MATERIALS AND METHODS

A total of 258 males and 258 females of the grasshopper *Eyprepocnemis plorans* were collected at Salobreña (Granada, Spain) during the 1992 season. In the laboratory, the insects were weighed and then dissected to extract the gonads, which were also weighed. Testes were fixed in 3:1 ethanol-acetic acid, where testis follicles were individualized and counted under a stereomicroscope (mean = 76.047, SE = 0.792, N = 255). Females were injected with 0.05 % colchicine in insect saline solution 6 h prior to fixation of ovarioles in 3:1 ethanol-acetic acid. Ovarioles were counted (mean = 74.328, SE = 0.550, N = 256) and the proportion of ovarioles with a developing egg (mean = 0.578, SE = 0.017, N = 201) was also recorded. Male and female bodies were stored in 70 % ethanol until measurements were performed. Cytological analyses to determine the number of B chromosomes and SCSs in each individual were performed by the C-banding technique described in CAMACHO et al. (1991).

The external traits measured were: thorax length and width, tegmina length, hind femur length, and hind tibia length. Due to allometry, these characters are strongly correlated, so that they were combined in a single trait indicating body size by means of a Principal Component Analysis (PCA). In males, PCA for the five traits measured gave rise to a single factor explaining 66.59% of total variance, with factor loadings ranging from 0.679 (tegmina length) to 0.897 (thorax length). In females, the single factor obtained explained 64.3 % of variance, and factor loadings ranged from 0.703 (tegmina length) to 0.869 (thorax length). These factors were used as a measure of body size in both sexes.

As a measure of body condition, we have used weight-based somatic condition. To define this, we obtained the somatic weight of each individual by subtracting gonadal weight from total weight, and

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Table 1. Comparison of three morphological traits between males with different constitutions for the B<sub>2</sub> chromosome and the SCS, by means of a parametric ANOVA. In the case of B effect on somatic condition, however, a Levene test indicated that variances were significantly different, for which a non-parametric Kruskal-Wallis ANOVA was used

Item	B <sub>2</sub> chromosome			SCS		
	F	df	p	F	df	p
Number of testis follicles	1.05	2, 232	0.351	1.70	2, 232	0.185
Body size	2.19	2, 234	0.114	1.08	2, 234	0.341
Somatic condition	4.81	2, 206	0.090	0.50	2, 206	0.609

then performed a regression analysis of this variable on body size (represented by the factor previously defined by PCA). Somatic weight has the advantage of being independent of the physiological state of the gonads. This is especially important in females where a new pod cycle is started about each week, so that total female weight is heavily dependent on whether an egg-laying cycle was beginning or ending. The results of the regression analyses were highly significant in both sexes, thus providing a good definition of somatic condition as the standardized residuals of this regression (males:  $r = 0.788 \pm 0.041$ ,  $F = 365.04$ ,  $df = 1, 223$ ,  $p < 0.0001$ ; females:  $r = 0.765 \pm 0.041$ ,  $F = 353.14$ ,  $df = 1, 250$ ,  $p < 0.0001$ ).

Since individuals with three or more Bs were rarely found, they were included in the same class as 2B individuals (noted as 2B<sup>+</sup>).

## RESULTS AND DISCUSSION

Table 1 shows the absence of significant differences between males with 0B, 1B and 2B<sup>+</sup> for the number of testis follicles, body size, or somatic condition, although the latter showed a significantly higher variance in 1B males (1.313) than in the other two classes (0.824 in 0B males and 0.681 in 2B<sup>+</sup> ones). In females, no significant differences were observed as well for the four characters analysed: the number of ovarioles, the proportion of ovarioles developing an egg, body size, and somatic condition (Table 2). Similar analyses also indicated absence of any effect of a SCS proximally located on the smallest autosome in either sex (Tables 1 and 2).

The absence of effects of supernumerary heterochromatin on morphological traits such as body size and the number of testis follicles or ovarioles is consistent with most previous studies, in which it has been apparent that both B chromosomes and SCSs rarely produce visible effects on the external phenotype of its carriers (for review, see JONES and REES 1982). On the other hand, it is not infrequent that supernumerary heterochromatin causes diverse physi-

ological effects, many detrimental to carrier fitness (for review, see JONES and REES 1982).

The most plausible evolutionary scenario for the presence of B chromosomes in *E. plorans* is that they were selfish (showed drive) when they first invaded natural populations, but the drive has been neutralized by the appearance of suppressor genes in the A chromosomes (CAMACHO et al. 1997a,b). The absence of significant effects on several fitness components (LÓPEZ-LEÓN et al. 1992c; CAMACHO et al. 1997b), and the morphological and physiological traits analysed here, suggests that the neutralization process not only involves a decrease in the B-transmission ratio but also phenotypic selection for grasshoppers less affected by the presence of Bs. This scenario is clearly reminiscent of host-parasite coevolutionary processes (FUTUYMA 1986). In fact, the only B variant showing drive hitherto known in *E. plorans*, a B<sub>2</sub>-derivative named B<sub>24</sub> located in Torrox (Málaga, Spain), showed drive through females and decreased female fertility, an effect not caused by B<sub>2</sub> in the nearby populations at Jete and Salobreña (Granada, Spain) (CAMACHO et al. 1997b). Accordingly, it seems that selfishness (drive) and parasitism (harmful effects on carriers) are parallel conditions.

The case of the SCS is slightly different because we have never observed drive for this heterochromatic element, although it cannot be ruled out that drive occurred at the beginning when it appeared in the population. On the contrary, heterozygous females carrying B chromosomes do tend to eliminate the SCS chromosomes (LÓPEZ-LEÓN et al. 1991, 1994), perhaps as result of genomic interaction between the two genome parasites. At present, however, we have no more information about the cause of the SCS undertransmission. In any case, the result is that, other factors being neutral, the SCS is doomed to go extinct. But its frequency is not decreasing in natural populations, at least in the short term, and the fitness components hitherto analysed are not affected by the presence of the SCS (LÓPEZ-LEÓN et al. 1995; this paper). Therefore, future efforts should be focused to

Table 2. Comparison of four morphological traits between females with different constitutions for the B<sub>2</sub> chromosome and the SCS, by means of a parametric ANOVA

Item	B <sub>2</sub> chromosome			SCS		
	F	df	p	F	df	p
Number of ovarioles	2.97	2, 253	0.053	0.27	2, 253	0.762
Eggs/ovarioles	0.08	2, 198	0.925	0.03	2, 198	0.975
Body size	1.64	2, 251	0.197	2.92	2, 251	0.056
Somatic condition	1.09	2, 249	0.337	0.13	2, 249	0.881

analyse other fitness components that might explain how this polymorphism persists in natural populations.

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