

## SHORT COMMUNICATION

**Evolutionary dynamics of a B chromosome invasion in island populations of the grasshopper *Eyprepocnemis plorans***

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**Abstract**

Four natural populations of the grasshopper *Eyprepocnemis plorans* in the Mallorca island were analysed for several years revealing the recent invasion of the B<sub>1</sub> chromosome from the south-west part of the island (Palma region) towards the north and to the east. In only 10 years, the mean number of Bs in the northern population at Pollença increased from 0.053 to 0.692. Therefore, B chromosome invasion seems to be very rapid and has recently arrived to the north of the island. The south-west (close to Palma) is the most likely point at which B invasion started in the Mallorca Island. Finally, the number of B chromosomes was significantly associated to an increase in chiasma frequency (and thus recombination) in A chromosomes.

**Introduction**

The evolutionary dynamics of parasitic B chromosomes seems to be characterized by very rapid invasions, by virtue of drive, followed by neutralization by means of the evolution of suppressor genes in A chromosomes which eliminate B chromosome drive (Camacho *et al.*, 1997). In the model developed by these authors, a driving B chromosome may invade a population in only some tens of generations. Therefore, the likelihood of witnessing B invasion episodes is rather low, which, in addition to the need of studying the same set of natural populations for several generations, explains why few cases of B invasion have been reported so far.

Only three well-known examples of spectacular B frequency increase are known, and these all occurred over very short time periods. In the grasshopper *Eyprepocnemis plorans*, the parasitic B<sub>24</sub> chromosome recently invaded the Torrox population (Málaga, south Spain) and showed a change in the mean number of Bs per individual from 0.34 in 1984 to 0.98 in 1992 and 1.53 in 1994, displacing the neutralized B<sub>2</sub> (which was still present in 1984 but not in the later samples) by its significant meiotic drive in females (Zurita *et al.*, 1998).

In the fish *Prochilodus lineatus*, B frequency increased from 1.443 to 2.766 in only 10 years (Cavallaro *et al.*, 2000). Finally, in the wasp *Trypoxylon albitarse*, B chromosome frequency increased from 0.133 to 0.962 in the Nova Ilha population (Porto Firme, Brazil) between 1997 and 1999 (four generations), suggesting a very fast B invasion (Araújo *et al.*, 2002).

A myriad of changes have been reported in individuals hosting B chromosomes (see Jones & Rees, 1982, for a review on B chromosome effects). One of the most frequently reported effects of B chromosomes is that on chiasma frequency (for review, see Hewitt, 1979 and Jones & Rees, 1982), which is of particular relevance as it implies changes in host recombination and, consequently, in the amount of genetic variation. The hypothesis of inducible recombination considers the increase in chiasma frequency as a response in the host provoked by the parasitic B, because natural selection would favour any emerging offspring being more resistant to the B, which is obviously facilitated by higher genetic variation derived from chiasma frequency increase (Bell & Burt, 1990). Recently, this hypothesis has gained strong empirical support as chiasma effects seem to be associated to the evolutionary status of the B chromosome polymorphism (Camacho *et al.*, 2002).

In the present work, we describe observations of a B chromosome invasion and frequency increase in the Mallorca Island (Balears, Spain), and report significant

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increase in chiasma frequency associated to the number of B chromosomes.

## Materials and methods

A total of 228 adult males of the grasshopper *E. plorans* were collected at four natural populations in the Mallorca Island (Balears, Spain) (Fig. 1). Testes were fixed in 3 : 1 ethanol-acetic acid and stored at 4 °C. Cytological preparations were performed by squashing a couple of testis

follicles in acetic orcein, for scoring B and chiasma number, or in 50% acetic acid, for performing C-banding by the method described in Camacho *et al.* (1984). The number of B chromosomes and the number of chiasmata were scored in 20 spermatocytes at diplotene per individual.

The frequency of B chromosomes was measured by the mean number of Bs and prevalence (the proportion of B-carrying individuals).

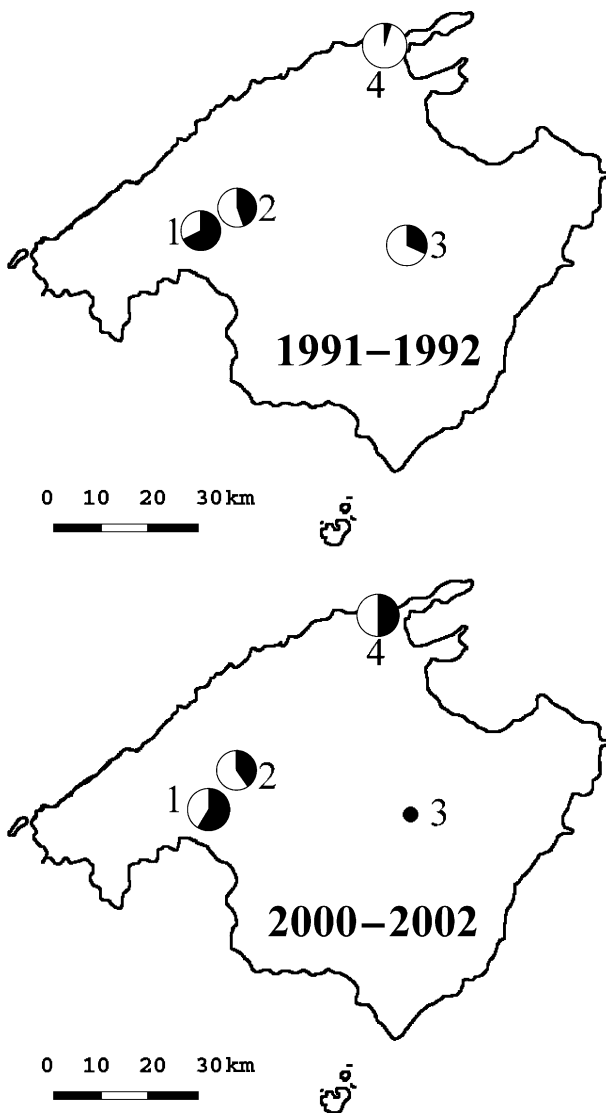
The two frequency parameters (mean Bs and prevalence) did not follow a normal distribution. For this reason, population comparisons were performed by the nonparametric Kruskal–Wallis ANOVA, with the sequential Bonferroni test to avoid statistical type I errors (Rice, 1989). However, chiasma frequency did follow a normal distribution so that ANCOVA was used to test B effects together with spatial and temporal variation in this trait. As individuals with more than two Bs were scarce in most samples, they were included for analysis within the 2B<sup>+</sup> class.

## Results

C-banding showed that B chromosomes in all populations are very similar to the B<sub>1</sub> chromosome, which, in the Iberian Peninsula, is the most abundant B, with two dark C-bands (proximal and interstitial), and a lighter distal region (Henriques-Gil *et al.*, 1984). Table 1 shows B chromosome frequency at all samples in the four populations analysed. Three populations (S'Esgleieta, Santa María and Pollença) were analysed in two or more years, with a temporal scope of about 10 years in each population. This permitted us to test temporal variation for B frequency (Table 2). Whereas the samples collected in S'Esgleieta and Santa María did not show significant differences among years, for any of the two parameters analysed (mean Bs and prevalence), the two samples collected in Pollença (in 1992 and 2002) differed significantly in both parameters.

Bearing in mind these significant temporal changes, spatial population differences were tested separately in the start (1991–92) and end (2000–02) of the analysed period. A comparison among the four populations in 1991–92 showed significant differences among them for both mean Bs (Kruskal–Wallis:  $H_3 = 22.26$ ,  $N = 99$ ,  $P < 0.001$ ) and prevalence ( $H_3 = 22.07$ ,  $N = 99$ ,  $P < 0.001$ ). However, these differences were not apparent in 2000–02 (mean Bs:  $H_2 = 0.05$ ,  $N = 65$ , n.s.; prevalence:  $H_2 = 1.11$ ,  $N = 65$ , n.s.) (Fig. 1).

Mean cell chiasma frequency scored in the A chromosomes of males with different number of B chromosomes, in each population, is summarized in Table 3. An ANCOVA performed on the four populations, with mean cell chiasma frequency as dependent variable, population as a random factor and number of B chromosomes as a covariate showed significant effects of both population ( $F = 8.85$ ,  $P < 0.0001$ ) and number of Bs ( $F = 10.00$ ,  $P < 0.01$ ) on chiasma frequency. In the two populations



**Fig. 1** Maps of Mallorca island showing B frequency at collection sites (1 = S'Esgleieta, 2 = Santa María, 3 = Petra, 4 = Pollença) at the beginning (1991–92) and end (2000–02) of the time period analysed. Black area in the pie diagrams shows B prevalence. Note that in 1991–92 B frequency was high in the west and the centre of the island, but it was very low in the north. This difference was not apparent in 2000–02 after the significant B frequency increase in Pollença.

Population	Year	Number of Bs					Total	Mean Bs	SE	Prevalence	SE
		0	1	2	3	4					
S'Esgleietta	1991	14	24	2	3	-	43	0.860	0.123	0.674	0.072
	1996	8	13	5	-	-	26	0.885	0.140	0.692	0.092
	1997	8	5	1	-	-	14	0.500	0.174	0.429	0.137
	1998	10	20	1	2	1	34	0.941	0.158	0.706	0.079
	1999	16	3	4	3	1	27	0.889	0.241	0.407	0.096
	2000	5	6	1	-	-	12	0.667	0.188	0.583	0.149
Santa María	1992	10	8	-	-	-	18	0.444	0.121	0.444	0.121
	2000	6	1	2	1	-	10	0.800	0.359	0.400	0.163
Petra	1992	13	5	1	-	-	19	0.368	0.137	0.316	0.110
Pollença	1992	18	1	-	-	-	19	0.053	0.053	0.053	0.053
	2002	13	10	1	2	-	26	0.692	0.173	0.500	0.100

**Table 1** B chromosome frequency in four populations from Mallorca island measured by the mean number of Bs per individual (mean Bs) and the proportion of individuals carrying B chromosomes (prevalence).

**Table 2** Analysis of temporal variation in three natural populations from Mallorca island, by means of the nonparametric Kruskal-Wallis ANOVA and the sequential Bonferroni test ( $P_b$ ).

Kruskal-Wallis	Population		
	S'Esgleietta	Santa María	Pollença
Mean Bs			
<i>H</i>	4.26	0.36	10.06
d.f.	5	1	1
<i>N</i>	156	45	45
<i>P</i>	0.5121	0.5493	0.0015
$P_b$			0.0045
Prevalence			
<i>H</i>	9.37	0.06	10.02
d.f.	5	1	1
<i>N</i>	156	45	45
<i>P</i>	0.0953	0.8075	0.0015
$P_b$			0.0045

where two or more samples were available (S'Esgleietta and Pollença), an ANCOVA, adding year of sampling as a random factor to the former design, showed no effect of population ( $F = 0.45$ , n.s.) or year ( $F = 0.37$ , n.s.) but a significant effect of B chromosome number ( $F = 9.82$ ,  $P < 0.01$ ) (Fig. 2).

## Discussion

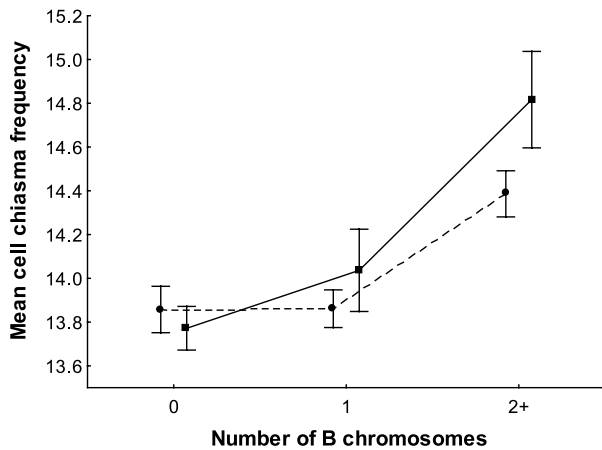
The B chromosome found in all populations analysed from Mallorca is similar to the B<sub>1</sub>, which is the principal, and most likely ancestral, B chromosome in the Iberian Peninsula (Henriques-Gil *et al.*, 1984; Cabrero *et al.*, 1997; Camacho *et al.*, 2000).

The sharp change in B frequency observed in Pollença clearly suggests the recent B chromosome invasion of this population, with B frequency, measured by any of the two parameters employed, increasing ten times in the 10-year period analysed. This rapid B chromosome frequency increase was presumably the result of an intense meiotic drive, in resemblance with the invasion

**Table 3** Chiasma scores in four natural populations from Mallorca island. The mean number of chiasmata was obtained from 20 diplotene cells per individual. *N* = number of males.

Population	Year	Number of Bs	Number of chiasmata		
			Mean	SE	<i>N</i>
S'Esgleietta	1991	0	14.00	0.22	10
		1	13.84	0.13	23
		2	14.25	0.10	2
		3	14.12	0.11	3
	1996	0	13.82	0.24	7
		1	13.77	0.17	11
		2	14.67	0.18	5
		1997	0	13.58	0.24
	1998	1	14.28	0.93	2
		0	13.92	0.19	10
		1	13.96	0.16	19
		2	14.10		1
	1999	3	13.98	0.38	2
		4	14.55		1
		0	13.70		1
		1	13.25	0.15	2
2000	2	14.38	0.53	2	
	3	15.20		1	
	4	14.20		1	
	Santa María	1992	0	14.32	0.33
1	14.88	0.30	6		
Petra	1992	0	14.51	0.19	10
	1	14.47	0.42	3	
	2	14.75		1	
Pollença	1992	0	13.72	0.15	17
		1	13.90		1
	2002	0	13.83	0.12	13
		1	14.04	0.21	10
		2	14.40		1
		3	15.03	0.13	2

shown in the Torrox population in this same species (Zurita *et al.*, 1998). In the two other populations analysed over time, S'Esgleietta and Santa María, B frequency was already high at the beginning of the time



**Fig. 2** Effect of B chromosome number on mean cell chiasma frequency in S'Esgleieta (dotted line) and Pollença (solid line). Bars indicate mean  $\pm$  SE.

period analysed. These facts strongly suggest that B chromosomes are spreading over the Mallorca island from the south-west towards the north and probably the east (Petra population).

B chromosome invasion in the Pollença population has seemed to be a very fast process, in agreement with theoretical studies predicting B invasions lasting in the order of tens of generations (Camacho *et al.*, 1997). This is also consistent with previous observations in *E. plorans* (Zurita *et al.*, 1998) and other taxa (Cavallaro *et al.*, 2000; Araújo *et al.*, 2002), and explains the scarcity of invasion examples in the literature. The frequency cline from the west to the east and the north, observed in 1991–92 suggests that B invasion most likely began in the west, close to Palma, the main port of the island, presumably associated to commercial interchanges with the Iberian Peninsula, as *E. plorans* is a species living in humid zones including cultivated plants of many commercial species.

The increase in chiasma frequency in the A chromosomes, and thus recombination, caused by the presence of the B<sub>1</sub> chromosome in Mallorca populations of the grasshopper *E. plorans*, is consistent with the predictions based on the hypothesis of inducible recombination (Bell & Burt, 1990). This recombination increase in the host genome is interpreted as an evolutionary response favoured by natural selection as some of the more variable offspring may be resistant to the parasitic B chromosome.

The chiasma effect pattern shown in Fig. 2 resembles that observed for the same B chromosome variant (B<sub>1</sub>) in Moroccan populations, but it suggests a rather weaker effect than that of B<sub>24</sub> in the Torrox population (compare Fig. 2 in this paper with Fig. 1 in Camacho *et al.*, 2002). It is thus likely that chiasma effects are, to some extent, dependent on the particular B chromosome variant, or else on whether the B invasion is the first (Mallorca and Morocco populations) or it is actually a B polymorphism regeneration, i.e. the replacement of a neutralized B (B<sub>2</sub>)

by a newly parasitic one (B<sub>24</sub>), which was indeed the situation in the Torrox population (see Zurita *et al.*, 1998). It is possible that regenerations are more aggressive, from the parasite point of view, than primary invasions, causing a higher response in the host (A chromosomes) to increase recombination.

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