

# The B chromosome polymorphism of the grasshopper *Eyprepocnemis plorans* in North Africa. I. B variants and frequency

M. BAKKALI, J. CABRERO, M. D. LÓPEZ-LEÓN, F. PERFECTTI & J. P. M. CAMACHO\*

*Departamento de Genética, Facultad de Ciencias, Universidad de Granada, E-18071 Granada, Spain*

Polymorphism for B chromosomes has been detected in all nine populations of the grasshopper *Eyprepocnemis plorans* ssp. *plorans* sampled in Morocco. The most frequent B chromosome in all populations showed a C-banding pattern and size similar to those of the B<sub>1</sub> variant found in the Iberian Peninsula. In addition, other B chromosome variants (B<sub>1iso1</sub>, B<sub>1iso2</sub>, B<sub>1d1</sub>, B<sub>1di1</sub>, B<sub>3</sub> and B<sub>1dd1</sub>) were discovered in these populations, although at a very low frequency. No significant differences in B chromosome frequency were found either in the nine populations or, for some of them, in up to three consecutive years. These results are discussed in the light of current hypotheses on the evolution of this B chromosome polymorphism in the Iberian Peninsula.

**Keywords:** B chromosomes, *Eyprepocnemis plorans* ssp. *plorans*, grasshopper, Orthoptera.

## Introduction

B chromosomes, also called accessory or supernumerary chromosomes, are large pieces of dispensable independently segregating DNA (Camacho *et al.*, 1997a,b). Reported in more than 1300 plant species and nearly 500 animal species (Jones & Puertas, 1993), variation in the number and morphology of B chromosomes is a frequent chromosomal polymorphism found in natural populations of many eukaryote organisms. B chromosomes show extensive variation in size and morphology, and most of them are heterochromatic with a variety of different responses to C-banding. Because of their harmful effects on carriers, most of them are considered genome parasites (Östergren, 1945; Müntzing, 1963; Nur, 1966, 1969; Puertas *et al.*, 1985) that maintain themselves by accumulation mechanisms (Jones, 1985, 1991).

B chromosomes are especially frequent in Orthoptera, where they sometimes constitute apparently stable polymorphisms (Hewitt, 1979). The grasshopper *Eyprepocnemis plorans* ssp. *plorans* shows a highly remarkable B chromosome polymorphism where the great majority of populations harbour B chromosomes (Camacho *et al.*, 1997a,b). This subspecies inhabits the Mediterranean region, the Caucasus, Turkey, Turkmenistan, Iran and south-western Arabia (Dirsh, 1958). More than 40 B

chromosome variants have been described in natural Spanish populations of this grasshopper species, according to size, morphology and C-banding pattern (Henriques-Gil *et al.*, 1984; Henriques-Gil & Arana, 1990; López-León *et al.*, 1993); however, only five variants have had success in establishing polymorphisms (B<sub>1</sub>, B<sub>2</sub>, B<sub>5</sub>, B<sub>6</sub> and B<sub>24</sub>), the remaining types being found at extremely low frequency (Henriques-Gil & Arana, 1990; López-León *et al.*, 1993). New B variants appear frequently (López-León *et al.*, 1993) and the replacement of a B variant by another one (Henriques-Gil & Arana, 1990) can be caused by a detectable meiotic drive favouring the substituting variant (Zurita *et al.*, 1998). Evidence for the monophyletic origin of most B chromosome variants, including the predominant types, has been provided by C-banding (Henriques-Gil *et al.*, 1984; López-León *et al.*, 1993) and fluorescent *in situ* hybridization (Cabrero *et al.*, 1999).

The B chromosome polymorphism of *E. plorans* has provided evidence for the long-term evolution of these genome parasites as a result of an 'arms race' between them and the A genome. Thus B chromosomes, starting as parasites (possessing drive and damaging host fitness), can be neutralized because of the evolution of drive-suppressor genes in the A genome and the parallel evolution both of less damaging B chromosome variants and of more tolerant A genomes (Camacho *et al.*, 1997a,b). This explains the existence of polymorphisms for B chromosome variants lacking drive (López-León *et al.*, 1992a) and apparent effects on host fitness

\*Correspondence. E-mail: jpmcamac@ugr.es

(López-León *et al.*, 1992b; Martín-Alganza *et al.*, 1997). These neutralized B chromosome variants, however, are condemned to random extinction and the B chromosome polymorphism would disappear unless replaced by newly arising selfish B chromosome variants (Camacho *et al.*, 1997b). Evidence for such a regeneration of the B chromosome polymorphism has also been found in *E. plorans* (Zurita *et al.*, 1998).

The geographical distribution of different B chromosome variants of this species has been extensively studied in Spain and the presence of B chromosomes has been reported in almost all natural populations analysed (Camacho *et al.*, 1980; Henriques-Gil *et al.*, 1984; Henriques-Gil & Arana, 1990), the only non-B populations being located in an inland region of the Segura River basin (Cabrero *et al.*, 1997). The single North African sample hitherto analysed (Melilla) showed the presence of a B chromosome very similar to those found in the Iberian Peninsula (Henriques-Gil & Arana, 1990).

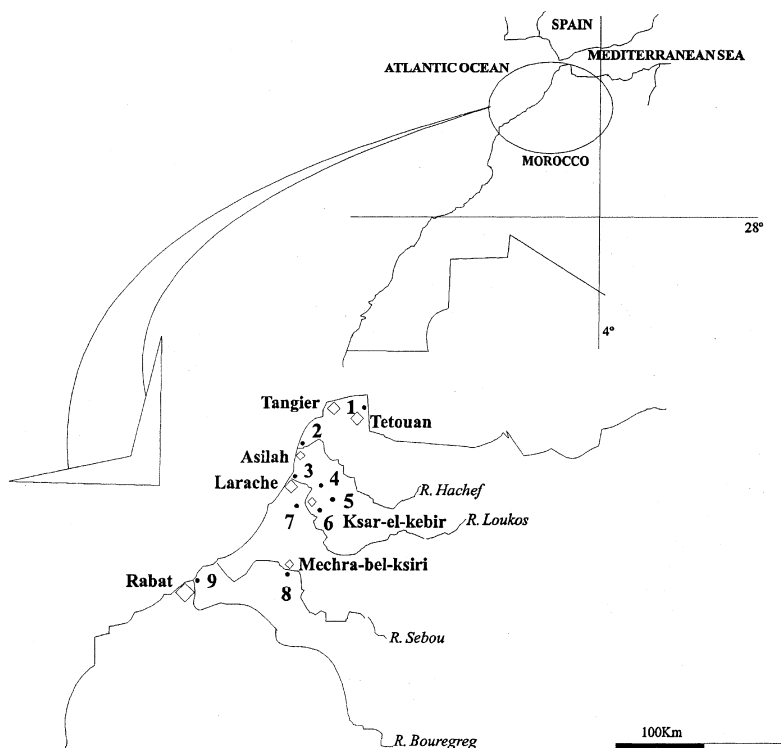
The present paper seeks to provide a more complete picture of B chromosome distribution in natural African populations of *E. plorans*. We report the presence of B chromosomes in all nine populations sampled from Morocco, and the existence of several low-frequency B chromosome variants. We also perform an analysis of B chromosome frequency variation in time and space.

## Materials and methods

Specimens of the grasshopper *E. plorans* ssp. *plorans* were collected at nine natural populations from north-western Morocco during September in 1995, 1996 and 1997. The geographical location and the number of specimens caught in each of these nine populations are shown in Fig. 1 and Table 1. The populations sampled in 1995 were Smir (between Ceuta and Tetouan), Frain and SO.DE.A (close to Ksar-el-Kebir), and Mechra (close to Mechra-bel-Ksiri). In 1996 we collected grasshoppers at Ain l'abid and Tatouft (near Ksar-el-Kebir), in addition to taking new samples of the same populations sampled in 1995. In 1997, we captured new specimens from the six populations sampled in previous years and collected grasshoppers in three new sites: Larache, Asilah and Rabat.

Testes were fixed in 1:3 acetic acid-ethanol without any pretreatment. Females were injected with 0.1 mL of 0.05% colchicine in insect saline solution 6 h before fixation of the ovaries. Fixed gonadal tissues were stored at 4°C for at least one month before analysis.

B chromosome presence was studied by squashing testis follicles and ovarioles in acetic orcein. For characterization on the basis of morphology and C-banding patterns, gonadal tissues of B-chromosome-carrying individuals were C-banded as described by Camacho *et al.* (1991). The B chromosome structure was



**Fig. 1** Geographical location of the nine populations of the grasshopper *Eyprepocnemis plorans* sampled in Morocco from 1995 to 1997. ◇, Cities; ●, Populations (1, Smir; 2, Asilah; 3, Larache; 4, Ain l'abid; 5, Tatouft; 6, Frain; 7, SO.DE.A; 8, Mechra; 9, Rabat). Principal rivers are also indicated.

**Table 1** Spatial and temporal distribution of B chromosome frequencies in specimens of the grasshopper *Eyprepocnemis plorans* ssp. *plorans* caught at nine Moroccan localities

Population	Year	0B	1B	2B	3B	Total	B <sub>1</sub> freq.	Total B freq.	Comparison of B <sub>1</sub> freq. between years			B <sub>1</sub> prevalence	Total B prevalence
									$\chi^2$	d.f.	P		
Smir	95	8	3	0	0	11	0.273	0.273				27.273	27.273
	96	18	5	0	0	23	0.217	0.217				21.739	21.739
	97	21	10†	2‡	1§	34	0.382	0.500				32.353	38.235
	Total	47	18	2	1	68	0.309	0.368	2.365	4	0.779	27.941	30.882
Asilah	97	7	1	0	0	8	0.125	0.125	—	—	—	12.500	12.500
Larache	97	26	13	1	0	40	0.375	0.375	—	—	—	35.000	35.000
Ain l'abid	96	15	9	1	0	25	0.440	0.440				40.000	40.000
	97	38	9¶	0	0	47	0.170	0.192				17.021	19.149
	Total	53	18	1	0	72	0.264	0.278	5.519	2	0.055	25.000	26.389
Tatouft	96	13	2	4	0	19	0.526	0.526				31.579	31.579
	97	26	12	2	0	40	0.400	0.400				35.000	35.000
	Total	39	14	6	0	59	0.441	0.441	5.346	2	0.080	33.898	33.898
Frain	95	13	5	2	0	20	0.450	0.450				35.000	35.000
	96	17	2	1	0	20	0.200	0.200				15.000	15.000
	97	28	10††	0	0	38	0.237	0.263				23.684	26.316
	Total	58	17	3	0	78	0.282	0.295	5.494	4	0.213	24.359	25.641
SO.DE.A	95	25	8‡‡	1	0	34	0.265	0.294				23.529	26.471
	96	49	14	1	0	64	0.250	0.250				23.438	23.438
	97	27	14§§	0	0	41	0.268	0.342				26.829	34.146
	Total	101	36	2	0	139	0.259	0.288	5.519	4	0.055	24.460	27.338
Mechra	95	34	9	1	0	44	0.250	0.250				22.727	22.727
	96	54	25¶¶	5†††	1‡‡‡	85	0.400	0.447				34.118	36.471
	97	14	5	2	0	21	0.429	0.429				33.333	33.333
	Total	102	39	8	1	150	0.360	0.387	2.795	4	0.596	30.667	32.000
Rabat	97	11	3§§§	2	0	16	0.375	0.438	—	—	—	25.000	31.250

†One individual carried one B<sub>1iso1</sub>.

‡One individual carried one B<sub>1iso1</sub> and one B<sub>1iso2</sub>.

§This individual carried two B<sub>1</sub> and one B<sub>1iso2</sub>.

¶One individual carried one B<sub>1iso2</sub>.

††One individual carried one B<sub>1iso2</sub>.

‡‡One individual carried one B<sub>1d,d1</sub>.

§§Three individuals carried one B<sub>1d,d1</sub>.

¶¶One individual carried one B<sub>1iso2</sub>.

†††One individual carried one B<sub>1d1</sub> and one B<sub>1d,et1</sub>.

‡‡‡This individual carried two B<sub>1</sub> and one B<sub>1iso1</sub>.

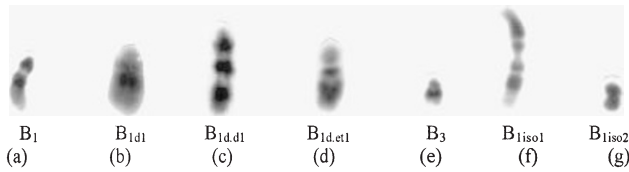
§§§One individual carried one B<sub>3</sub>.

examined in male pachytene–diplotene cells, because of the less condensed state of B-chromatin in these meiotic stages.

## Results

The standard chromosome complement of males and females of the grasshopper *E. plorans* is composed of 23 (22 + X0) and 24 (22 + XX) telocentric chromosomes, respectively.

Our study of gonadal tissues of *E. plorans* ssp. *plorans* specimens revealed the presence of B chromosomes in all nine Moroccan populations sampled. Only two individuals (Mechra-1996 and Smir-1997) were found bearing three B chromosomes, this being the highest number of B chromosomes found in the same individual (Table 1). The most frequent B chromosome in all nine populations was an acrocentric chromosome, about half the size of the X chromosome, with two proximal C-bands of similar sizes. Fluorescent *in situ* hybridization



**Fig. 2** C-banded B chromosome variants found in the nine Moroccan *Eyprepocnemis plorans* ssp. *plorans* natural populations sampled. (a) The most frequent B chromosome found in all the nine populations studied; (b) found in one individual in Mechra 1996; (c) found in one individual in SO.DE.A 1995 and in three from the same population in 1997; (d) present in one individual in Mechra 1996; (e) observed in one individual in Rabat 1997; (f) described as B<sub>12</sub> and B<sub>1iso</sub> by Henriques-Gil *et al.* (1984) and López-León *et al.* (1993), respectively. One individual carrying this B variant was found in Mechra 1996 and two in Smir 1997; (g) one individual carried this B variant in Mechra 1996, four in Smir 1997, one in Ain l'abid 1997, and another one in Frain 1997.

analysis shows that this B chromosome is made up of the same two DNA types (rDNA and a 180-bp repeat DNA) present in B chromosomes from the Iberian Peninsula (Cabrero *et al.*, 1999). All these data suggest that the predominant B chromosome in Morocco is similar (in terms of size, morphology, C-banding pattern and DNA content) to the predominant one in the Iberian Peninsula, which was named B<sub>1</sub> by Henriques-Gil *et al.* (1982) (Fig. 2). Temporal analysis of B<sub>1</sub> frequency variation in Moroccan populations revealed no significant difference between years of collection in any of the six populations where this comparison could be made (Table 1). Likewise, a comparison of the frequency of individuals with different numbers of B<sub>1</sub> chromosomes in the nine populations also failed to detect significant differences ( $\chi^2_{16} = 18.292$ ;  $P = 0.309$ ). The mean prevalence (percentage of individuals carrying at least one B chromosome) was  $28.32\% \pm 2.26$ , and there were no significant differences between populations ( $\chi^2_8 = 3.983$ ;  $P = 0.861$ ) or years within populations ( $P$  ranging from 0.093 to 1).

In addition to the principal type (B<sub>1</sub>), we found six other B chromosome variants (Fig. 2), for which the structure and possible mode of origin are summarized in Fig. 3.

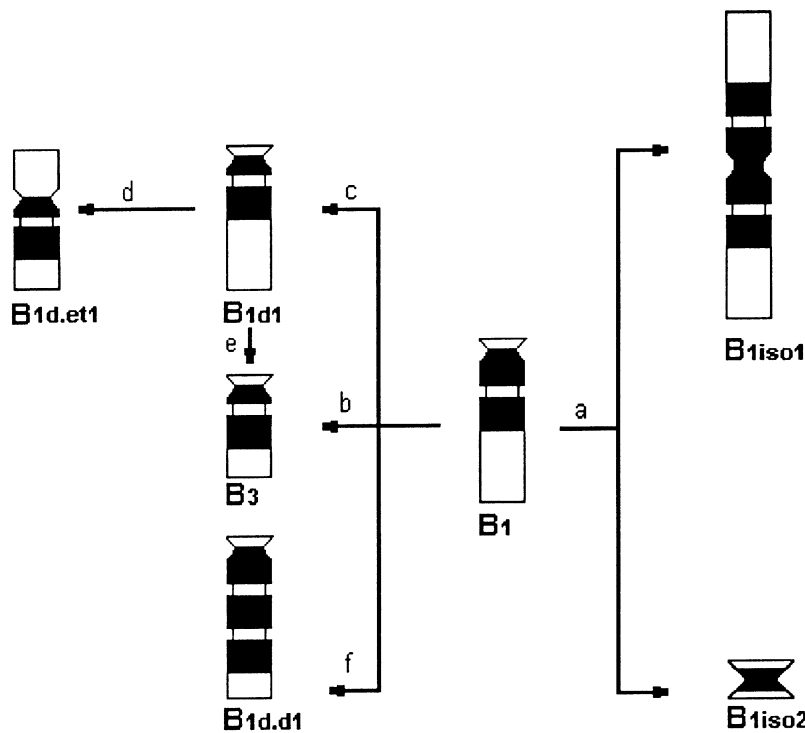
## Discussion

With the exception of the grasshoppers *Atractomorpha bedeli* and *Acrida lata* inhabiting sites heavily disturbed by human activity (Sannomiya & Kayano, 1968), and the grasshopper *Myrmeleotettix maculatus*, which shows a cline movement in East Anglia (Shaw, 1983), the frequencies of most plant and animal B chromosomes that have been studied over several years have remained stable (Jones & Rees, 1982; Cano & Santos, 1989;

Parker *et al.*, 1991). In the case of the grasshopper *E. plorans*, Camacho *et al.* (1997b) reported nonsignificant temporal variation in B chromosome frequency at two Spanish localities (Jete and Salobreña), sharply contrasting with another Spanish population (Torrox) where rapid changes in B<sub>24</sub> frequency were demonstrated (Zurita *et al.*, 1998). The nonsignificant interannual changes of the B chromosome frequency observed in the six Moroccan populations of *E. plorans* sampled at least twice (Smir, Ain l'abid, Tatouft, Frain, SO.DE.A and Mechra) corroborate that the time needed to record significant changes in B chromosome frequency depends on the B variant, the population, and the evolutionary status of the polymorphism at the time of sampling.

The proportion of individuals carrying B chromosomes was similar in the nine Moroccan populations studied. However, Spanish populations of this species show different local B chromosome frequency (Henriques-Gil *et al.*, 1984; Henriques-Gil & Arana, 1990; Camacho *et al.*, 1997b). The low spatial differentiation in Morocco could be explained by a rapid invasion of the North African populations by the B chromosome polymorphism, together with high gene flow between the nine populations studied. This scenario is supported by the flying capacity of this grasshopper species and the absence of major geographical barriers between the nine localities. The presence of some B variants at very low frequency in some populations, and their absence in others, does not diminish the importance of gene flow as a possible cause of the spatial stability of B chromosome frequency through the Moroccan populations analysed, because these are, presumably, short-lived variants usually with low transmission efficiency (López-León *et al.*, 1993). Thus, it is unlikely that they will migrate to other populations before disappearing. It is remarkable that the only two B variants detected in more than one population (B<sub>1iso1</sub> and B<sub>1iso2</sub>) are those produced by a recurrent phenomenon (centromere misdivision); they are also frequently found in Spanish populations at a very low frequency because of their low transmission ratio (López-León *et al.*, 1993). B<sub>1iso1</sub> has been described by Henriques-Gil *et al.* (1984) as B<sub>12</sub> and as B<sub>1iso</sub> by López-León *et al.* (1993), and according to the code proposed by López-León *et al.* (1993) B<sub>1iso2</sub> can be considered to be a B<sub>mini</sub>.

The highly dynamic character of this B chromosome polymorphism (Camacho *et al.*, 1997b) is supported by the presence of other B variants detected in some of the nine Moroccan populations studied. This dynamism of B chromosomes is an important characteristic for the maintenance of the polymorphism, because it means that a new B variant with drive can replace the principal neutralized B chromosome, thereby regenerating and prolonging the polymorphism life cycle (Zurita *et al.*, 1998).



**Fig. 3** Hypothetical origin of the six minority B variants arising from the most widespread variant,  $B_1$ . (a) Centromere misdivision and subsequent chromatid nondisjunction; (b) deletion of part of the proximal dark C-band and part of the distal light region; (c) deletion of part of the proximal dark C-band; (d) extra-radial transposition; (e) deletion of part of the distal light region; (f) duplication of a dark C-band and deletion of part of the distal light region.

In the chive *Allium schoenoprasum*, Bougourd & Parker (1979) traced with high precision the sites where B chromosomes probably originated and their mode of spreading. Despite the extensive study of many Iberian *E. plorans* populations, such results have not yet been achieved in this grasshopper, partly because of almost universal B chromosome presence in Spanish populations, and partly because of the scarcity of information from African populations.

Henriques-Gil *et al.* (1984) postulated two possibilities for the origin of the B chromosomes of *E. plorans*: (i) B chromosomes originated prior to the colonization of the Iberian Peninsula by this grasshopper (in this case the North African populations should have B chromosomes) or (ii) B chromosomes originated in a Spanish population and spread throughout Spain. We can add another hypothesis: (iii) B chromosomes of *E. plorans* originated independently in Spain and Morocco. Our study shows that B chromosomes are present in all the Moroccan populations studied, and the fact that the principal B chromosome found in all the studied populations is a very similar B variant ( $B_1$ ) indicates that we can consider this B variant as the original B chromosome in Moroccan populations. The observed north–south continuity of B chromosome presence can be interpreted as the result of a migration of  $B_1$ -carrying individuals through the studied populations.  $B_1$  is also considered the original B chromosome variant in Spanish natural populations (Henriques-Gil *et al.*, 1984; López-León

*et al.*, 1993). López-León *et al.* (1994) proposed the X chromosome as the ancestor of the B chromosomes of the Jete and Salobreña Spanish populations. Moreover, FISH studies show that B chromosomes from Moroccan and Spanish *E. plorans* populations have a common origin because both are made up of the same DNA sequences (Cabrero *et al.*, 1999). All these data lead us to deduce the possible intercontinental migration of these B chromosomes and to discard the third hypothesis.

The common origin of B chromosomes from Spain and Morocco, with  $B_1$  as the original B in both countries, their similar response to C-banding, their similar DNA content (Cabrero *et al.*, 1999) and the existence of different B variants in both Spain and Morocco, indicate that the near-neutral model developed for Spanish populations (Camacho *et al.*, 1997a,b) might be useful to apply to the polymorphism in Morocco. An inland region in the Iberian Peninsula lacking B chromosomes has been interpreted as evidence of relict populations preceding B origin, as they are isolated by geographical barriers from the B-carrying ones (Cabrero *et al.*, 1997). This indicates that B chromosomes most likely originated after the species colonized the Iberian Peninsula, and this would run counter to hypothesis (i). The demonstration of several Spanish populations where  $B_1$  has been substituted by other B variants ( $B_2$  in Granada province,  $B_5$  in Fuengirola; see Henriques-Gil & Arana, 1990), and the recent polymorphism regeneration shown in Torrox (see

Zurita *et al.*, 1998), indicate that the polymorphism is presumably old in Spain, so that it has reached the near-neutral stage in many populations and the regeneration stage in a few localities. In Morocco, the polymorphism shows lower B frequencies than in Spain, only the original B (B<sub>1</sub>) constituting a polymorphism, with no observed regeneration. The fact that B<sub>1</sub> shows drive in some populations (Bakkali *et al.* unpubl. data) suggests that the B polymorphism is still in the selfish stage (the beginning) and thus it is still young in Morocco. All these facts suggest that the B polymorphism most likely arose in the Iberian Peninsula after the colonization of this species, and B<sub>1</sub> has recently colonized the north of Africa where the polymorphism is in the selfish starting stage of the near-neutral cycle.

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