The B chromosome polymorphism of the grasshopper *Eyprepocnemis plorans* in North Africa: III. Mutation rate of B chromosomes

M Bakkali¹ and JPM Camacho

Departamento de Genética, Facultad de Ciencias, Universidad de Granada, Granada 18071, Spain

B chromosome variation in nine Moroccan populations of the grasshopper *Eyprepocnemis plorans* was analysed for 3 consecutive years. In addition to B₁, which was the predominant B chromosome in all nine populations, we found 15 other B variants, albeit at very low frequency. Eight variants were found in adults caught in the wild, four appeared in adults reared in the laboratory and seven were found in embryo progeny of controlled crosses between a 0B male and a B-carrying female. Some variants were found in more than one kind of material. At least the seven B variants that appeared in embryo progeny of females carrying a different B type arose *de novo* through mutation of the maternal B chromosome. The mutation rate of B chromosomes was 0.73%, on average, which explains the high

variety of morphs and banding patterns found. The most frequent *de novo* mutations observed in these chromosomes were centromere misdivision with or without chromatid nondisjunction, which generates iso-B-chromosomes or telocentric Bs, respectively, as well as translocations with A and B chromosomes and deletions. But the whole variation observed, including that found in adult individuals, suggests that other mutations such as duplications, inversions and centric fusions do usually affect B chromosomes. Finally, B chromosome mutation rate was remarkably similar in both Moroccan and Spanish populations, which suggests that it might be dependent on B chromosome intrinsic factors.

Heredity (2004) 92, 428–433, advance online publication, 3 March 2004; doi:10.1038/sj.hdy.6800437

Keywords: B chromosome; *Eyprepocnemis plorans*; grasshopper; mutation rate; parasitic elements; Orthoptera

Introduction

Eukaryote genomes are structured in a number of standard chromosomes (A) and, in many species, a variable number of supernumerary, B, chromosomes that behave as genome parasites (Camacho *et al.*, 2000). B chromosomes have been reported in more than 1300 plant and 500 animal species (Jones and Rees, 1982; Jones and Puertas, 1993; Jones, 1995), and Beukeboom (1994) estimated that about 15% of described living beings harbour polymorphisms for B chromosomes. The nature of these chromosomes is heterogeneous, although they are predominantly heterochromatic and show a high variety of morphologies, chromosomal banding patterns and molecular compositions (Henriques-Gil *et al.*, 1984; Green *et al.*, 1987; Green, 1988; López-León *et al.*, 1993; Bakkali *et al.*, 1999; Cabrero *et al.*, 1999). In Orthoptera, which is the animal group where B chromosome polymorphisms have been more frequently reported (Jones and Rees, 1982), B chromosomes are commonly telocentric, but there are also some iso-B chromosomes originated through centromere misdivision and chromatid nondisjunction, or by centric fusion between two B chromosomes (see López-León *et al.*, 1993).

The karyotype of the grasshopper *Eyprepocnemis plorans* is composed of $2n = 22 + X0$ in males and $2n = 22 + XX$ in females. The autosomes may be classified into three size groups: Long (L₁–L₂), Medium (M₃–M₈) and Small (S₉–S₁₁), the X chromosome size being intermediate between those of L₂ and M₃. Previous studies have shown that almost all *E. plorans* populations analysed from the Mediterranean region and the Caucasus show the polymorphism for B chromosomes (Camacho *et al.*, 1980; Henriques-Gil *et al.*, 1984; López-Fernández *et al.*, 1992; López-León *et al.*, 1993; Cabrero *et al.*, 1997; Bugrov *et al.*, 1999). This polymorphism has revealed the existence of an arms race between A and B chromosomes leading the system through successive stages with B chromosomes passing from parasitic to near-neutral (Camacho *et al.*, 1997) and *vice versa*, thus facilitating B-polymorphism regeneration (Zurita *et al.*, 1998). A crucial requisite for this regeneration is a high mutability of B chromosomes, a property that has been observed in Spanish B chromosomes (López-León *et al.*, 1993).

All North African *E. plorans* populations hitherto analysed showed the presence of the B chromosome polymorphism. Henriques-Gil and Arana (1990) reported the presence, in Melilla, of a predominant B variant that they named B₁₆. Later, Bakkali *et al.* (1999) analysed nine Moroccan populations where the predominant B variant was similar, in morphology and C banding pattern, to the Spanish B₁ described by Henriques-Gil *et al.* (1984). The present work analyses the mutability of B chromosomes in nine Moroccan

Correspondence: JPM Camacho, Departamento de Genética, Facultad de Ciencias, Universidad de Granada, Granada 18071, Spain, E-mail: jpmcamacho@ugr.es

¹Current address: Department of Zoology, the University of British Columbia, 6270 University Boulevard, Vancouver, BC, V6T 1Z4 Canada. Received 3 February 2003; accepted 3 December 2003; published online 3 March 2004

populations of the grasshopper *E. plorans*, and estimate B chromosome mutation rate in controlled crosses between B-carrying females and OB males.

Materials and methods

Adult specimens of the grasshopper *E. plorans* were collected for 3 consecutive years (1995–1997) at nine natural populations in North-Western Morocco: Smir, Asilah, Larache, Ain l'abid, Tatouft, Frain, SO.DE.A., Mechra Bel Ksiri (to which we call Mechra) and Rabat (see map locations in Bakkali *et al.*, 1999). Most animals were fixed for cytological analysis in Morocco to avoid death risks during transport to Spain. Some specimens caught at Smir, SO.DE.A. and Mechra were taken alive to the laboratory to perform controlled crosses between B-carrying females and OB males. Live specimens were bred in cages similar to those described in Clemente *et al.* (1985), in a culture room with 10:14 light:dark photoperiod, and 28°C. Culture conditions were similar to those described in Herrera *et al.* (1996). To facilitate controlled crosses, we performed an *in vivo* analysis of B chromosome presence in males, which were vivisected for the extraction of some testis follicles by means of a little cut in the median dorsal part of the abdomen. Owing to mortality risks, *in vivo* study of females was not carried out. For this reason, and to increase the likelihood that females were B-carriers, we firstly obtained a stock of animals with increased B frequency (the progeny of B-carrying males) (see details in Bakkali, 2001; Bakkali *et al.*, 2002). Female virginity in this stock was preserved, for controlled crosses, by separating males and females in the preadult nymph stage. Egg pods were collected from females used in the controlled crosses.

For cytological analysis of adults, testes were fixed in 1:3 acetic acid–ethanol and stored at 4°C, and females were injected with 0.2 ml of 0.05% colchicine in insect saline solution 6 h prior to anaesthesia, dissection and extraction of ovaries, which were fixed and stored like testes. For the cytological analysis of embryos, eggs were dissected in insect saline solution, after 11 days of incubation at 28°C, and embryos were immersed in 1 ml of 0.05% colchicine in insect saline solution for 45 min. Hypotonic treatment was performed by adding 1 ml of distilled water for 10 min. Finally, embryos were fixed in 1:3 acetic acid–ethanol.

Cytological preparations from adult materials were carried out by squashing two testis follicles (or two ovarioles) in acetic orcein for B presence detection, or in 50% acetic acid (and then removing the coverslip after freezing in liquid nitrogen) to perform C banding. Embryo preparations were made by a method similar to that used by Meredith (1969) for the study of mammal meiosis (see details in Camacho *et al.*, 1991). C banding was performed by the technique described in Camacho *et al.* (1984).

To name the different B chromosome variants found in Morocco, and avoid confusion with the names used in Spain (B_1 , B_2 , etc), we have added an M to the subscript indicating order of finding (B_{M2} , B_{M3} , etc). Excepting B_1 , which was essentially similar to the Spanish one (see Cabrero *et al.*, 1999; Bakkali *et al.*, 2002), coincidence in the classificatory number between Moroccan and Spanish B chromosomes does not necessarily reflect

similarity between them. Table 1 shows the equivalence (wherever possible) between Moroccan and Spanish B variants.

Mutation rate was calculated from the *de novo* origin of B variants in controlled crosses. For this purpose, we scored the number of embryos carrying the maternal B chromosome as well as those carrying a different B variant. The detectable mutation rate per B chromosome (u_B) depends on the total number of embryos analysed. It is calculated as the reciprocal of the product between the total number of embryos analysed (N) and the mean number of Bs found in the sample (M_B): $u_B = 1/(N M_B)$. The mutation rate of a B chromosome is u_B times the number of embryos carrying mutations involving this B chromosome.

Results

In addition to the principal B chromosome, B_1 , the only one present at a relatively high frequency in all Moroccan populations analysed by us (see Bakkali *et al.*, 1999), we found 15 other minority B variants that were distinguishable on the basis of their morphology and C banding pattern (Figure 1 and Table 1). Eight variants were found in adults caught in the wild, four appeared in adults reared in the laboratory and seven were found in embryo progeny of controlled crosses. As Table 2 shows, some of these variants were found in more than one kind of material. All B variants showed very low frequency, and seven of them (shown in Table 3) were found in embryos whose mother carried a different B variant. The only conceivable mode of origin for these variants is through mutation of the maternal variant, which can be considered the ancestral one.

Several individuals were additionally found to carry chromosomal translocations involving B and A chromosomes. An adult male carried a reciprocal translocation between the B_{M8} variant and a medium-sized autosome (M_4) (see a detailed study in Bakkali *et al.*, 2003), two embryos carried B–A Robertsonian translocations (with the simultaneous observation of a mini-chromosome) and another embryo carried a B–A centric fusion (with no mini-chromosome) (Figure 2). One of the Robertsonian translocations occurred between B_1 and the M_5 autosome and the other involved B_{M3} and the smallest autosome (S_{11}). The centric fusion occurred between B_{M7} and the third autosome (M_3).

The average mutation rate for all types of maternal B chromosomes analysed in controlled crosses was 0.73% (Table 4), but there were conspicuous differences between variants, with B_{M7} , an isochromosome, being the most unstable B chromosome (9.64%) giving frequently rise to the B_{M12} variant through centromere misdivision (see Tables 1 and 3) and being involved in a B–A translocation (see Figure 2). In total, 20 mutations were found involving B chromosomes, 20% of which were translocations with A chromosomes, 20% were translocations between B chromosomes, and the remaining 60% were mutations involving a single B chromosome. Most single B mutations were centromere misdivisions with or without later chromatid non-disjunction to give rise, respectively, to iso-B-chromosomes (eg B_1 becoming B_{M4} , or B_{M3} becoming B_{M13})

Table 1 Description and possible origin of the 15 B chromosome variants found in the six *Eyprepocnemis plorans* Moroccan populations

Variant	Description	Ancestral B	Putative mutations from B ₁ or the ancestral B (when known, see Table 3)	Spanish equivalent
B _{M2}	Similar to B ₁ , with a smaller proximal C band. Formerly named B _{1d1}	B ₁	Deletion of satDNA	—
B _{M3}	Same size as B ₁ , but the interstitial dark C band is in a distal location	B ₁	Paracentric inversion	—
B _{M4}	Iso-B chromosome whose arms are similar to B ₁ . Formerly named B _{1iso1}	B ₁	Centromere misdivision and chromatid nondisjunction	B ₁₂ ^b = B _{1iso}
B _{M5}	Mini-iso-B chromosome whose arms are similar to B ₁ short arm. Formerly named B _{1iso2}	B ₁	Centromere misdivision and chromatid nondisjunction	—
B _{M6}	It shows an additional dark C band and a smaller distal light band. Formerly named B _{1d,d1}	B ₁	Duplication of satDNA and deletion of rDNA	B ₂₄ ^b
B _{M7}	Iso-B chromosome with a single dark C band in each arm	B ₁	Breakage between the two satDNA blocks and chromatid nondisjunction with gaining of neocentric activity	—
B _{M8}	Same size and C bands as B _{M7} but in different order	B _{M2}	Breakage between the two satDNA blocks, gaining of neocentric activity and tandem fusion of both chromatids	—
B _{M9}	Iso-B chromosome lacking satDNA	Unknown	Deletion of satDNA and centromere misdivision with chromatid nondisjunction	—
B _{M10}	Two dark C bands like those of the B _{M2} chromosome, and a larger short arm. The light distal region is shorter. Formerly named B _{1d,et1}	B _{M2}	Extra-radial transposition	—
B _{M11}	Two dark C bands like those of the B _{M2} chromosome, and a smaller light region. Formerly named B ₃	B _{M2}	Partial deletion of most rDNA	B ₃ ^b
B _{M12}	Similar to one arm of the B _{M7} chromosome	B _{M7}	Centromere misdivision	—
B _{M13}	Iso-B chromosome whose arms are similar to the B _{M3} chromosome	B _{M3}	Centromere misdivision and chromatid nondisjunction	—
B _{M14}	Metacentric chromosome with arms similar to the B ₁ y B _{M3} chromosomes	B ₁ and B _{M3}	Robertsonian translocation between the B ₁ and B _{M3} chromosomes	—
B _{M15}	Sub-metacentric chromosome whose arms are similar to the B _{M2} y B _{M10} chromosomes	B _{M2} and B _{M10}	Robertsonian translocation between the B _{M2} and B _{M10} chromosomes	—
B _{mini}	Mini-B chromosomes. In this group are included all the small B chromosomes, including B _{M5}	Acrocentric B chromosomes	Deletions, centromere misdivisions and Robertsonian translocations	B _{mini} ^c

The proximal and interstitial regions of C-banded B chromosomes contain the 180bp tandem repeat DNA (satDNA) and the distal light region contains ribosomal DNA (rDNA).

^aBakkali *et al* (1999).

^bHenriques-Gil *et al* (1984).

^cLópez-León *et al* (1993).

or telocentric Bs (eg B_{M7} becoming B_{M12}) (see Tables 3 and 4). The only exception was an embryo from the Smir population carrying B_{M11} presumably arisen from the maternal B, B_{M2}, through deletion of most rDNA (see Figure 1).

Discussion

On the basis of morphology and C banding pattern, more than 50 B variants have been described in natural populations of *E. plorans* (Henriques-Gil *et al*, 1984; López-León *et al*, 1993). The B₁ chromosome was considered the original B in Spanish populations (Henriques-Gil *et al*, 1984). Given its predominance in all nine Moroccan populations analysed, we also considered B₁ as the original B in Moroccan populations (Bakkali *et al*, 1999). In Melilla, however, the most

frequent B chromosome variant (B₁₆), reported by Henriques-Gil and Arana (1990), was different from B₁. Since this population is separated from the Moroccan populations analysed in Bakkali *et al* (1999) by the Rif mountains, and from Spanish populations by the Mediterranean sea, it is likely that the B chromosome polymorphism has followed an independent evolutionary pathway in Melilla, although this subject requires further study.

Although B₁ is well established in all nine populations analysed, our present results show that new B chromosome variants are arising through different mutations, at least in six out of these populations. Bearing in mind that only B chromosomes showing drive, or being beneficial for the host, are expected to become frequent in natural populations (Camacho *et al*, 1997), it is likely that most of the rare B variants found in

Morocco, at very low frequency, are either of very recent origin or else neutral or deleterious (it is reasonable to assume that it is unlikely that a beneficial B variant could appear by mutation of a parasitic B). Since B chromosomes lack a regular meiotic behaviour (they do not go in pairs that pair and segregate during meiosis, as A chromosomes do), a neutral B variant cannot reach fixation by drift, but only be eliminated. In this scenario, the observation of many B variants at low frequency is explained by high mutation rate (0.73% on average) compensating loss by drift, although we cannot rule out that any of these rare variants could prosper through drive.

The most frequently observed B mutation was centromere misdivision with chromatid nondisjunction giving rise to iso-B-chromosomes. Translocations with A or B chromosomes were also frequent, most of them implying centromere breakpoints, but only one deletion of rDNA was observed. The complete range of B variants (see Figure 1 and Table 1) suggests that other mutations, such as duplications, inversions and centric fusions, can also affect B chromosomes.

A very similar situation has been reported in Spanish populations, where the *de novo* formation of 14 B chromosome variants was found in the progeny of

controlled crosses and gravid females, the formation of iso-B-chromosomes being also the most frequently observed mutation, but tandem and centric fusions, pericentric inversions and deletions were also observed (López-León *et al*, 1993). Since these 14 B variants were found among a total of 3012 embryos whose average B frequency was 0.787, the detectable mutation rate per B chromosome was 0.042 in that sample, and the average mutation rate for B chromosomes (excluding B–A

Table 3 Frequency of B chromosome variants arisen in controlled crosses performed in the laboratory between a 0B male and a B-carrying female

Ancestral B in the mother	New B Variant	Population	Embryos carrying the new B variant
B ₁	B _{M4}	Smir	1
B ₁	B _{M4}	SO.DE.A.	2
B _{M2}	B _{M11}	Smir	1
B _{M7}	B _{M12}	Smir	7
B _{M3}	B _{M13}	SO.DE.A.	1
B ₁ and B _{M3}	B _{M14}	SO.DE.A.	1
B _{M2} and B _{M10}	B _{M15}	Mechra	1
B ₁	B _{mini}	SO.DE.A.	1
B _{M2}	B _{mini}	Mechra	1

These variants were found in some embryos whose mother carried a different B chromosome type. Each line refers to a mating of a virgin female to a single 0B male.

Figure 1 Morphology and C banding pattern of the 16 B chromosomes found in the nine Moroccan populations studied in this work. B₁ is the principal B chromosome found in all of these populations, the remaining are minority B chromosomes found in some adults from the natural populations or in embryos and adults obtained in our laboratory (see Table 2).

Figure 2 C banding of the chromosomes produced through A–B chromosome translocations. M₄–B_{M8} and B_{M8}–M₄ were found in and adult male caught at the population of Smir, the remaining had arisen in controlled crosses performed in the laboratory. The dotted line joins the centromeres of all chromosomes. The vertical lines indicate the A chromosome region in each mutant chromosome.

Table 2 Frequency of 10 B variants found in adult specimens from Moroccan populations of *Eyprepocnemis plorans*

Population	Year	N	Number of specimens carrying the variant:									
			B _{M2}	B _{M3}	B _{M4}	B _{M5}	B _{M6}	B _{M7}	B _{M8}	B _{M9}	B _{M10}	B _{M12}
Smir	1997	34	1 ^a	—	2 ^b	2 ^b	1 ^a	1 ^a	1 ^b	1 ^b	—	—
Ain l'abid	1997	47	—	—	—	1 ^b	—	—	—	—	—	—
Frain	1997	38	—	—	—	1 ^b	—	—	—	—	—	—
SO.DE.A.	1995	34	—	—	—	—	1 ^b	—	—	—	—	—
	1997	41	—	1 ^a	—	—	3 ^b	—	—	—	—	—
Mechra	1996	85	1 ^b	—	1 ^b	1 ^b	—	—	—	—	1 ^b	—
Rabat	1997	16	—	—	—	—	—	—	—	—	—	1 ^b

N = Number of wild adults examined from the wild.

^aVariants found in adult specimens reared in the laboratory.

^bB chromosome variants found in adults caught in the natural population.

Table 4 Mutation rate of B chromosomes in populations from Morocco, as deduced from the analysis of embryo progeny raised in controlled crosses between a 0B male and a B-carrying female

Maternal B	Population	N _c	Embryo progeny with				M _B	μ _B (%)	Type of mutation						Total	Freq (%)
			0B	1B	2B	N _e			Translocations							
									B–A		B–B		Single B			
									No.	Freq (%)	No.	Freq (%)	No.	Freq (%)		
B ₁	Smir	9	420	412	19	851	0.529	0.222					1	0.222	1	0.222
	SO.DE.A	6	226	243	2	471	0.524	0.405	1	0.405	1	0.405	2	0.810	4	1.619
	Mechra	23	616	811	99	1526	0.661	0.099								
	Total	38	1262	1466	120	2848	0.599	0.059	1	0.059	1	0.059	3	0.176	5	0.293
B _{M2}	Smir	7	214	436	20	670	0.710	0.210					1	0.210	1	0.210
	Mechra	1	40	30	0	70	0.429	3.333			1	3.333			1	3.333
	Total	8	254	466	20	740	0.684	0.198			1	0.198	1	0.198	2	0.395
B _{M3}	SO.DE.A	6	150	409	2	561	0.736	0.242	2	0.484	1	0.242	1	0.242	4	0.969
B _{M7}	Smir	3	107	83	0	190	0.437	1.205	1	1.205			7	8.434	8	9.639
B _{M10}	Mechra	1	33	37	0	70	0.529	2.703			1	2.703			1	2.703
	Total	56	1806	2461	142	4409	0.623	0.036	4	0.146	4	0.146	12	0.437	20	0.729

N_c = number of crosses, N_e = total number of embryos analysed, M_B = mean number of B chromosomes, μ_B = mutation rate per B chromosome. The mutations observed were B-A and B-B translocations, and several other mutations involving a single B chromosome.

translocations that were not reported) was 0.59%, a figure being remarkably similar to the observed in Morocco: $0.437 + 0.146 = 0.583\%$ (see Table 4). This similarity suggests that B chromosome mutability depends more on B intrinsic factors than on A-chromosome or environmental factors.

These possible factors might include centromere instability, breakage of repetitive DNA (rDNA or satDNA) and transposable elements. The prevalence of centromere misdivision among the observed chromosome mutations suggests that centromere is the most unstable region in the *E. plorans* B chromosomes. This instability might derive from its heterochromatic nature and/or the presence of repetitive DNA in its vicinity, that is, the 180 bp sat DNA (López-León *et al.*, 1994, 1995). This would be consistent with the finding that centromere is a repeated structure that can be split into smaller fragments that are still able to work properly (Zinkowski *et al.*, 1991). In maize, the size of B-chromosome centromeres, produced by misdivision, is strongly correlated to meiotic transmission and the presence of B-specific DNA repeats in the centromere (Kaszás and Birchler, 1996, 1998). However, it is also possible that centromere propensity to breakage is associated to the presence of mobile elements, which have been shown to be frequent in B chromosomes, for example, in *Nasonia vitripennis* (McAllister, 1995). Preliminary data, however, suggest that mobile elements such as *Gypsy* and *Mariner* are absent from repetitive DNA and centromeric regions of *E. plorans* B chromosomes (López-León *et al.*, unpublished), although it does not rule out the possible implication of other unknown elements associated to B centromere instability. In fact, *Gypsy* and *Mariner* are located between the satDNA blocks in Spanish B₁, B₂ and B₂₄ and, remarkably, the Moroccan B_{M7} arose by breakage at this level presumably followed by neocentric activity, which may be associated to the presence of

repetitive DNA (Alfenito and Birchler, 1993; Manzanero *et al.*, 2002).

The high mutability of *E. plorans* B chromosomes is tolerated because of their dispensable nature, but it promotes their evolution by increasing the likelihood of generating new parasitic variants and thus constitutes one of the main B-chromosome weapons in their evolutionary arms race with A chromosomes (Camacho *et al.*, 1997). Our present work suggests that this high mutability is most likely due to B-chromosome intrinsic factors, the nature of which will be the subject for future research.

Acknowledgements

M Bakkali thank the Universities of Abdelmalek Essaadi (Morocco) and Granada (Spain), the 'Agencia Española De Cooperación Internacional, Instituto de Cooperación con el Mundo Árabe, Mediterráneo y Países en Vías de Desarrollo' (Spain) and the 'Ministère de l'Enseignement Supérieur, de la Formation des Cadres et de la Recherche Scientifique' (Morocco) for conceding studentships. We thank Mr Soulaïmane Bakkali for his help in capturing specimens. This study was supported by grants from the Spanish Ministerio de Ciencia y Tecnología (BOS2000-1521) and Plan Andaluz de Investigación, Grupo no. CVI-165.

References

- Alfenito MR, Birchler JA (1993). Molecular characterization of a maize B chromosome centric sequence. *Genetics* **135**: 589-597.
- Bakkali M (2001). *Evolución de los cromosomas B del saltamontes Eyrepocnemis plorans en Marruecos*. PhD thesis. Universidad de Granada: Spain.
- Bakkali M, Cabrero J, López-León MD, Perfectti F, Camacho JPM (1999). The B chromosome polymorphism of the

- grasshopper *Eyprepocnemis plorans* in north Africa. I. B variants and frequency. *Heredity* **83**: 428–434.
- Bakkali M, Cabrero J, Camacho JPM (2003). B–A interchanges are an unlikely pathway for B chromosome integration into the standard genome. *Chromosome Res* **11**: 115–123.
- Bakkali M, Perfectti F, Camacho JPM (2002). The B-chromosome polymorphism of the grasshopper *Eyprepocnemis plorans* in North Africa: II. Parasitic and neutralized B1 chromosomes. *Heredity* **88**: 14–18.
- Beukeboom LW (1994). Bewildering Bs: an impression of the 1st B-Chromosome Conference. *Heredity* **73**: 328–336.
- Bugrov A, Warchalowska-Sliwa E, Vysotskaya L (1999). Karyotypic features of *Eyprepocnemidinae* grasshoppers from Russia and Central Asia with reference to the B chromosomes in *Eyprepocnemis plorans* (Charp). **47**: 97–104.
- Cabrero J, López-León MD, Bakkali M, Camacho JPM (1999). Common origin of B chromosome variants in the grasshopper *Eyprepocnemis plorans*. *Heredity* **83**: 435–439.
- Cabrero J, López-León MD, Gómez R, Castro AJ, Martín-Alganza A, Camacho JPM (1997). Geographical distribution of B chromosomes in the grasshopper *Eyprepocnemis plorans*, along a river basin, is mainly shaped by non-selective historical events. *Chromosome Res* **5**: 194–198.
- Camacho JPM, Carballo AR, Cabrero J (1980). The B chromosome system of the grasshopper *Eyprepocnemis plorans* sub. *plorans* (Charpentier). *Chromosoma* **80**: 163–166.
- Camacho JPM, Cabrero J, Viseras E, López-León MD, Navas-Castillo J, Alché JD (1991). G-banding in two species of grasshoppers and its relationship to C, N and fluorescence banding techniques. *Genome* **34**: 638–643.
- Camacho JPM, Viseras E, Navas J, Cabrero J (1984). C-heterochromatin content of supernumerary chromosome segments of grasshoppers: detection of an euchromatic extra segment. *Heredity* **53**: 167–175.
- Camacho JPM, Shaw MW, López-León MD, Pardo MC, Cabrero J (1997). Population dynamics of a selfish B chromosome neutralized by the standard genome in the grasshopper *Eyprepocnemis plorans*. *Am Nat* **149**: 1030–1050.
- Camacho JPM, Sharbel TF, Beukeboom LW (2000). B chromosome evolution. *Philos Trans Roy Soc London B* **355**: 163–178.
- Clemente ME, García MD, Presa JJ (1985). Estudio sobre la biología en cautividad de Caelifera (Insecta, Orthoptera). In: Gosálvez J, López-Fernández C, García de la Vega C (eds) *Orthoptera*. Vol 1. UAM: Madrid, pp. 256–261.
- Green DM (1988). Cytogenetics of the endemic New Zealand frog, *Leiopelma hochstetteri*: extraordinary supernumerary chromosome variation and a unique sex-chromosome system. *Chromosoma* **97**: 55–70.
- Green DM, Kezer J, Nussbaum RA (1987). Supernumerary chromosome variation and heterochromatin distribution in the endemic New Zealand frog, *Leiopelma hochstetteri*. *Chromosoma* **95**: 339–344.
- Henriques-Gil N, Arana P (1990). Origin and substitution of B chromosomes in the grasshopper *Eyprepocnemis plorans*. *Evolution* **44**: 747–753.
- Henriques-Gil N, Santos JL, Arana P (1984). Evolution of a complex polymorphism in the grasshopper *Eyprepocnemis plorans*. *Chromosoma* **89**: 290–293.
- Herrera JA, López-León MD, Cabrero J, Shaw MW, Camacho JPM (1996). Evidence for B chromosome drive suppression in the grasshopper *Eyprepocnemis plorans*. *Heredity* **76**: 633–639.
- Jones RN (1995). Tansley review no. 85: B chromosomes in plants. *New Phytol* **131**: 411–434.
- Jones RN, Puertas MJ (1993). The B-chromosomes of rye (*Secale cereale* L). In: Dhir KK, Sareen TS (eds) *Frontiers in Plant Science Research*. Bhagwati Enterprises: Delhi, pp 81–112.
- Jones RN, Rees H (1982). *B Chromosomes*. Academic Press: New York.
- Kaszás É, Birchler JA (1996). Misdivision analysis of centromere structure in maize. *EMBO J* **15**: 5246–5255.
- Kaszás É, Birchler JA (1998). Meiotic transmission rates correlate with physical features of rearranged centromeres in maize. *Genetics* **150**: 1683–1692.
- López-Fernández C, Mezzanotte R, Gosálvez J (1992). Autosomal, sex and B chromosomes in *Eyprepocnemis plorans* (Orthoptera) viewed with restriction endonuclease *in situ* digestion. *Heredity* **68**: 365–372.
- López-León MD, Cabrero J, Pardo MC, Viseras E, Camacho JPM, Santos JL (1993). Generating high variability of B chromosomes in the grasshopper *Eyprepocnemis plorans*. *Heredity* **71**: 352–362.
- López-León MD, Neves N, Schwarzacher T, Heslop-Harrison TS, Hewitt GM, Camacho JPM (1994). Possible origin of a B chromosome deduced from its DNA composition using double FISH technique. *Chromosome Res* **2**: 87–92.
- López-León MD, Vázquez P, Hewitt GM, Camacho JPM (1995). Cloning and sequence analysis of an extremely homogeneous tandemly repeated DNA in the grasshopper *Eyprepocnemis plorans*. *Heredity* **75**: 370–375.
- Manzanero S, Vega JM, Houben A, Puertas MJ (2002). Characterization of the constriction with neocentric activity of 5RL chromosome in wheat. *Chromosoma* **111**: 228–235.
- McAllister BF (1995). Isolation and characterisation of a retroelement from B chromosome (PSR) in the parasitic wasp *Nasonia vitripennis*. *Insect Mol Biol* **4**: 253–262.
- Meredith R (1969). A simple method for preparing meiotic chromosomes from mammalian tests. *Chromosoma* **26**: 254–258.
- Zinkowski RP, Meyne J, Brinkley BR (1991). The centromere-kinetochore complex: a repeat subunit model. *J Cell Biol* **113**: 1091–1110.
- Zurita S, Cabrero J, López-León MD, Camacho JPM (1998). Polymorphism regeneration for a neutralized selfish B chromosome. *Evolution* **52**: 274–277.