Generating high variability of B chromosomes in *Eyprepocnemis plorans* (grasshopper)

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Twenty-eight progeny analyses (PAs) performed on specimens of *E. plorans* collected from four natural Iberian populations have been informative about the transmission of rare B chromosome types or the *de novo* origin of some of them. At least 11 rare B-types have been found in addition to the predominant ones: B₁ in Daimuz, B₂ in Jete and Salobreña, and B₅ in Fuengirola. The presence in two controlled crosses of one embryo carrying a B-type which was absent in the parents suggests that these B variants (B_{2iso} and B_{1f1}) have originated *de novo*. Eleven other PAs suggest that new B derivatives are recurrently arising in these populations. The most frequent B chromosome mutation was centromere misdivision that originated four different B-types (B_{2m1}, B_{1iso}, B_{2iso} and B_{mini}). Other rearrangements were pericentric inversions (B_{2i1}, B_{2i2} and B_{2i3}), inverse tandem fusion (B_{2it1}), centric fusion (B_{1f1}) and deletions (B_{2d1} and B_{2d2}). The four B derivatives produced by centromeric misdivision are significantly eliminated during sexual transmission, most probably owing to deficiencies in the control of chromosome movement by their hemicentromeres. Those derived from translocations showed Mendelian transmission but deletion B variants showed a tendency to elimination. Our results suggest that B chromosome substitution of B₁ by B₂ in the Salobreña and Jete populations could be achieved by differences in relative transmission efficiency, as in one controlled cross, where the female carried 1 B₁ plus 1 B₂, B₂ was significantly overtransmitted and B₁ eliminated.

Keywords: B chromosomes, coevolution, drive, genomic parasitism.

Introduction

B chromosomes constitute one of the most intriguing polymorphisms present in a large number of natural populations of plant and animal species (Jones & Rees, 1982). Some properties of Bs have been investigated, mainly from the cytological point of view, although questions about their origin and their maintenance in natural populations remain largely unsolved.

One of the most striking B chromosome polymorphisms described to date is that shown by the grasshopper *Eyprepocnemis plorans*. Bs are present in almost all natural populations from the entire geographical distribution in the Iberian Peninsula, i.e. the Mediterranean and Atlantic coastal regions from Huelva to Tarragona. About 30 different B variants have been distinguished according to different morphology and C-banding patterns (Henriques-Gil &

Arana, 1990) although three types are prevalent: B₁, B₂ and B₅. B₁ is the most frequent in all the populations of the eastern (from Tarragona to Almería) and western zones (Cádiz and Huelva) of distribution, and for this reason it has been considered the original B in the Iberian Peninsula (Henriques-Gil *et al.*, 1984). B₂ is the main type in the province of Granada and eastern Málaga, and B₅ is restricted to western Málaga.

The evolution of Bs implies their origin from the chromosomes of the standard complement and subsequent differentiation to their present form. Furthermore, in populations of a given species different but related B variants can also be generated and the establishment and maintenance of these variants will depend on their transmission and their effects on the fitness of B-carriers. Most Bs investigated to date show accumulation mechanisms that may be enough to explain their maintenance even though the Bs are detrimental to carrier fitness (Nur, 1977). The three main types of Bs of *E. plorans* constitute an exception to this

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general rule as they are transmitted in a Mendelian ratio in populations where they are the most frequent B variant: B_1 at Daimuz (Valencia), B_2 at Jete and Salobreña (Granada) and B_5 at Fuengirola (Málaga). The possible causes of maintenance of this widespread polymorphism have been discussed by López-León *et al.* (1992). The present paper deals with the transmission rates of rare B variants that have appeared in the populations mentioned above and demonstrates the *de novo* origin of some of them and their possibilities of maintenance in subsequent generations.

Materials and methods

Specimens of the grasshopper *E. plorans* were collected at Daimuz (Valencia), Jete and Salobreña (Granada) and Fuengirola (Málaga). A large number of progeny analyses (PAs) were performed on controlled crosses (single or double) and gravid females, from which 28 showed the transmission mode of rare B chromosome types or else the *de novo* origin of new B variants. All methods employed in the present investigation are similar to those previously described (López-León *et al.*, 1991, 1992).

Seven progeny analyses were obtained by means of single (2) or double (5) crosses in which a female was mated to one or two males, respectively. Two double crosses were made by substitution so that they are separated into two independent PAs, one for each male, according to López-León *et al.* (1991). In the

remaining one, both males were placed simultaneously with the female and the frequency of mating exhibited by each male recorded, for which purpose one of the males was marked on the pronotum with nail varnish. The other 20 PAs came from females collected in the field when they were gravid. The effective paternity displacement existing in *E. plorans* (López-León *et al.*, 1993) means that gravid females may be considered as controlled crosses because the constitution of the parental male can be inferred from the offspring with a high degree of confidence on the basis of Mendelian transmission exhibited by B_1 , B_2 and B_5 chromosomes. PAs are coded as C (single crosses), DC (double crosses) or ♀ (gravid females), followed by the code number, the initial of the population (D, J, S or F) and the year of sampling.

Results

Figure 1 shows the C-banding pattern of all types of Bs found in the progeny analyses (PAs) performed in this work. They are in addition to the standard complement of $22 + X\sigma/22 + XX\text{♀}$ chromosomes.

The three main Bs show the following characteristics:

- (1) B_1 shows two similar sized C-bands on the long arm, the light distal region being conspicuously larger than the two C-bands as a whole.
- (2) B_2 in Daimuz also shows two C-bands of similar size on the long arm but in the Granada populations

Fig. 1 Three main types of B chromosomes in *E. plorans*: B_1 from Daimuz (Valencia), B_2 from Daimuz (the first), B_2 from Salobreña and Jete (Granada) (the second) and B_5 from Fuengirola (Málaga) together with 13 other B variants found in these populations. B_{mini} of at least two different sizes were found, which indicates that this is a heterogeneous category.

the interstitial band is larger than the proximal one. The main difference between B_2 and B_1 is the smaller size of its light distal region.

(3) B_5 is an acrocentric chromosome similar in size to B_1 but with a much larger C-band near the centromere and smaller light distal region.

In addition to these three main types, 12 other derivatives have appeared in the four populations sampled. The number of B variants is very high (more than 40, with those described in the present paper) so that it is necessary to codify their names properly in order to avoid confusion. We propose that each B variant should be named by the number of the main B from which it is presumed to have been derived, followed by the initial of the chromosomal mutation from which it arose and an order number. For example, B_{2i2} means that this B type is derived from the B_2 through an inversion and that it is the second B variant risen from this type of rearrangement.

Besides B_1 and B_2 , in Daimuz two other B variants appeared:

(1) B_{1iso} , which is an iso B_1 derived from centromere misdivision and subsequent chromatid non-disjunction. This B type was originally described as B_{12} by Henriques-Gil *et al.* (1984).

(2) B_{mini} , which is small, about the size of the short arm plus a hemicentromere, and has also arisen through centromere misdivision. This B-type must be heterogeneous as mini Bs can be derived from many other B variants, specifically from any Bs possessing a short arm.

Nine other B-types have appeared in Granada populations besides B_1 , B_2 and B_{mini} :

(1) B_{2m1} is a B_2 that has lost the short arm as a consequence of centromere misdivision. It could also have arisen from deletion of the short arm but the propensity of B_2 to suffer centromere misdivision generating B_{2iso} and B_{mini} chromosomes with low transmission rates, as we will see later, indicates that B_{2m1} is most probably derived from misdivision as it also shows a marked tendency to elimination.

(2) B_{2i1} shows part of the light distal zone of the long arm located on the short arm as the result of a pericentric inversion.

(3) B_{2i2} shows the entire light distal region of the long arm located on the short arm.

(4) B_{2i3} in which the proximal C-band is very small and the remaining long arm has changed its order, C band material being distally located and the light zone being interstitially located.

(5) B_{2d1} is a B_2 without the light distal region which has been deleted.

(6) B_{2d2} has lost the interstitial C-band and part of the light region.

(7) B_{1f1} is the result of a centric fusion between two B_1 chromosomes.

(8) B_{2iso} is an isochromosome derived from the B_2 by means of centromere misdivision and subsequent chromatid non-disjunction.

(9) B_{2it1} is the result of an inverse tandem fusion between two B_2 chromosomes with the loss of the centromere and the short arm in one of them.

Finally, one B variant appeared in Fuengirola besides B_5 and B_{mini} : B_{5t1} which shows the C-banded region located at the end of the long arm as a result of an intrachromosomal translocation.

Inheritance of rare B chromosome variants

The results of all the progeny analyses are shown in Table 1. Details concerning them are the following.

B_1 , B_{2m1} and B_{2iso} . Previous reports (Henriques-Gil *et al.*, 1984; Henriques-Gil & Arana, 1990) indicated that B_1 was apparently absent from the provinces of Granada and eastern Málaga. The two populations analysed by us in this area (Jete and Salobreña) have shown the presence of this type of B although in a low number of individuals. Thus, seven of our PAs have permitted the analysis of the mode of inheritance of this chromosome in four of which it was transmitted simultaneously with another B variant (B_{2m1} or B_2) through the same parent.

In ♀43J90, the female lacked Bs and the male presumably possessed 1 B_1 which was slightly, but not significantly, undertransmitted (transmission rate, $K=0.364$). The ♀31J90 female carried 1 B_2 and the inferred male 1 B_1 +1 B_2 , both Bs being transmitted according to a Mendelian ratio. The ♀24S90 was the third progeny analysis in which the male parent presumably carried a B_1 accompanied, in this case, by the B_{2m1} . Both Bs were significantly eliminated.

The four remaining progeny analyses showed B_1 transmission by the female side. The female ♀6S87 carried 1 B_2 that was binomially transmitted ($K=0.559$). Its progeny also showed the presence of one B_{2iso} in 35.6 per cent of the embryos so that it may be inferred that the male parent probably carried 1 B_{2iso} which was significantly undertransmitted. The three remaining progeny analyses correspond to two double crosses in which the female parent carried 1 B_1 plus 1 B_2 . In DC10J89a, the male parent carried 1 B_2 , so that the mean numbers of B_2 s (1.024) and B_1 s (0.595) in the offspring were consistent with Mendelian expectations (1 and 0.5, respectively). In DC10J89b the same female as in the preceding PA, now crossed to a 0B male, again transmitted her B_1 and B_2 in rates consistent with binomial expectations (0.523 and 0.538,

Table 1 Transmission and *de novo* origin of 12 B chromosome variants derived from the three main types (B_1 , B_2 and B_3) in three Spanish natural populations of the grasshopper *Eyprepocnemis plorans*

Progeny analysis	No. of Bs in parents		Embryo/eggs	Embryo offspring analysed	Total	$\bar{x}_B$	Z
	♀	♂					
C5J88	$1B_2$	$1B_2 + 1B_{2d2}$	42/54	$5(0B):9(1B_2):2(1B_{2d2}):11(2B_2):6(1B_2 + 1B_{2d2}):8(2B_2 + 1B_{2d2})$	41	$B_2:1.293$ $B_{2d2}:0.390$	1.88 -1.41
C26S89	$2B_2$	$1B_2 + 1B_{2d2}$	62/67	$1(0B):4(1B_2):14(2B_2):8(1B_2 + 1B_{2d2}):8(2B_2 + 1B_{2d2}):3(3B_2 + 1B_{2d2}):1(3B_2)$	39	$B_2:1.744$ $B_{2d2}:0.487$	1.01 -0.16
DC4J88a	$1B_2 + 1B_{2d1}$	OB	94/96	$9(0B):12(1B_2):17(1B_{2d1}):14(1B_2 + 1B_{2d1})$	52	$B_2:0.500$ $B_{2d1}:0.596$	0 1.39
DC4J88b	$1B_2 + 1B_{2d1}$	$1B_2$	55/57	$7(0B):10(1B_2):8(1B_{2d1}):17(1B_2 + 1B_{2d1}):3(2B_2):5(2B_2 + 1B_{2d1})$	50	$B_2:0.860$ $B_{2d1}:0.600$	-0.99 1.41
DC10J89a	$1B_2 + 1B_1$	$1B_2$	48/50	$3(0B):11(1B_2):5(1B_1):5(2B_2):1(2B_1):11(1B_1 + 1B_2):5(2B_2 + 1B_1):1(2B_1 + 1B_2)$	42	$B_2:1.024$ $B_1:0.595$	0.16 1.23
DC10J89b	$1B_2 + 1B_1$	OB	88/99	$8(0B):23(1B_2):21(1B_1):12(1B_1 + 1B_2):1(1B_{2iso} + 1B_1)$	65	$B_2:0.538$ $B_1:0.523$	0.61 0.37
DC3J90	$1B_1 + 1B_2$	OB $1B_2$	81/86	$19(0B):14(1B_1):27(1B_2):12(1B_1 + 1B_2):9(2B_2)$	81	$B_2:0.704$ $B_1:0.321$	— —
Parthenogenic			36/54	$5(0B):8(1B_1):14(1B_2):7(1B_1 + 1B_2):1(1B_{in} + 2B_2)†$	35	$B_2:0.629$ $B_1:0.457$	1.53 -0.51
	Total		117/140	$24(0B):22(1B_1):41(1B_2):19(1B_1 + 1B_2):9(2B_2):1(1B_{in} + 2B_2)$	116	$B_2:0.629†$ $B_1:0.362$	2.77* -2.97*
♀6S87	$1B_2$	$1B_{2iso}$	63/66	$19(0B):18(1B_2):1(2B_2):8(1B_{2iso}):13(1B_2 + 1B_{2iso})$	59	$B_2:0.559$ $B_{2iso}:0.356$	0.91 -2.21*
♀3S90	$1B_2$	$2B_2$	40/70	$4(0B):12(1B_2):12(2B_2):8(3B_2):1(1B_2 + 1B_{2iso})$	37	$B_2:1.649$	0.60
♀24S90	$1B_2$	$1B_1 + 1B_{2m1}$	73/76	$18(0B):24(1B_2):5(1B_1):6(1B_{2m1}):9(1B_2 + 1B_{2m1}):6(1B_1 + 1B_2):2(2B_2)$	70	$B_2:0.614$ $B_1:0.157$ $B_{2m1}:0.214$ $B_1 + B_{2m1}:0.371$	1.91 -5.74* -4.79* -2.16*
♀38S90	$3B_2$	$2B_2$	25/33	$2(1B_2):10(2B_2):11(3B_2):1(1B_2 + 1B_{2m1})$	24	$B_2:2.333$	-0.32
♀43S90	$2B_2$	$1B_2$ or § $2B_2$	38/38	$10(1B_2):20(2B_2):3(3B_2):1(2B_2 + 1B_{2iso})$	34	$B_2:1.794$	1.14 -0.60
♀62S90	$2B_2$	$2B_2 + 1B_{2d1}$	30/30	$4(1B_2):13(2B_2):5(3B_2):2(4B_2):1(1B_{2d1}):2(2B_2 + 1B_{2d1}):2(3B_2 + 1B_{2d1}):1(1B_2 + 1B_{2m1})$	30	$B_2:2.133$ $B_{2d1}:0.167$	0.36 -3.65*
♀27S91	$1B_2$	$1B_2 + 1B_{2d3}$	64/67	$8(0B):14(1B_2):11(1B_{2d3}):16(1B_2 + 1B_{2d3}):9(2B_2):3(2B_2 + 1B_{2d3})$	61	$B_2:0.885$ $B_{2d3}:0.492$	-0.89 -0.13

Table 1 Continued

Progeny analysis	No. of Bs in parents		Embryo/eggs	Embryo offspring analysed	Total	$\bar{x}_B$	Z
	♀	♂					
Q7J90	1B ₂	1B ₂	40/40	5(0B):23(1B ₂):9(2B ₂):1(3B ₂):1(1B ₂ + 1B _{2t})	39	B ₂ : 1.154	0.96
Q3J90	1B ₂	1B ₁ + 1B ₂	37/37	4(0B):11(1B ₂):3(1B ₁):4(2B ₂):1(3(1B ₁ + 1B ₂):1(2B ₂ + 1B ₁)	36	B ₂ : 0.944 B ₁ : 0.472	-0.34 -0.34
Q42J90	1B _{mini}	1B ₂	20/20	7(0B):9(1B ₂):1(1B _{mini})	17	B ₂ : 0.529 B _{mini} : 0.059	0.24 -3.64*
Q43J90	0B	1B ₁	35/35	21(0B):12(1B ₁)	33	B ₁ : 0.364	-1.56
Q47J90	2B ₂	1B ₂	39/39	3(0B):16(1B ₂):15(2B ₂):4(3B ₂):1(1B ₂ + 1B _{2t})	39	B ₂ : 1.513	0.05
Q55J90	3B ₂	0B	36/36	5(0B):11(1B ₂):17(2B ₂):2(3B ₂):1(1B ₂ + 1B _{mini})	36	B ₂ : 1.444	-0.23
Q86J90	0B	2B ₂ + 1B _{2t}	40/40	1(0B):10(1B ₂):2(2B ₂):6(1B _{2t}):9(1B ₂ + 1B _{2t}):4(2B ₂ + 1B _{2t}):1(1B _{2t} + 1B _{mini})	33	B ₂ : 0.939 B _{2t} : 0.576	-0.36 0.87
Q109J90	1B _{mini}	0B	51/51	41(0B):6(1B _{mini})	47	B _{mini} : 0.128	-5.10*
Q115J90	2B ₂	2B ₂	45/45	3(0B):10(1B ₂):24(2B ₂):7(3B ₂):1(2B ₂ + 1B _{2t})	45	B ₂ : 1.800	-0.67
Q116J90	1B ₂ + 1B _{2t}	0B	45/45	3(0B):15(1B ₂):19(1B _{2t}):5(1B ₂ + 1B _{2t})	42	B ₂ : 0.476 B _{2t} : 0.571	-0.31 0.92
Q23F88	1B ₅	1B _{5t}	35/36	7(0B):10(1B ₅):10(1B _{5t}):8(1B ₅ + 1B _{5t})	35	B ₅ : 0.514 B _{5t} : 0.514	0.17 0.17
Q12F89	0B	1B ₅	48/48	21(0B):26(1B ₅):1(1B _{mini})	48	B ₅ : 0.542	0.56
Q18D89	0B	1B ₁ + 1B ₂ + 1B _{1t} + 1B _{mini} §§	88/90	58(0B):6(1B ₁):5(1B ₂):7(1B _{1t}):9(1B _{mini}):1(1B ₁ + 1B ₂):1(1B _{1t} + 1B _{mini}):1(1B ₂ + 1B _{1t})	88	B ₁ : 0.080 B ₂ : 0.080 B _{1t} : 0.102 B _{mini} : 0.114	— — — —
Q25D89	1B ₁	1B ₁	34/34	10(0B):15(1B ₁):8(2B ₁):1(1B _{1t})	34	B ₁ : 0.912	-0.51

Z was calculated by the approximation of binomial to normal distribution. For this purpose, the transmission rate per B chromosome (the quotient between $\bar{x}_B$ for a B variant and the total number of Bs of such a type present in the parents) was the parameter tested, and the expected value was 0.5, as is predicted by Mendelian segregation.

B variants presumably arisen *de novo* are noted in bold-type letters.

$\bar{x}_B$: mean number of B chromosomes.

†In the parthenogenetic offspring of DC3J90, frequencies of ova produced by the female (as deduced from the haploid cells in the embryos) are expressed instead of embryo frequencies, with the only exception of the last embryo which was diploid and mosaic for the presence of 2B₁ + 2B₂ or else 1B_{1t} + 2B₂ (see Figs 2 and 3). For calculations, however, this embryo was considered as derived from an ovum with 1B₁ + 1B₂.

‡This figure results from the assumption that females transmitted B₂ to all their ova in the ratio observed for parthenogenetic embryos.

§In this PA it is uncertain whether the male parent possessed 1B₂ (Z = 1.14, P > 0.05) or 2B₂ (Z = -0.60, P > 0.05).

§§Male parent was presumably a rare mosaic for the four types of B chromosomes, so that it is not clear what is the appropriate hypothesis to calculate Z.

respectively). The progeny reflects exactly the composition of the ova produced by the female: 8 (0B): 21 (1B₁): 23 (1B₂): 13 (1B₁ + 1B₂) as the male lacked Bs. The last class included one embryo with 1B₁ + 1B_{2iso}, which will be considered later. These figures allow us to infer the segregation of these B-types during female meiosis. If they segregate at random, similar frequencies of the four types of embryos would be expected (16.25). A Chi-square test showed that B₁ and B₂ did not segregate randomly during female meiosis ($\chi^2 = 9.03$, d.f. = 3, $P = 0.01-0.05$), owing to an excess of migrations to opposite poles (44 embryos with 1B₁ or 1B₂) to the detriment of migrations to the same pole (21 embryos with 1B₁ + 1B₂ or without Bs). This indicates that B₁ and B₂ segregate in 67.69 per cent of the first meiotic divisions, as would be expected if B₁ and B₂ formed a bivalent in 35.38 per cent of cells, and this determined their migration to opposite poles.

The last progeny analysis in which B₁ transmission could be analysed is DC3J90. As Table 1 shows, B₁ was carried exclusively by the female and the mean number of B₁ chromosomes among the progeny (0.362) showed significant elimination through the female ($Z = -2.97$, $P < 0.05$). B₂, however, was carried by the female and also by one of the two males placed with her. Both males copulated with the female and so paternal B₂ inheritance cannot be analysed with confidence. Maternal B₂ transmission, however, could be ascertained because the two latter egg-pods laid by the female contained 35 haplo-diploid embryos produced by accidental thelytoky. The number of B₂ chromosomes in the haploid cells of these embryos should correspond to the number in ova (0.629, see Table 1). If this figure were assumed to be valid for the 116 ova produced, then this transmission ratio showed significant B₂ accumulation ($Z = 2.77$, $P < 0.05$). Hence, this female showed significant tendencies to B₂ accumulation and B₁ elimination.

B_{mini}. Two progeny analyses (♀42J90 and ♀109J90) comprising a gravid female and her offspring showed the transmission of the B_{mini} chromosome. Both females, but a significant minority of her offspring, carried the B_{mini} chromosome (Table 1). As a whole, only 7 of 64 embryos analysed from both females carried the B_{mini} so that its mean transmission ratio (0.109) was significantly lower than the expected one ($Z = -6.26$, $P < 0.05$).

B_{2d1}. Three progeny analyses showed the transmission mode of this chromosome. Two of them corresponded to the DC4J88 cross in which the female carried 1B₂ plus 1B_{2d1}, the first male lacked B chromosomes and the second male carried 1B₂. Transmission rates of B₂

and B_{2d1} were consistent with Mendelian expectations (Table 1). The other progeny analysis was ♀62S90 in which the female carried 2B₂ and, on the basis of offspring karyotypes, the male was inferred to possess 2B₂ plus 1B_{2d1}. Thus, the transmission rate (0.167) of B_{2d1} was significantly lower than expected.

B_{2d2}. The only progeny analysis for this B chromosome type was performed by means of a single cross, C5J88, in which the female carried 1B₂ and the male 1B₂ plus 1B_{2d2}. This cross showed a tendency to an accumulation of B₂ and elimination of B_{2d2} although the results did not reach statistical significance (Table 1).

B_{2i1}. Two PAs, ♀86J90 and ♀116J90, showed Mendelian transmission of this B chromosome variant through both sexes.

B_{2i2}. One PA, C26S89, showed that the transmission mode of this B type by the male side was consistent with Mendelian expectations.

B_{2i3}. One PA, ♀27S91, showed Mendelian transmission rate of this B variant by the male side.

B_{5i1}. The female ♀23F88 carried 1B₅ that was binomially transmitted. In the offspring one B_{5i1} also appeared, so it can be inferred that the male parent carried it, and that this B variant was transmitted according to Mendelian rules.

De novo origin of B chromosome variants

Thirteen PAs have demonstrated the appearance of B chromosome types that were not present in the parents, or else their presence was unlikely. Two of them were double crosses in which the new type was absent from the female and the two males crossed with her. Thus, one embryo out of 107 yielded by the DC10J89 female carried 1B_{2iso} chromosome (plus 1B₁) which most likely arose by centromere misdivision and chromatid non-disjunction of the maternal B₂. A female embryo mosaic showing cells with either two B₁ chromosomes (plus 2B₂) (Fig. 2) or with 1B_{1fl} (plus 2B₂) (Fig. 3) was found among the offspring produced by parthenogenesis in DC3J90. B_{1fl} had a larger centromere region than the B_{2iso} (compare both in Fig. 1), suggesting an origin by centric fusion of two B₁ chromosomes. This embryo presumably came from an ovum with 1B₁ plus 1B₂ which was subsequently diploidized, the centric fusion arising in one of the first diploid cells produced.

We can be confident in these two PAs of the *de novo* origin of both B types whereas in the remaining 11 this

Figs 2–4 Mitotic metaphase cells of embryos of *E. plorans*. **Fig. 2** Cell from a mosaic embryo carrying 2B₁ + 2B₂ chromosomes. **Fig. 3** Cell from the same embryo as in Fig. 2 showing 1B_{1f1} + 2B₂ chromosomes. **Fig. 4** Cell from an embryo possessing 1B_{2iso} plus 1B_{mini} (arrowhead).

Table 2 Frequency of different types of B chromosomes found in embryos yielded by gravid females collected at Jete (55 females) and Salobreña (29) populations. Mutation rates have been calculated assuming that a single embryo in a PA carrying a B type that was not present in the mother, has arisen *de novo*

B type	Population	Mean number of Bs in				Mutation rate
		Embryos	N	Adults	N	
B ₁	Jete	0.0139	2091	0.0182	110	—
	Salobreña	0.0119	921	0.0172	58	—
B _{2iso}	Jete	0.0014	2091	0	110	0.0014
	Salobreña	0.0022	921	0.0172	58	0.0021
B _{mini}	Jete	0.0042	2091	0.0182	110	0.0009
	Salobreña	0	921	0	58	—
B _{2i1}	Jete	0.0202	2091	0.0182	110	—
	Salobreña	0.0010	921	0	58	0.0010
B _{2i2}	Jete	0.0005	2091	0	110	0.0005
	Salobreña	0	921	0	58	—
B _{2d1}	Jete	0	2091	0	110	—
	Salobreña	0.0052	921	0.0172	58	—
B _{2it1}	Jete	0	2091	0	110	—
	Salobreña	0.0010	921	0	58	0.0010
B _{2m1}	Jete	0	2091	0	110	—
	Salobreña	0.0098	921	0.0172	58	—

possibility has to be inferred because we did not analyse the male parent. ♀3S90, ♀43S90, ♀47J90 and ♀115J90 showed the presence of a single embryo with one B_{2iso}. On the other hand, one B_{1iso} was found in a descendant from ♀25D89 (Table 1). Significantly, in ♀86J90 one embryo carried two very infrequent types of Bs (1B_{2iso} plus 1B_{mini}) (Fig. 4). The probability of carrying both Bs from independent origins is $0.0014 \times 0.0042 = 5.88 \times 10^{-6}$ (see Table 2), so that the expected number of embryos (among the 2091 analysed in Jete) carrying both types of Bs is 0.0125. It is likely that in this embryo B_{2iso} and B_{mini} have a

common origin by means of the same process of centromere misdivision and subsequent non-disjunction of long arm chromatids to constitute the B_{2iso}. A single embryo carrying 1B_{mini} was observed in the offspring from ♀55J90 and ♀12F89. B_{2i1} and B_{2i2} each appeared in a single embryo from ♀62S90 and ♀7J90, respectively. The case of ♀38S90 is remarkable where one embryo carried a B variant (B_{2it1}) that has never been found in adults, so it is likely to have arisen *de novo* by telomere fusion of two B₂ chromosomes during male or female gametogenesis.

The progeny from ♀18D89 are of special interest as four types of Bs (B_1 , B_2 , B_{mini} and B_{iso}) have been found (Table 1). The female parent was a non-B carrier so they must, undoubtedly, have been transmitted by the male side. The presence of $1B_1$ plus $1B_2$ in the same embryo indicates that both Bs must be present in the same male parent. The same deduction can be made from the observation of one embryo possessing $1B_{\text{iso}}$ plus $1B_{\text{mini}}$ and from another embryo with $1B_2$ plus $1B_{\text{iso}}$. Consequently, it is likely that the four B types were carried by a single mosaic male parent. In this case, it was not clear what transmission hypothesis must be tested because we did not know the proportion of germ cells in this male carrying each type of B chromosome.

Discussion

Some progenies shown in Table 1 constitute a clear demonstration of *de novo* origin of B chromosome variants as they were carried by a single embryo in PAs whereas the parents did not carry them. These observations suggest both that new B-types are arising (i.e. $B_{2\text{it}1}$ and $B_{2\text{it}2}$ that were present only in embryos) and that some types of Bs are recurrently arising in each generation (i.e. B_{iso} , $B_{2\text{iso}}$, B_{mini} and $B_{2\text{it}1}$ that were present in both embryos and adults). This indicates that the B chromosome polymorphism in *E. plorans* is being fed by the recurrent formation of new B variants with rates ranging from 0.0005 ($B_{2\text{it}2}$) to 0.0021 ($B_{2\text{iso}}$) (Table 2). This is also supported by the appearance in a gravid female from the Salobreña population (♀27S91) of a B variant ($B_{2\text{it}3}$) that was not observed in extensive samplings of this same population in precedent generations. In plants, the origin of new types of Bs from controlled crosses and experimental populations has also been reported (Bougourd & Parker, 1979; Holmes & Bougourd, 1991; M. Jiménez, personal communication).

Henriques-Gil *et al.* (1984) suggested that deletions, duplications and misdivisions are the most likely mechanisms responsible for B chromosome polymorphism in *E. plorans*. In this work, the most frequent chromosomal mutation yielding new types of Bs was centromeric misdivision that gave rise to $B_{1\text{iso}}$, $B_{2\text{iso}}$, B_{mini} and $B_{2\text{m}1}$ (Fig. 5). Also relevant are putative inversions ($B_{2\text{it}1}$, $B_{2\text{it}2}$ and $B_{2\text{it}3}$), inverse tandem fusion ($B_{2\text{it}1}$), centric fusion ($B_{1\text{f}1}$) and deletion ($B_{2\text{d}1}$ and $B_{2\text{d}2}$). This indicates that the Bs of *E. plorans* seem to be prone to undergoing any type of chromosomal rearrangement. Translocations between Bs and members of the standard chromosome complement have been reported in natural populations (Henriques-Gil *et al.*, 1983; Cabrero *et al.*, 1987). It is highly signi-

Fig. 5 Chromosomal rearrangements involved in the generation of nine B chromosome variants derived from B_2 in natural populations of the province of Granada. In all cases the origin of the arrows point to the presumed breakage points on the B_2 chromosome. a: Centromere misdivision; b: chromatid non-disjunction; c and d: pericentric inversions; e: paracentric inversions; f: inverse tandem fusion; g and h: deletions.

ficant that all chromosomal mutations affecting Bs depend, as a whole, on breakage at seven main points: the short arm, the centromere, a region in the proximal C-band, the secondary constriction, the joining region between the interstitial C-band and the light distal region, and the mid and distal zones of the latter (Fig. 5).

Several chromosome rearrangements have also been proposed to explain Bs' diversity in other species. For instance: (i) B derivatives could originate as deletions and centric misdivisions in the plants *Centaurea scabiosa* (Fröst, 1957), *Secale cereale* (Lima-de-Faria, 1963), *Aster ageratoides* (Matsuda, 1970), *Hypochoeris maculata* (Parker, 1976; Parker *et al.*, 1982), *Allium schoenoprasum* (Bougourd & Parker, 1979) and *Crepis capillaris* (Parker *et al.*, 1989). Remarkably, the rate of centromere misdivision giving rise to iso Bs reported in *C. scabiosa* (1.76×10^{-3} to 2.6×10^{-3}) and *H. maculata* (1.33×10^{-3} to 1.86×10^{-3}) was very similar to those we have observed in *E. plorans* (1.4×10^{-3} to 2.1×10^{-3} for $B_{2\text{iso}}$; Table 2). (ii) Three break insertions which are indistinguishable from inversions and addition or deletion of bands could be responsible for the differences found among some of the 23 distinct G-banding B variants observed in the grasshopper *Chortioicetes terminifera* (Webb & Nehaus, 1979). (iii) C-banding patterns displayed by Bs of the rodents *Perognatus baileyi* (Patton, 1977) and *Uromys caudimaculatus* (Baverstock *et al.*, 1982) are consistent

with an evolutionary model in which additions or deletions in the C-banded regions could play an important role. As Jones & Rees (1982) have pointed out, the existence of different types of Bs within a given species is not surprising on the grounds of their relative 'inertness' because we might expect a greater tolerance to different chromosome rearrangements. It has recently been suggested (Green, 1990) that a mechanism like Muller's Ratchet could account for the evolution of some Bs as it would allow the accumulation of deletions and heterochromatinizations.

At this point, we may speculate about the scarcity of some B chromosome variants in natural populations, for instance B_{1iso} , B_{2iso} and B_{mini} , which are recurrently formed in each generation. The maintenance of newly arisen Bs depends mainly on their efficiency during sexual transmission and their effects on carrier fitness. Whereas the latter is unknown, it seems clear that Bs with a hemicentromere, as those which resulted from misdivision, show a very high tendency to elimination probably from a loss in the efficiency of the hemicentromere to control chromosome movement at meiotic anaphases during gametogenesis and even at mitotic anaphase during zygote cleavage divisions. At present, the reason for the tendency to eliminate Bs with deletions affecting the light distal region on the male side is not evident. It is conceivable that it may be related to some type of unknown function of the lost chromatin as the relocation of this region on the short arm (B_{2il} and B_{2iz}) or on the end of the long arm (B_{5il}) does not affect the characteristic Mendelian inheritance of B_2 and B_5 , respectively. The increase in frequency of those B variants lacking accumulation mechanisms is difficult unless they improve the fitness of individuals carrying them, a fact that is unknown at present.

The case of the B_1 chromosome found in Granada province merits special mention. Most observations suggest that B_1 is the original B chromosome in the Iberian Peninsula from which the remaining Bs have derived, including some types (i.e. B_2 and B_5) that are much more frequent than B_1 in certain localities (Henriques-Gil *et al.*, 1984). This indicates that a substitution of Bs must have taken place in at least two regions, namely in Fuengirola (western Málaga) where B_5 is the predominant type and the province of Granada where B_2 has substituted B_1 (see for discussion Henriques-Gil & Arana, 1990). In Granada province, B_1 showed a trend to elimination by both sexes although it was only significant in two of the seven PAs studied. The most remarkable fact is that in the female DC3J90 ($1B_1 + 1B_2$), B_2 was accumulated and B_1 eliminated, although total B transmission by this female was that expected for a 2B carrier ($0.362 + 0.629 = 0.991$).

The contrary situation (accumulation of B_1 and elimination of B_2) has not been observed as the female DC10J89 transmitted B_1 and B_2 at Mendelian rates. Consequently, the substitution of B_1 by B_2 in the Salobreña and Jete populations could be based on transmission efficiency differences between both variants, B_2 being favoured. If a B chromosome substitution is effectively taking place, and populations are near the complete substitution of B_1 by B_2 , we can deduce that even hypothetical improvements of individual fitness exerted by B_1 will not be enough to counteract its elimination during transmission. The evolution of this B chromosome polymorphism at Málaga and Granada provinces will be the purpose of future study.

For B chromosome polymorphisms that appear to be at or near equilibrium, it is generally accepted that selective forces acting against individuals with a high number of Bs are counteracted by cytological accumulation mechanisms (Shaw & Hewitt, 1990). In these situations mutations decreasing the transmission of the B will be favoured. In fact, the existence of genes in the A genome suppressing meiotic drive of Bs has been reported in the grasshopper *Myrmeleotettix maculatus* (Shaw *et al.*, 1985), the mealy bug *Pseudococcus affinis* (Nur & Brett, 1988) and rye (Romera *et al.*, 1991). On these grounds, the B chromosome polymorphism displayed by *Eyprepocnemis plorans* is quite unusual because the three main variants (B_1 , B_2 and B_5) showed a Mendelian transmission ratio, at least in the central areas of their distribution (López-León *et al.*, 1992). One of the most feasible hypotheses to explain the existence of the widespread B chromosome polymorphism in *E. plorans* is the existence of drive in the beginning (López-León *et al.*, 1992). If drive suppressor genes were to have evolved in this species, the current situation of the polymorphism might be explained by a majority implantation in different populations of allelic variants in A chromosomes responsible for the Mendelian transmission ratio observed at present. In any case, a certain coadaptation between A and B genomes appears to have been achieved. Thus, data from the present work indicate that differences in transmission rate could play an important role in the substitution of B_1 by B_2 in Granada populations, as postulated by Henriques-Gil *et al.* (1984). Moreover, other different B variants are continuously arising, perhaps as a coevolutionary response to the challenges imposed by the host genome, thus being a reflection of the relationships that take place between A and B genomes. Whereas most of these new B variants will disappear from the populations in which they originate, owing to their low transmission rate (Table 3), the future of others showing

Table 3 Transmission ratios observed for 12 B chromosome variants

B type	Transmitted by	Progeny analysis	Transmission ratio
B ₁	♂	♀24S90	0.157
		♀31J90	0.472
		♀43J90	0.364
		Total	0.331
	♀	DC10J89a	0.595
		DC10J89b	0.523
		DC3J90	0.362
		Total	0.493
	♂ + ♀	♀25D89	0.456
	B ₂	♂	♀42J90
♀86J90			0.470
Total			0.500
♀		DC4J88a	0.500
		DC10J89b	0.538
		♀6S87	0.559
		♀24S90	0.614
		♀55J90	0.481
		♀116J90	0.476
		Total	0.528
♂ + ♀		C5J88	0.647
		C26S89	0.581
		DC4J88b	0.430
		DC10J89a	0.512
		♀3S90	0.550
		♀38S90	0.476
		♀62S90	0.533
		♀27S91	0.443
		♀7J90	0.577
		♀31J90	0.472
		♀47J90	0.504
		♀115J90	0.450
		Total	0.515
B ₅	♂	♀12F89	0.542
	♀	♀23F88	0.514
B _{mini}	♀	♀42J90	0.059
		♀109J90	0.128
		Total	0.094
B _{2m1}	♂	♀24S90	0.214
B _{2i1}	♂	♀86J90	0.576
	♀	♀116J90	0.571
B _{2i2}	♂	C26S89	0.487
B _{2i3}	♂	♀27S91	0.492
B _{2d1}	♂	♀62S90	0.167
	♀	DC4J88a	0.596
		DC4J88b	0.600
		Total	0.598

Table 3 Continued

B type	Transmitted by	Progeny analysis	Transmission ratio
B _{2d2}	♂	C5J88	0.390
B _{2iso}	♂	♀6S87	0.356
B _{5t1}	♂	♀23F88	0.514

Mendelian transmission ratio is at present unpredictable.

Another situation is worthy of mention in which B chromosomes could avoid the influence of the A genome, namely their integration in this genome by means of centric fusions with regular members of the complement. This type of rearrangement has been reported to occur spontaneously in natural populations of *E. plorans* (Henriques-Gil *et al.*, 1983; Cabrero *et al.*, 1987) and has recurrently appeared in the last years either in adult individuals or in single embryos among offspring of crosses where the parents did not show such chromosomal mutation (M. D. López-León *et al.*, unpublished data). In fact, Bs fused to an autosome do not show any cytologically detectable methylation, in contrast to free Bs, which produce the reactivation of a silent nucleolar organizer region present in the Bs (López-León *et al.*, 1991). Therefore, both the generation of new variants and the integration in the A genome are the main evolutionary strategies displayed by B chromosomes in natural populations of *E. plorans*.

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