Sex-dependent meiotic behaviour of B chromosomes in the grasshopper *Eyprepocnemis plorans*

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The meiotic behaviour of B chromosomes differs strongly between males and females of *Eyprepocnemis plorans*. In anaphase I of oogenesis the Bs always divide equationally though this segregation may become more or less aborted and a number of different secondary oocytes can be formed. In contrast, equational divisions of accessories in male meiosis are rare. This peculiar behaviour of B chromosomes in the females does not seem to be associated with accumulation or loss.

INTRODUCTION

B chromosomes differ from the members of the normal karyotype in several features but specially because they may be present in different numbers, with an absence of clear effects on the carrier individual, and because they display non-mendelian modes of transmission. The peculiar modes of inheritance are in part a consequence of atypic behaviour during the meiotic divisions or, in the case of many plant species, in the postmeiotic mitoses (for review see Jones and Rees, 1982).

The fact that B chromosomes may be present in different numbers leads to a great range of possible configurations in the first meiotic division and, even when a pair of Bs exist in a cell, bivalents are not always formed. These phenomena result in many different patterns of inheritance.

The atypical behaviour of accessories is, in many cases, clearly associated with mechanisms of accumulation that tend to increase the frequency of Bs in the population. This is particularly so in the female where only one of the products of meiosis will form a functional gamete. For instance, in the grasshoppers *Myrmeleotettix maculatus* (Hewitt, 1973), *Melanoplus femur-rubrum* (Lucov and Nur, 1973) and *Heteracris littoralis* (Cano and Santos, 1988) Bs undergo a process of meiotic drive that increases the probability of inclusion in the egg. In these cases, the behaviour of Bs is probably not qualitatively different in males and females. Hewitt (1976) concluded that

meiotic drive in *Myrmeleotettix* females was a consequence of spindle asymmetry with the B chromosome orientating towards the egg pole more often. Likewise, Cano and Santos (1988) observed in *H. littoralis* that in anaphase I cells one of the poles was more condensed than the other suggesting an asymmetric spindle in this species also.

Recent advances in the technique for the analysis of female meiosis after metaphase I in grasshoppers (Henriques-Gil *et al.*, 1986) have allowed us to contrast B chromosome behaviour between male and female meiosis in the grasshopper *Eyprepocnemis plorans* and our findings are described in this report.

MATERIAL AND METHODS

Male and female *E. plorans* were collected during September 1985 near the Spanish localities of Fuengirola (Málaga), Salobreña (Granada) and Daimuz (Valencia). The males were vivisected and the testes fixed in acetic-ethanol 1:3. The preparations of fixed material were made by the traditional squash technique with acetic-orcein staining.

The females were maintained in laboratory conditions in order to obtain mature eggs for meiotic observations. Female meiosis was analysed following the technique of Perry and Jones (1974) as modified by Henriques-Gil *et al.* (1986). The number of B-carrying females from each popula-

Table 1 Number of females analysed from each population and the number of cells scored for each stage of meiosis

Population	No. of females	No. of cells					
		M I	A I	T I	M II	A II	T II
Daimuz	10	11	10	16	10	5	8
Salobreña	8	8	12	10	8	9	6
Fuengirola	12	11	14	8	6	10	7
Total	30	30	36	34	24	24	21

tion and the number of cells analysed are given in table 1.

RESULTS

The natural populations analysed here were selected because of their B chromosome differences. Thus, in Daimuz accessory chromosomes are of the B₁ type, in Salobreña B₂ is usually found and in Fuengirola most accessories are of B₅ type. Other rare variants are also occasionally found in all three populations. The B chromosomes can be easily distinguished after C-banding staining having an obvious monophyletic origin (Henriques-Gil *et al.*, 1984a).

These three types of B chromosomes show similar cytological behaviour in male meiosis (Henriques-Gil *et al.*, 1982, 1984a). After an heteropycnotic state in the first meiotic prophase, at metaphase I univalent B chromosomes are usually placed outside of the equatorial plate (like the X chromosome; see fig. 1). If two Bs are present in the same individual, bivalents can be formed with variable frequencies showing an equatorial position similar to the autosomal bivalents. In anaphase I the B moves undivided towards one of the poles (fig. 3) giving rise to perfectly normal behaviour during the second division where each chromatid segregate to a pole. However, there is a low frequency of cells (less than 5 per cent) where orientation of univalent Bs in the equatorial plate is followed by equational segregation at anaphase I. Isolated chromatids in the second meiotic division are unable to move towards either pole and may become excluded from the rest of the chromosome complement forming a micronucleus.

The observations of female meiosis provide a quite different picture. Stages prior to the first metaphase could not be analysed due to the diffuse stage commonly occurring in grasshopper oocytes. At metaphase I the univalent B chromosomes

orientate amphitelicly in or near to, the equatorial plate (fig. 2).

In anaphase I the accessories divide in an equational manner in *all cells* analysed so far, and this applies for females from any of the natural populations sampled, presumably carrying, in each case, different types of B chromosomes. This can be clearly observed in figs 4 and 5.

However, the late consequences of this phenomenon are not necessarily the same: in some cells the dividing univalent is placed near one of the anaphase poles suggesting that this division may not always be accomplished. This hypothesis is proved by the fact that the B chromosome constitution of secondary oocytes is variable. In addition to second division cells with one B-chromatid (the expected result after equational division), in a female having one B, different metaphase II cells may show zero chromatids or a two chromatid chromosome (see figs 6, 7 and 8). Unfortunately, with our technique the number of cells available for analysis declines as meiosis progresses. Nevertheless, the limited quantitative study of the different types of second metaphases or their consequences in anaphase II indicates a random distribution of the B chromosome to the secondary oocyte: excluding the cells with 1B-chromatid, the number of secondary oocytes with one B chromosome and those without B is not statistically significant with respect to a 1:1 ratio (table 2).

DISCUSSION

It is well known that chromosome behaviour usually show some differences between male and female meiosis. For example, the rates of recombination are not the same for both sexes and there are some extreme cases like *Drosophila* males or *Bombyx* females with achiasmatic meiosis. In the species analysed here, the grasshopper *E. plorans*, a similar phenomenon was detected by Cano *et al.* (1987): the frequency of chiasmata is significantly lower in the females than in the males. Accordingly, we could also observe that the frequency of B-bivalents in two-B females is much lower than the frequencies commonly observed in two-B males (see Henriques-Gil *et al.*, 1984b). (Later stages of meiosis could not be obtained in two-B females.)

The results reported here point out an active role of B chromosomes in determining their anaphase I behaviour depending on sex. The 100 per cent equational first anaphases in the females contrast with a very low frequency of this type of

Figure 1. Metaphase I cell from a 1B male. The B chromosome is outside of the equatorial plate. In all cases, arrows indicate B chromosomes when they can be undoubtedly identified.

Figure 2. Metaphase I cell from a 1B female. The B is at the equatorial plate.

Figure 3. Anaphase I cell from a 1B male, with 11 chromosomes in one pole and 13 in the other (11 autosomes plus the X and the B).

Figures 4–5. Anaphase I (4) and telophase I (5) cells from a 1B female showing the B chromosome dividing in an equational manner.

Figures 6–8. Metaphase II cells from a 1B female containing 13 chromosomes, 12 chromosomes plus 1B chromatid and 12 chromosomes respectively.

Table 2 The distribution of B chromosomes in secondary oocytes of females of *E. plorans* having one B chromosome

Population	Metaphase II and anaphase II cells		
	Without B	1 B-chromosome	1 B-chromatid
Daimuz (B ₁)	7	8	—
Salobreña (B ₂)	9	7	1
Fuengirola (B ₃)	9	5	2
Total	25	20	3
Expected after random segregation	22.5	22.5	—

$$\chi^2 = 0.556; 1 \text{ df}; P > 0.4.$$

division in the males. Whatever mechanisms are involved, they do not affect the normal chromosomes which segregate identically in both sexes (except the X chromosome).

In *M. maculatus* and *H. littoralis* the behaviour of B chromosomes in male and female meiosis is similar but, in the latter, Bs tend to migrate towards the egg pole due to an asymmetry of the spindle which makes more probable the localization of the B in the egg side than in the polar body side (Hewitt, 1976; Cano and Santos, 1988). The first meiotic division spindle of *Eyprepocnemis* may also be asymmetric since at late anaphase I and telophase I one pole is more condensed than the other (see figs 4 and 5), however the fact that Bs orientate in the equatorial plate indicates that there is no parallel with the cases of *Myrmelotettix* or *Heteracris*. In *Eyprepocnemis* a clear relationship between the capacity for equational division of B univalents and their position in the nucleus is observed (see Ostergren, 1951): if the B univalents are near to the metaphase I plate, they divide equationally whereas a polar disposition is followed by reductional division. In consequence, their behaviour must be probably determined when the plate is formed, that is, from diakinesis to metaphase I. Thus, either B-kinetochores have different properties in female than in male meiosis or a same B distinguishes some differences of the meiotic spindle of each sex.

It is important to note that in *Eyprepocnemis* some rare B variants are occasionally found (with a probable origin from the main ones) that show a high rate of equational segregation in anaphase I of males (Henriques-Gil *et al.*, 1982, 1984a). This could therefore result from a loss in the mutant Bs of the ability to distinguish male from female meiosis exhibiting a high rate of equational segregations in male anaphase I. Although we have no reasons to suppose other kinds of behaviour,

females carrying such derived types of B were not available for analysis at meiosis in order to observe if their behaviour is still the same or not. In maize, nondisjunction of B chromosomes in the second pollen grain mitosis also seems to be controlled by specific factors of the B chromosome located near the centromere (Carlson and Chou, 1981).

In a number of B chromosome systems studied in detail, atypical behaviour of accessories is often associated with accumulation mechanisms that probably counterbalance a decrease in frequency in natural populations produced by a lower fitness of B carriers (Jones and Rees, 1982). Accordingly, the most obvious meaning of the phenomena reported here could be to imply an accumulation of Bs in the female gametogenesis of *Eyprepocnemis*. However, observations do not seem to support this hypothesis. In first place, there is no evidence for such a mechanism because the frequency of Bs in second oocytes is not different to a 1:1 ratio of cells without B and cells with one B, expected after a random segregation of the accessory in the first anaphase (the slight deviation is towards a lower number of Bs; see table 2). Secondly, and more importantly, Cano (1988) observed that the three types of B chromosomes analysed here are inherited in the offspring in a random manner with no tendencies to accumulation or loss from *either sex*. This argues against meiotic drive of B chromatids in anaphase II.

The significance of the behaviour described here is, therefore, far from clear. Hewitt (1974) suggested that after the origin of a B chromosome system it could become gradually more coadapted with the A-genome so as to produce a minimum of deleterious effects on the carrier individuals; in more neutral situations the rate of accumulation of Bs should also decrease avoiding an excessive increase in B frequency. Indeed, Blackman (1976) observed that in *Eucraphis* both males and

females always have two B chromosomes. Both accessories pass to the functional spermatocytes but this apparent accumulation is compensated by some elimination, probably during oogenesis, to form the offspring again with a pair of B chromosomes. However, at this point, additional data are obviously needed in the case of *Eyprepocnemis*. Nevertheless, the fact that the different types of B chromosomes share the same peculiar behaviour during the female meiosis suggests strongly some importance in their maintenance.

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