B CHROMOSOMES IN *RATTUS FUSCIPES* I. MITOTIC AND MEIOTIC CHROMOSOMES AND THE EFFECTS OF *B* CHROMOSOMES ON CHIASMA FREQUENCY

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Received 11.x.83

SUMMARY

The small heterochromatic *B* chromosomes of *Rattus fuscipes assimilis* are similar to those described by others in the related species *Rattus rattus*. They are apparently stable in somatic tissues, but in male meiosis they are liable to non-disjunction. The *B* chromosomes in *R. f. assimilis* are associated with an increase in the mean cell chiasma frequency, at least in males.

1. INTRODUCTION

A large number of plant and animal species have some individuals which carry apparently non-essential chromosomes in addition to their normal complement. These chromosomes have been described variously as "super-numerary," "accessory" or "*B*" chromosomes (see Battaglia 1964, Muntzing 1974, Jones 1975, and Volobujev 1981 for reviews).

B chromosomes (*Bs*) are frequently characterised by unconventional disjunction in germ line cells, although the precise pattern of behaviour varies between species (Jones, 1975). This is not only true of the oft-studied grain-bearing plants and orthopteran insects, but also of the relatively poorly known mammalian species with *Bs*. There are six mammalian species in which detailed studies have been reported of germ line disjunction or transmission of *Bs* to offspring. These are: the harvest mouse, *Reithrodontomys megalotis* (Shellhammer, 1969), the pocket mouse, *Perognathus baileyi* (Patton, 1977), the black rat, *Rattus rattus* (Pretel and Diaz de la Guardia, 1978; Yosida, 1977), the red and silver foxes, *Vulpes vulpes* and *V. fulvus* (Volobujev, 1981), and the hooved lemming, *Dicrostonyx torquatus* (Gileva and Chebotar, 1979). In each of these species there is some evidence of a non-Mendelian accumulation mechanism which allows the production of more *B* chromosome-bearing gametes than expected. The consequences of such an accumulation at the population level are unknown and because of this, the present project was initiated. The aim was to tie-in a study of the cytogenetics of a *B* chromosome system with observations on the transmission rates of *Bs* from parents to offspring and to use this data in conjunction with information from a study of the population genetics of *B* chromosomes.

The Australian bushrat, *Rattus fuscipes* is a species ideally suited to such a study. Baverstock *et al.*, (1977) have described the *B* chromosomes of *R. f. assimilis*, a subspecies which is native to southeastern Australia. It is one of the most common small mammals in this region, and its biology

is fairly well known. It has the added advantage of being reasonably easily bred in the laboratory, which allows directed crosses to be made for transmission studies.

The present paper describes the structure of the *B* chromosomes in *Rattus fuscipes assimilis* and their behaviour at mitosis and meiosis. It forms the background for a population study (Thomson, 1984) which gives an assessment of the fitness of *B* chromosome-bearing individuals in natural populations.

2. MATERIALS AND METHODS

(i) *Culture methods for karyotypes*

(a) *Short term leukocyte culture*

Two techniques were used, either that of Baverstock *et al.*, (1977) or that of de Boer *et al.*, (1977). The latter technique is notable for the use of Concanavalin A as a mitogen, and the higher dose of Glutamine.

(b) *Long term tissue culture*

Initiation of monolayer cultures from various tissues was achieved by the method of Jovanovic and Atkins (1969), modified by the use of foetal calf serum in place of chicken plasma and serum for adhering tissue fragments to the bottom of culture bottles.

(c) *Short term tissue culture*

Liver from newborn rats and spleen from adult rats could be cultured to make chromosome preparations within two hours of sacrificing the animal with modification of the methods of Evans *et al.*, (1972) and Stock *et al.*, (1972).

Bone marrow chromosome preparations were made using the methods of Yosida and Sagai (1973), and corneal epithelium by the method of Fredga (1964).

(ii) *Chromosome banding techniques*

G and C bands were obtained by the methods of Yosida and Sagai (1973). For Nucleolus Organizer Region (NOR) bands, the method of Munke and Schmiady (1979) was used.

(iii) *Meiotic chromosomes*

Chromosome spreads were obtained from testes using a technique similar to that of Stock *et al.*, (1972), the critical steps being the dissolution of the seminiferous tubules with 60 per cent acetic acid and the careful spreading of cells onto hot (60°) slides by using a 1 ml syringe and 23 g needle. The suspension is expelled onto the slide, then the droplet drawn back into the syringe, leaving a circular film. This process is repeated several times on the one slide, the slides are allowed to dry, and are then stained with Giemsa or aceto-orcein.

PLATE 1. The unbanding mitotic metaphase chromosomes of *Rattus fuscipes assimilis*. 1(a) Male. 1(b) Female. B chromosomes are indicated.

PLATE 2. (a) The *G* banded karyotype. Note the virtual absence of banding of the *B* chromosome. (b) The *C* banded karyotype. *B* chromosomes in this species are *C* band positive, as seen here.

3. RESULTS AND DISCUSSION

(i) *The somatic chromosomes*

The standard karyotype of *Rattus fuscipes assimilis* has been described by Yosida *et al.*, (1971) and is superficially similar to the $2n=38$ ("Oceanic") form of *Rattus rattus*. As indicated in plate 1 the *B* chromosomes are small metacentrics, similar to those found in both the $2n=38$ and $2n=42$ forms of *Rattus rattus*.

Mitotic chromosome spreads were examined from leukocyte culture, bone marrow, corneal epithelium and from fibroblast culture from various sources (lung, heart, kidney, spleen, testis, ovary and body-wall). In rats where karyotypes were examined for more than one tissue, the number of *B* chromosomes was constant within an individual (total numbers of *B* chromosome-bearing rats examined for three or more tissues were: seven males, five females). Direct preparations from the livers of newborn rats also showed numbers of *B* chromosomes identical to those from fibroblast cultures derived from the same individuals (3 males; 5 females). Anaphase/telophase spreads from corneal epithelium showed no evidence of unusual segregation of the *B* chromosomes, that is, there was no obvious lagging of the *Bs*.

Thus the *Bs* can be said to be "stable" in somatic tissues, unlike those in some other mammals, *e.g.*, *Echymipera kalabu*, a marsupial bandicoot (Hayman *et al.*, 1969).

The *G* banded karyotype of *Rattus fuscipes* has been described by Yosida and Sagai (1973) and shown to differ from the $2n=42$ form of *Rattus rattus* by two Robertsonian interactions. It is notable that these interactions are different from those relating the $2n=38$ form of *Rattus rattus* to the $2n=42$ form. As can be seen in plate 2(a) the *B* chromosome is distinct from all the *A* chromosomes in that it lacks strong bands, but shows a minor band at the distal end of the shorter arm.

The *C* banded karyotype (plate 2(b)) is characterised by very small centromeric bands on all the autosomes and the *X* chromosomes, with the *Y* chromosome and the *B* chromosomes being almost completely *C* positive. In the literature the term "*C* positive" chromatin is generally used as a synonym for constitutive heterochromatin, which is in turn accepted as consisting largely of multiple copies of simple sequence DNA, with few genes coding for proteins (Pardue and Gall, 1970; Peacock *et al.*, 1977). Although this implies that the *B* chromosomes may contain DNA of different sequence arrangement to the standard ("*A*") chromosome complement, there is no biochemical evidence that the range of repeated sequence DNA in *B* chromosomes is different to that found in the *As*.

Detailed studies have now been done on the DNA of individual *R. f. assimilis* males with and without *B* chromosomes, and no clear differences have been detected. Only a small proportion of the genome is made up of highly repeated sequences, which is consistent with the very small *C* bands seen at metaphase. Whilst there is a consistent skewness towards the heavy (*G+C* rich) side of the buoyant density profile in neutral Cesium Chloride gradients ($\rho=1.699 \text{ g/cm}^3$), no cryptic satellite sequences have been resolved following use of Ag^+ , Hg^{++} , Actinomycin-DI or Hoechst 33258. Restriction endonuclease patterns for DNA from males with or without *B* chromosomes were identical and indistinguishable from those published by

Miklos *et al.*, (1980). It would thus appear that the *B* chromosomes of *R. fuscipes assimilis* do not contain any DNA sequences substantially different from those present in the standard chromosome complement. This is consistent with studies on *B* chromosomes in other species (e.g., rye; Rimpau and Flavelt, 1976 and the grasshopper *Myrmeleotettix maculatus*; Dover and Henderson, 1976), and indicates that the *Bs* have not arisen from uncontrolled multiplication of a few simple sequence DNAs attached to a centric fragment. However, there is no evidence to show whether the *Bs* arose from a single *A* chromosome or from segments of various *A* chromosomes translocated onto a centric fragment.

Silver staining of the *R. f. assimilis* metaphase chromosomes showed that there are no active Nucleolus Organiser Regions present on the *B* chromosomes (plate 3). This is not unexpected, since *B* chromosomes have only rarely been reported to be associated with nucleoli.

(ii) *The meiotic chromosomes*

Meiosis was examined in twenty-seven male *R. f. assimilis*. The *A* complement behaves as expected, with the formation of 19 bivalents during the first meiotic prophase. The *B* chromosomes, like the XY pair, are positively heteropycnotic in prophase I but slightly negatively pycnotic in metaphase I (plate 4(a)). In individuals with the two *B* chromosomes (2*Bs*), the *Bs* appear to be paired at leptotene and zygotene (plate 4(b)) but are seen as univalents at diplotene/diakinesis (plate 4(c)). In the single 3*B* individual examined, there were usually three dark staining bodies at leptotene and zygotene, presumably the XY pair, a *B* chromosome pair and a *B* univalent. In all metaphase spreads, the *Bs* were seen as univalents.

These observations suggest that although the *B* chromosomes may pair, there is almost certainly no chiasma formation between *Bs*. Similar observations were made by Yong and Dahliwal (1972) and Pretel and Diaz de la Guardia (1978) for the *B* chromosomes of *Rattus rattus*. Thus, for both species non-disjunction of *B* chromosomes is likely to occur at the first division of male meiosis. Unfortunately, metaphase II spreads could not be scored accurately so all observations are limited to the first division. It is interesting to note that the *Bs* do not seem to associate with any *A* chromosome pair, particularly in the light of observations by Solari and Ashley (1977) and de Boer and Branje (1979) that in some mammals (namely *Psammomys obesus* and *Mus musculus*) unpaired chromosomes, particularly the extra chromosomes in trisomics are usually seen in close association with the XY-pair at prophase I.

(iii) *B Chromosome effects on chiasma frequency*

One of the most intriguing effects of *B* chromosomes in many organisms is an apparent change in the chiasma frequency in *B* chromosome individuals (Jones, 1975). The results presented in table 1 show that there is also a significant effect on chiasma frequency related to the presence of *B* chromosomes in *R. f. assimilis*.

The Analysis of Variance (table 2) shows that there is fairly wide variation of chiasma frequency between animals. Despite this, there is a highly significant difference between the three chromosome groups (0*B*,

PLATE 3. Nuclear Organizer Region banding.

PLATE 4. Male meiosis. (a) Metaphase I in a 1*B* individual. *B* indicates the *B* univalent. The XY pair has separated, as indicated. (b) Zygotene in a 2*B* male. *BB* indicates the heavily staining *B* chromosome pair. XY indicates the sex chromosome pair. (c) Diplotene in a 2*B* male. The *B* chromosomes (labelled *B*) are not paired.

TABLE 1

Mean number of chiasmata per cell within individuals, averaged over a number of individuals in three groups; those with no *B* chromosomes (0*B*), those with one *B* chromosome (1*B*) and those with more than one *B* chromosome (2*B*+3*B*)

<i>B</i> Chromosome Groups (N)	0 <i>B</i> (12)	1 <i>B</i> (9)	2 <i>B</i> +3 <i>B</i> (5+1)
Mean cell chiasma frequency $\pm$ S.D.	28.38 ± 0.59	29.75 ± 0.79	29.80 ± 1.24

TABLE 2

Analysis of variance of chiasma frequency data

Source of variation	Degrees of freedom	Mean square	Variance ratio
Between chromosome groups	2	66.05	8.641***†
Between individuals within group	24	6.97	5.174***
Within individuals	158	1.35	

*** Significant at the 0.1 per cent level.

† Variance ratio for between chromosome groups/between individuals within groups is corrected for unequal sample sizes (Sokal and Rohlf, 1969).

1*B* and 2*B*+3*B*). Student's *t*-tests show that the difference between the 1*B* and 2*B*+3*B* groups is not significant, ($t = 0.077$, $df = 13$, $p > 0.05$), but that the difference between 0*B* and 1*B* groups is highly significant ($t = 4.387$, $df = 19$, $p < 0.01$).

Thus, in *Rattus fuscipes assimilis* males, the presence of *B* chromosomes is associated with an increase of mean cell chiasma frequency. This effect is not additive: 2*B* and 3*B* individuals have apparently similar mean chiasma frequencies to 1*B* individuals. A similar "plateau" effect is seen in other *B* chromosome-bearing species, e.g. *Myrmeleotettix maculatus* (John and Hewitt, 1965), *Phaulacridium* spp. (Westerman, 1977) and *Perognathus baileyi* (Patton, 1977).

Female meiosis was not studied because of the difficulties of obtaining oocytes from each individual. It cannot be assumed that chiasma frequencies would be the same in females as in males, nor that any effect would be in the same direction. Speed (1977) has shown that in the mouse, chiasma frequencies are different in males and females, and that ageing effects (chiasma frequencies tend to decrease with age in females) are different in males and females. Hewitt (1976) has shown that, in *Myrmeleotettix maculatus*, *B* chromosomes do have different effects on chiasma frequencies in males and females. Whilst this point should be kept in mind, it would seem unlikely that the *B* chromosome effects on mean cell chiasma frequency in females would negate those in males.

The mechanism by which the chiasma frequency is increased in the presence of *B* chromosomes is unknown. Although a genic mechanism is

often inferred, it should also be considered that a purely mechanical mechanism is possible as suggested by Rhoades and Dempsey (1972). One hypothesis can be made by extrapolating from the fact that *B* chromosomes (unlike extra *A* chromosomes—Verma and Lin, 1979) are correlated with an increase in the mitotic cell cycle time in some species (Jones 1975). If meiotic prophase I is also lengthened in the presence of *B* chromosomes, this may permit additional chiasmata to be formed. However, experiments by Fox *et al.*, (1974) suggest that the meiotic cell cycle time is not lengthened by *B* chromosomes in *Myrmeleotettix maculatus*. Unfortunately, no other studies have been published which throw further light on this problem, and so the mechanism by which *B* chromosomes affect *A* chromosome chiasma frequency remains unclear.

What effect an increased chiasma frequency in male *R. fuscipes* may have at the population level is difficult to assess. If the extra chiasmata are not located telomerically, it can be assumed that *B* carrying individuals would produce more recombinants, on average, than *0B* individuals, and thus would produce more phenotypic variability in their offspring. Moss (1966) showed that the offspring of *B* carrying plants in experimental populations of rye did, indeed, show greater variability than the offspring of *0B* plants. However, because crossing was uncontrolled (*i.e.*, the *B* chromosome constitutions of the male parents were unknown) and because most of the factors measured (seed weight, number of grains per ear, growth rate etc.) are inter-correlated and influenced by maternal effects, the conclusions which can be drawn from this data are unclear.

Even if a change in the variability of offspring can be demonstrated, problems arise when interpreting the significance of such a change in the context of the natural population. Rees and Dale (1974), in their analysis of interpopulation chiasma frequency differences in some *Lolium* and *Festuca* species, suggested that an increase in the mean chiasma frequency of the population leads to a breakdown of adapted gene complexes with a concomitant release of variability in the population, but that much of the variability is then lost through selection acting against extreme recombinants. This process, they suggest, ultimately produces a specialised population with low variability and a high level of homozygosity in spite of high recombination frequency. However, it is implied that the increased recombination rate is maintained in the face of low comparative fitness by inter-population or inter-deme selection. The necessary conditions for such selection to be effective are highly restrictive (small deme sizes, high rates of deme extinction and very low gene flow between demes; Levin and Kilmer 1973), and for this reason it seems unlikely that the model proposed by Rees and Dale (1974) is widely applicable. In general, any model for situations where there is advantage for individuals possessing high rates of recombination requires complex, varying environments. The theoretical manipulations become somewhat involved, and the role which *B* chromosomes may play is difficult to determine. This is particularly so because of the general deleterious effect that *B* chromosomes have on their hosts in many species, including *Rattus fuscipes* (Thomson, 1984).

Acknowledgments. Part of this work was done whilst one of us (R.T.) was in receipt of a LaTrobe University Postgraduate Research Scholarship. The capture, holding and occasional sacrifice of *R. fuscipes* was carried out under permits issued by the Fisheries and Wildlife

Division of the Ministry for Conservation (Victoria). We would like to thank Dr Chris Collet, who either performed or assisted with most of the DNA analysis.

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