Karyological Studies on Black Rat (*Rattus rattus* L.) from Bulgaria

Rossitsa Belcheva and Vulko Bisserkov

Chair of Genetics, Faculty of Biology, University
of Sofia, Sofia, Bulgaria

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Karyological investigations on the black rat (*Rattus rattus*) have shown that it is a unique species because of frequent occurrences of chromosome polymorphism and geographical variations due to Robertsonian fusions, pericentric inversions and supernumerary chromosomes. The black rats (*R. rattus*) are divided into the following four geographical variants according to the difference of chromosome number and karyotypes: the Asian ($2n=42$), Ceylones ($2n=40$), Oceanian ($2n=38$), and Mauritius ($2n=42$) (Yosida *et al.* 1971, 1974, Yosida 1979a, b). They distribute occupying different geographical areas (Yosida *et al.* 1980). It is suggested that the Asian karyotype is the ancestral form and that the Ceylonese and the Oceanian karyotypes developed sequentially by centric fusions (Yosida *et al.* 1971, 1974, Yosida 1973). The Mauritius karyotype developed from the Oceanian by the Robertsonian fission type mechanism (Yosida *et al.* 1979). All *R. rattus* subspecies distributed outside of Asia have a diploid chromosome number of 38 (Gamperl 1980).

European black rats have been studied karyologically by many investigators (Capana *et al.* 1970, 1971, Kral 1974, Pretel *et al.* 1978, Vistorin *et al.* 1978, Dulic 1978, De La Guardia *et al.* 1979, Gamperl 1980). Polymorphism caused by supernumerary chromosomes was only described for a Spanian population of European black rat (Pretel *et al.* 1978). De La Guardia *et al.* (1979) have found a structural polymorphism caused by changes in the morphology of the chromosomes in three pairs.

The present paper reports the karyological studies of *R. rattus* collected from different areas of Bulgaria, which reveal a structural rearrangement of some autosomes, B-chromosomes, C-band polymorphism and the location of active ribosomal cistrons.

Materials and methods

The specimens of *R. rattus* (13 male and 12 female) used for this study were collected from 7 localities in Bulgaria (Fig. 1). Chromosome preparations were made from colchicine-blocked metaphases of bone marrow cells of the femur. Preparations were also made from the testes of all the male animals to give both mitotic and meiotic metaphases. The technique was that of Evans *et al.* (1964). G-banding was accomplished with the method of Seabright (1971), using Giemsa instead of Leischmann's stain and C-banding was done by the method of Sumner

(1972). For selective staining of the active nucleolar organizing regions (NORs) the technique of Goodpasture and Bloom (1975) was applied.

Results and discussion

The karyotypes in 25 black rats, *R. rattus*, from 7 localities in Bulgaria have been analyzed. Among them 14 showed the typical Oceanian type with 38 chromosomes and among the remaining 11 rats from 4 localities 8 were characterized by having 39 chromosomes and 3 rats had 40 chromosomes. They included one and two supernumerary chromosomes respectively (the B-chromosomes are described in the end of this paper). The Oceanian karyotype is characterized by two large metacentric pairs (M_1 and M_2). According to Yosida *et al.* (1971, 1974) the larger metacentric pair (M_1) has originated in the Robertsonian fusion of acrocentric pairs no. 4 and 7, and the other smaller one (M_2) in that of the acrocentric pairs no. 11 and 12 in the Asian type of black rat. There are six small metacentric pairs (nos.

Fig. 1. A map of Bulgaria showing the localities from where rats were trapped.

14, 15, 16, 17, 19 and 20), two subtelocentric chromosome pairs (nos. 1 and 9), seven acrocentric pairs (nos. 2, 3, 5, 6, 8, 10 and 13) in the Oceanian karyotype, and acrocentric X and Y.

In the karyotypes analysed we found a polymorphic pair no. 13 in respect to acro- and subtelocentrics. This polymorphism of chromosome no. 13 is due to a pericentric inversion. Among the 25 specimens of *R. rattus* karyologically studied 17 have an acrocentric pair no. 13 (A/A), 2 have a subtelocentric pair no. 13 (S/S), and 2 have a heteromorphic pair no. 13 (A/S) with one member acrocentric and one member subtelocentric (Figs. 2, 3, 4). Four individuals from 4 localities (1, 2, 3, 4—Fig. 1) show a mosaicism in respect to the shape of chromosome no. 13. Some metaphases have an acrocentric pair no. 13 (A/A), and some metaphases have a heteromorphic pair no. 13 (A/S). Yosida (1977a) described a wide distribution of pair no. 13 chromosome polymorphism in the rats and suggested that the inversion of the pair has occurred independently in several places of the world. Our findings are in agreement with this suggestion.

In four individuals from 3 localities (2, 4 and 7—Fig. 1) we found a polymor-

phism in chromosome pair no. 10 due to a pericentric inversion. These four individuals show a mosaicism in respect to the type of chromosome no. 10. Some metaphases have an acrocentric chromosome pair no. 10 (A/A), and some metaphases have a heteromorphic pair no. 10 with one acrocentric and one subtelocentric chromosome (A/S) (Fig. 5).

In two individuals from two localities (5 and 7—Fig. 1) a pericentric inversion in chromosome pair no. 9 was established. These two individuals show a mosa-

Figs. 2-5. 2, karyotype of female *R. rattus* with acrocentric pair no. 13, with Robertsonian fission in one member of M_1 resulted in the formation of two acrocentric chromosomes, with a heterozygous M_2 and one acrocentric B-chromosome. 3, karyotype of female *R. rattus* with subtelocentric pair 13 and with two metacentric B-chromosomes ($2n=38+2Bs$). 4, karyotype of a male *R. rattus* with heteromorphic pair no. 13 consisting of acrocentric and subtelocentric chromosomes. 5, karyotype of female *R. rattus* with heteromorphic pair no. 10 consisting of acrocentric and subtelocentric chromosomes, and heteromorphic pair no. 9 consisting of subtelocentric and metacentric chromosomes.

icism in respect to the type of the chromosome no. 9. Some metaphases have a subtelocentric chromosome pair no. 9 (S/S), and some metaphases have a heteromorphic chromosome pair no. 9 consisting of one subtelocentric and one metacentric chromosome (S/M) (Fig. 6). Polymorphism in pairs no. 1, 9 and 13 chromosomes have been described in black rats (Yosida 1977a). The pair no. 1 and 9

Figs. 6-9. 6, karyotype of male *R. rattus* with heteromorphic pair no. 9 consisting of subtelocentric and metacentric chromosomes. 7, karyotype of male *R. rattus* with Robertsonian fusion in chromosome pairs nos. 3 and 6. 8, C-band karyotype of male *R. rattus*. 9, C-band karyotype of male *R. rattus*. Note the small amount of C-band material on chromosome pairs 3 and 8 and the absence of C-band on chromosome pair 13.

polymorphism was found only in Asian type black rats. Recently new cases of structural polymorphism were discovered in this species caused by pericentric inversions affecting new chromosome pairs nos. 5 and 7 (according to Yosida's numeration) (De La Guardia *et al.* 1979), and pair no. 8 (Kasahara *et al.* 1981). Our data indicate two new inversions in chromosomes nos. 9 and 10 in black rat. These inversions were not established in all metaphases analysed per individual and we still refrain for commenting their importance.

In one individual we found metaphases with 37 chromosomes because there was Robertsonian fusion in chromosome pairs nos. 3 and 6 (Fig. 7). In one individual a metaphase with 39 chromosomes due to Robertsonian fission-type mechanism in M_1 was established (Fig. 2).

C-banding. Fig. 8 presents a C-band karyotype of a male *R. rattus*. The C-band is invariably centromeric. The sex chromosomes carry constitutive heterochromatin at the centromere region also (Figs. 8, 9, 10). The centromere regions are not always stained uniformly. In the majority of chromosomes the centromere region is uniformly intensive, whereas in some preparations in a few chromosomes this dense region is resolved, chromosome pairs nos. 3, M_1 and 8 (Fig. 9). The C-band technique sometimes revealed dimorphism in certain chromosome pairs. We have found this most evident in chromosome pairs nos. 14 and 16 (Figs. 8, 9, 10). One member of these pairs may have a large centromeric block, whereas that on its homologue is much smaller. Yosida (1979a) pointed out that the polymorphism in *R. rattus* is also remarkable by a variation of heterochromatic C-bands. Our data reveal a C-band polymorphism too.

Gamperl (1980) has not described C-band in chromosome pair no. 13. Our data showed distinct C-band in centromeric region of pair 13, no centromeric heterochromatin in this pair and dimorphism: one member of pair 13 has centromeric C-band and its homologue has not (Figs. 8, 9, 10). Pair 13 is polymorphic for acrocentric and subtelocentric, and it shows no correlation between the chromosome type and C-banding pattern. Yosida (1975), Vistorin *et al.* (1978), Gamperl (1980) have found that Y-chromosome of black rat is totally heterochromatic. The Y-chromosome of black rat in Bulgaria has only centromeric heterochromatin.

G-banding. Fig. 11 presents G-band karyotype of male *R. rattus*. The analyses of G-band patterns demonstrated that there are no differences between *R. rattus* in Bulgaria and the other black rats with 38 chromosomes (Yosida *et al.* 1973, Gamperl 1980).

Ag-NORs. As Yosida (1978, 1979b) already reported there is a consistent pattern of Ag-NOR distribution in the Asian, Ceylones and Oceanian types of black rats *R. rattus*. This species has 3 Ag-NORs bearing chromosome pairs nos. 3, 8 and 13. In our material, chromosome analysis after application of silver staining technique (Goodpasture and Bloom 1975) indicated that there are four chromosome pairs with silver stained NORs. Silver staining occurred at the telomere region of the short arms of chromosome pairs 3, 8 and 13, and at the telomere region of the long arm of one acrocentric chromosome which is identified as chromosome 10 (Fig. 12). The NORs in chromosomes 3, 8 and 13 are localized close but distally to the

centromeric heterochromatin. Chromosomes of *R. rattus* show silver stained NORs in one or both of the homologues. The number of chromosomes per cell on which Ag-NORs could be detected vary (Fig. 13). NOR association is observed among chromosomes nos. 3 and 8 (Fig. 12).

The ammoniacal silver staining procedure which stains the chromosome regions containing the rRNA genes indicates only active NOR (Hsu *et al.* 1975, Miller *et al.* 1976). It seems likely that silver staining involves non-histon proteins (Howell and Denton 1976) which are acidic proteins involved in rRNA transcription (Olert *et al.* 1979) as a part of the ribosomal nucleoprotein complex remaining around the active NORs (Schwarzacher *et al.* 1978). With respect to the total number of NORs in *Rattus rattus* genome one has to await the results of in situ hybridization with radioactively labeled rRNA.

Supernumerary, or B-chromosomes. As was already pointed out there are 11 specimens of black rats from 4 localities (1, 2, 3 and 7—Fig. 1) with a presence of 1 or 2 supernumerary chromosomes, respectively. The size and the shape of these chromosomes are different. In 6 specimens supernumeraries are metacentric. These additional metacentric chromosomes in size belong to the category of the 20th pair of metacentric (Figs. 3, 10). Four individuals have one metacentric B-chromosome, and two have 2 metacentric B-chromosomes. In 5 specimens from two localities (3 and 7—Fig. 1) there are acrocentric B-chromosomes (Fig. 2). Four individuals have one acrocentric B-chromosome and the remaining one has 2 acrocentric B-chromosomes.

In male meiotic prophase-I B-chromosomes appear in a univalent condition. At pachytene it is possible to observe that the B-chromosome is strongly contracted and it seems to a metaphase chromosome (Fig. 14). The observations made in meiosis clearly show the supernumerary character of these chromosomes.

In the Oceanian-type black rat, supernumerary chromosomes have been reported for the black rats in Southern India (Yosida 1977b) and for European black rats from a Spanish population (Pretel *et al.* 1978). The supernumerary chromosomes in the black rats are metacentric only (Yosida 1977b, Pretel and De La Guardian 1978). In the individuals with extra chromosomes each of the additional metacentric or acrocentric supernumerary chromosomes show a clear centromeric heterochromatin (C-band) only (Fig. 10). Raman and Sharma (1974) reported no centromeric C-band in the B-chromosomes of black rats. Yosida found that "... The supernumeraries ... stained heavily along their entire length by C-band staining" (Yosida 1977b, p. 149). Our findings about C-band in B-chromosome in *R. rattus* are not in agreement with Yosida's data. Evidently there are different ways in which B-chromosomes in black rats might have arisen, and they might have differed in size, shape, C-bands and in function as well.

Figs. 10–14. 10, C-band karyotype of male *R. rattus* ($2n=38/2Bs$). Note the small amount of C-band material on M_1 and clear C-band in centromeric regions on the B-chromosomes and Y. 11, G-band karyotype of male *R. rattus*. 12, silver-stained metaphase plate of male *R. rattus*. Note NOR in telomeric region of the long arm of chromosome 10 (long arrow) and association between NORs of chromosomes 3 and 8 (short arrow). 13, silver-stained metaphase plate of male *R. rattus*. Note Ag-NORs of different size. 14, pachytene stage from male meiosis of *R. rattus*. Arrow indicates B-chromosome.

Summary

The black rat (*Rattus rattus* L.) from Bulgaria belongs to the Oceanian-type ($n=38$). Chromosomal analyses show that a polymorphism occurs in chromosomes nos. 9, 10 and 13 due to pericentric inversions. A polymorphism concerning size difference of centromeric heterochromatin (C-band) in some of the chromosomes is observed. The analyses of G-band patterns demonstrate that there are no differences between our material and the other black rats with 38 chromosomes. The NORs of the black rat (*R. rattus*) are found in telomeric regions of the short arms of chromosome pairs 3, 8, 13 and in telomeric region of the long arm of chromosome pair no. 10. A polymorphism due to supernumerary chromosomes is detected. The supernumeraries are metacentric and acrocentric. They show centromeric C-band after C-band staining.

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