CHAPTER IV

A CHROMOSOMAL STUDY OF RATTUS RATTUS (L.) POPULATION
OF NORTH WESTERN INDIA

Recent karyological studies on populations of Rattus rattus in different parts of the world have generated considerable interest. Many subspecies of Rattus rattus show high incidence of chromosomal polymorphism (Yosida et al., 1965; Yosida et al., 1969; Bianchi et al., 1969; Capanna and Civitelli, 1969; Gropp et al., 1970; Ray-chaudhuri and Pathak, 1970; Badr and Badr, 1970). Majority of these chromosomal variations between different populations have been attributed to Robertsonian translocations and pericentric inversions (Yosida et al., 1969; Yosida and Sagai, 1972; 1973a).

Contrary to the earlier concepts (Yosida et al., 1971; Gropp et al., 1971; Capanna and Civitelli, 1970; 1971), it is now known that the Indian subcontinent, particularly the Southern India too has Rattus rattus subspecies with 2n=38 (Lakhotia et al., 1973; T. Sharma and Rajiva Raman, 1972; Satyaprakash and Aswathanarayana, 1972a; 1972-1973). Thus the North Indian Rattus rattus population has 2n=42 or more (Ray-chaudhuri and Pathak, 1970; T. Sharma and Rajiva Raman,
whereas South India has both the populations of 2n*38 and 2n*42 (Lakhotia et al., 1973; T. Sharma and Rajiva Raman, 1972). The variability in chromosome number even within the members of a subspecies provides a useful material for evolutionary studies and also to ascertain the taxonomic status of various populations. In recent years chromosome banding techniques have been used to understand and clarify the taxonomic and karyological relations of many mammalian groups including Rattus rattus (Yosida and Sagai, 1972; 1973a). The chromosomes and various banding patterns of Rattus rattus from Ahmedabad in North Western India have been studied to shed more light on the karyology of Indian Rattus rattus. The results are presented in this chapter.

MATERIALS AND METHODS

Animals:

Animals were collected from the highly populated domestic areas as well as from the fields around the University campus. A total of 38 animals were used for karyological studies and for kidney cultures.

Bone marrow chromosomes:

Direct bone marrow chromosomes were obtained using the standard air drying technique (Rothfels and Siminovitch, 1958). The method is same as the one described in Chapter I. But in the case of rats, the femur bones were used instead of humerus bones from the colchisinised animals.
Kidney culture chromosomes:

Kidney cultures were set with kidneys from adult animals and were grown in TC 199 medium and 20% bovine serum with penicillin and streptomycin at normal concentrations (Chapter I). The cell density for primary explantation was 1 to $2 \times 10^6$ cells per ml. The cultures were harvested with mild trypsinization and hypotonic treatments. The other details of the technique used are same as in Chapter I.

C-band staining:

For C-banding both the techniques of Arrighi and Hsu (1971) and Sumner (1972) were used with some modifications. Two to three days old slides were denatured either in 0.01N NaOH for 1 to 1½ minute (Arrighi and Hsu, 1971) at room temperature or in 0.5% BaOH at 56°C for 30 minutes (modification of Sumner, 1972). No pretreatment with RNase and HCl was used. The slides from the both treatments were then incubated for 12 to 24 hour in 2xSSC or in Sorensen's buffer at 65°C at pH 7.0. The slides were rinsed and dehydrated through alcohol grades. They were stained with Giemsa (E. Merck) stain diluted 1:10 in Sorensen's buffer (pH 7.0) for ½ to 1 hour.

G-band staining:

For studying G-bands, either trypsin treatment (Sea-bright, 1971) or saline sodium citrate (SSC) incubation (Sumner et al., 1971) were used. A 0.005% solution of 2x crystalline trypsin (Worthington, U.S.) in Sorensen's buffer
(pH 7.5) was used at room temperature for 10 to 60 seconds. Alternatively, the slides were incubated in 2xSSC at 65°C for 1 or 2 hours. The slides from either treatment were stained with 1:50 diluted Giemsa (E. Merck) in Sorensen's buffer (pH 7.0) for 10 minutes.

**Hoechst 33258 staining:**

Fresh slides were stained with Hoechst 33258 in Sorensen's buffer (0.05μg per ml) at pH 7.0 for 10 minutes in darkness (Hilwig and Gropp, 1972). The slides were observed on Carl Ziess Fluorescence microscope with HBO 200 lamp with excitor filters BG 3/2 and UG 14 and suppressor filters OG 1 and GG 9. Photographs were taken on high contrast ORWO DK-5 documentary film.

**OBSERVATIONS**

A total of 38 animals including 19 males and 19 female adults were used for karyological studies. Coat colour was seen to vary in the individuals. Majority of them had a dull black dorsal skin and almost white belly, some had dull black skin but belly had some black pigmentation and in one individual both the back and belly are typically dull black as in *Rattus rattus rufescens*. The subspecific identification was doubtful, hence are considered as *Rattus rattus* (L.).

All the individuals have 2n=38 chromosomes and display identical karyotypes (figs. 1 to 4). The karyotype consists of
two large pairs of metacentrics, one large subtelocentric pair, 8 pairs of graded telocentrics including the sex chromosomes, 7 pairs of small metacentrics and a pair of small submetacentrics. The X chromosome is one of the large telocentrics and the Y chromosome is the smallest telocentric.

**C-bands:**

Centromeric regions of the chromosomes stain intensely after C-band staining (figs. 5 to 7). The C-band positive regions are presented in an idiogram (fig. 8). None of the chromosomes show any intercalary C-bands. The centromeric C-band region vary in different chromosomes with respect to their size and intensity. Metacentrics and large subtelocentrics have medium sized C-bands. The telocentric pairs four and six show relatively large C-bands but the second pair of telocentrics only a faintly stained small C-band region. The X chromosome carries a distinctly stained centromeric heterochromatin. Y chromosome, the smallest of the telocentrics often does not show any specific C-band pattern. It stains uniformly but heavily than euchromatin (figs. 5 and 7). All the small metacentrics and submetacentrics show more prominent C-bands.

**G-bands:**

G-bands obtained by either trypsin treatment or SSC incubation were nearly similar. However, trypsin digestion gave clearer and sharper bands.

The typical G-bands observed after trypsin digestion
are shown in figures 9 and 10. A diagrammatic representation of the different G-bands seen in the various chromosomes of the complement is shown in figure 11. The homologous chromosomes usually show identical G-band positive regions though sometimes minor variations may be seen between the homologues. It is seen that in almost every chromosome the C-band positive regions are also G-band positive though the staining intensity of these regions is sometimes less in G-banded chromosomes. It seems, however, that in the submetacentric chromosome pair \((SM_1)\) the C-band region remains unstained in G-band treated chromosomes. It is also to be noted that the Y chromosome does not show any bandedness and it remains uniformly stained. Thus, the Y chromosome lacks clear C- and G-bands. The three chromosome constrictions noted by Yosida and Sagai (1973a) on the subteloentric and the largest telocentric chromosome pairs of different populations of \textit{Rattus rattus} were seen in our material as well.

**Hoechst 33258 fluorescence:**

The chromosomes of \textit{Rattus rattus} fluoresced brightly and uniformly when stained with Hoechst 33258 at pH 7.0, (fig. 12). However, the small Y chromosome showed the brighter fluorescence than the rest of the complement. There was no indication of banding on any chromosome in the karyotype (fig. 12).
<table>
<thead>
<tr>
<th>Chromosome type</th>
<th>R. r. wroughtoni*</th>
<th>R. rattus (L.)</th>
<th>R. r. rufescens*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sagar</td>
<td>Ahmedabad</td>
<td>Sagar</td>
</tr>
<tr>
<td></td>
<td>Karnataka State</td>
<td></td>
<td>Karnataka State</td>
</tr>
<tr>
<td></td>
<td>2n=42</td>
<td>2n=38</td>
<td>2n=38</td>
</tr>
<tr>
<td>Large subtelo-centric</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Acrocentric</td>
<td>13</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Small metacentric</td>
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<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Submetacentric</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

*from Lakhotia et al. (1973).*
DISCUSSION

The domestic black rat, *Rattus rattus* has a worldwide distribution and is divided into a large number of subspecies on the basis of various morphological and anatomical features (Ellerman, 1961). Chromosomally, however, two main types can be identified on the basis of diploid number. One group is characterised by $2n=38$ and the other $2n=42$. It is interesting to note that for many of the *Rattus rattus* subspecies both these diploid chromosome numbers have been reported (Lakhotia et al., 1973; Rajiva Raman and Sharma, 1974).

All the individuals of *Rattus rattus* from Ahmedabad examined in the present study consistently had chromosome number of $2n=38$ (Shah et al., 1973) and identical karyotypes (figs. 1 to 4). The karyotype, however, differs slightly from other published karyotypes of *Rattus rattus* with 38 chromosomes. In table 1 the karyotype of the present population is compared with the karyotypes of *Rattus rattus wroughtoni* with $2n=42$ and *Rattus rattus rufescens* with $2n=38$ from South Western India (see Lakhotia et al., 1973). It can be seen that the present karyotype with 38 chromosomes resembles the karyotype of *Rattus rattus rufescens* which also has 38 chromosomes, except that in the former there is only one small submetacentric while in *Rattus rattus rufescens* from South there are two such chromosome pairs. In the present *Rattus rattus* the acrocentrics are 8 pairs while in *R. r. rufescens* there are only 7 pairs. Two small submeta-
centrics are also observed in the published karyotypes of European population of *Rattus rattus* (Capanna and Civitelli, 1970) and the African population of *Rattus rattus* (Capanna and Civitelli, 1971). The present karyotype with one sub-metacentric pair may be due to a single pericentric inversion of the medium sized acrocentric pair instead of two as in other 38 chromosome karyotypes.

Earlier studies indicated that in India, *Rattus rattus* with 2n=38 is probably confined to the southern peninsular region. Thus Lakhotia et al. (1973) reported *Rattus rattus rufescens* from South Western India to have 2n=38. T. Sharma and Rajiva Raman (1972) have reported 2n=38 both for *Rattus rattus rufescens* from Quilon and Nagpur and *Rattus rattus wroughtoni* from Quilon and Ettumanore in South India. All these places are south of Ahmedabad from where the present *Rattus rattus* have been collected. It is not known whether further north also *Rattus rattus* with 2n=38 can be found. So far all the reports have shown only 2n=42 or more for *Rattus rattus* from North India (Ray-choudhuri and Pathak, 1970; G.P. Sharma et al., 1970; T. Sharma and Rajiva Raman, 1971). It is significant, however, that not even a single *Rattus rattus* could be obtained from Ahmedabad with more than 2n=38 chromosomes, although the occurrence of supernumerary chromosomes or hypodiploid number has been reported for many of the *Rattus rattus* populations (Satyaprakash and Aswathanarayana, 1972b; Rajiva Raman and Sharma, 1974; Yosida and Sagai, 1975; Yosida et al., 1974).
A comparative study of chromosomes of various *Rattus rattus* populations with $2n=38$ and $2n=42$ or more is useful particularly using the recently developed chromosome banding techniques since this would permit tracing the homologies of different chromosomes in various populations. It has been suggested that the $2n=38$ and $2n=42$ chromosome types can be derived from each other by Robertsonian translocation (Bianchi et al., 1969; Capanna and Civitelli, 1969; Badr and Badr, 1970) and a recent study by Yosida and Sagai (1972; 1973a) using G-band techniques has provided evidence for the origin of the two pairs of large metacentrics characteristic of $2n=38$ population, from 4 pairs of acrocentrics in $2n=42$ types, since the G-bands are clearly comparable in these chromosomes. A linking population of *Rattus rattus* with $2n=40$ with only one Robertsonian translocation resulting in a single large metacentric pair is reported from Ceylon by Yosida et al. (1972).

The techniques of Arrighi and Hsu (1971) and Sumner (1972) were used to obtain the C-band patterns of *Rattus rattus* chromosomes (Aravinda Babu et al., 1975). However, the treatment of BaOH (Sumner, 1972) being much prolonged facilitated better control over the denaturation process than with NaOH (Arrighi and Hsu, 1971). The C-band pattern of this population presents usual features, characteristic of mammalian chromosomes (Yunis and Yasmineh, 1971), the heterochromatin being located exclusively in centromeric regions of all the autosomes and the X chromosome. The Y chromosome showed no
clear C-bands but showed characteristic uniformly medium intense stain as seen in other mammalian species eg., mouse, man and cattle (Arrighi and Hsu, 1971; Hsu and Arrighi, 1971; Hansen, 1972; Seth and Kunze, 1974).

The trypsin technique of Seabright (1971) gave sharper and better resolved G-bands than the SSC technique of Sumner et al. (1971). The G-bands obtained by both the techniques were similar as reported by Cooper and Hsu (1973). The G-banding revealed that the heterochromatic C-band positive regions of *Rattus rattus* chromosomes are G-band positive except for the small submetacentric pair, where the C-band positive is G-negative as seen in the human chromosome number 9 (Hsu et al., 1972).

The C- and G-banding pattern of various populations from different geographical regions have been studied by many (Yosida and Sagai, 1972; 1973a; 1975; Bradshaw, 1971; Rajiva Raman and Sharma, 1974). A great variation of C-band pattern of *Rattus rattus* population was reported by Yosida and Sagai (1975) from large centromeric blocks in Asian type to the complete absence of C-bands on atleast seven pairs of Japanese black rat. However, the present *Rattus rattus* population studied has medium sized distinct C-bands on all the autosomes and the X chromosome and shows similarity with the North American black rat reported by Bradshaw (1971) and with those described as Oceanian type with 2n=38 by Yosida and Sagai (1975). The comparison of the G-band patterns revealed the close kinship of the present population with *Rattus rattus rattus* from
Australia and *Rattus rattus rufescens* from South India, both with 2n=38 described by Yosida and Sagai (1972, 1973a). Many of the reported populations have polymorphic C-bands within the homologous pairs of the species. Such polymorphism of C-bands were not encountered in the present study.

A bis-benzimidazol derivative "Hoechst 33258" revealed the centromeric constitutive heterochromatin in mouse chromosomes (Hilwig and Gropp, 1972) as bright fluorescent regions. On the other hand, in cattle (Seth and Kunze, 1974) and in Algerian hedgehog (Hilwig and Gropp, 1972) chromosomes, the Hoechst 33258 stained centromeric heterochromatin was dull. The centromeric C-band positive regions of *Rattus rattus* chromosomes showed uniform staining with Hoechst 33258 in eu- and heterochromatin unlike mouse, cattle and Algerian hedgehog chromosomes where the heterochromatin is differently intense. However, the Y chromosome exhibited a bright fluorescence like many other mammalian species (Hilwig and Gropp, 1972; Seth and Kunze, 1974).

It is noteworthy that the present population studied had individuals with black as well as white belly, besides the different individuals studied also differ amongst themselves with respect to certain morphoanatomical features traditionally used for subspecific identification (Ellerman, 1961). However, inspite of these morphological differences, no chromosomal differences have been noted so far. All the individuals examined showed similar C- and G-band patterns.

It is expected that a comprehensive analysis of
different Giemsa and fluorescence banding patterns of Rattus rattus may elucidate the true taxonomy of this complex group.

**SUMMARY**

Rattus rattus population from Ahmedabad, situated in North West of India has diploid chromosome number of 38, unlike the Northern Rattus rattus population which have 2n=42 as reported by several workers. Majority of the specimen were similar to Rattus rattus wroughtoni, and a few resembled Rattus rattus rufescens in their morpho-anatomical features. However, all the individuals studied have identical karyotype: autosomes consist of two large metacentrics, seven pairs of large to small graded telocentrics, seven pairs of small metacentrics and a pair of small submetacentrics. The X chromosome is medium sized telocentric, and the Y chromosome in male is the smallest telocentric of the complement.

The centromeric regions of all the autosomes and the X chromosome are C-band positive, though some autosomal pairs differ in size and staining intensity of C-band region. The Y chromosome did not show any clear banding but stained uniformly with medium intensity.

The G-banding pattern helped to identify all the chromosome pairs of the metaphase complement. In the karyotype all the C-band positive regions are G-band positive except for the small submetacentric pair.
All the chromosomes of black rat showed bright fluorescence when stained with Hoechst 33258. There is no indication of banding on many of the chromosomes. However, Y chromosome is the brightest of the complement.

The chromosomes of the present population of Rattus rattus are compared with the population from various parts of the world and with different chromosome numbers, mainly using the C- and G-banding patterns.

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PLATE XIX

Fig. 1. The metaphase complement of male Rattus rattus L. with 2n=38 chromosomes from Ahmedabad (Primary kidney culture).

Fig. 2. The karyotype of male Rattus rattus with two pairs of large metacentrics (M₁ and M₂), a large subtelocentric pair (ST₁), 7 pairs of telocentrics (T₁ to T₇), 7 pairs of small metacentrics (M₃ to M₉) and a pair of small submetacentrics (SM₁). The X is medium sized telocentric and Y is the smallest telocentric.
PLATE XX

Fig. 3. The metaphase chromosomes of a female *Rattus rattus* (bone marrow) from Ahmedabad.

Fig. 4. The karyotype of female *Rattus rattus*.  
M = metacentric, ST = subtelocentric,  
T = telocentric and SM = submetacentric chromosomes.
PLATE XX
PLATE XXI

Fig. 5. The C-band staining of a male metaphase chromosomes of *Rattus rattus* from kidney culture (technique of Sumner, 1972). Note the heteropycnotic small Y with medium intensity (→).

Fig. 6. The C-band staining of a female metaphase complement from bone marrow (technique of Arrighi and Hsu, 1971).

Fig. 7. The karyotype of the male C-banded metaphase shown in fig. 5. The centromeric regions of all the chromosomes are C-band positive with a characteristic staining on Y chromosome.
Fig. 8. The idiogram showing the C-band regions on various chromosomes of \textit{Rattus rattus} from Ahmedabad.
PLATE XXIII

Fig. 9. The G-band staining of a metaphase from female black rat bone marrow (trypsin technique of Seabright, 1972).

Fig. 10. The karyotype showing the G-banded chromosomes from the metaphase shown above.
PLATE XXIV

Fig. 11. The idiogram showing the G-band patterns on *Rattus rattus* somatic chromosomes. Different staining intensities are shown in black or cross lines.
Fig. 12. The karyotype of male *Rattus rattus* metaphase chromosomes stained with 'Hoechst 33258' (from kidney culture). All the chromosomes showed the uniform fluorescence. The Y chromosome is the brightest of the complement.