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Distribution of chromosomes in metaphase plates of *Mesocricetus newtoni**

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SUMMARY

Fifty-one metaphase figures from male *Mesocricetus newtoni* bone-marrow cells were analysed with respect to the distribution of chromosomes. The peripheral location of the sex chromosomes (XY) and that of the chromosome pairs 3 and 9, as well as the non-peripheral location of the chromosome pairs, 16, 17, 18 was revealed. The significance of the peripheral location of the X chromosome and a possible explanation of the characteristic distribution of the chromosomes are discussed.

1. INTRODUCTION

While the relative constancy of the position of chromosomes in meiosis may not occur in somatic cell division, there is increasing evidence that a rather non-random distribution of the chromosomes in metaphase plates occurs during mitosis too. Schneiderman & Smith (1962) have shown that certain homologous chromosomes tend to lie together more frequently than would be expected by chance. Morishima, Grumbach & Taylor (1962) found that the late-replicating X chromosome displays rather peripheral locations in flattened metaphase figures, although German (1962) did not find any differences between the frequency of the peripheral location of the late-replicating X chromosome and that of the other chromosomes. Peripheral location of the Y chromosome in metaphase figures from cultured human leucocytes was reported by Miller *et al.* (1963*a*) as well as specific location of some other chromosomes (Miller *et al.* 1963*b*). The suggestion was made (Miller *et al.* 1963*a*, *b*) that perhaps all the chromosomes tend to occupy specific positions.

The distribution of chromosomes in flattened metaphase spreads may reflect the distribution of chromosomes in the nuclear spindle equator, assuming that the colchicine and the hypotonic pretreatment do not have differential effects on specific chromosomes. If the chromosomes in the somatic cells undergo little relative movement during interphase, it may have a functional significance (Miller *et al.* 1963*a*, *b*).

The present paper is an attempt to reveal any non-random distribution of chromo-

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somes in male metaphase plates of the Rumanian hamster (*Mesocricetus newtoni*). The karyotype of *M. newtoni* consists of 18 pairs of chromosomes: 2 pairs of metacentrics, 5 pairs of submetacentrics and 11 pairs of subtelocentrics. The *X* chromosomes are the biggest subtelocentrics of the complement, while the *Y* is the smallest submetacentric (Raicu & Bratosin, 1966; Raicu, Hamar, Bratosin & Borsan, 1968).

2. MATERIALS AND METHODS

Male Rumanian hamsters from the Department of Genetics, University of Bucharest, were used in this study. Metaphase figures were obtained from bone marrow cells of animals previously injected with 0·06% colchicine solution 2 h before killing. Hypotonic pretreatment was performed in sodium citrate, and fixation in a 3:1 mixture of alcohol and acetic acid. Aliquots of the suspension were dropped on clean slides and cells were quickly flattened and dried. The slides were stained in Giemsa solution and rinsed with water.

Metaphase figures were photographed and copies were made with a final magnification of × 2700. Only 51 metaphase plates with nearly equal diameters (about 60 mm) were selected to minimize somewhat the effect of the dispersion on the chromosome distribution.

The location of chromosomes was established by estimating the distance of the centromere of each chromosome from the centre of the metaphase plate, as determined from the mean of the co-ordinates of all the centromeres in the figure, and ascribing thus each chromosome to one of four equal concentric areas into which the metaphase figure was divided. The four equal concentric areas, designated as I, II, III, IV from the centre to the periphery, correspond each to 25% of the total area of the metaphase figure.

The chromosomes in each metaphase figure were also classified as peripheral or non-peripheral in location by the method described by Miller *et al.* (1963*a*), and the results obtained by the two procedures were compared.

3. RESULTS AND DISCUSSION

The distribution of the chromosomes in the four equal concentric areas is shown in Table 1. Heterogeneity χ^2 calculated for the 80 (4×20) observations indicates a significant heterogeneity between chromosomes.

Some of the chromosomes in *Mesocricetus newtoni* metaphase figures appear to be distributed in a non-random fashion, as revealed by the χ^2 calculated for each chromosome, using the column totals to give expected values. Thus, *X* and *Y* chromosomes and the chromosome pairs 3 and 9 have a statistically significant tendency to occupy a peripheral position. On the other hand, chromosome pairs 16, 17 and 18 are located rather near the centre of the metaphase plate.

In comparing the distribution of chromosomes in the four areas with their peripheral or non-peripheral location, as established by the method of Miller *et al.* (1963*a*) (Table 2), some chromosomes which tend to lie in the outer part of the

Table 1. *Test of significance of the distribution of individual chromosomes**

(I, II, III, IV designate the four concentric areas into which the metaphase plate was divided.)

Chromosome	I	II	III	IV	Total	χ^2†	$P <$
X	6	13	10	22	51	16·86	0·01
Y	8	14	11	18	51	8·49	0·05
1	30	30	20	22	102	1·01	0·80
2	35	20	20	27	102	2·27	0·70
3	21	25	25	31	102	8·45	0·05
4	32	28	24	17	101	2·22	0·70
5	32	22	22	26	102	1·17	0·80
6	29	25	15	33	102	6·79	0·10
7	31	20	24	27	102	2·83	0·50
8	25	26	26	25	102	3·50	0·50
9	22	23	22	35	102	10·85	0·02
10	36	24	25	17	102	2·80	0·50
11	37	23	25	17	102	3·21	0·50
12	36	29	18	19	102	1·66	0·70
13	35	27	20	20	102	0·54	0·95
14	39	26	19	18	102	2·28	0·70
15	42	25	22	12	101	7·75	0·10
16	36	36	14	15	101	9·22	0·05
17	37	33	18	11	99	9·04	0·05
18	47	26	16	8	97	17·23	0·01
Total	616	495	396	420	1927	—	—

* Heterogeneity χ^2 for the 80 (4×20) observations = 118·5; D.F. = 57; $P < 0{\cdot}01$.
† D.F. = 3.

Table 2. *Test of significance of the peripheral or non-peripheral location of individual chromosomes, as established by the method of* Miller *et al.* (1963*a*)

Chromosome	Total no.	Peripheral (%)	χ^2*	$P <$
X	51	50·98	14·08	0·01
Y	51	39·21	3·42	0·10
1	102	32·35	1·13	0·30
2	102	34·31	2·30	0·20
3	102	34·31	2·30	0·20
4	101	30·69	0·45	0·70
5	102	37·25	4·84	0·05
6	102	33·33	1·66	0·20
7	102	36·27	3·89	0·05
8	102	26·47	0·09	0·80
9	102	38·23	5·88	0·02
10	102	23·52	0·96	0·50
11	102	22·54	1·46	0·30
12	102	19·60	3·57	0·10
13	102	27·45	0·005	0·95
14	102	18·62	4·62	0·05
15	101	18·81	4·25	0·05
16	101	23·76	0·75	0·50
17	99	12·12	12·73	0·01
18	97	11·34	13·50	0·01
Total	1927	27·76	—	—

* With Yates' correction, D.F. = 1.

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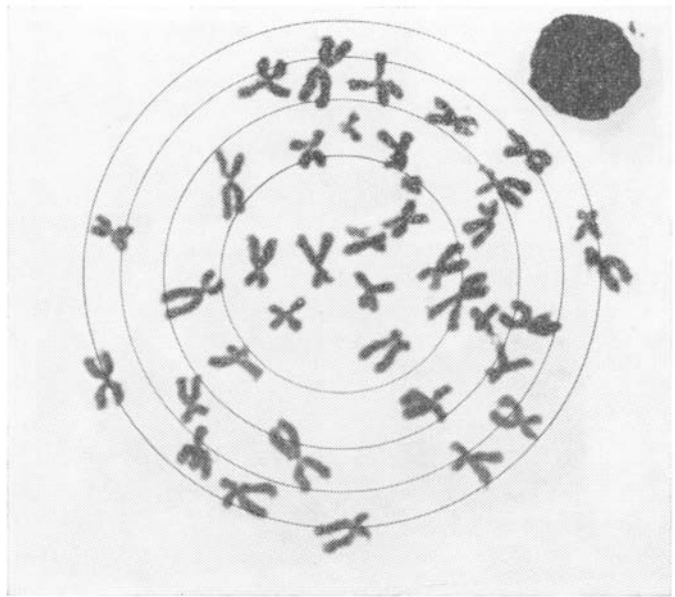

Fig. 1. The method used by us for the location of *Mesocricetus newtoni* chromosomes in four equal concentric areas.

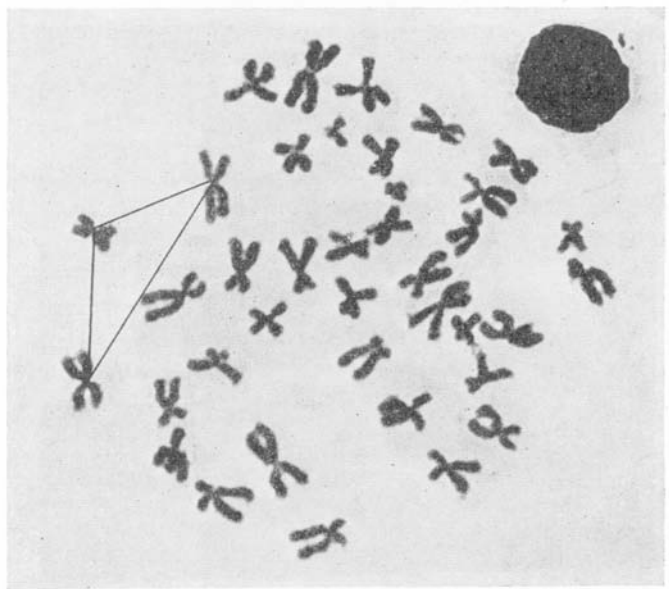

Fig. 2. The method of Miller *et al.* used for the classification of chromosomes as peripheral or non-peripheral. A chromosome is defined as peripheral if it lies outside the line connecting the peripheral chromosomes on either side of it.

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metaphase plate (X, chromosome pair 9) appear to be peripheral according to Miller *et al.* too, and chromosomes scored as non-peripheral by this latter method appear to be located in the inner part of the plate (chromosome pairs 17 and 18).

There are, nevertheless, some discrepant cases, as the two methods do not always pick out the same chromosomes as peripheral or non-peripheral. It should be pointed out that a method based on counting the frequency of occurrence of chromosomes in four areas should be more reliable than a method such as Miller's, which only judges the position of each chromosome in regard to its neighbours.

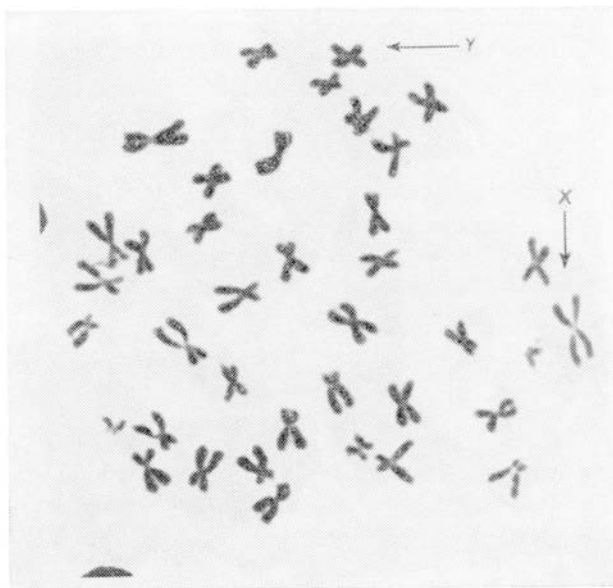

Fig. 3. The peripheral distribution of sex chromosomes in the metaphase plate of *Mesocricetus newtoni*.

It should be mentioned that the nomenclature in the *Mesocricetus newtoni* karyotype (Raicu & Bratosin, 1966; Raicu *et al.* 1968) is based only on the size of the chromosomes. The results reported here seem to suggest that the small chromosomes (16, 17, 18) are located at the inner part of the metaphase plate, while the big ones are rather peripheral in location. An exception is the Y chromosome, which, although rather small in size, is peripheral in location. The peripheral location of the Y chromosome suggests that the observed distribution of chromosomes is not a technical artifact, due to colchicine and hypotonic pretreatment, as chromosomes comparable in size with Y differ from it in location.

Miller *et al.* (1963*b*) suggested that the peripheral chromosomes are the late-replicating ones. This is probably true for the Y and X chromosomes in *Mesocricetus newtoni* too. As to the other chromosomes, no indication is yet available in this species.

The location of the X chromosome in flattened metaphase figures has not been established with certainty. However, there is some evidence supporting its peri-

pheral location (Morishima *et al.* 1962; Grumbach, Morishima & Taylor, 1963; Miller *et al.* 1963*a*, *b*). In our study, the highly significant peripheral location of the *X* chromosome in the metaphase figures of *Mesocricetus newtoni* is in good agreement with these observations.

As the inactivation of an *X* chromosome proceeds at random (Ohno & Cattanach, 1962; Lyon, 1963), it may be inferred that the male *X* corresponds to either of the two female *X*, and thus its location may suggest the peripheral location of the *X* chromosomes in both sexes. The tentative suggestion was made (Miller *et al.* 1963*a*, *b*) that a correlation exists between the peripheral location of the *X* chromosomes and the sex chromatin, which is usually found at the periphery of the interphase nucleus in female mammalian cells (Barr, 1959).

If the peripheral location of chromosomes is dependent upon the later termination of replication, as heterochromatin terminates DNA replication later than euchromatin (Lima de Faria, 1961), it is likely that the functional differences which are responsible for the characteristic distribution of chromosomes may consist in their amount of heterochromatin (Miller *et al.* 1963*a*, *b*).

The lack of information concerning DNA replication in *Mesocricetus newtoni* does not allow any conclusion about the connexion between the time of DNA replication and the location of chromosomes in this species.

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