

Chromosome Preparations of Opossum Leucocytes

Chromosomes of opossum have been studied either directly from bone marrow or other tissues established in culture¹⁻³. For the last year, both common (*Didelphys virginiana*) and woolly (*Caluromys derbianus*) opossums have been used in this laboratory for chromosome replication studies. The following technique for chromosome preparation from cultured leucocytes of opossum has proven very satisfactory; a modification of the method suggested by MOORHEAD et al.⁴ for human chromosome preparation.

Procedure. (1) Draw 2-5 ml of blood from the femoral vein in a heparin wet sterile syringe. (2) Transfer the blood sample to sterile culture tubes and keep the sample at 4°C for 1½ h. (3) Withdraw the plasma along with buffy coat and mix it with tissue culture medium (TC-199 Microbiological Associates Inc.; 15% fetal calf serum; addition of antibiotic is not necessary) in a ratio of 1 drop of plasma (by a sterile pasteur pipette) to 1 ml of the tissue culture medium. Crude extract from red kidney beans or commercial phytohemagglutinin (Difco) is added 2 drops (pasteur pipette) per ml of the mixture; crude bean's extract yields better results. (4) Set up the culture in a 1 ounce prescription bottle and incubate at 37°C. Shake the bottle after 4 h of incubation. (5) Add colchicine (1 µg/ml) after 72 h of incubation and then reincubate

for an additional 4 h prior to the time of harvest. (6) Centrifuge at 800 rev/min for 6 min and discard the supernatant. (7) Treat the cells with 1% sodium citrate (stored at 37°C) for 16 min. (8) Pour off the supernatant, fix the cells with acetic-alcohol (1:3) for 16 min at room temperature. Wash in 45% acetic acid, resuspend the cells in the fixative and spread on slides by igniting the cell suspension⁵. (9) Stain the slides with 2% Orcein in 45% acetic acid.

Comments. Because of its simplicity and its ability to facilitate good metaphase spreads (Figures 1 and 2), this

¹ E. L. SHAVER, Can. J. Genet. Cytol. 4, 62 (1962).

² J. D. BIGGERS, H. I. FRITZ, W. C. D. HARE and R. A. McFEELY, Science 148, 1602 (1965).

³ M. LEGATOR, C. JACOBSON, M. PERRY and D. DOLIMPIO, Life Sci. 5, 397 (1966).

⁴ P. S. MOORHEAD, P. C. NOWELL, W. J. MELLMAN, D. N. BATTIPS and D. A. HUNGERFORD, Expl Cell. Res. 20, 613 (1960).

⁵ E. SAKSELA and P. S. MOORHEAD, Cytogenetics 1, 225 (1962).



Fig. 1

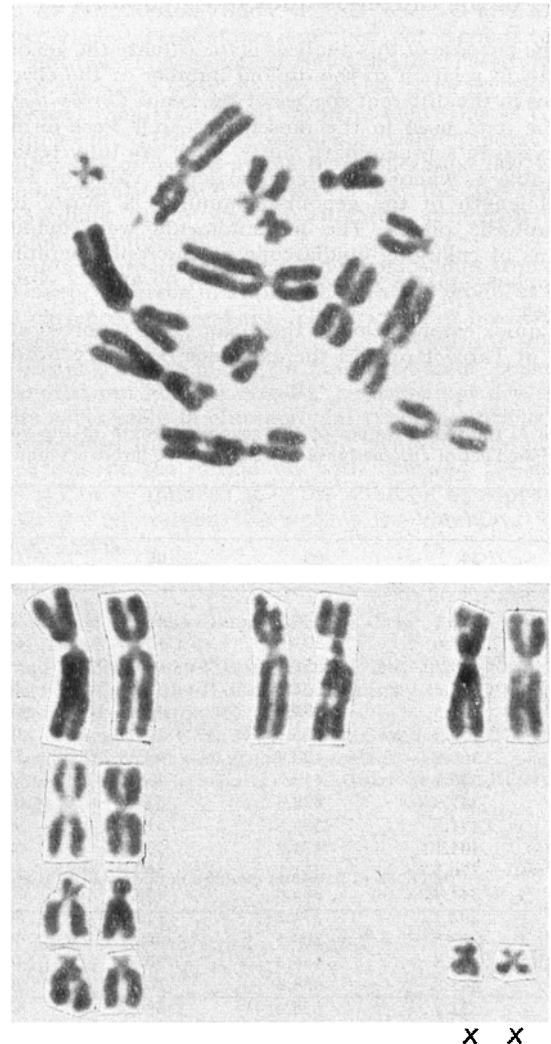


Fig. 2

Figs. 1 and 2. Leucocyte metaphase figures obtained by the technique described; final magnifications on the film were at $\times 100$. 1. Karyotype of a female common opossum (*Didelphys virginiana*). 2. Karyotype of a female woolly opossum (*Caluromys derbianus*).

method has been well adopted for our purpose. In addition, this has clarified morphological details of certain opossum chromosomes; the X-chromosomes of female woolly opossum are easily identified as the smallest sub-metacentric pair of the complement⁸.

Résumé. C'est la première fois que l'on décrit une technique pour la préparation des chromosomes de leucocytes d'opossum. Cette technique a l'avantage d'être simple et elle permet en outre de préciser les détails morphologiques de certains chromosomes de l'opossum. Par exemple, les chromosomes X de la femelle de l'opossum laineux (*Caluromys derbianus*) sont aisément identi-

fiés comme étant la plus petite paire submétacentrique du complément.

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Statistical Analysis of the Relation Between the Length of the Genoma and the Diploid Number of the Chromosomes in *Cercopithecus*

The purpose of this analysis is to evaluate the genoma's length in relation to the diploid number of the chromosomes in the different species of the genus *Cercopithecus*¹.

The data used in the present research were formerly analyzed in a preliminary way². They are fully reported in Table I, where, for every particular value of $2n$ the total length of the genoma examined is shown in 20 metaphasic plates. The measurements were made by means of enlarging epidiascope on microphotographs of metaphasic plates with $2n = 54, 60, 66$ and 72 chromosomes.

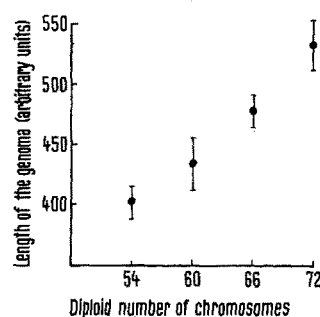
A quick examination of the mean value reported at the foot of Table I reveals the existence of a very probable

Table I. Individual values of the genoma's length in the various diploid set of chromosomes in *Cercopithecus* (arbitrary units)

$2n$				
No.	54	60	66	72
1	508.1	324.5	483.7	456.1
2	381.6	307.9	404.0	493.4
3	426.3	552.7	403.9	591.8
4	476.3	352.3	511.4	461.7
5	521.3	385.0	468.5	491.0
6	469.9	753.1	517.5	485.4
7	366.8	426.4	485.3	558.4
8	396.1	449.7	468.1	598.4
9	347.3	428.5	524.6	619.4
10	471.8	387.6	515.0	543.3
11	404.1	474.2	533.8	628.2
12	370.5	341.1	549.9	519.1
13	341.4	365.6	453.8	734.3
14	292.7	371.2	413.7	384.5
15	387.8	360.5	476.7	661.1
16	393.8	460.3	508.0	485.2
17	445.8	388.0	367.5	561.7
18	331.7	538.0	384.2	417.1
19	338.8	549.3	546.8	503.4
20	363.1	446.5	516.2	426.9
Totals	8033.2	8656.4	9532.6	10630.4
means	401.66	432.82	476.63	532.0
	± 14.08	± 23.25	± 12.40	± 19.77

linear 'trend' in the increasing of the length of the genotype in relation to the diploid number of chromosomes (see Figure). Since such an increase might lead to interesting cytological and cytogenetical considerations and interpretations, a careful analysis has been carried out to control this within the limits of the above remarks.

A variance analysis has been performed. The total variability has been broken down to the components due to the variations between groups and within groups. The results obtained are summarized in Table II.



Diagrammatic representation of the total length of the chromosomes in species with different number of chromosomes of the genus *Cercopithecus*.

Table II. Variance analysis of length of the genoma

Source of variation	Degree of freedom	Sums of squares	Variance	Variances ratio
Between groups	3	190,644.92	63,548.31	9.90 ^a
Within groups	76	487,837.54	6,418.91	
Total	79	678,482.46		

^a $p < 0.01$

¹ B. CHIARELLI, *Cytologia*, in press (1966).

² B. CHIARELLI and C. VACCARINO, *Atti Ass. genet. ital.* 9, 328 (1963).