

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 complement<sup>8</sup>.

**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*<sup>1</sup>.

The data used in the present research were formerly analyzed in a preliminary way<sup>2</sup>. 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.

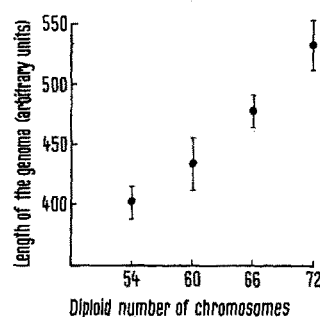
A quick examination of the mean value reported at the foot of Table I reveals the existence of a very probable

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
	$\pm 14.08$	$\pm 23.25$	$\pm 12.40$	$\pm 19.77$

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 <sup>a</sup>
Within groups	76	487,837.54	6,418.91	
Total	79	678,482.46		

<sup>a</sup>  $p < 0.01$

<sup>1</sup> B. CHIARELLI, *Cytologia*, in press (1966).

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

Since the variation between the means is statistically significant at the 10% level<sup>3</sup>, it is possible to conclude the actual existence of a heterogeneity of the material and, therefore, a real increase of the genome in relation to the diploid number of the chromosomes in this group of species.

The linearity of this increase has been controlled by means of polynomial analysis; and it has been found that the mean value calculated fits very well with the observed means<sup>4</sup>. We can therefore suppose an additive mechanism in the increase of the number of chromosomes.

**Riassunto.** Utilizzando metodiche statistiche è stato analizzato l'incremento della lunghezza totale del genoma rispetto al numero dei cromosomi in piastre a diversa ploidia nel genere *Cercopithecus*. È stato riscontrato che

la relazione numero dei cromosomi e lunghezza del genoma è significativamente lineare. È supposto un meccanismo additivo per interpretare l'incremento del numero dei cromosomi.

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<sup>3</sup> R. A. FISCHER and F. YATES (Oliver and Boyd, Edinburgh and London, 1949).

<sup>4</sup> For this part of the analysis the cooperation of Prof. F. SALVI is warmly acknowledged.

## Mutagenesis with Ethyl Methanesulphonate in *Nigella damascena* L.

The effects of ethyl methanesulphonate (EMS) on living cells are still controversial. Evidence indicates that this compound has a high mutagenic ability but its chromosome breaking ability is somewhat dubious. Many modifying factors appear to influence its effects on chromosomes<sup>1-5</sup>. The mechanisms by which these modifications occur are not well understood, although some enzyme inhibitions could possibly play a role. Because of the variable effects on chromosomes, it is not surprising that the origin of the sterility is still questionable. Previous experiments with higher plants, mainly barley and broad bean, suggested a chromosomal origin, at least partially. It seemed however that this assertion should be verified. For this reason the present experiments were performed with a quite different plant material: *Nigella damascena*<sup>6,7</sup>, which has shown a high sensitivity to ionizing radiations and mutagenic chemicals. Some peculiarities of this material make the analysis of the effects relatively simpler.

**Material and methods.** *N. damascena* L. seeds (var. Miss Jekyll blue double) were treated with EMS (Eastman Kodak) at concentrations ranging from 0.1 to 0.3 g/100 ml bidistilled water for 4 and 5 h.

Since an increase in the number of chromosome aberrations was described when copper salts are added to EMS solutions, another experiment was carried out with solutions containing  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (0.5 mg/100 ml).

For the cytological investigation, both dry and 20 h presoaked seeds were treated as mentioned above, and germinated in Petri dishes on wet filter paper at 21°C.

Root tips were fixed (Carnoy) about 50 h after the onset of germination which, in this material and under the experimental conditions described, corresponds to the first mitosis. For genetical investigation, seeds were treated the same way, planted in clay pans for about 2 weeks and then transferred into the field. Blocks corresponding to different treatments were randomized. Sterility was measured at the first generation (M1). The second generation (M2) was grown and the first leaf of the seedling stage was investigated principally for chlorophyll mutations.

**Results.** Comparative effects on chromosomes after seed treatments are given in Table I. EMS alone produces very little chromosome breakage. The amount of breakage is increased considerably when copper sulphate is added. The breakage is also higher for presoaked seeds. It should be pointed out that in *Nigella*, treatment of dry seeds results exclusively in chromosomal type aberrations, i.e. those induced (in G1) before DNA synthesis. From the comparison of the effects on both dry and presoaked seeds it can be inferred that the addition of copper increases the chromosome as well as the chromatid class of aberrations.

<sup>1</sup> J. and M. MOUTSCHEN-DAHMAN, XI Int. Congr. Genet. (La Haye) 1, 87 (1963).

<sup>2</sup> J. and M. MOUTSCHEN-DAHMAN, Radiat. Bot. 3, 297 (1963).

<sup>3</sup> J. and M. MOUTSCHEN-DAHMAN, Experientia 19, 144 (1963).

<sup>4</sup> G. BARI, Caryologia 16, 619 (1963).

<sup>5</sup> C. R. BHATIA and K. R. NARAYANAN, Genetics, 52, 577 (1965).

<sup>6</sup> J. and M. MOUTSCHEN-DAHMAN, Naturwissenschaften 52, 9 (1965).

<sup>7</sup> J. and M. MOUTSCHEN-DAHMAN, J. GILOT and M. REEKMAN, Cellule 66, 83 (1966).

Table I. Modification of chromosomal effects with EMS and  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (300 anaphases analysed in each case)

		Control	Control + Cu	EMS 0.1	EMS 0.1 + Cu	EMS 0.2	EMS 0.2 + Cu	EMS 0.3	EMS 0.3 + Cu
Dry seeds	Bridges	0	0	1	4	0	6	1	8
	Fragments	0	2	2	8	2	11	2	13
	% aberrations	0	0.66	1	4	0.66	5.66	1	7
Presoaked seeds	Bridges	0	0	0	4	1	6	2	10
	Fragments	2	2	1	10	3	20	2	26
	% aberrations	0.66	0.66	0.33	4.66	1.33	8.66	1.33	12