Since the variation between the means is statistically significant at the 10% level[3], it is possible to conclude the actual existence of a heterogeneity of the material and, therefore, a real increase of the genoma 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[4]. 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 adittivo per interpretare l'incremento del numero dei cromosomi.

B. CHIARELLI

Centro di Primatologia, Istituto di Antropologia, Università di Torino (Italy), 26th September 1966.

[3] R. A. FISCHER and F. YATES (Oliver and Boyd, Edinburgh and London, 1949).

[4] 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[1-5]. 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*[6,7], 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 $CuSO_4 \cdot 5H_2O$ (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.

[1] J. and M. MOUTSCHEN-DAHMEN, XI Int. Congr. Genet. (La Haye) *1*, 87 (1963).
[2] J. and M. MOUTSCHEN-DAHMEN, Radiat. Bot. *3*, 297 (1963).
[3] J. and M. MOUTSCHEN-DAHMEN, Experientia *19*, 144 (1963).
[4] G. BARI, Caryologia *16*, 619 (1963).
[5] C. R. BHATIA and K. R. NARAYANAN, Genetics, *52*, 577 (1965).
[6] J. and M. MOUTSCHEN-DAHMEN, Naturwissenschaften *52*, 9 (1965),
[7] J. and M. MOUTSCHEN-DAHMEN, J. GILOT and M. REEKMANS, Cellule *66*, 83 (1966).

Table I. Modification of chromosomal effects with EMS and $CuSO_4 \cdot 5H_2O$ (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

Table II. Reduction of fertility induced by increased concentrations of EMS and EMS – Cu^{++}

	Control		EMS-4 h treatment						EMS-5 h treatment					
	Without Cu	With Cu	Without Cu			With Cu			Without Cu			With Cu		
			0.05	0.1	0.2	0.05	0.1	0.2	0.05	0.1	0.2	0.05	0.1	0.2
Fruit per plant	14.2 ± 5.2	17.5 ± 2.8	12.9 ± 3.2	15.3 ± 2.7	9.3 ± 2.3	11.2 ± 2.6	21.3 ± 3.7	18.3 ± 3.9	8.4 ± 2.0	9.2 ± 3.2	8.3 ± 2.2	12.5 ± 5.9	9.6 ± 2.3	10.4 ± 4.5
Non-abortive seeds per fruit	39 ± 3.7	48.8 ± 2.0	26.0 ± 2.0	18.2 ± 1.2	8.2 ± 1.0	30.6 ± 2.3	24.8 ± 0.9	10.1 ± 0.9	17.9 ± 2.0	10.1 ± 1.5	2.7 ± 0.6	21.8 ± 1.6	12.4 ± 1.6	5.3 ± 1.3
Abortive seeds per fruit	32.9 ± 3.5	28.9 ± 1.5	42.4 ± 1.7	67.3 ± 1.7	69.2 ± 2.7	66.0 ± 3.0	71.5 ± 1.8	73.0 ± 2.0	48.6 ± 2.6	59.2 ± 3.1	61.5 ± 3.2	56.0 ± 2.0	59.0 ± 2.6	60.8 ± 4.5

Confidence limit $= \pm t_{0,05}\,\sigma$ for $\sigma = \sqrt{\frac{\Sigma\,(x-x)^2}{n\,(n-1)}}$

Table III. Mutation rate of the M2 generation

	Control		EMS-4 h treatment						EMS-5 h treatment					
	Without Cu	With Cu	Without Cu			With Cu			Without Cu			With Cu		
			0.05	0.1	0.2	0.05	0.1	0.2	0.05	0.1	0.2	0.05	0.1	0.2
Mutation rate (%)	0.42	0.43	4.53	8.4	10.34	7.11	11.24	16.15	8.74	13.51	18.75	10.82	15.82	20.0
No. of M2 plants investigated	716	925	706	594	232	661	418	260	286	74	32	545	158	15

In preliminary genetic investigations, 0.1 g/100 ml/7 h of EMS was found to induce complete sterility. Pollen appeared normal and fruits ripened but there was no seed observed. This indicated that the major part of the sterility was diplontic rather than haplontic. Data for the reduction of fertility obtained at lower doses are reported in Table II. Mosaics were observed sometimes in the M1 generation at a rather high rate (up to 20%). The origin of these mosaics is not readily explainable.

A high proportion of *viridis* chlorophyll mutations was found in the M2 generation. Data are summarized in Table III. The number of mutations generally increased with EMS dose and there is a significant increase of mutations when copper is added. Morphological mutants were found to be less common than chlorophyll ones. However, it should be kept in mind that only the early stages of the life cycle of this species is being observed, and the production of changes not detectable at this early stage may have been produced. When copper was added to the EMS solution it did not appear to alter the spectrum of chlorophyll mutations.

Discussion and conclusion. In this investigation, it is quite apparent that *N. damascena* is sensitive to EMS compared with other higher plants, e.g. barley and broad bean. The dose of EMS utilized could not be increased without inducing a very high sterility.

The occurrence of fruits without seeds or with abortive seeds shows that a major part if not all the induced sterility is diplontic. This is in contrast to seed treatments in barley in which chromosomal rearrangements, as observed in M1 meiosis, may be involved in the decreased fertility. A plausible explanation could be that in some biological materials the action of EMS is more greatly influenced by modifying factors. Such modifying factors could possibly be inherent in the material itself rather than due to the experimental conditions of the treatment. However, in *Nigella* modifying factors like copper influence the chromosome breakage and the M2 mutation rate and slightly the M1 fertility. The difference could be explained on the basis of a differential selection of aberrations[8].

Résumé. Des graines de *Nigella damascena* ont été traitées par des doses d'EMS allant de 0,1 à 0,3 g/100 ml/4–5 h. On a pu confirmer qu'aux doses utilisées, l'EMS n'exerce que peu d'effet sur les chromosomes. Cependant, l'adjonction d'une certaine quantité de cation cuivre accroît très fortement l'effet au niveau chromosomique. On a étudié la stérilité induite par les différents traitements à la génération traitée et le taux de mutations au cours de la génération ultérieure. L'adjonction d'une certaine quantité de cuivre n'influence que faiblement la fertilité mais accroît le taux de mutations. Comparée à d'autres plantes, la nigelle est particulièremen sensible aux traitements.

J. GILOT, M. and J. MOUTSCHEN-DAHMEN

Laboratoire de Génétique, Université de Liège (Belgium), 2nd March 1967.

[8] The present work was aided by the Centre National d'Etude des Mutations (Belgium). The authors wish to thank Dr. R. W. BRIGGS (Brookhaven National Laboratory) for valuable discussions and criticism of the manuscript. They are grateful to Mrs. N. LABOURY, Mr. F. GILARD and Mr. S. KOZDROWSKI for skillful and efficient assistance.