Although it is not reflected in table 2, in some of the progeny I have found some individuals which carry the reversion of the cn^{rbr} allele (phenotype b^+, cn^{+c}, vg^+) and some others which show the reversion of the *cn* allele (Phenotype *b*, cn^{+c}, *vg*). This suggests that in the same individual progenitor there may occur more than 1 reversion.

Through appropiate crosses, I substituted the 3rd chromosome in the unstable cn^{rbr}/cn heterozygote, which then remained unstable (table 3). I obtained similar results when I substituted the X chromosome. These results lead us to suppose that the cause of instability is located in the 2nd chromosome which also contains the *cinnabar* locus and the marker loci used.

In order to know whether cn^{+c} revertants alleles are stable or not, I have crossed flies carrying a cn^{+c} allele with a balanced strain (*Cy0, Curly* derivative of Oster, whose genotype is In(2LR), 0, dp^{ive}, *Cy, pr,* cn^2/Bl, *cn, bw).* From the offspring I have selected males with curly wings and wild type eye color (carrying the *Cy*0 chromosome and the cn^{+c} allele in its homologue). These males were mated with *cn/cn* homozygous females. In the offspring of those crosses, 2 types of reversions were noted: 1st, from cn^{+c} to cn^{rbr} and 2nd, from cn^2 to cn^{+c} (this 2nd reversion in interesting because it is a new *cn* allele that reverts by the action of the mutator agent). Without reversions, the offspring expected will be divided into 2 classes: one, with curly wings and cinnabar eyes, another showing wild type wings and eyes. But if reversions take place, 1 or 2 more phenotypes may occur: a) curly wings, wild-type eyes, indicating that a cn^2 allele has reverted to cn^{+c}, and b) wild type wings and cinnabar eyes indicating that a cn^{+c} allele has back-reverted to a *cn* allele. The 3rd and 4th columns in table 2 present the results obtained from such crosses (revertants of both are presented only), and we can see, firts that the mutator agent acts against cn^2 alleles, and secondly that the cn^{+c} alleles are not stable since they can rever again. Appearance in clusters leads us to suppose that both events occur in phases prior to meiosis.

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Gene controlled condensation in individual chromosomes[1]

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Summary. When cells were irradiated with variable doses of gamma rays, 0.33% showed the appearance of single decondensed chromosomes (SDC) at the moment at which all the other chromosomes of the complement exhibited the normal condensed state corresponding to metaphase stages. Several hypotheses are discussed to explain the origin of SDC. It appears that the most reasonable mechanism to explain our observations is to assume that the process of chromosome condensation is independently controlled in each individual chromosome by a gene/s located in each one of the chromosomes of the complement. A radiation-induced deficiency in one of these genes may produce an impairement in the normal process of condensation of the carrier chromosome which would give rise to SDC.

It is known that changes in the extent of chromosome condensation have a great influence on gene transcription and replication[3]. Genetically active regions are usually decondensed, whereas chromatin condensation is usually found in genetically inactive areas[4]. Likewise, an extended state of the chromatin also seems to be necessary for the chromosome replication to occur[5]. Some time ago, Mazia[6] suggested the existence of orderly changes in the degree of chromosome condensation through the cell cycle. However, due to the difficulties inherent in the analysis of chromosomes in interphase nuclei this suggestion remained to be confirmed. Recently, by using the technique of premature chromosome condensation[7,8] it became possible to visualize the chromosome cycle in interphase HeLa cells[8]. The data obtained demonstrate that there is a progressive chromosome decondensation during the G1 and a progressive chromosome condensation during the G2 phase.

Though the cycle of decondensation-condensation is a general process involving the whole of the chromatin, it is not necessarily simultaneous for all chromosomes or chromosome regions. Since the classical paper of Heitz[10] on heterochromatin it has been well known that certain chromatin areas have an asynchronous cycle of condensation (allocycly) which gives rise to the phenomenon of positive and negative heteropycnosis[11]. Hence, it seems evident that different chromosomes or different intrachromosomal areas may have independent mechanisms controlling the phenomenon of coiling.

In this report evidence is given suggesting that gamma radiation may produce an impairment in the process of chromosome condensation, and the probable causes of this phenomenon are discussed.

Material and methods. Two series of experiments were performed. In the 1st series a total of 8 *Akodon molinae* (Rodentia Cricentidae) were irradiated with 400 R. At 8, 12, 16 and 20 h after irradiation the animals were sacrificed. Colchicine (0.1 µg/g of b.wt) was injected 1.5 h, before

Correlation between the frequency of SDC and gene mutation rates

Gamma-ray doses	Expected mutation rates*	Observed frequency of SDC	Cells analyzed	Relative frequency of SDC
0	-	-	1600	-
100	0.7×10^{-3}	0	400	0
200	1.4×10^{-3}	1	400	2.5×10^{-3}
400	2.8×10^{-3}	3	900	3.3×10^{-3}
800	5.6×10^{-3}	5	700	7.0×10^{-3}

* Estimated with the equation in the text.

killing the specimen. Bone marrow chromosome spreads were prepared and stained with Giemsa.

In a 2nd set of experiments a total of 16 *A. molinae* were sorted out in 4 groups of 4 animals each. The 1st group received 100 R, the 2nd 200 R, the 3rd 400 R and the 4th 800 R of gamma radiation respectively. 14.5 h after irradiation all the animals were i.p. injected with colchicine and sacrificed 1.5 h later. Chromosome spreads were prepared and stained with Giemsa.

Irradiation was performed with a Theratron 780 cobalt 60 source at a dose of 200 R/min. The animals were placed in special cardboard boxes and simultaneously irradiated in groups of 4 animals.

Four nonirradiated *A. molinae* were injected with colchicine and sacrificed 1.5 h later. Bone marrow spreads from these animals were used as controls. All the animals used were adult females from the laboratory colony of the IMBICE.

Results. In the 1st set of experiments it was possible to determine that 16 h after the irradiation with 400 R, 48.2% of the metaphases showed chromosome-type aberrations. This figure was lower at 8, 12 and 20 h. Moreover, at 8- and 12-h intervals there was an increase in chromatid-type aberrations which later fell off (fig. 1). Therefore, it was assumed that most of the cells irradiated 16 h before mitosis were in G1 at the moment of irradiation. Accordingly, this time interval was employed in the experiments with increasing doses of gamma radiation.

Over a total of 2400 irradiated cells analyzed, 8 showed the image depicted in figures 2–4. Most chromosomes of these cells exhibited the usual condensation pattern corresponding to metaphase stages; yet, 1 chromosome in 7 cells and 2 chromosomes in 1 cell were unusually extended exhibiting a characteristic pattern of G-banding. These lengthened elements were considered to result from an impairment in the process of chromatin condensation. Since these decondensed chromosomes had 2 chromatids (figs 1–4) it was assumed that the phenomenon of deficient condensation occurred after the period of chromosome replication.

The appearance of single decondensed chromosomes (SDC) was not observed in control cells (1600 cells analyzed). It was therefore concluded that chromosome uncoiling was the direct result of gamma radiation on the cell.

One of the 8 cells with SDC was found following the treatment with 200 R, 3 cells (belonging to 2 different specimens) were detected after the treatment with 400 R and the remaining 4 cells (obtained from 3 different specimens) appeared after the treatment with 800 R. Yet, the number of cells with SDC was too small (0.33%) to establish a correlation between the frequency of decondensation and the radiation dose.

Five out of the 9 decondensed chromosomes could be identified with accuracy on the basis of their G-banding patterns. The remaining 4 chromosomes did not show a band distribution clear enough to allow a correct characterization. Decondensed chromosomes were: 2 cases of chromosomes No. 1 (figs 2 and 3); 1 chromosome No. 5 (fig. 4); 1 chromosome No. 3 (fig. 4) and 1 chromosome No. 5 (not shown in the figures).

Discussion. Three different mechanisms can be considered to explain the appearance of SDC: a) multiple breakage of the DNA molecule, b) damage of the protein scaffold involved in the process of chromosome condensation, c) induced-gene mutation.

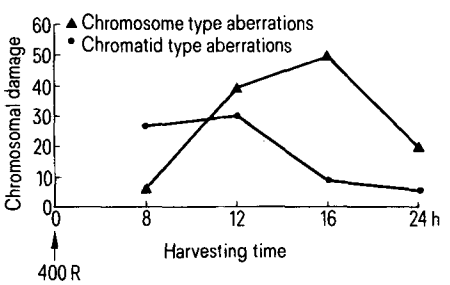
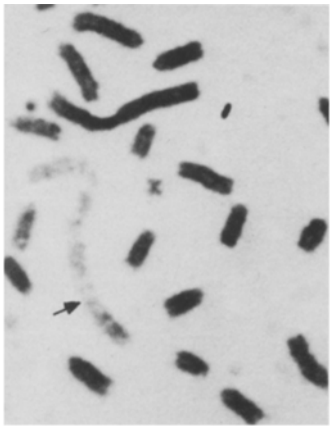
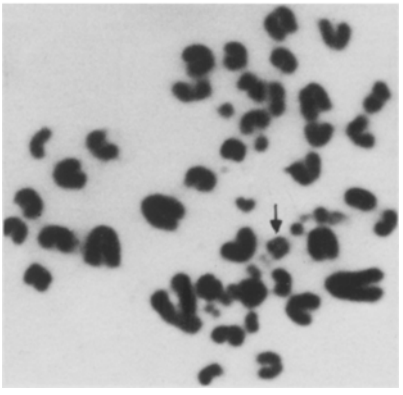


Figure 1. Frequency of chromosome- and chromatid-type aberrations at various time-intervals after irradiation with 400 R of gamma-rays.





Figures 2 and 3. Metaphases showing SDC (chromosomes No. 1). × 1200.

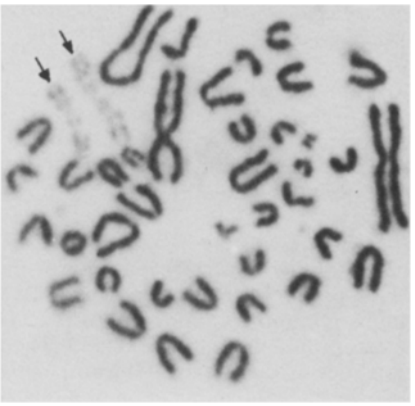


Figure 4. Metaphase showing 2 SDC (chromosomes No. 3 and No. 5). × 1200.

a) Recently it has been shown that the induction of DNA breaks by radiation inhibits the appearance of the supercoiling characteristic of the mitotic chromosome[12]. Therefore, SDC might result from the persistence of open breaks in the chromatin fibril. However, the existence of chromosome and chromatid breakages not accompanied by decondensation, and the lack of breakages in the 9 decondensed chromosomes described here, make this hypothesis improbable.

b) There is growing evidence suggesting that the folding and assembly of the long interphase chromatin fibril to give rise to the condensed metaphase chromosome depends on a chromosome scaffold formed by non-histone proteins[12,13]. Lately, it has been proposed that scaffold proteins become associated with the nuclear membrane in interphase cells and that the regions of DNA-scaffold protein association are particularly radiosensitive[14]. Thus, it is possible to speculate that the radiation-damage induced in the G1 phase in the scaffold-DNA complexes may be expressed in metaphase stages as chromosome decondensation.

There are data indicating that about 10% of each chromosomal DNA molecule is attached to the nuclear membrane, probably in the form of DNA-scaffold complexes[14]. The gamma-ray doses employed in this experiment probably have the limited capability of producing only scattered damage in just a few of the DNA-scaffold protein complexes. Thus, a phenomenon of decondensation restricted to discrete areas of one or perhaps only a few metaphase chromosomes could be expected to result from the above radiation-induced lesion. Conversely, the decondensation found in this experiment comprises the entire length of one or more chromosomes.

c) As a 3rd alternative to explain the appearance of SDC it may be proposed that a gene/s controls the degree of condensation of each chromosome. Thus, when ionizing radiation or any other agent induces a mutation in this gene the carrier chromosome will suffer a condensation impairment.

In mice it has been determined[15] that the mutation rate per R per locus is 1.7×10^{-7}. *A. molinae* has approximately the same amount of DNA as mice[16]. Accordingly, it may be assumed that the mutation rate in these 2 species is approximately similar[17]. Therefore, the mutation rate of the gene/s for chromosome condensation can be estimated with the following equation: $1.7 \times 10^{-7} \times D \times n$; in which D = radiation dose in R, and n = diploid number (42 for *A. molinae*). The table gives the expected frequencies of mutation, the absolute and relative frequencies of SDC found and the total number of cells analyzed in each radiation dose. It can be seen that an acceptable coincidence exists between the expected mutation rates and the observed relative frequencies of SDC.

Although the findings in this report fit the predictions of the mutation hypothesis, it is worth bearing in mind that this hypothesis is still speculative and that further experimental work will be needed to confirm its validity.

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Chromosomal findings on eight species of European *Cryptocephalus*

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Summary. Chromosome studies on 8 species of European *Cryptocephalus*; *C. aureolus* Suffr., *C. capucinus* Suffr., *C. globicollis* Suffr., *C. hypochoeridis* L., *C. moraei* L., *C. rugicollis* Ol., *C. sexpustulatus* Vill., and *C. violaceus* Laich. have shown an identical karyotypic formula, $14^{II} + Xy_r$, 2n = 30. Most species of *Cryptocephalus* share 2n = 30 chromosomes. The only interspecific differences are in the size of bivalents and in the sex-determining systems. The chromosomal interrelationships of *Cryptocephalus* with other allied groups are also discussed.

The genus *Cryptocephalus* Geoffr. (Coleoptera, Chrysomelidae) is one of those groups of beetles particularly rich in species, with more than 1600 taxa described[2]. Its geographical distribution is almost cosmopolitan; it is represented everywhere except in the Australian region. The morphology and systematics of the nearly 200 European species has been well known since the publication of a relatively recent monograph[3]. The species of *Cryptocephalus* differ in size, color, punctuation, shape of pronotum ... etc., but they look very similar in general features. Our aims are to provide chromosomal data on this genus for a better understanding of its cytotaxonomy and karyological evolution.

Materials and method. Eight species of *Cryptocephalus* from different Spanish sources were chromosomally analyzed in 1979–1981, and only male adult specimens were used in our study. The species, number of specimens and localities were as follows: *C. aureolus* 2 specimens from Vallcebre (Barcelona), *C. capucinus* 7 specimens from La Garriga (Barcelona), *C. globicollis* 2 specimens from Vallcebre (Barcelona), *C. hypochoeridis* 2 specimens from Vallcebre (Barcelona)