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)

and 2 specimens from Espinelves (Girona), *C. moraei* 1 specimen each from Planoles (Girona), Sant Marçal and Aiguafreda (Barcelona) and 2 specimens from Vidrà (Girona), *C. rugicollis* 2 specimens from Moral de Calatrava (Ciudad Real) and 2 specimens from Sils (Girona), *C. sexpustulatus* 5 specimens from Vallcebre (Barcelona), 2 specimens from Sant Marçal and 1 specimen from Aiguafreda (Barcelona), and 2 specimens from Vidrà (Girona), and *C. violaceus* 4 specimens from Vallcebre (Barcelona) and 2 specimens from La Riba (Tarragona).

The specimens captured in the field were immediately analyzed in the laboratory. From each individual, testes were removed and teased on a slide covered with a few drops of acetic orcein (from Gurr's), stained for 15 minutes and subsequently squashed. The best metaphase spreads were photographed using a Zeiss Photomicroscope. 5 cells at least were counted in each individual checked.

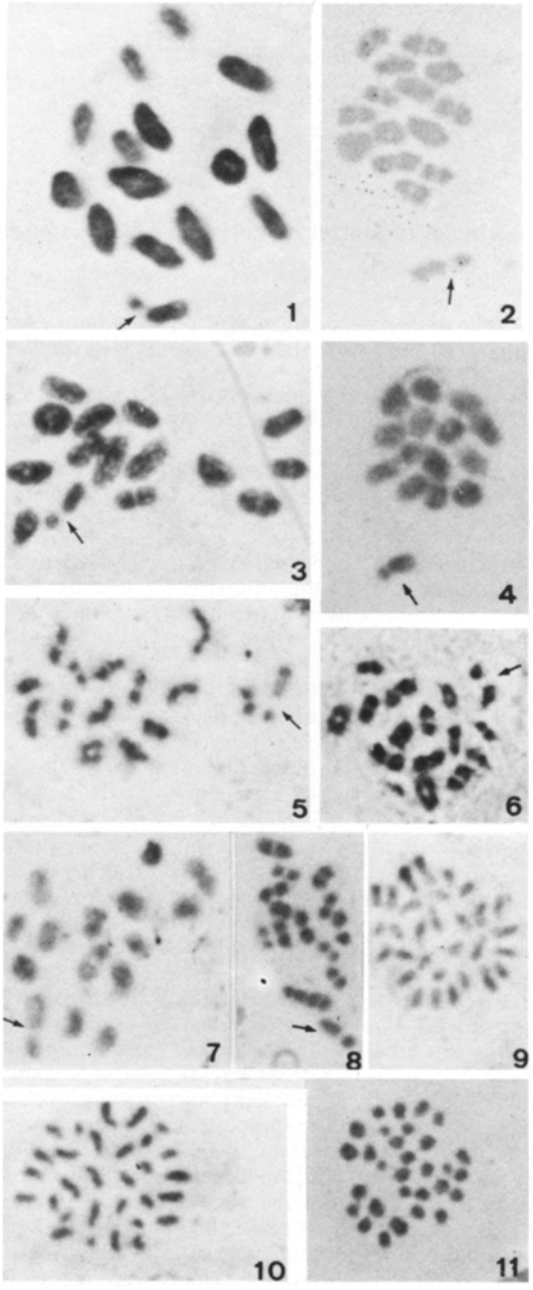
Results. All the 8 species of *Cryptocephalus* examined share an identical number of chromosomes, 2n = 30, and the same karyotypic formula, $14^{II} + Xy_r$, that is a sex-determining system of the 'rod' type. Metaphases I were obtained in all the 8 species (figs 1–8), and spermatogonial metaphases in *C. moraei* (fig. 9), *C. sexpustulatus* (fig. 10), and *C. capucinus* (fig. 11). In spite of the identity in number and sex-determining system, the eight species of *Cryptocephalus* may be separated into 2 rough groups according to the size of meiotic bivalents because those of *C. globicollis* and *C. violaceus* (figs 1 and 3, respectively) were slightly larger than any of the remainder. The mitotic chromosomes of the few available metaphases were of small size and metacentric shape though the position of each centromere was not clearly apparent (figs 9–11).

Discussion and conclusions. The number of chromosomes in the species of *Cryptocephalus* is characterized by a quite striking homogeneity (table). Only 4 out of the 18 species studied deviate from 2n = 30 chromosomes, a number which should undoubtedly be taken as the modal value. However, identity in number of chromosomes among allied species does not imply absence of chromosomal differentiation. Some of our species of *Cryptocephalus* show differences in the size of bivalents which could possibly be attributed to the amount of heterochromatin, like those reported among the species of *Dermestes* beetles[11,12]. Besides that, there are also differences in the sex-determining systems. The species analyzed here all have the 'rod' type of association between sex-chromosomes, Xy_r, while most of the previously checked ones had the 'parachute' type, Xy_p. The discrepancy between both kinds of results should prompt further analyses on more species of *Cryptocephalus* to find the modal sex-system for the genus. The low chromosomal variability found in the species of *Cryptocephalus* could be accounted for by the substantial capacity for dispersal due to their flying abilities. Bush et al.[13] reported in this sense that the groups of vertebrates with

Chromosome numbers and male sex-systems in *Cryptocephalus*

Species	2n	Karyotypic formula	References
C. analis Ol.	30	$14^{II} + Xy_p$	Yadav[4]
C. aureolus Suffr.	–	$14^{II} + Xy_r$	*
C. capucinus Suffr.	30	$14^{II} + Xy_r$	*
C. globicollis Suffr.	–	$14^{II} + Xy_r$	*
C. hypochoeridis L.	–	15^{II}	Barabas and Bezo[5]
C. hypochoeridis L.	–	$14^{II} + Xy_r$	*
C. moraei L.	30	$14^{II} + Xy_r$	*
C. octopilosus Baly	32	$15^{II} + Xy_p$	Sharma and Sood[6]
C. oppositus Jac.	30	$14^{II} + Xy_p$	Sharma and Sood[6]
C. quadruplex Newn.	–	$11^{II} + Xy_p$	Smith[7]
sp.	30	$14^{II} + Xy_p$	Yadav[4]
sp.	30	$14^{II} + Xy_p$	Yadav[4]
C. rugicollis Ol.	–	$14^{II} + Xy_r$	*
C. sexpunctatus L.	16	$7^{II} + Xy_p$	Takenouchi and Shiitsu[8]
C. sexpustulatus Vill.	30	$14^{II} + Xy_r$	*
C. sexsignatus F.	30	$14^{II} + XY$	Kacker[9]
C. triangularis Hope	32	$15^{II} + Xy_p$	Sharma and Sood[6]
C. venustus F.	–	c 12^{II}	Smith[10]
C. violaceus Laich.	–	$14^{II} + Xy_r$	*

* Present paper.



Figures 1–8. Metaphases I of *C. globicollis* (1), *C. rugicollis* (2), *C. violaceus* (3), *C. hypochoeridis* (4), *C. moraei* (5), *C. sexpustulatus* (6), *C. aureolus* (7), and *C. capucinus* (8). All of these metaphases show $14^{II} + Xy_r$, and the 'rod' type of sex-determining system is arrowed.

Figures 9–11. Spermatogonial metaphases of *C. moraei* (9), *C. sexpustulatus* (10), and *C. capucinus* (11) × 2000.

great vagility generally have much more homogeneous karyotypes than those of reduced vagility because of the smaller probability of any chromosome mutation being fixed. On the other hand, the modal chromosome number of *Cryptocephalus*, 2n = 30, enables a clear separation with respect to other species of Cryptocephalinae, like those of *Pachybrachis*, with 2n = 16, or those of the close Clytrinae subfamily, with 2n = 22-24 mostly[14,15]. A common karyological trait of the 2 major groups of Camptosomata chrysomelids, Cryptocephalinae and Clytrinae, is the small size of their chromosomes, a characteristic which is also shared with those of Megalopodinae[16], the presumed ancestor subfamily of both groups.

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The nucleolar organizer in meristem cells of *Allium cepa* L. bulbs

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Summary. The nucleolar organizer (NOR) was studied in nucleoli of dormant meristems cells of *Allium cepa* L. bulbs by means of electron and light microscopy. Nucleoli are mainly fibrillar and they present a clear segregation between fibrillar and granular components. In these cells, the NOR, whose electron density is lower than the NOR heterochromatin, appears as a compact single mass located inside the nucleolus. These ultrastructural characteristics make NOR visualization possible at the light microscopic level by the complementary use of both silver impregnation and Feulgen staining techniques.

The structure of the nucleolar organizer (NOR) has been extensively studied at the ultrastructural level, both in animal and plant cells[2,3]. Basically, it is formed from a bundle of chromatin fibers penetrating into the nucleolar mass[4-6].

The nucleolar ultrastructure pattern varies according to the functional stage of the cell[7,8], appearing segregated in the dormant cells of *Allium cepa* L. seeds and bulbs[9-11]. It has been suggested that the nucleolar organizer itself participates in the mechanism of segregation[3,6,12]. However optical visualization of the NOR in interphase nuclei, in relationship with other nucleolar components, has not been extensively studied. The present report deals with the visualization of the NOR at light microscopy level in dormant meristem cells of *Allium cepa* L. bulbs. These optical images are compared with those observed at the ultrastructural level.

Material and methods. Root primodia were dissected from the crowns of *Allium cepa* L. bulbs. Silver impregnation, according to Fernández-Gómez et al.[13] and Feulgen staining, with hydrolysis in 5 N HCl at 20 °C for 1 h, were used for the NOR study at the light microscopic level. After staining, squashes were prepared from the terminal 2 mm of each root. Permanent mounting was carried out following Conger and Fairchild's method[14]. For electron microscopy, the 2nd mm from the root tips were fixed in 2% glutaraldehyde in 0.025 M cacodylate buffer pH 7.2 for 2 h and postfixed in 1% osmium tetroxide in the same buffer for 1 h. Afterwards, the root segments were embedded in Epon 812 according the current procedures. Ultrathin sections were stained with uranyl acetate and lead citrate. The preparations were examined with a Phillips EM-300 electron microscope.

Results. Figures 1 and 2 show ultrastructural views of the nucleolus in the dormant meristem cells. It can be seen that the nucleolus is very compact, and the segregation of the nucleolar components is plainly apparent where the fibrillar part is surrounded by a very thin rim of granules. Penetration of a less dense material into the nucleolar mass is clearly seen. The morphological pattern of this region coincides with the one previously described as the NOR in *Allium cepa* L.[6,11]. The NOR zones are continuous with the adjacent chromatin mass, called NOR-associated chromatin or NOR heterochromatin by Phillips[15]. In some cases, 2 of these masses are associated with a single intranucleolar NOR (fig. 2).

Figures 3 and 4 show meristem cell nuclei as they appear in dormant unsprouted roots after silver impregnation, which preferentially contrasts the fibrillar nucleolar component. A very compact nucleolus is penetrated by a clear zone corresponding to a less stained material. This material crosses the nucleolus either completely (fig. 3) or, more often, partially (fig. 4).

Figures 5 and 6 show nuclei from similar meristem cells as they appear after Feulgen staining. The nucleoli are unstained though Feulgen positive masses are detected within them. In some cases, a Feulgen-positive mass seems to penetrate the nucleolus (see arrows, fig. 5 and 6).

When 2 nucleoli are present, a Feulgen positive mass is present in each of them (fig. 6). However, in nuclei where both nucleoli have fused in a single nucleolus 2 such Feulgen-positive masses are detected (fig. 5).

The Feulgen-positive areas correspond to the NOR heterochromatin while intranucleolar NOR is not evident. On the other hand, silver impregnation allows us to follow the channel occupied by intranucleolar NOR which remains unstained.

Discussion. The nucleolar organizer (NOR) has been described in meiotic cells[15,16] and in somatic metaphase chromosomes by silver impregnation[17] and by the N-banding technique[18]. However these techniques do not provide information about the structural relationships between the