Table 2. Esterase 3 and acid phosphatase 2 alleles for different species (bracket means a low allele frequency)

	Enzymes EST 3								ACPH 2				
Species \ Alleles	A	B	C	D	E	F	G	H	A	B	C	D	E
santonensis, France						+	+	+				+	+
(lucifugus) grassei, France, North-Spain				+	+				+	+			
(lucifugus) banyulensis, East-Spain	+	+	(+)						+	+			
(lucifugus) grassei × *banyulensis*, South-West Spain, Portugal	+	+	+	+	+				+	+			
(lucifugus) lucifugus, Italy	+	(+)	+						+	+			
(lucifugus) balkanensis, Yougoslavia, Greece	+	+									+		

and Greece (fig. 4 and table 2). Intermediate populations in the circular overlap[5] *(banyulensis x grassei)* have 5 alleles for esterase 3. The true *banyulensis* and *lucifugus* species can be recognized by their different allelic frequencies of esterase 3 (table 2). In Italy *[(lucifugus) lucifugus]* the C allele is present at a 0.56 frequency and in Eastern Spain at a 0.15 frequency and the B allele is present at 0.05 in Italy and at 0.36 in Eastern Spain. Differences between *Apis mellifera ligustica* in Italy and *A.m. mellifera* in France[10] are very similar.

In *Reticulitermes*, a study of the enzymatic polymorphism is the only method for discriminating between European species using workers, but the specific level separating these species was studied using mixiologic analysis: sexual attraction[2] and social group recognition.

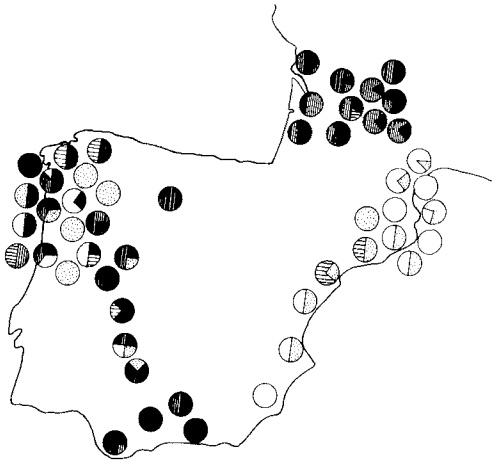

Figure 4. Distribution of esterase 3 genotypes from the Iberian Peninsula. Est 3 allele frequencies are given as segments of circles for populations: -white: allele A, -dotted: allele B, -horizontal hatching: allele C; -vertical hatching: allele D, -black: allele E.

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Chromosome arrangement throughout mitosis and interphase in *Allium sativum* (Liliaceae)

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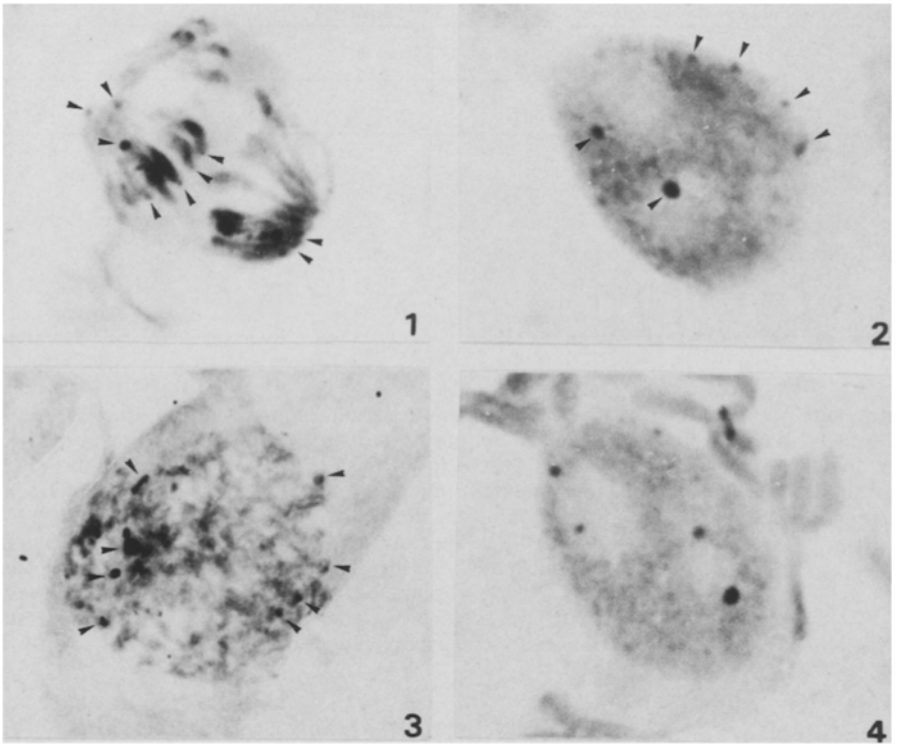
Summary. Allium sativum (garlic) root-tip chromosomes were subjected to a C-banding procedure. In addition to the nucleolar bands reported previously in this species, bands which are telomeric or close to the telomeres have been detected in some pairs. This has allowed us to analyze the arrangement of chromosomes during interphase.

Due to the uncoiled and extended condition of the greater part of the chromosomes during interphase, the chromosomal arrangement at this stage is always more difficult to investigate than that during mitosis. Nevertheless, it can be studied by preferential staining of constitutive heterochromatin or by cytological detection of a specific replication pattern (mainly late replication) after labeling of DNA. In both cases, a comparison between the distribution of chromocenters observed at interphase and the position of chromosome regions preferentially stained at mitosis can be carried out.

According to a number of reports, the spatial arrangement of chromosomes during interphase is quite similar to that observed at telophase[1-8], and this situation has been compared with the attachment of the bacterial chromosome to a specific region of the cell membrane[9].

In a cytovariety of *A. sativum*, Ghosh and Roy[4] reported C-bands in only 2 submetacentric nucleolar chromosomes,



Figures 1–4. Giemsa C-banding at mitosis and in interphase nuclei of *Allium sativum*. *1* Cell at telophase showing polarized nucleolar and telomeric C-bands (arrowheads). *2* Interphase nucleus in which the polarization is also evident. *3* Maintenance of the chromosomal arrangement at prophase. *4* Fused nucleoli as a consequence of the association of nucleolar C-bands.

close to the secondary constrictions. In the present report we have carried out C-banding in this species and, in addition to the nucleolar bands reported previously, bands which are telomeric or located close to the telomeres have been detected in 3 chromosome pairs. This fact has allowed us to analyze the orientation of interphase chromosomes, using the advantage provided by the 2 different locations of C-bands.

Material and methods. Root meristems of *Allium sativum* obtained from commercial sources were used. The garlic bulbs were placed in tap water at 25 ± 0.5 °C to facilitate root growth. The tap water was renewed every 24 h and continuously aerated. Only the bases of the bulbs remained submerged. The fixation of roots was carried out when they reached a length of 10–20 mm. Fixation was at 4 °C in a mixture of ethanol-acetic acid (3:1). C-banding was performed as follows. The fixed roots were subjected to an enzyme maceration with 0.5% pectinase (Sigma, from *Aspergillus niger)* dissolved in citrate buffer adjusted to pH 4.2 at 37 °C for 1 h. Squashing of the roots was carried out in 45% acetic acid on gelatined slides, and then the coverslips were removed by the dry ice method, the preparations were dehydrated by passing them through ethanol, and eventually air dried. Subsequently, the slides were treated with 5% barium hydroxide at room temperature for 15 min, washed in acetic acid to eliminate residues of carbonate and placed in 2× SSC (standard saline citrate) at 60 °C for 1 h. Finally, they were washed in 0.15 M Sörensen phosphate buffer at pH 6.8, stained with 3% Giemsa (Fluka) dissolved in the same buffer for 10 min, air dried and mounted with DPX.

Results and discussion. Telophase chromosomes of *A. sativum* showed a characteristic C-banding pattern (fig. 1). The Giemsa C-bands telomeric or located close to the telomeres appeared to be arranged facing the cell plate in formation. On the other hand, the larger C-bands adjacent to the nucleolar organizers of the 2 submetacentric chromosome pairs were located near the opposite pole of the nucleus. As a result, a clear polarity in the banding pattern of the nucleus is visible.

In interphase cells, this polarity was also evident (fig. 2), suggesting that the mode of arrangement of the chromosomes at telophase tends to remain during interphase. In the same way, when the cells enter prophase, the C-banding pattern reveals a similar mode of distribution of the chromosomes (fig. 3). These results agree with those reported by different authors about the maintenance of the spatial chromosomal arrangement and orientation at interphase.

Another important feature observed has been the variation in the relative position of the nucleolar C-bands (figs. 2 and 4), as was reported by Ghosh and Roy[4]. As a result of the association of these bands, fused nucleoli were found in many cases (fig. 4). On the other hand, the distribution-range of the telomeric bands was found to be nearly constant. In our opinion, this provides evidence in support of the idea that telomeric or centromeric C-blocks are possibly attached in some way to the nuclear membrane[2,4,6,8,10–12] whereas the nucleolar C-bands located interstitially could remain free in the nucleoplasm[4]. These latter heterochromatic blocks sometimes appear to be fused during interphase.

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