60 blastomeres), is composed of 7 chromosomes which are clearly recognizable and comparable with the chromosomes which are found in the female embryos. Only in embryos which pertained to very later stages, a few diploid metaphases were observed. It is therefore possible to establish, by using quite simple techniques, that, at least in one heterogonic Rotifer, male somatic cells bear a haploid chromosome set.

The blastomeres of male embryos are all haploid as far as the 5th–6th cleavage-division at least, and only afterwards can one find a few diploid metaphases, which presumably correspond to the tetraploid sets that are found in female embryos.

TAUSON's results concerning diploidy in males of *Asplanchna intermedia* are possibly based on artefacts. As regards the different numbers (n-8, $2n$-16) of STORCH[6], we can either assume that the population observed by STORCH had a different number of chromosomes, or that the technique employed in 1924 led to faulty countings.

Riassunto. Mediante l'impiego di tecniche appropriate è stato possibile definire il corredo cromosomico di *Asplanchna priodonta* Gosse 1850 (Rotatoria) e dimostrare almeno per una specie di Rotiferi, che i maschi sono aploidi.

C. ROBOTTI

Istituto di Zoologia dell'Università degli Studi, Via Accademia Albertina 17, I–10123 Torino (Italy), 13 May 1975.

Giemsa Banding and Heterochromatin Distribution in *Ornithogalum*

In recent years, linear differentiation of chromosomes, by staining with fluorochrome compounds or Giemsa, have facilitated the qualitative study of heterochromatin distribution in the genome of various organisms[1–4]. Further, the heterochromatic regions have been shown to be the preferential sites for radiation and chemically induced chromosomal aberrations[5–8]. In the present paper, we report the distribution of heterochromatin on the chromosomes of a Liliaceous plant, *Ornithogalum virens*. This plant has fairly large chromosomes but few in number ($2n = 6$); it therefore seems to be ideal material for studying the relationship between heterochromatin distribution and the localization of induced breaks in the chromosomes.

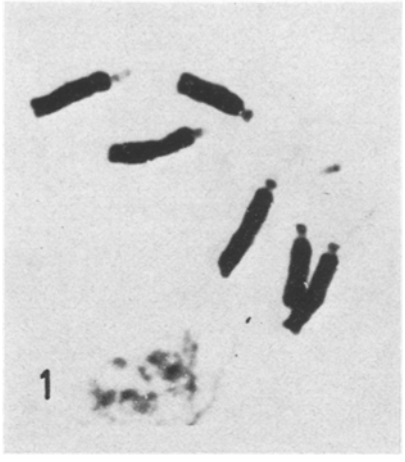

Fig. 1. Karyotype of *Ornithogalum virens* (Feulgen stained). × 2,000.

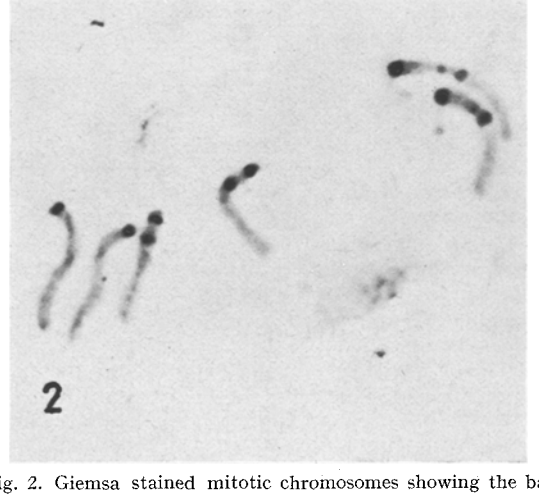

Fig. 2. Giemsa stained mitotic chromosomes showing the banding pattern. × 2,000.

Material and methods. The procedure adopted for Giemsa staining is similar to that described by SARMA and NATARAJAN[9] and SCHWEIZER[10]. Chromosome preparations were made from the root meristem of germinating seeds or sprouting bulbs of *Ornithogalum*. Root tips of about 1–2 cm length were pretreated with saturated aqueous solution of α-bromonaphthelene for 30 min and fixed in 3:1 alcohol-acetic acid mixture. Fixed root tips were hydrolyzed for 2–5 min at 60 °C in 0.1 *N* HCl and squashed in 45% acetic acid on gelatinized slides. The cover-slips were removed by freezing the slide in liquid nitrogen. The slides were washed in 90% and in absolute ethanol, air dried and treated with saturated aqueous solution of barium hydroxide at 40–50 °C for 6 min for alkali denaturation. Alkali treatment was terminated by 2 washes for 2 min each in warm distilled water followed by washing in running water for 30 min. The slides were then incubated in 2 × SSC (0.3 *M* NaCl + 0.03 *M* trisodium citrate, pH 7.0) at 60 °C for 2 h. The preparations were stained in Giemsa solution (2 ml of Giemsa stock solution, prepared from E. Merck Giemsa powder, in 100 ml of Sörensens phosphate buffer, pH 6.8) for 1 h at room temperature. Excess stain was removed by repeated washing in distilled water till a clear differential staining of chromosomes was achieved. Preparations were made permanent by mounting the air-dried slides in euparol.

[1] T. CASPERSSON, S. FABER, G. E. FOLEY, J. KUDYNOWSKI, E. J. MODEST, E. SIMONSSON, U. WAGH and L. ZECH, Expl Cell Res. *49*, 219 (1968).
[2] A. T. NATARAJAN, R. P. SHARMA and G. AHNSTRÖM, Hereditas *69*, 217 (1971).
[3] A. T. NATARAJAN and N. P. SARMA, Genet. Res. Cambridge *24*, 103 (1974).
[4] P. DOBEL, R. RIEGER and A. MICHAELIS, Chromosoma *43*, *409* (1973).
[5] H. J. EVANS, Int. Rev. Cytol. *13*, 221 (1961).
[6] A. T. NATARAJAN and G. AHNSTRÖM, Chromosoma *28*, 48 (1969).
[7] S. TAKEHISA, Mutation Res. *17*, 267 (1973).
[8] C. A. BOURGEOIS, Chromosoma *48*, 203 (1974).
[9] N. P. SARMA and A. T. NATARAJAN, Hereditas *74*, 233 (1973).
[10] D. SCHWEIZER, Experientia *30*, 570 (1974).

Results and discussion. Karyotype analysis made from normal feulgen stained preparations showed that all the chromosomes in this species are sub-telocentric (Figure 1). Of the 3 pairs, one is satellited and almost equal in length to the 2nd pair, approximately measuring 8.5 and 7.75 μm respectively. The 3rd pair is the smallest of the three (6 μm).

Giemsa staining revealed that all the 3 pairs of chromosomes possess a distinct C-banding pattern (Figure 2). Of the total 6 bands observed in a haploid complement, the satellited chromosome, chromosome I has 3 conspicuous bands as compared to the 2nd chromosome which has only a single band. Chromosome III, the smallest one in the genome, revealed 2 bands. Since these bands are distinctly localized along the length of the each chromosome, an accurate and easy identification of the chromosomes is possible at a glance. Each of these 6 bands is designated by serial number starting from 1 to 6. The distribution of the bands in relation to their relative position and size of the normal chromosome complement, centromere being the reference point, is given in the Table and represented diagramatically in Figure 3. Based on the measurement of C-band regions in relation to the total haploid chromosome complement, approximately 13.5% of the genome is heterochromatic.

Relative position of C-bands on the long arms of respective chromosomes of *Ornithogalum virens*

Serial number of the band	Chromosome on which located	Location with reference to centromere of the carrier chromosome (distance in μm)	Size of the band (μm)
Band 1	Chromosome I	< 0.01	0.75
2	Chromosome I	2.00	0.25
3	Chromosome I	3.00	0.50
4	Chromosome II	< 0.01	0.50
5	Chromosome III	< 0.01	0.50
6	Chromosome III	1.00	0.50

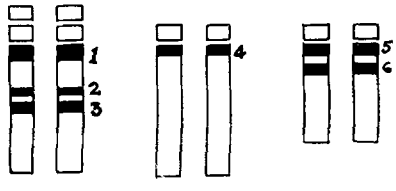

Fig. 3. Diagramatic representation of the distribution of bands in relation to their relative position and size of the chromosome complement. Bands are numbered from 1–6. Scale: 1 μm = 4 mm.

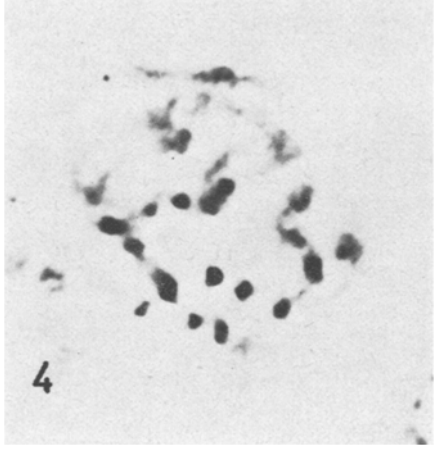

Fig. 4. Giemsa stained interphase nucleus showing the chromocentres. × 2,000.

Observations made on the interphase nucleus in Giemsa stained preparations show 12 darkly stained bodies corresponding to the number of C-bands on metaphase chromosomes (Figure 4). It is generally believed that the number of darkly stained regions or chromocentres in an interphase nucleus is less than the number of C-bands in a genome, indicating that homologous chromosomes, probably form common chromocentres. Even in *Haplopappus gracilis*, known to have smallest number of chromosomes in plant kingdom ($2n = 4$), AMES and MITRA[11] reported less number of blocks of condensed chromatin in interphase nucleus as compared to the number of heterochromatic segments observed at mitosis. Chromocentric association between the chromosomes has been implicated in the involvement of heterochromatin in radiation and chemically induced exchange formations[12–14]. In the present study, the observations on the occurrence of individual chromocentres in interphase nucleus and their correspondence to the number of C-bands in metaphase chromosomes, offer unique opportunity for studying the relationship between the distribution of heterochromatin and mutagen-induced chromosomal aberrations.

Summary. In *Ornithogalum virens*, following Giemsa staining, the mitotic chromosomes revealed distinct C-banding pattern. Interphase nucleus also showed 12 chromocentres corresponding to the number of C-bands. Based on the measurements of C-bands, about 13.5% of the genome is heterochromatic.

R. P. SHARMA and N. P. SARMA[15]

Indian Agricultural Research Institute, Division of Genetics, 128, Nuclear Research Laboratory, New Delhi 110012 (India), and Department of Botany, Delhi University, Dehli 110007 (India), 18 June 1975.

[11] I. H. AMES and J. MITRA, Can. J. Genet. Cytol. *10*, 433 (1968).
[12] A. T. NATARAJAN and G. AHNSTRÖM, Chromosoma *30*, 250 (1970).
[13] A. T. NATARAJAN and W. SCHMID, Chromosoma *33*, 48 (1971).
[14] A. T. NATARAJAN and R. P. SHARMA, Mutation Res. *22*, 73 (1973).
[15] Acknowledgments: We are grateful to Miss SHASHI BHALLA for excellent technical help and to Drs. V. L. CHOPRA, S. BHASKARAN and S. KUMAR for valuable suggestions during the course of this investigation.