13 Ferguson, A., and Parrott, D.M.V., Transplantation *15* (1973) 546.
14 Messer, M., and Dahlqvist, A., Analyt. Biochem. *14* (1966) 376.
15 Koldovsky, O., Asp, N.G., and Dahlqvist, A., Analyt. Biochem. *27* (1969) 409.
16 Garen, A., and Levinthal, C., Biochim. biophys. Acta *38* (1960) 470.
17 Schmitz, J., Preiser, H., Maestracci, D., Ghosh, B.K., Cerda, J.J., and Crane, R.K., Biochim. biophys. Acta *323* (1973) 98.
18 Ferguson, A., Gerskowitch, V.P., and Russell, R.I., Gastroenterology *64* (1973) 292.
19 Kendall, K., Jumawan, J., Koldovsky, O., and Krulich, L., J. Endocr. *74* (1977) 145.
20 Montgomery, R.K., Sybicky, M.A., and Grand, R.J., Devl Biol. *87* (1981) 76.
21 Martin, G.R., and Henning, S.J., Endocrinology *111* (1982) 912.

0014-4754/83/101150-03$1.50+0.20/0

Formation of ring chromosomes by diethyl sulphate and gamma-rays

R.N. Gohil and Asha Kaul

Cytogenetics Laboratory, Department of Botany, University of Kashmir, Srinagar-190006 (India), January 4, 1982

Summary. Ring chromosomes were formed in the root tip cells of *Allium sativum* and *A. cepa* var. *viviparum* after treatment with diethyl sulphate and gamma-rays. Both centric and acentric types of ring chromosomes were observed. The behavior of these chromosomes during different stages of somatic cell division is discussed.

Ring chromosomes have been reported to occur spontaneously both in plants (maize[1-3] and barley[4]) and in animals[5]. In some organisms, they have been produced upon exposure to environmental stress. Both centric and acentric ring chromosomes have been observed. Although the mode of their origin has been hypothesized by a number of workers, the cause of breakage and reunion in nature is not fully understood. In order to induce somatic mutations, cultivated species of *Allium* were treated with some chemical and physical mutagens. We observed ring chromosomes in addition to other chromosomal anomalies in the root tip cells of the treated material.

Materials and methods. Propagules of *A. sativum* and *A. cepa* var. *viviparum* were treated with 2 chemical and 1 physical mutagen as shown in the table.

Mutagen used	Concentration/ doses	Time of treatment
1. Ethyl methane sulphonate (EMS)	100 mM 200 mM 300 mM	2, 4 and 8 h
2. Diethyl sulphate	20 mM 25 mM 30 mM	1, 2 and 3 h
3. Gamma rays	3 Gy 4 Gy 5 Gy	

Root tips from the treated propagules and the controls were fixed directly in 1:3 acetic alcohol. Root tips were hydrolyzed in 1N HCl for 10 min at 60 °C, stained in feulgen and squashed in 1% acetocarmine.

Observations and discussion. Ring chromosomes were observed in the root tip cells of *A. sativum* treated with 25 mM of diethyl sulphate and 4 and 5 Gy of gamma-rays and *A. cepa* var. *viviparum* treated with 4 Gy of gamma-rays whereas, they were absent in the controls. The frequency of cells with ring chromosomes was higher in tips exposed to gamma-rays. These chromosomes were observed at mitotic prophase (fig. 1), metaphase and anaphase (figs 2 and 3). The rings observed in *A. sativum* were of small size and in none of these could the centromere be located. At anaphase they remained as laggards either in the center of the cell or moved towards one of the poles. Their behavior during cell division was typical of acentric fragments. In gamma-ray-treated bulbils of *A. cepa* var. *viviparum*, in addition to small-sized acentric ring chromosomes, large dicentric ring

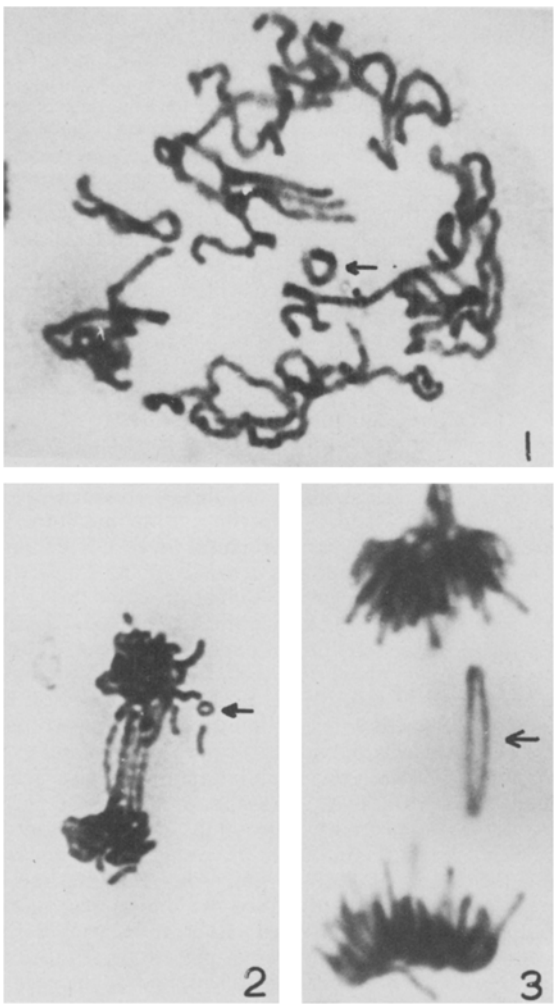

Figure 1. Root tip cell of *A. sativum* at prophase of mitosis with one acentric ring chromosome.
Figure 2. Root tip cell of *A. sativum* at anaphase of mitosis with one acentric ring chromosome which has moved towards one of the poles. Note the acentric fragments also.
Figure 3. Root tip cell of *A. cepa* var. *viviparum* at anaphase of mitosis with a dicentric ring chromosome lying in the center. (Arrows point towards ring chromosomes).

chromosomes were also observed. These dicentric ring chromosomes also remained in the center of the cell at anaphase with the 2 centromeres stretched towards the 2 poles (fig. 3). In all of these cells, in addition to the ring chromosomes, some chromosome fragments were also invariably observed. The ring chromosomes, centric and acentric, as well as fragments formed laggards at mitotic anaphase, leading to the deletion of some chromosome segments. The laggards formed micronuclei at telophase.

Since the formation of ring chromosomes involves breakage-reunion (erratic) cycle and the hard rays (gamma) are known for their chromosome-breaking properties, the relationship between the formation of ring chromosomes and exposure to gamma-rays is quite clear. The present investigations have revealed that, like gamma-rays, diethyl sulphate too can induce breakages in the chromosomes resulting in the production of ring chromosomes and fragments.

1 McClintock, B., Proc. natl Acad. Sci. *18* (1932) 677.
2 McClintock, B., Genetics *23* (1938) 315.
3 McClintock, B., Cold Spring Harb. Symp. quant. Biol. *9* (1941) 72.
4 Singh, R.J., and Tsuchiya, T., Chromosoma *82* (1981) 133.
5 White, M.J.D., Animal cytology and evolution. Cambridge University Press, Cambridge 1973.

0014-4754/83/101152-02$1.50 + 0.20/0

Unusual heteromorphic sex chromosomes in a marsupial frog[1]

M. Schmid, T. Haaf, Beatrix Geile and S. Sims

Institut für Humangenetik, University of Würzburg, Koellikerstr. 2, D-8700 Würzburg (Federal Republic of Germany), and Department of Zoology, University of Leicester, Leicester (England), April 11, 1983

Summary. Heteromorphic sex chromosomes of the XY/XX-type were found in the karyotypes of the South American marsupial frog *Gastrotheca riobambae* (Anura, Hylidae). The Y chromosome is considerably larger than the X chromosome and almost completely heterochromatic. The only nucleolus organizer region is localized in the X chromosome; this leads to a sex-specific difference in the number of nucleolus organizers. In the male meiosis, X- and Y chromosomes form a sex bivalent which can be readily distinguished from the autosomal bivalents.

Cytogenetic analyses have been performed in about half of the extant species of the Amphibia[2-4]. The chromosomes of salamanders, frogs and toads are very attractive for cytogenetic studies, because most of the species are distinguished by very long chromosomes and a low diploid chromosome number. Furthermore, the pairing arrangements of the chromosomes can be studied not only in the male stages of meiosis, but also in the fine-structured lampbrush chromo-

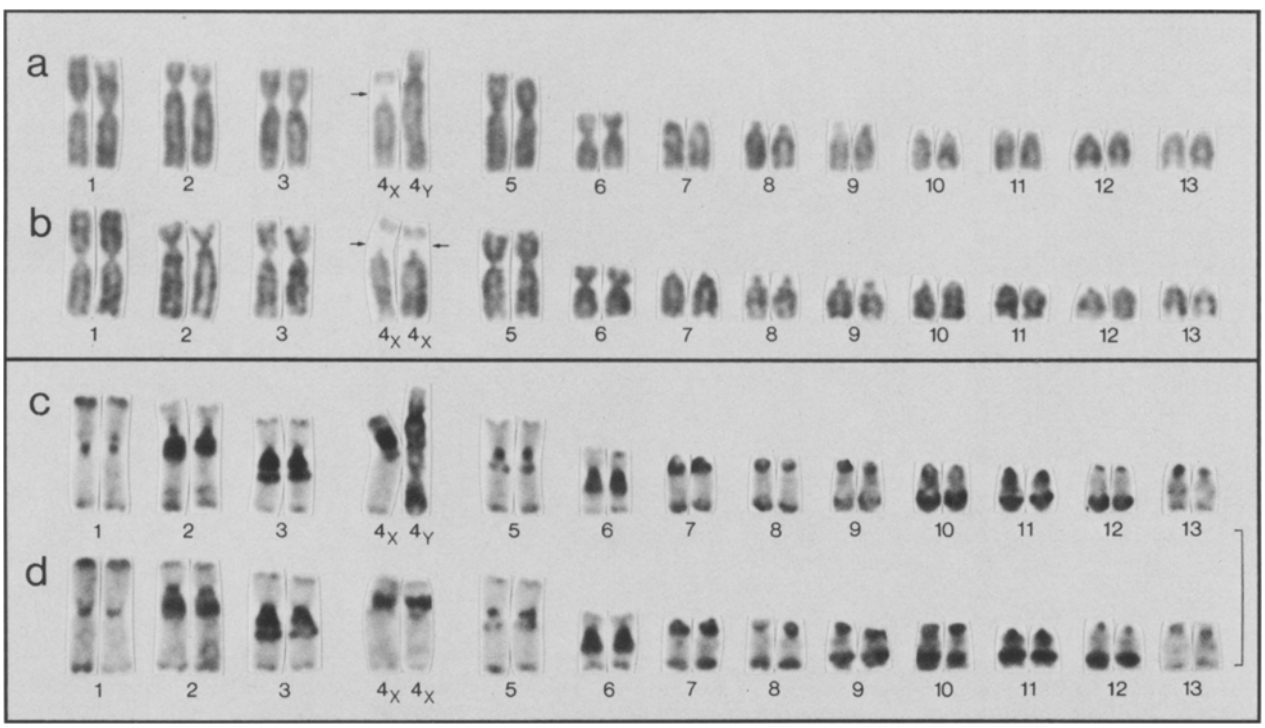

Figure 1. Karyotypes of male (a, c) and female (b, d) animals of *Gastrotheca riobambae*. *a* and *b* Conventional orcein staining demonstrating the highly heteromorphic XY sex chromosome pair No. 4 in the male (a), and the homomorphic XX pair in the female (b). The arrows indicate the conspicuous secondary constriction (nucleolus organizer region) in the short arm of the X chromosome. *c* and *d* Constitutive heterochromatin stained according to the C-band-technique[9]. Note the large number of constitutively heterochromatic regions in the karyotype and the almost completely heterochromatic Y chromosome (c). The bar in the figure represents 10 µm.