Genetic Breakdown of Chromosome Behaviour of *Tribulus terrestris*

A fertile polyploid series has been known to exist within *Tribulus terrestris* (Zygophyllaceae) with plants having $n = 12$ (diploid), 18 (triploid)and 24 (tetraploid) chromosomes[1,2]. Recently TANDON and RAO[3] have discussed the mechanism of evolution of higher chromosomal forms in *Tribulus terrestris* and indicated its significance for control measures of certain weeds. The present study deals with the genetic control of chromosome behaviour of *Tribulus terrestris*.

In a naturally occurring triploid population of *T. terrestris*, a plant with morphological variation was spotted. This plant was vigorous in growth, densely hairy and bore larger flowers and more pairs of leaflets per rachis than the normal triploids. A cytological study of microsporogenesis was, therefore, considered desirable. Flower buds were fixed in Carnoy's fluid (6 parts absolute alcohol + 3 parts chloroform + 1 part glacial acetic acid) for less than 1 h. They were then transferred for 24 h to propionic alcohol (1:3) where propionic acid had been saturated with ferric acetate. Squash preparations of anthers were prepared in propionocarmine[4].

In naturally occurring triploids, meiosis was normal with 18 bivalents at diakinesis and at metaphase I (Figure 1). At metaphase I the chiasma frequency per cell and per bivalent was 20.15 and 1.11 respectively. The disjunction of chromosomes at anaphase I was normal with 18:18 distribution of chromosomes to the poles. Metaphase II and anaphase II were regular. Normal tetrads were formed after completion of the second division. The pollen fertility was 78.3. A meiotic study in pollen mother cells of the morphological variant showed that it was at triploid level. At diakinesis and metaphase I, a large number of univalents was observed (Figure 2). The mean number of bivalents, univalents and multivalents per cell at metaphase I was 10.53, 14.54 and 0.09, respectively. The chiasma frequency per cell and per bivalent was 12.81 and 0.71, respectively; 20% of the cells showed laggards at telophase I. At anaphase II, 6% of the cells showed micronuclei. The plant was sterile. The pollen fertility was as low as 0.19%.

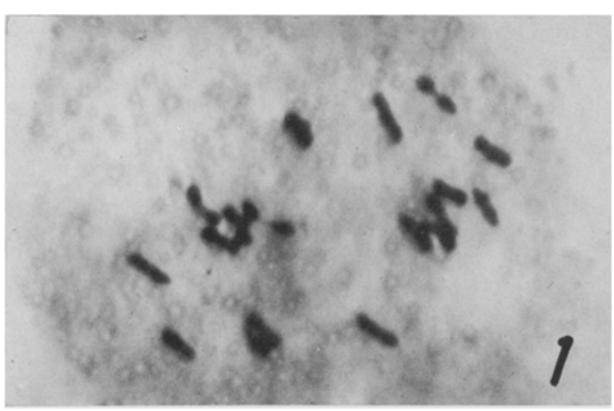

Fig. 1. Metaphase I in naturally occurring triploid showing 18 bivalents.

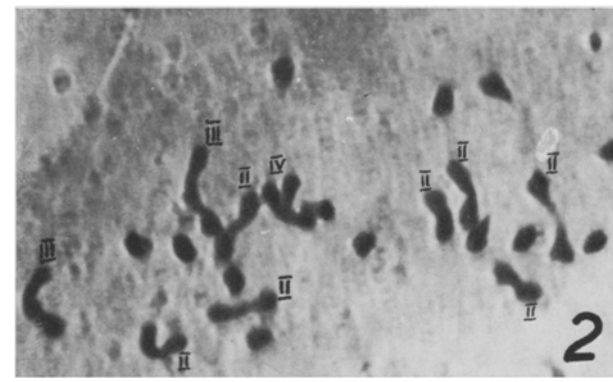

Fig. 2. Metaphase I in sterile triploid showing 7 bivalents (marked as II), 1 quadrivalent (marked as IV), 2 trivalents (marked as III); the remaining 12 chromosomes, which are unmarked, are all univalents.

At present sufficient evidence is available on genetic control of chromosome pairing in polyploids[5–7]. Experiments have indicated that this genetic control is confined to a single locus[7]. It is known that gene mutation of recessive nature is responsible for variation in pairing behaviour of chromosomes[8]. Disruption or weakening of the association of homologs has been ascribed to the homozygosity of recessive alleles[7]. The occurrence of a sterile triploid plant, showing failure of normal chromosome pairing, in a fertile population of triploids, is probably due to a spontaneous gene mutation. It is also likely that, as reported in other cases, the mutated gene, in the homozygous condition, led to a genetic breakdown of normal meiosis. The sterility caused by genetic breakdown of meiosis is not an adaptive evolutionary advantage in case of *Tribulus terrestris*.

Zusammenfassung. Das Vorkommen einer fruchtbaren Polyploidie-Serie innerhalb *Tribulus terrestris* war bekannt ($n = 12$, Diploid, $n = 18$, Triploid, und $n = 24$, Tetraploid). Untersuchung über die Zytologie eines unfruchtbaren, natürlich vorkommenden Triploides deutet auf genetische Kontrolle bei der Paarbildung der Chromosomen von *Tribulus terrestris*.

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Giant Sex Chromatin in Endopolyploid Trophoblast Nuclei of the Rat

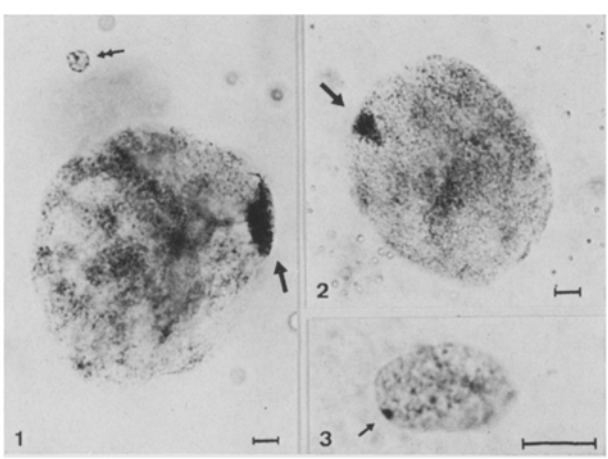
Secondary giant cells in the trophoblast of white rat placenta originate by endomitotic processes[1–4]. Because this is an unique event in mammals, the degrees of endopolyploidy (DNA contents) of the giant nuclei were reexamined, and the nuclear structure was studied with special reference to the behavior of the inactivated *X* chromosome in female embryos.

White rats were killed when pregnancy had reached 14 days, and the uteri were fixed in ethanol-acetic acid (3:1). For a first orientation, sections were made and

stained with hematoxylin-eosine. Feulgen-stained squash preparations were performed for the determination of the nuclear DNA content (hydrolysis: 10 min in N HCl of 60 °C; pararosaniline: 1.5 h at 25 °C; permanent slides were made by the dry-ice technique). A Zeiss microspectral photometer was used in combination with the two-wavelengths-method ($\lambda_1 = 4800$ Å, $\lambda_2 = 5040$ Å) of Patau[5]. For the rapid structural analysis of a great stock of material, carmine-strained squash preparations were preferred.

The endopolyploid trophoblast nuclei can easily be found, even in squash preparations, due to their enormous size (Figure 1). The degrees of endopolyploidy of the giant nuclei were determined by means of their Feulgen-DNA content. The basic value 2C was obtained by measurements of telophase nuclei and of $2n/G_1$ nuclei of the embryo. The giant nuclei of the trophoblast differ in their DNA contents, but fall into 6 classes (Table). The highest values found give evidence for 4.096-ploidy, which is more than was hitherto known in mammals (including earlier reports on the rat trophoblast[1–4]). Most of the nuclei may have levels of $512n$, $1.024n$, and $2.048n$, and they are equally distributed among these 3 classes. Nuclei of a lower degree of endopolyploidy seem to be the result of amitotic fragmentation of giant nuclei, at least in part.

The trophoblast nuclei of both sexes exhibit different structures: Either the chromatin is homogeneously distributed or it is organized in form of chromatin-bundles, like the polytene chromosomes of several plants[6]. However, the giant trophoblast nuclei of female embryos (known by the occurrence of sex chromatin-positive nuclei, Figure 3) possess 1 large heterochromatic body mostly situated at the periphery of the nucleus. This chromocenter is larger than all other chromocenters, and it is present in all nuclei of female embryos, but in none of male ones (Figures 1 and 2). Therefore this body is assumed to be the multiple sex chromatin, formed by all the inactivated X chromosomes which originate by endomitotic processes from the 1 inactivated X chromosome of the diploid cell. On the other hand, all the X chromosomes which are endomitotic descendants of the euchromatic X evidently remain in the active state. This corresponds with older findings in the rat, though no sex chromatin was seen in the *highly* endopolyploid nuclei[7]. The behavior of the X chromosomes indicates that dosage compensation occurs relative to the number of X within 1 chromosome complement only, but not relative to the absolute number of X. Although the phenomenon cited is already known from tetraploid and octoploid nuclei[8], the giant sex chromatin of the rat trophoblast nuclei may be an excellent source for studies on the function of inactivated X chromosomes in mammals.



Sex chromatin in diploid and endopolyploid nuclei of female rat. Aceto-carmine; scale indicates 10 μm.

Fig. 1. Endopolyploid trophoblast nucleus (arrow indicates giant sex chromatin), and diploid nucleus, probably of a blood cell (double-arrow); Fig. 2. Endopolyploid trophoblast nucleus (arrow indicates giant sex chromatin); Fig. 3. Nucleus of a fibroblast cell showing normal sex chromatin (arrow), at higher enlargement.

DNA content and level of endopolyploidy of nuclei in the rat embryo and trophoblast (average values of 18 slides made from 3 embryos)

Source of nuclei	Number of nuclei	DNA in arbitrary units and S.E.	Indicated Level of endopolyploidy
Embryo telophase	10	21± 2.2	$2n$
Embryo class 1	25	21± 1.0	$2n/G_1$
Embryo class 2	20	44± 2.8	$2n/G_2$
Trophoblast class 1	18	1 675± 11.8	$128n$
Trophoblast class 2	15	3 200± 36.5	$256n$
Trophoblast class 3	27	6 092± 29.1	$512n$
Trophoblast class 4	32	11 684±122.7	$1\,024n$
Trophoblast class 5	15	22.684±440.5	$2\,048n$
Trophoblast class 6	4	40 031±405.9	$4\,096n$

Zusammenfassung. Der DNA-Gehalt und die Chromatinstruktur der hoch endopolyploiden Zellkerne des Rattentrophoblasten wurden untersucht. Cytophotometrische Messungen des Feulgen-DNA-Gehalts weisen auf Polyploidiegrade bis zu $4.096n$. Die Riesenkerne von weiblichen Embryonen weisen einen grossen heterochromatischen Körper auf, der als Sex-Chromatin, gebildet durch einige Hundert oder Tausend inaktivierte X-Chromosomen, gedeutet wird.

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[9] Acknowledgment. I thank Dr. W. SCHNEDL, Institute of Histology and Embryology, University of Vienna, for kindly supplying us with fixed material and with stained sections.