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Sex chromatin in *Rumex acetosa* L.

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A b s t r a c t — The author found in cell nuclei of *Rumex acetosa* L. a chromatin grain (or grains) analogical to the sex chromatin of animals. She considers correct to determine it as sex chromatin, too. In zoological material it is characteristic for the female sex, in the studied plant species it is on the contrary only in male individuals.

In 1949 M. L. Barr & E. G. Bertram discovered in the nuclei of the cat's neuron cells a body, which was possible to stain by nuclear stains. The body occurred in 30—40% of the nuclei of female cats, but in males the occurrence did not exceed 4%. Later this body was discovered even in other mammals as well as in human beings and was determined as the sex chromatin. This discovery of the nuclear sexual dimorphism gave the basis for relatively simple tests to examine the disorder of somatosexual and psychosexual development of man in medical practise.

Sex chromatin is a special chromocenter, which is larger than other chromatin particles and is usually located either on the inner surface of the nuclear membrane or it lies close to the main nucleolus. Probably it is derived from the heterochromatic regions of chromosomes. M. A. Graham & M. L. Barr (1952) and K. L. Moore & M. L. Barr (1953) deduced, that the sex chromatin may represent heterochromatic regions of homologous X-chromosomes and that the XY complex failed to form a distinct and striking chromocenter with regard to the small size of the Y-chromosome. S. Ohno, W. D. Kaplan & R. Kinosita (1959) studying the resting nuclei of rat liver cells found, that only one of the two X-chromosomes of the female sex was positively heteropycnotic while both X and Y-chromosomes in similar cells of males were isopycnotic. Ohno and his collaborators have therefore deduced, that the sex chromatin may be derived from a single X-chromosome. This was confirmed by I. L. Kosin's & H. Ishizaki's studies (1959) on nuclei of the domestic chicken. They found characteristic sex chromatin by the female, which has the constitution ZO and therefore must be—if related to the sex chromosomes at all—derived from a single Z-chromosome. On the other hand an alternative hypothesis was put forward by S. J. Segal & W. O. Nelson (1957), who suggested that the sex chromatin may also be derived from a pair of autosomes, which bears sex determiners.

This obvious sex dimorphism in interphase nuclei of animal cells influenced my work in which I tried to state the similar nuclear dimorphism in interphase nuclei of dioecious plants.

First of all I traced the possibility to determine various structures in nuclei, by which the male would differ from the female. I observed the structures in nuclei on slides made by applying various fixatives and staining procedures. Studying the slides, I fixed my attention mainly on the problem whether plants contain sex chromatin like animals, too. This was connected with the shape and number of chromocenters and their possible connection with heterochromosomes.

I followed the sexual dimorphism of *Rumex acetosella* L. (2n), *Rumex acetosa* L., *Melandrium album* (MILL.) GARCKE and *Ginkgo biloba* L. I observed the nuclei of these plants in the cells o the elongation region in roots, further the nuclei of the somatic cells of stem, leaves and flower buds. Although I found everywhere distinct chromocenters in nuclei, and in the leaves of *Ginkgo* and the roots of *Melandrium* even nuclear bodies which by their shape and position could correspond to sex chromatin, I did not find the difference between males and females. Only in *Rumex acetosa* I found the body with characteristic features for one sex.

I discovered it in the material fixed in Bouin's fluid and stained with Heidenhain's iron haematoxylin. This method was not satisfactory, because it was difficult to distinguish the chromatin grains, which could hardly be differentiated sufficiently and distinctly. Simultaneously the nucleolus was strongly stained, so in some cases it would be possible to mistake it with the equally stained chromatin grains. Therefore I tried to distinguish the structures inside the nuclei according to H. R. GUARD (1959). The method was determined for distinguishing the sex chromatin of animals. I fixed this material in fixing fluid containing alcohol (FAA). The sections were brought down to 70% alcohol, then they were stained for 2 to 4 minutes in the following solution: 1 g. ponceau S, 0,3 g. phosphomolybdic acid, 5 ml. glac. acetic acid, 100 ml. 50% alcohol. After the staining the sections were counterstained with 1% aqueous solution of light green FS for 10 minutes and were dehydrated, cleared in xylol and mounted in methylmetacrylate mounting medium ("Solakryl"). As Biebrich scarlet, given in the original shedule, was not available for me, I used for the preparation of the staining solution ponceau S, which is chemically most similar. For the same reason I counterstained with light green instead of fast green FCF. In Guard's shedule the phosphotungstic acid is added. I tried this as well as phosphomolybdic acid, which proved to be better.

The staining effect was very good, because by this method the chromatin grains were evidently different. The cell walls and chloroplasts were stained lightly green; the nucleoli mainly of female plants were green and of male plants reddish, the other nuclear structures were light green to pink, chromatin bodies in male nuclei being bright red.

The best material were the leaves where the chromatin bodies could be easily distinguished. In the stem they could be seen best in the nuclei of the cortex cells, then in the vascular tissues and worst in the pith. In the majority of male plants nuclei a homogeneous body appears which is distinctly delimited from other nuclear structures. It is often lens-shaped, almost hemispheroidal (Tab. I — 2, 3, 6), otherwise elongated (Tab. I — 1) or approximately triangle-like (Tab. I — 5). Sometimes there are two such grains in the nuclei, rarely even several. In the female plants there are also more intensively stained structures, but these ones differ clearly from the chromatin grains in male nuclei. They look more like granular clumps, do not form homogeneous formations and are not clearly delimited from other nucleolar structures, on the contrary, they rather merge together. As far as the chromatin grains occur here, they are much smaller than those in the male nuclei (Tab. II).

The differences in nuclei structures of both sexes are distinct. With regard to the analogy between the chromatin grain in female nuclei of animals and that of *Rumex acetosa*, I consider it correct to determine this chromatin grain or grains as sex chromatin. In the described case it is characteristic for the male sex, in whose chromosome set are three sex chromosomes (XYY). The mechanism of sex determination may be based like the *Drosophila* scheme on the balance between the X-chromosomes and autosomes. T. ONO (1935) and Y. YAMAMOTO (1938) found out that Y-chromosomes do not carry male determinants; they seem to be almost genetically inert (Á. LÖVE 1957). To this fact the occurrence of sex chromatin of the male sex would fully correspond, where it would represent the heterochromatic regions of Y-chromosomes.

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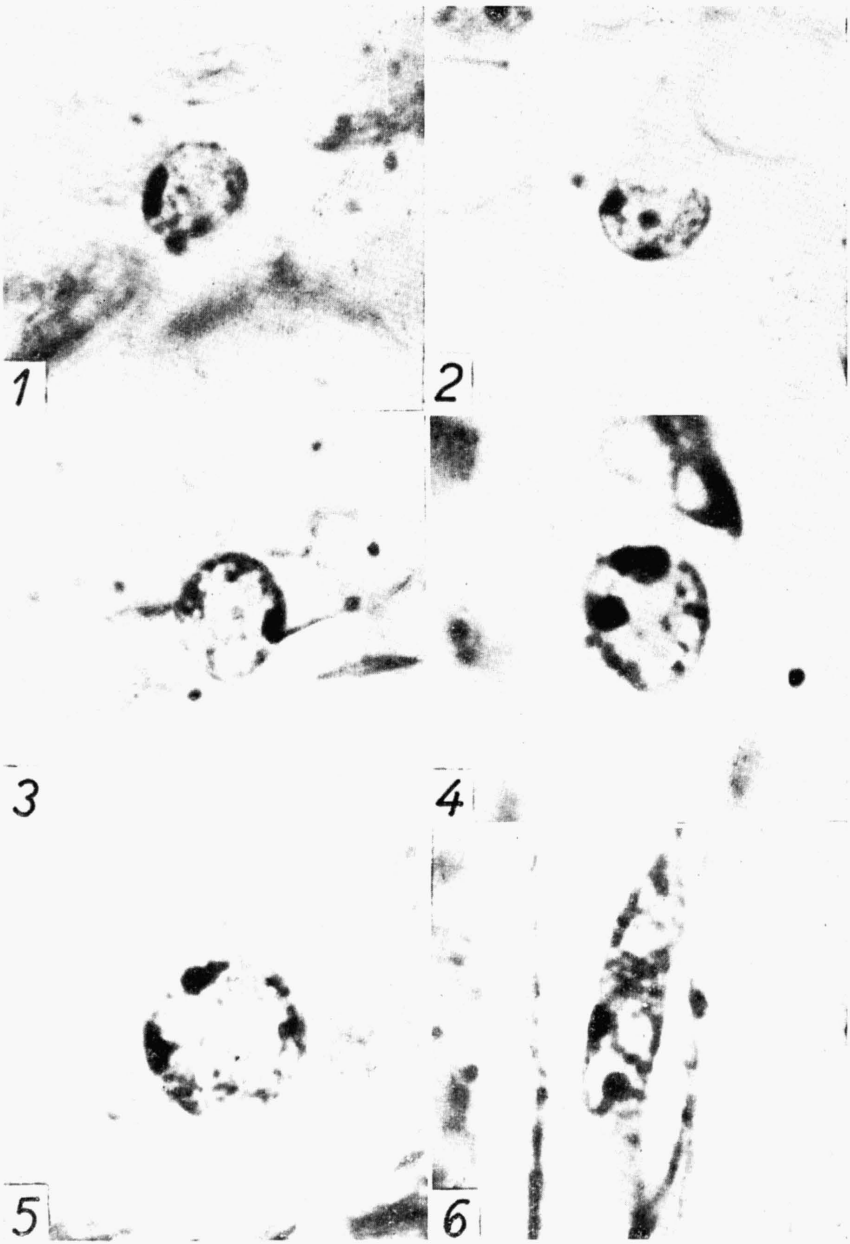
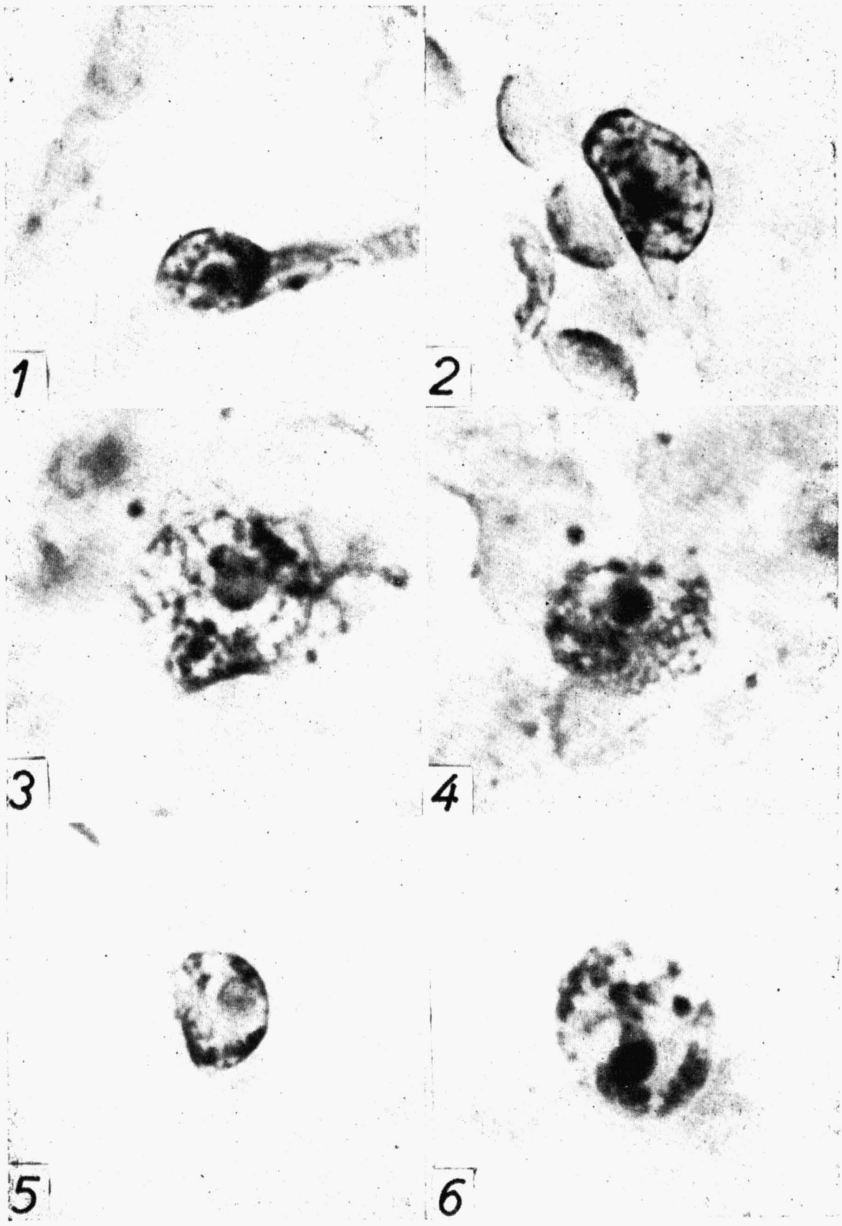
Explanations of the plates:

Tab. XXII. — *Rumex acetosa* L., nuclei in cells of leaves (1, 2, 3, 5) and stems (4, 6) of the male plants.

Tab. XXIII. — *Rumex acetosa* L., nuclei in cells of leaves (1—4) and stems (5, 6) of the female plants.



Z. Pazourková: Sex chromatin in *Rumex acetosa* L.



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