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Studies on the Sex Chromatin in Various Tissues of the Vegetative Organs of *Rumex acetosa* L.

Studium pohlavního chromatinu v různých pletivech vegetativních orgánů *Rumex acetosa* L.

Vladimír Váňa

VÁŇA V. (1972): Studies on the sex chromatin in various tissues of the vegetative organs of *Rumex acetosa* L. — Preslia, Praha, 44 : 100—111. — The sex chromatin of *R. acetosa* L. is produced by the heterochromatin of two Y chromosomes present in the diploid set of the male plant. In interphase nuclei it can be observed either as a chromocentre, as a persisting chromosome, or as a transitional form between these two types. The incidence of nuclei with a different number of persisting bodies was studied in the tissues of the stem, leaf and elongation zone of the root and the position of persisting bodies in the nuclei was evaluated. — *Department of Genetics, Microbiology and Biophysics, Caroline University of Prague, Viničná 5, Praha 2, Czechoslovakia.*

Introduction

In the last 20 years, and especially after the discovery of sexual dimorphism in interphase nuclei of somatic mammalian cells (BARR et BERTRAM 1949), considerable progress has been made in studies of differences of interphase nuclei in both sexes of the same species. As regards plants, the first studies of sexual dimorphism of interphase nuclei were performed on *Rumex acetosa* L. (SHIMIZU 1961, PAZOURKOVÁ 1964). All earlier papers (HEITZ 1928, SHIMOTOMAI et KOYAMA 1932 a.o.) described the presence of morphologically not clearly differentiated chromocentres in the interphase nuclei of the male and female gametophytes of Bryophytes. PAZOURKOVÁ (1964) observed that the interphase nuclei of the male plant contained one or several chromocentres of identical staining properties as those of the sex chromatin of mammals. These chromocentres could not be identified in the female plant. It was assumed that these chromocentres are formed by heterochromatin of two Y chromosomes present in the diploid set of chromomes of the male plant. This hypothesis was confirmed by the results of studies on the localization of the heterochromatic segments in chromosomes of this species (VÁŇA ined.). Similar observations have been made in the closely related species *Rumex thyrsiflorus* FINGERH. (ŻUK 1969a, 1969b).*)

PAZOURKOVÁ (1964) found interphase nuclei with a varying number of chromocentres similar to sex chromatin in the differentiating tissues of the root and also in the differentiated tissues of them stem and leaf. In addition, she found also nuclei in which these structures were absent. She described persisting chromosomes from both types of tissues. The present study was performed in order to obtain information on the incidence of nuclei containing different numbers of these persisting bodies in various tissues of the vegetative organs of the male plant of *Rumex acetosa* L. Using a modification of

*) Recently M. KURITA et Y. KUROKI published a series of cytogenetic studies on *Rumex acetosa* L. These papers arrived, alas, too late to be considered by the present author.

Guard's (GUARD 1959) staining method (PAZOURKOVÁ 1964) it was possible to differentiate the sex chromatin from the other chromocentres in the interphase nucleus.

Material and methods

The plants employed were grown from seed in the genetical garden of the Caroline University. Seed was collected in natural populations in the vicinity of Chomutov (about 380 m a. s. l.). Root-tips were cut off from each plant to study the karyotype. After pretreatment in oxiquinoline the root-tips were fixed in 1 : 3 acetic alcohol and squashed in acetorceine.

The sex chromatin in the interphase nuclei of the vegetative organs was studied in tissues from the elongation zone of the roots, from the central part of mature leaves and from part of the stem close under the inflorescence. All material was fixed in FAA, dehydrated in alcohol and embedded in paraffin. Sections were stained using a modification of Guard's method (PAZOURKOVÁ 1964) or the Feulgen reaction (NĚMEC et al. 1962) (staining in Schiff's reagent for 1—1.5 hrs; the sections were washed three times for 15 min. each in water saturated with SO_2).

Since results may often be distorted by the thickness of the section, we tried to determine experimentally a thickness which would offer a typical view of the nuclei in the tissue under consideration. We compared the results obtained from 6—14 μm thick sections and those of the evaluation of 200—500 nuclei in each tissue. The results indicated that the most suitable thickness of the section was as follows: about 12 μm for transverse section of the leaf; 10—12 μm for longitudinal sections of the root; 12—14 μm for longitudinal sections of the stem. We evaluated 300 nuclei per tissue and plant investigated.

Results

The karyotype of all plants examined was: 2n = 8i + 2j + 2v + XX (for the female); 2n = 8i + 2j + 2v + XYY (for the male).

Samples of each plant were taken from the central portion of the mature leaf (evaluating separately the interphase nuclei of cells of the epidermis, the vascular tissues and the parenchyma), from part of the stem close under the inflorescence (evaluating separately nuclei in the cells of the cortex, of the vascular tissues and of the pith) and from the elongation zone of the roots (evaluating separately the nuclei of the outer part in which the primary cortex differentiates, and of the inner part in which differentiation of the tissues of the central cylinder occurs; to simplify the text we shall refer to these parts as to the primary cortex and the central cylinder).

The samples were stained using two staining methods:

a) a modification of Guard's method by which sex chromatin can be differentiated from all other chromocentres in the interphase nucleus; sex chromatin stains an intensive red, the nucleolus orange to reddish, the other structures inside the nucleus feebly red or feebly green;

b) Feulgen reaction — chromocentres stain an intensive bluish-violet, the coloration of the diffuse chromatin of the interphase nucleus is feeble, the nucleolus remains unstained.

Chromocentres, persisting chromosomes and transitional forms between these two types were observed in all organs and tissues of the male plant examined. For these three types of structures we have chosen the collective term "persisting bodies".

The morphology of persisting bodies

The chromocentres (Pl. V : 1—3): shape oval, ellipsoid to hemispherical, occasionally triangular on cross section. Chromocentres located at the inner surface of the nucleus were flattened at the site of contact with the nuclear membrane. Chromocentres in the nuclei of pith cells are frequently of irregular shape. Sometimes, a thin thread can be seen protruding from the chromocentre to the inside of the nucleus. The length of the chromocentres ranges from 1 to 2 μm.

Transitional forms from chromosomes to chromocentres (Pl. VI : 1—4). This group comprises structures of considerable morphological variation of which several basic types could be distinguished:

a) a chromocentre elongated towards one side in a club-shaped protrusion of varying length; its diameter does not surpass that of the chromocentre itself;

b) a club-shaped body of equal width throughout (by contrast to persisting bodies designated

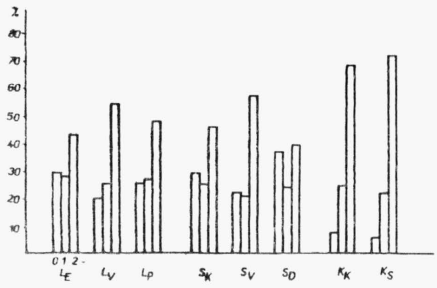

Fig. 1. — Mean values of the incidence of nuclei without persisting bodies, with one and with two and more persisting bodies in the tissues of the vegetative organs of male plants of *Rumex acetosa*. — Stained with Guard's modified method. Explanation of the lettering: L — leaf, L_E — epidermis, L_V — vascular tissues, L_P — parenchyma; S — stem, S_K — cortex, S_V — vascular tissues, S_D — pith; K — elongation zone of the root, K_K — primary cortex, K_S — central cylinder.

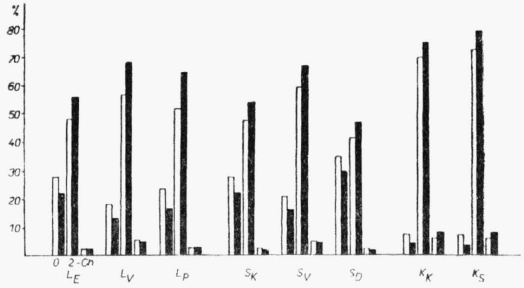

Fig. 2. — Mean values of the incidence of nuclei without persisting bodies, with two and more persisting bodies and with persisting chromosomes (Ch) in the tissues of the male plants of *Rumex acetosa*. — Stained with Feulgen reaction (black) and Guard's modified method.

"persisting chromosomes without a distinct constriction", these bodies are shorter, and are never "L"- or "V"-shaped;

c) a chromocentre connected by a thin thread with one or several distinctly smaller chromocentres;

d) two chromocentres of about the same size joined together by a thin thread;

e) three or more chromocentres of approximately the same size joined together by thin threads frequently arranged into "V"- or "L"-shaped patterns;

f) a club-shaped body connected with one or several chromocentres of the same diameter by a thin thread;

g) bodies similar to one of the described types, but with an irregular outline. The occurrence of such bodies, however, is extremely rare.

Persisting chromosomes (Pl. VI : 5—7). These are mostly elongate bodies without a visible constriction; sometimes, when bent they resemble the letter "V" or "L". If there is a constriction, this is mostly median or submedian, occasionally subterminal. Very rarely it was possible to distinguish, apart from the centromere, the longitudinal cleavage of a chromosome to chromatids. An occasional nucleus with two persisting chromosomes may also be found. I myself found only seven of these in the differentiated tissues of stems and leaves.

In the cells of the female plant we found neither persisting chromosomes nor transitional forms; but even structures considered to be chromocentres differ mostly from chromocentres in the nuclei of the male plants. Chromocentres of the female plant staining as sex chromatin with Guard's method, are of irregular shape and mostly smaller than those in the male plants

(Pl. V : 6). The majority of nuclei of the male plant contain only diffuse or fibrous chromatin structures (Pl. V : 4, 5). An evaluation of the chromocentres of the female plant is greatly influenced to the observer's subjective view. In this paper chromocentres of the female plant classified as such were only those which resembled, at least by their size, the chromocentres of nuclei of the male plant.

The incidence of nuclei with a different number of persisting bodies

The incidence of nuclei with a different number of persisting bodies was studied in preparations stained according to a modification of Guard's method.

Ten male plants were used for this study. All results are given in Tab. 1; the mean values of the individual tissues examined are shown in Fig. 1. In order to obtain an exact proof that the evaluated persisting bodies are chromatin structures containing DNA, a simultaneous evaluation was made of material composed of all organs of five plants and stained with the Feulgen reaction. The results are given in Tab. 1; the mean values obtained from both staining methods are compared in Fig. 2. Thus, the mean values of material stained with Guard's method were calculated only from the values of material of the five plants stained also with the Feulgen reaction. This accounts for certain differences in the mean values given in Tab. 1. The data recorded in the tables for each type of nuclei in the tissue concerned represent the lowest and highest value determined in the complex and the mean value calculated for material stained with Guard's method (from 10 male plants) and that stained with Feulgen reaction (5 male plants). The data for the individual tissues are given in three separate columns. The first column contains data on the incidence of nuclei without and that of nuclei with one, two or more persisting bodies regardless of the type of these bodies (i.e. chromocentre, transitional form or persisting chromosome). The second column contains data on the incidence of nuclei with persisting chromosomes; the third shows the incidence of nuclei with either persisting chromosomes or transitional forms (i.e. the incidence of nuclei containing persisting bodies of another type than that typical of the sex chromatin known, e.g., from mammals, which, in this paper, have been designated as chromocentres).

The percentage of nuclei without persisting bodies being generally lower in material stained with the Feulgen reaction, I assumed that this difference must have been caused by the fact that chromocentres produced by autosomal heterochromatin may, sometimes, be very similar in shape and size to the typical chromocentres of interphase nuclei of the male plant (i.e. sex

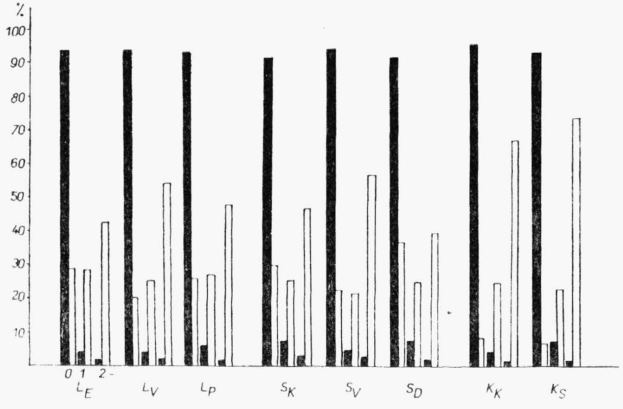

Fig. 3. — Mean values of the incidence of nuclei with various numbers of persisting bodies in the tissues of the female (black) and the male plants of *Rumex acetosa*. — Stained with Guard's modified method.

Tab. 1. — The incidence of the interphase nuclei with various numbers of persisting bodies in the tissues of the vegetative organs of the male plants of *Rumex acetosa*. — Stained with Guard's modified method (G — material from 10 plants) and Feulgen reaction (F — material from 5 plants). The values are given in percentage, the upper numbers giving the lowest and the highest values, the bottom number the mean value.

Organ, tissue	Staining method	Number of persisting bodies in the nucleus			Persisting chromosomes	Persisting chromosomes and transitional forms
		0	1	2 and more		
Leaf epidermis	G	18.7—36.3 29.2	20.3—35.0 27.8	29.0—51.0 43.0	0.7—4.0 2.2	2.3— 8.7 5.2
	F	14.7—29.0 22,5	20.3—26.3 22.3	50.3—63.3 55,2	1.0—3.9 2.4	2.7— 6.3 4.7
vascular tissue	G	12.0—30.7 20.2	18.4—30.0 25.6	42.3—63.7 54.2	2.0—7.7 4.6	5.0—11.3 7.8
	F	11.0—17.3 13.3	10.7—22.3 18.5	59.0—78.0 68.2	3.2—6.0 4.9	6.0—10.3 8.4
parenchyma	G	16.0—34.0 25.3	21.4—34.7 26.7	37.0—58.6 48.0	0.7—5.7 2.8	3.7— 6.3 5.3
	F	9.3—24.0 17.1	14.3—24.0 18.3	52.7—79.0 64.6	1.3—3.7 2.8	5.0— 7.7 6.2
Stem cortex	G	23.0—36.0 29.2	19.0—29.4 24.9	34.6—52.7 45.9	0.3—4.0 1.9	1.3— 7.0 4.4
	F	20.3—25,7 22.3	20.3—28.3 24.3	51.0—58.0 53.4	0.3—3.0 1.8	3.0— 6.7 4.6
vascular tissues	G	16.7—30.0 22.1	12.7—26.3 21.0	47.3—70.6 56.9	1.3—6.7 4.1	5.0—11.0 7.9
	F	13.3—19.7 16.1	11.7—22.0 17.5	58.3—73.0 66.4	3.0—5.7 4.5	7.0—13.3 9.7
pith	G	27.0—51.6 36.6	17.7—29.4 24.1	26.7—49.3 39.3	0.7—5.7 2.9	3.7— 9.3 6.3
	F	25.3—35.7 29.3	19.7—27.7 23.7	36.6—53.6 47.0	0.3—3.6 2.0	2.7— 8,0 5.3

Organ, tissue	Staining method	Number of persisting bodies in the nucleus			Persisting chromosomes	Persisting chromosomes and transitional forms
		0	1	2 and more		
Root primary cortex	G	5.3 – 12.0 7.8	18.7 – 29.0 24.6	61.3 – 74.0 67.6	2.0 – 8.3 5.8	7.0 – 17.0 12.6
	F	2.3 – 6.7 4.2	16.0 – 25.3 21.1	72.0 – 77.7 74.7	7.0 – 9.7 8.4	10.7 – 19.3 14.5
central cylinder	G	3.7 – 9.0 6.1	17.0 – 26.4 22.2	64.7 – 79.3 71.7	4.3 – 9.3 7.2	10.7 – 19.0 15.2
	F	2.3 – 5.7 3.7	11.7 – 24.3 17.5	70.7 – 84.0 78.8	5.7 – 9.7 8.3	14.0 – 20.7 18.7

chromatin) and may even be classified as such. Chromocentres produced by autosomal heterochromatin do not stain with Guard's method, but stain with the Feulgen reaction. The suggestion that chromocentres produced by autosomal heterochromatin and stained with the Feulgen reaction may be classified as sex chromatin is supported by the finding that almost an identical number of nuclei containing persisting chromosomes was found in the same tissues of the same plants after staining with both methods. The difference in the results of both methods is found in the incidence of nuclei with chromocentres (see Tab. 1, Fig. 2). Chromocentres formed by heterochromatin of autosomes should be observed also in the nuclei of the female plant. Therefore, five female plants were evaluated by a similar method (Tab. 2). The results showed that also in the female plant the Feulgen reaction revealed a higher frequency of nuclei with chromocentres.

Fig. 3 gives a comparison of the incidence of nuclei with a different number of persisting bodies in the male and female plant stained with Guard's method. This difference is even more striking if we consider the earlier discussed differences in the appearance of these bodies in plants of both sexes. It may, therefore, be concluded that the sex chromatin is present in the interphase nuclei of all tissues examined of the male plant of *Rumex acetosa* L. It may be observed as a chromocentre, as a transitional form, or as a persisting chromosome. Since heterochromatic segments of both Y chromosomes do not appear to form common persisting bodies, two persisting bodies should correspond to the sex chromatin of this species. In none of our investigations of different tissues did we find sex chromatin in all nuclei; the number of nuclei without sex chromatin was higher in tissues of differentiated and no longer growing organs (stem, leaf) than in still differentiating tissues (elongation zone of the root). Also in the nuclei of undifferentiated, still growing tissues persisting chromosomes and transitional forms are frequently present.

Tab. 2. — The incidence of the interphase nuclei with various numbers of chromocentres in the tissues of the vegetative organs of the female plants of *Rumex acetosa*. — Stained with Guard's modified method (G — material from 5 plants) and Feulgen reaction (F — material from 5 plants). The values are given in percentage, the upper numbers giving the lowest and the highest values, the bottom number the mean value.

Organ, tissue	Staining metod	Number of chromocentres in the nucleus		
		0	1	2 and more
Leaf				
epidermis	G	89.0—98.0 94.1	2.0— 9,7 4.2	0.0— 2.7 1.7
	F	83.3—91.0 87.9	5.4—14.0 8.3	2.7— 6.0 3.8
vascular tissues	G	90.0—97.0 94.3	1.0— 5.3 3.9	0.0— 4.3 1.8
	F	79.0—89.7 85.6	6.7—16.3 9.5	3.0— 7.3 4.9
parenchyma	G	89.0—98.7 93.8	1.3— 9.7 5.1	0.0— 3.0 1.1
	F	77.3—90.3 84.6	4.0—16.7 10.8	0.0—10.0 4.6
Stem				
cortex	G	86.0—97.0 91.5	3.0—12.0 6.2	0.0— 4.3 2.3
	F	80.3—90.6 86.4	7.3—15.7 10.6	1.7— 4.0 3.0
vascular tissues	G	90.0—96.6 93.8	1.4— 7.0 4.1	1.0— 3.0 2.1
	F	77.0—87.7 81.9	8.3—13.7 11.1	4.0—11.7 7.0
pith	G	86.0—97.0 91.9	2.0—14.0 6.9	0.0— 2.0 1.2
	F	73.3—90.0 83.3	7.7—18.0 13.2	1.0— 8.7 3.5
Root				
primary cortex	G	94.0—97.0 95.5	3.0— 5.3 3.9	0.0— 2.0 0.6
	F	78.3—90.3 85.8	5.0—15.0 8.5	3.0— 6.0 5.7
central cylinder	G	87.7—98.0 92.5	3.0—10.0 6.6	0.0— 2.3 0.9
	F	82.0—90.0 86.3	5.4—12.0 9.0	2.7— 6.0 4.7

Tab. 3. — The position of persisting bodies in the interphase nuclei of the male plants of *Rumex acetosa*. — Stained with Guard's modified method; the values are given in percentage. Explanation of the letterings: M — the portion of the nuclei with persisting bodies adjacent to the nuclear membrane; N — nuclei with persisting bodies adjacent to the nucleolus; F — nuclei with persisting bodies free inside the nucleus.

Number of persisting bodies	Position	Organ, tissue							
		Leaf			Stem			Root	
		epid.	vasc. t.	par.	cor.	vasc. t.	pith	pr. c.	c. cyl.
1	M	76.1	89.5	84.7	86.8	90.1	65.4	79.9	89.9
	F	19.6	8.6	13.1	10.1	8.3	29.5	12.5	6.1
	N	4.3	1.9	2.2	3.1	1.6	5.1	7.6	4.0
2	MM	68.0	86.3	75.8	72.1	83.2	59.0	74.8	88.6
	MF	25.3	8.9	19.1	18.1	8.0	30.1	14.8	8.1
	MN	4.4	3.6	2.3	4.0	4.6	3.2	8.3	2.0
	FF	1.2	1.0	2.8	4.6	3.3	5.9	1.9	1.2
	FN	1.1	—	—	1.0	0.9	1.8	0.2	—
	NN	—	0.2	—	0.2	—	—	—	0.1

The position of the persisting bodies

In all ten male plants stained with Guards method an evaluation was made of the position of persisting bodies in all tissues. In each tissue we evaluated 100 nuclei containing one persisting body each and 100 nuclei containing two persisting bodies, and recorded the position of these structures — adjacent to the nuclear membrane; adjacent to the nucleolus; free inside the nucleus. The mean values are given in Tab. 3. Our results indicate that the majority of persisting bodies are situated at the inner margin of the nucleus in contact with the nuclear membrane.

Discussion

The incidence of nuclei with a different number of persisting bodies

The sex chromatin of *Rumex acetosa* L. is produced by the heterochromatin of two Y chromosomes present in the diploid set of chromosomes of the male plant. In interphase nuclei it has been observed either as a chromocentre, as a persisting chromosome, or as a transitional form between these two types. All in all, these three types of structures should be designated as persisting bodies. In view of the fact that the Y chromosomes do not pair in meiosis, Żuk (1969c) suggested that they do not form a common chromocentre. Therefore, there should be two persisting bodies to each sex chromatin. In the tissues examined the incidence of nuclei containing two (or more than two) persisting bodies ranged from 30 to 70% in the differentiated tissues of the stem and leaf, and from 60 to 75% in the undifferentiated tissues of the elongation zone of the roots. In all other nuclei we found either one persisting body or none. The point requiring clarification is the finding of less than two persisting bodies and, occasionally, more than two persisting bodies in some of the nuclei.

Until the present, extensive studies on the incidence of the sex chromatin have been performed only on interphase nuclei of several tissues of mammals, including man. The results of these studies brought forth a number of hypotheses suggesting explanations of the fact that the sex chromatin cannot be identified cytologically in some nuclei of the tissue, while its presence can be demonstrated in other nuclei of the same tissue (e.g. COMINGS 1967, LEVIJ et CAREL 1968, JAMES 1964 a.o.). These hypotheses take into consideration that the cytological detection of the sex chromatin may depend on the stage of the cell cycle, on the metabolic state of the cell, on the methods employed for making preparations etc. Taking these hypotheses as a guidance in an attempt to disclose why persisting bodies could not be found in all interphase nuclei of the male plant, we should bear in mind several basic differences between these two groups of organisms. The sex chromatin of mammals is produced by one of the two X chromosomes of homogametic sex which means that heterochromatisation is facultative; by contrast, the sex chromatin of *Rumex acetosa* L. is produced by the heterochromatin of the Y chromosomes and, hence, heterochromatisation is constitutive (BROWN 1966). In addition, there are also purely morphological differences among these structures. The X chromosome of most mammals represents 5% of the haploid set of chromosomes, while both Y chromosomes of *Rumex* represent more than 17% of the diploid set. Therefore, the chromocentres formed by the latter are larger in relation to the size of the nucleus and thus more evident than those of mammals. Moreover, neither persisting chromosomes nor transitional forms have been described from mammals. It appears, however, that these types of persisting bodies may be present in some rodent species of the subfamily *Microtinae*, in which sex chromosomes are larger than those of the other mammals. They could be identified in several photographs in studies on this material (e.g. SCHMID 1967, SIEGER et al. 1970 a. o.), but they had been identified only as "large chromocentres" by the respective authors.

The results of ŻUK's (1969a) autoradiographic studies on the replication of chromosomes in the related species *R. thyrsiflorus* FINGERH. confirm that the chromocentres formed by the heterochromatin of the Y chromosomes are in a condensed state during DNA replication. Sectioning of the nuclei, particularly of large nuclei, may distort the results. If sections are not thick enough the portion of the nucleus containing the sex chromatin may be cut off. This may occur mainly with the nuclei of the pith which are largest.

The metabolic state of the cell (and also that of the nucleus) appears to be the most important factor in cytological detection of the persisting bodies. The incidence of nuclei without persisting bodies is always lower in undifferentiated tissues of the elongation zone of the root than in the fully differentiated tissues of the stem and leaf. This accounts also for the different appearance of the nuclei without persisting bodies in these two types of tissues. The nuclei in differentiated tissues of the stem and leaf have a fine granular or fibrous structure and the same appearance as most of the nuclei of the female plant. By contrast the nuclei of the elongation zone of the root, evaluated as nuclei without persisting bodies, should mostly be considered to be nonevaluable. The coloration of these nuclei is of almost identical intensity and their inside may show a dense fibrous or meshwork-like structure, or several irregular, indistinctly outlined and more intensively stained clusters.

In differentiated tissues, i.e. mainly in those of the cortex and pith of the stem, polyploidization may be assumed to have occurred in view of differences in the size of nuclei in the adjacent cells. Polyploidization of nuclei in differentiated tissues of plant organs has been observed in a number of plants (JOHN et LEWIS 1968). It seems, therefore, that the decreased incidence of persisting bodies in the nuclei of differentiated tissues is due to the aging of the nuclei and to degenerative processes occurring in connection with polypoidization of these nuclei.

In view of the fact that the Y chromosomes of the dioecious species of the genus *Rumex* sect. *Acetosa* do not pair in meiosis, ŻUK (1969c) assumes that these do not form a common chromocentre in the interphase. This assumption has been supported also by another observation: if one chromocentre only is found in an interphase nucleus, this is not twice as big as any of the two chromocentres present in another nucleus of the same tissue, but is of the same size.

This finding suggests that one of the processes mentioned above occurred only in one of the two persisting bodies.

More than two persisting bodies have been found in some interphase nuclei. In the differentiating tissues of the root it has frequently been possible to observe several chromocentres of similar size clearly connected by a thin thread. Sometimes, two chromocentres morphologically similar to the sex chromatin and visibly connected with one another, have been observed even in differentiated tissues (Pl. V : 3). These findings indicate that, sometimes, one Y chromosome may form several chromocentres. The behaviour of the Y chromosomes at the transition to the interphase confirms this assumption (VÁŇA ined).

A comparison of the incidence of nuclei with a different number of persisting bodies in the tissues under consideration suggests that there are considerable differences between the differentiated tissues of the stem and leaf on the one hand and the tissues of the elongation zone of the root on the other hand. This difference is supported by the assumption that the aging of the nuclei and degenerative processes are chiefly responsible for a decreased incidence of persisting bodies in the nuclei of differentiated tissues. The values given for the individual tissues within these two groups are very similar to one another. The incidence of nuclei without persisting bodies is lower and that of nuclei with two persisting bodies higher only in the vascular tissues of the stem and leaf. The spindle-shaped nuclei of these tissues offer a better general view of the whole nucleus and, hence, there is less danger that part of the nucleus may be cut off in longitudinal sections. A special position is held by the nuclei of the pith cells. In these, the incidence of persisting bodies was lowest and their morphology differed frequently from that of the persisting bodies in the other tissues under consideration. Moreover, the influence of the various factors obscuring the possibility of cytological detection of the pesisting bodies (see above in the text), is highest in this tissue.

The position of the persisting bodies

The results obtained in the evaluation of the position of persisting bodies in the nuclei of the male plant indicate that these bodies are usually located at the nuclear membrane. Occasionally they may be found freely inside the nucleus or adjacent to the nucleolus. The preferential position of the sex chromatin against the inner surface of the nuclear membrane has been observed in the nuclei of somatic mammalian cells (BARR 1966) and confirmed by electron microscopic studies (JAMES 1960, WOLSTENHOLME 1965).

Assuming theoretically that the persisting bodies are always located at the nuclear membrane and considering the fact that the microscope shows a planar projection of the spatial structure of the interphase nucleus, it may be expected that at a random distribution of these bodies at the inner surface of the nuclear membrane, the microscopic picture may reveal only a certain part of these bodies in contact with the membrane, while the other bodies will appear as if situated inside the nucleus. The number of bodies observed inside the nucleus will depend on the size of the nucleus and that of the bodies and on the resolving power of the microscope (LEVIJ et CAREL 1968). The theoretical value of this quotient can be obtained by geometrical reflexion but this may not always be consistent with the actual finding (MILES 1961).

Although we did not make any theoretical calculation it is evident that the incidence of nuclei with persisting bodies situated at the nuclear membrane is higher than anticipated from a random position of these structures at the

inner surface of the nuclear membrane. Only in the nuclei of the spindle-shaped cells of vascular tissues and of differentiating tissues of the central cylinder of the root the theoretical estimation may be consistent with the actual finding of a high incidence of persisting bodies located at the nuclear membrane, because the width of the nucleus is mostly no more than twice or three times larger than the width of the chromocentre. It is also interesting that in most tissues the decrease of these values in nuclei containing two persisting bodies has been found to be less significant than anticipated.

It may be extremely difficult to explain this phenomenon; the influence of the thickness of the sections may be considered only with large nuclei of the stem pith. It is evident that if part of a nucleus with a persisting body is cut off this is mostly a structure which would have been observed inside the nucleus.

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Souhrn

Bylo studováno zastoupení pohlavního chromatinu v interfázových jádrech pletiv vegetativních orgánů *Rumex acetosa*. Materiál byl zpracován barvicí metodou podle Guarda, umožňující cytologické odlišení pohlavního chromatinu od ostatních chromocenter v interfázovém jádře. Ve všech studovaných pletivech byly u samčích rostlin nalezeny v interfázových jádrech struktury, barvící se jako pohlavní chromatin. Jsou popisovány jako perzistující útvary a zahrnují chromocentra, přechodné formy od chromozómů k chromocentrům a perzistující chromozómy. Kromě jader, obsahujících různý počet perzistujících útvarů (většinou jeden až tři), byla ve všech sledovaných pletivech zjištěna i jádra, v nichž tyto útvary pozorovány nebyly. Zastoupení perzistujících útvarů bylo studováno jednak v diferencovaných pletivech stonku a listu, jednak v diferencujících se pletivech elongační zóny kořínků. V prvé skupině pletiv byl obecně zjištěn vyšší podíl jader bez perzistujících útvarů a nižší podíl jader, obsahujících perzistující chromozómy a přechodné formy. Perzistující útvary jsou většinou pozorovány na vnitřním okraji jádra v dotyku s blánou jadernou, vzácně volně uvnitř jádra nebo v kontaktu s jadérkem. Pro ověření chromatinové povahy perzistujících útvarů bylo u části materiálu provedeno i hodnocení při použití Feulgenovy nukleální reakce. Uvedenými barvicími metodami byl zpracován i materiál z vegetativních orgánů samičích rostlin. Perzistující chromozómy a přechodné formy zde nebyly zjištěny vůbec a i chromocentra, barvící se jako pohlavní chromatin a nalezená v malé části interfázových jader, se většinou liší morfologií od chromocenter v jádrech rostlin samčích.

References

Barr M. L. (1966): The significance of the sex chromatin. — Intern. Rev. Cytol., New York, 19 : 35–95.

Barr M. L. et E. G. Bertram (1949): A morphological distinction between neurons of the male and female and the behaviour of the nucleolar satellite during accelerated nucleoprotein synthesis. — Nature, London, 163 : 676–677.

Brown S. W. (1966): Heterochromatin. — Science, Washington, 151 : 417–425.

Commings D. E. (1967): Sex chromatin, nuclear size and the cell cycle. — Cytogenetics, Basel, 6 : 120–144.

Guard H. R. (1959): A new technic for differential staining of the sex chromatin and the determination of its incidence in exfoliated vaginal epithelial cells. — Amer. J. Clin. Pathol., Baltimore, 32 : 145–151.

Heitz E. (1928): Das Heterochromatin der Moose I. — Jahrb. Wiss. Bot., Leipzig, 69 : 762–818.

James J. (1960): Observations on the so-called sex chromatin. — Zeitschr. Zellforsch., Berlin, 51 : 597–616.

— (1964): Intermitotic transformation of the Barr body in cultured cells of human female. — Zeitschr. Zellforsch., Berlin, 64 : 178–180.

John B. et K. R. Lewis (1968): The chromosome complement. — Wien-New York. [Protoplasmatologia VI A.]
Levij I. S. et R. Carel (1968): Variations in the incidence of sex chromatin — a reappraisal. — Acta Cytol., Baltimore, 12 : 352—356.
Miles C. P. (1961): Peripheral position of sex chromatin. — Nature, London, 191 : 626—627.
Němec B. et al. (1962): Botanická mikrotechnika. — Praha.
Pazourková Z. (1964): Sexuální dimorfismus interkinetického jádra některých dvoudomých rostlin. — Ms. [Kand. Pr. — Knihovna Kat. Fyziol. Rostl. Genet. Mikrobiol. Biofyz. Přírod. Fak. UK Praha.]
Schmid W. (1967): Heterochromatin in mammals. — Arch. Klaus-Stift. Vererb. Forsch., Zürich, 42 : 1—60.
Shimizu Y. (1961): Sex chromatin in Rumex acetosa. — Kromosomo, Tokyo, 49 : 1521—1523. [Jap. with Engl. summary.]
Shimotomai H. et Y. Koyama (1932): Geschlechtschromosomen bei Pogonatum inflexum Lindb. und Chromosomenzahlen bei einigen anderen Laubmoosen. — J. Sci. Hiroshima Univ. 1 : 95—102.
Sieger M., F. Pera et H. G. Schwarzacher (1970): Genetic inactivity and heteropyknosis in Microtus agrestis. — Chromosoma, Berlin, 29 : 349—364.
Váňa V. (ined.): The localization of heterochromatic segments in the chromosomes of Rumex acetosa L. — Preslia. [In the press.]
Wolstenholme D. R. (1965): Electron microscopic identification of sex chromatin bodies of tissues culture cells. — Chromosoma, Berlin, 16 : 453—462.
Žuk J. (1969a): Analysis of Y chromosome heterochromatin in Rumex thyrsiflorus. — Chromosoma, Berlin, 27 : 338—353.
— (1969b): Autoradiographic studies in Rumex with special reference to sex chromosomes. — In: Darlington C. D. et K. R. Lewis [ed.]: Chromosomes today. Vol. 2, p. 183—188. — Edinburgh.
— (1969c): The additional heterochromatic chromosome and its influence on sex chromosome pairing in Rumex. — Heredity, Lund, 24 : 69—74.

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See also plates V.—VI. in the appendix.

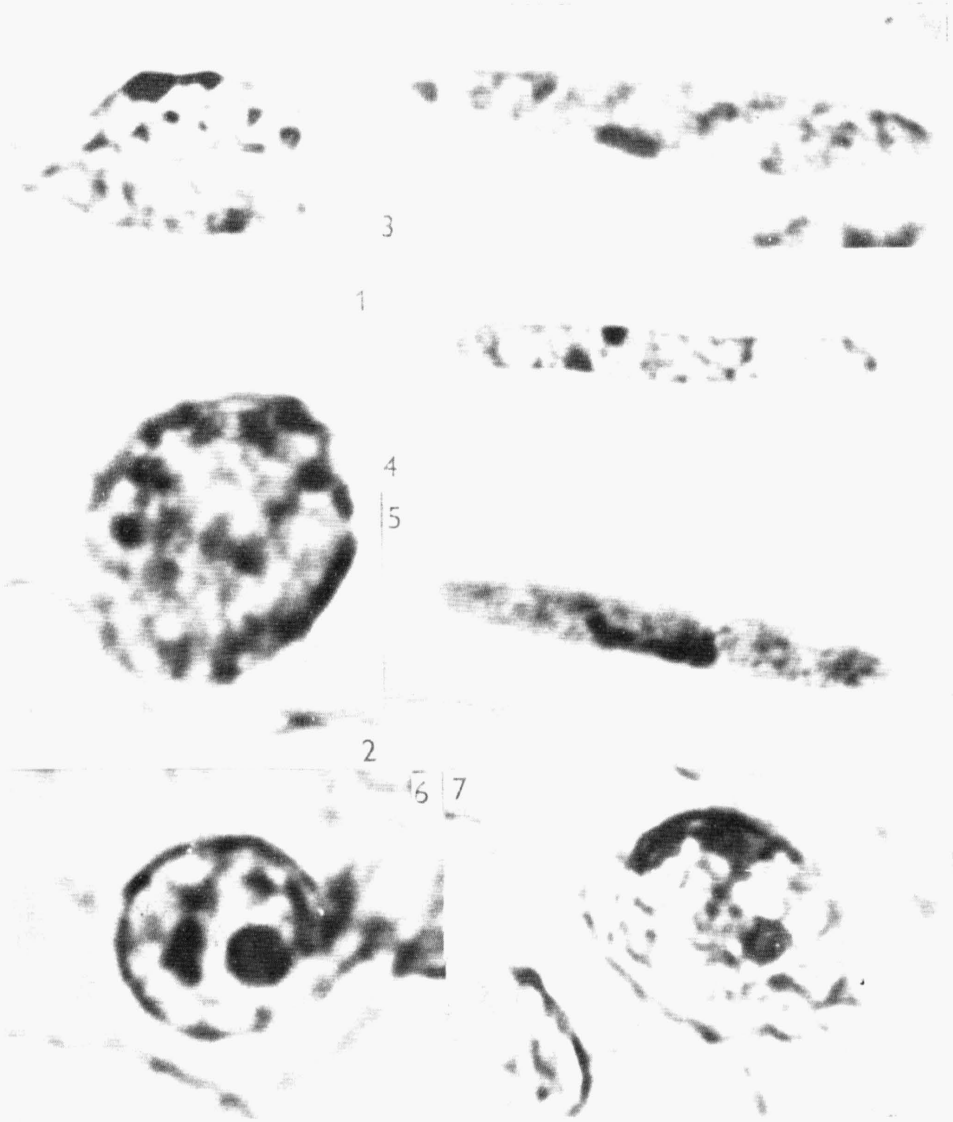

Plate V. — Interphase nuclei from various tissues of the vegetative organs of the male and female plants of *Rumex acetosa*. — 1,6: leaf parenchyma; 2: central cylinder of the root; 3: vascular tissue of the stem; 4: pith, and 5: cortex of the stem. — Stained with Feulgen reaction (2, 3) and modified Guard's method (others). Magnification approx. 4500×.

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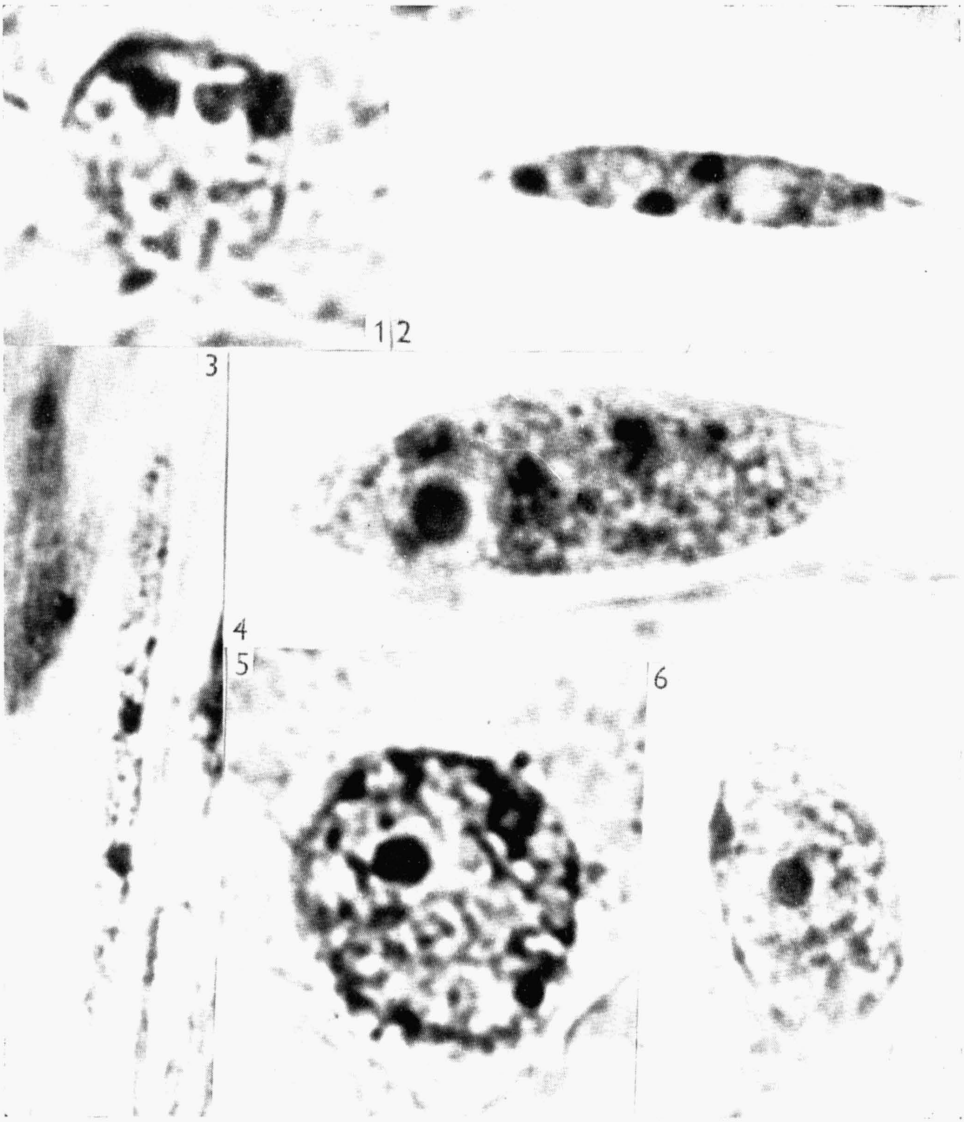

Plate VI. — Transitional forms (1—4) and persisting chromosomes (5—7) in interphase nuclei from various tissues of the vegetative organs of the male plants of *Rumex acetosa*. — 1, 2, 6, 7: leaf parenchyma; 3: vascular tissue of the stem; 4: vascular tissue of the leaf; 5: central cylinder of the root. — Stained with Feulgen reaction (1, 4, 5) and modified Guard's method (others). Magnification approx. 4500×.

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