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The Localization of Heterochromatic Segments in the Chromosomes of *Rumex acetosa* L.

Umístění heterochromatinových úseků v chromozómech *Rumex acetosa* L.

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VÁŇA V. (1972): The localization of heterochromatic segments in the chromosomes of *Rumex acetosa* L. — Preslia, Praha, 44 : 316—326. — The heterochromatic segments in the chromosomes of *Rumex acetosa* were studied using three cytological methods with special regard the sex chromosomes. Both Y chromosomes of the male plant are largely heterochromatic and persist as the sex chromatin bodies in the interphase nuclei. A description is given of the behaviour of these chromosomes during mitosis and of changes in their morphology during the formation of sex chromatin. — *Department of Genetics, Microbiology and Biophysics, Caroline University of Prague, Viničná, 5, Praha 2, Czechoslovakia.*

Introduction

Rumex acetosa L. is dioecious with a diploid chromosome number of 14 in the female and 15 in the male plant (KIHARA et ONO 1923, YAMAMOTO 1938, LÖVE 1957 a.o.). Responsible for the different number of chromosomes in the sexes is the unusual arrangement of the sex chromosomes of this species. The female plant is homogametic and its diploid set of chromosomes contains, in addition to six pairs of autosomes, two X chromosomes. The male plant is heterogametic and has three sex chromosomes — one X and two morphologically different Y chromosomes. The study of aneuploid plants occurring frequently in natural populations indicated that the sex of this species is determined by the ratio of the number of X chromosomes to the number of haploid sets of autosomes. Y chromosomes are not important in sex determination (YAMAMOTO 1938). LÖVE (1957) considered them to be genetically inert. Therefore, after the discovery of sex chromatin in the male plant of this species (SHIMIZU 1961, PAZOURKOVÁ 1964a, PAZOURKOVÁ 1964b) it was suggested that sex chromatin in this species is formed by the heterochromatin of the Y chromosomes.

An attempt has been made in this study to determine the localization of the heterochromatic segments in the chromosomes of this species. The author employed several methods to differentiate heterochromatin and euchromatin in metaphase chromosomes. In addition, he studied the behaviour of the Y chromosomes during the cycle of cell division.

The classical method employed for the differentiation of heterochromatin in metaphase chromosomes is pretreatment with low temperatures (from 0—4° C) for several days ("starvation method", DARLINGTON et LACOUR 1940). After this pretreatment, the chromatids in the heterochromatic segments of metaphase chromosomes are reduced to about half the size of those in the euchromatic segments. No changes were observed in the size of the segments occupied by heterochromatin. By contrast, changes were found in their staining properties — they were negatively heteropycnotic in the metaphase in comparison with the euchromatic segments. In the prophase, the heterochromatic segments were positively heteropycnotic similar as those in not pretreated material. Sometimes, the differentiation of these segments in the prophase was better after pretreatment with low temperatures as observed by AMES et MITRA (1968) in *Haplopappus*.

Another method of pretreatment enabling a differentiation of heterochromatin in metaphase chromosomes is the application of a water solution of salts of some heavy metals (LEVAN 1946). The best results were obtained using mercuric nitrate. In this procedure, heterochromatin stains more intensively than euchromatin within the course of the complete cell cycle including the metaphase. This offers perfect conditions for studying the nondividing nuclei, because the diffuse chromatin structure remains practically unstained. Thus, the intensively stained chromocentres can be well-distinguished from the other structures in the nucleus.

The last method applied was that of treatment with HCl-acetic acid under increased temperatures (TAKEHISA 1968). Heterochromatin appeared in the metaphase chromosomes as negatively heteropycnotic segments and frequently their diameter was smaller than that of euchromatic segments.

Differences in the stainability of heterochromatin and euchromatin in some phases of the mitotic cycle revealed properties which were used as a basis for distinguishing between these two types of chromatin (HEITZ 1928). The heterochromatic segments of the chromosomes seem to stain more intensively than the euchromatic segments in the prophase and telophase and retain this increased stainability in the interphase during which the chromocentres are formed. Although it has often been reported in the literature that in some organisms whole chromosomes (chiefly the sex and B chromosomes) are heterochromatic, the first photographs of interphase nuclei with chromosomes persisting in the metaphase state are found in papers dealing with dioecious species of the genus *Rumex* Sect. *Acetosa*. In *Rumex acetosa* L. these structures were described by PAZOURKOVÁ (1964a), in *R. thyrsiflorus* FINGERH. by ŽUK (1969a, 1969b). These persisting chromosomes identified as Y chromosomes are discussed in detail in the part of this study.

Materials and methods

Seeds collected in a natural population in the vicinity of Chomutov (380 m above sea level) were germinated in moist filter paper in Petri dishes at room temperature. Root-tips were excised, pretreated with 0,03 % aqueous solution of oxiquinoline for 4 hrs. at 11—14° C, fixed in Carnoy's mixture (ethanol : glacial acetic acid = 3 : 1) for 12—48 hrs. and squashed in acetorceine according ŽUK (1963), or after a short maceration (3 min.) in 1 : 1 (ethanol : conc. HCl) mixture squashed in 1 % acetorceine.

The localization of heterochromatic segments was studied using three methods: a) cold treatment — seedlings with roots about 0.5 cm long were grown for 3 or 4 days at 0—3° C. After pretreatment with oxiquinoline root tips were squashed in acetorceine; b) pretreatment with mercuric nitrate — root tips were treated with 0,005 M aqueous solution of mercuric nitrate for 3—4 hrs, thoroughly washed in water and after fixation squashed in acetorceine; c) treatment with HCl-acetic acid — root tips were pretreated with oxiquinoline, washed in water and transferred to a mixture of 9 parts of 2 % orceine in 45 % acetic acid and 1 part of 1 N HCl for 4—5 min. at 90° C and squashed in 1 % acetorceine.

Some photographs (Pl. XVIII : 5—7) were taken from permanent preparations stained with Feulgen reaction or Guard's modified method. These methods were described in a previous paper (VÁŇA 1972).

Results and discussion

The karyotype of all *Rumex acetosa* plants studied was 2n = 8i + 2j + + 2v + XX (female plants) and 2n = 8i + 2j + 2v + XYY (male plants); Pl. XIX: 1.

The localization of the heterochromatic segments in metaphase chromosomes of *Rumex acetosa* L.

Of the three methods employed for the differentiation of heterochromatin in metaphase chromosomes, treatment with HCl-acetic acid under increased temperatures proved to be the most suitable one. Using this method a distinct differentiation of negatively heteropycnotic (i.e. heterochromatic) segments in the metaphase chromosomes was frequently observed, the diameter of

the chromatids was even smaller than that of the other (i.e. euchromatic) segments of the chromosomes.

A method to be recommended for studies on structures of non-dividing nuclei is pretreatment with mercuric nitrate. The chromocentres are more distinct and more sharply outlined than those of untreated material in view of the fact that all other structures of the interphase nucleus remain almost unstained. In addition, in the telophase, when euchromatic segments of the chromosomes fail to stain, it is possible to observe the localization of heterochromatic segments chiefly in chromosomes with high contents of heterochromatin. A decreased staining ability has frequently been observed in euchromatic segments of metaphase chromosomes, but the differences in stainability of euchromatin and heterochromatin were less distinct than those found with the preceding method. By contrast to other methods, this method discloses more frequently the heterochromatic nature of chromosome segments situated near the centromeres ("prochromosomes"). The increased stainability of these chromosomal parts in the metaphase and telophase was described by LEVAN (1946), who drew attention also to the disadvantage of this method. Mercuric nitrate solution employed for pretreatment is, in fact, a fixation which is slow in penetrating the object and hence is responsible for frequent imperfect fixation of cells in the central part of the root meristem; consequently, these cannot be evaluated.

Pretreatment with low temperatures appears to be the least suitable of all methods employed. Differences in the staining capacity of the segments in metaphase chromosomes could either not be observed or these differences were too indistinct to distinguish exactly the individual segments with different staining properties. Negatively heteropycnotic segments disclosed by this method differed frequently in their localization in the chromosomes on the various metaphase plates. Since a comparison with the results obtained using the other methods was extremely difficult, we refrained from including data obtained from pretreatment with a low temperature in the final evaluation of the localization of heterochromatic segments (Pl. XIX : 2 and Pl. XX : 3). In recent years, objections have been raised by several authors to consider negative heteropycnosis following pretreatment with a low temperature to be a property of heterochromatin, and negatively heteropycnotic segments in the metaphase to be heterochromatic segments (LIMA-DE-FARIA et JAWORSKA 1968). AMES et MITRA (1968) observed in *Haplopappus* that negatively heteropycnotic segments are those located in the metaphase chromosomes at the point of junction between euchromatin and heterochromatin.

Pl. XIX: 2 illustrates the results of this preliminary study of the localization of heterochromatic segments in the chromosomes of *Rumex acetosa*. Pl. XX: 3 lists all other types of localization of heterochromatin in the Y chromosomes. Heterochromatic segments in j- and v-autosomes were identified with both methods, as were the segments in Y chromosomes figured in Pl. XIX: 2. Smaller heterochromatic segments in other autosomes and in X chromosome were identified employing pretreatment with mercuric nitrate and most of these also by employing treatment with HCl-acetic acid. In view of the fact that the three pairs of i-autosomes are very similar to one another and could not be differentiated in the individual metaphase plates, it was difficult to determine which of these pairs contained a heterochromatic segment around the centromere. This, however, was always present in one of the three pairs. The fourth pair of i-autosomes is slightly longer and can be differentiated from the other three pairs on the individual metaphase plates. In part of the roots examined, treatment with HCl-acetic acid revealed in one chromosome of this pair a heterochromatic segment occupying about one third of the distal part of the long arm (in Pl. XIX: 2

this segment is marked with hatching in one chromosome of this pair). In two roots only this segment was observed in both homologous chromosomes.

ŻUK (1969a) studied replication of chromosomes in *Rumex thyrsiflorus* by autoradiography. This species is closely related to *R. acetosa* and its karyotype contains morphologically identical autosomes and sex chromosomes. The plant populations of both species collected from different sites show variation in the representation of the individual types of autosomes (ŻUK 1963, ZABOROVSKA 1969). Żuk investigated the localization of late replicating (i.e. heterochromatic) segments in plants with a karyotype containing the same number of individual types of autosomes as that in our material. He found late replication in both Y chromosomes, in the short arm of the j-autosome and in one arm of the v-autosome. After pretreatment with oxiquinoline this writer found that in addition to late replicating parts of the autosomes, the two Y chromosomes were almost completely heterochromatic, too.

The localization of heterochromatic segments in the chromosomes of *Rumex acetosa* is consistent with ŻUK's data as regards the heterochromatic nature of parts of the j- and v-autosomes. In addition to these, we observed smaller heterochromatic segments in X chromosome and some autosomes. Both Y chromosomes contained several different types of heterochromatin localization. In these chromosomes we observed always smaller segments of euchromatin (one to three) in addition to larger heterochromatic segments. Several other observations confirming the assumption that Y chromosomes are not completely heterochromatic will be discussed in the section dealing with the Y chromosome behaviour during the cycle of cell division.

The problem of finding various types of localization of heterochromatic segments in the Y chromosomes not only employing different methods, but also using one method only (Pl. XIX: 2 and Pl. XX: 3) will have to be solved. Our studies on this problem are only of a provisional character, because this problem will have to be examined on extensive material to find a definitive answer. A comparison of Pl. XIX: 2 and Pl. XX: 3 shows that the differences occur chiefly in the number of small euchromatic segments, the size and position of which is almost identical. On the other hand it has been indicated by several observations made while studying nuclei at the end of cell division (see below) that despiralization of these chromosomes does not always start in the same portion of the chromosome. Consequently, attention should be drawn to the fact that pretreatment with oxiquinoline was employed before treatment with HCl-acetic acid but not before pretreatment with mercuric nitrate. Therefore, in view of the earlier disclosed differences in chromosome contraction in metaphase plates with and without pretreatment and in the contraction of metaphase chromosomes after pretreatment in various plates (this applies especially to the long chromosomes — see SASAKI 1961), the localization of heterochromatic segments has been ascertained on the basis of a comparison of chromosome length in a certain metaphase plate with the same chromosome figured in the idiogram.

Special attention should be paid to the localization of heterochromatic segments in the sex chromosomes of the male plant. ŻUK (1969a) observed in this autoradiographic studies of DNA replication in the chromosomes of *R. thyrsiflorus* that both Y chromosomes are completely heterochromatic, while the X chromosome is completely euchromatic. The sex chromosomes of the male plants of these species produce a trivalent with an X chromosome in its centre during pairing in meiosis. The end of each arm of the X chromosome pairs with one Y chromosome ("end-to-end pairing") (ŻUK 1969b). Later ŻUK (1970) suggested that both Y chromosomes must contain small euchromatic segments at the end of the arms to be able to pair with an exclusively euchromatic X chromosome. On these lines we made an interesting observation in *Rumex acetosa*: we identified a small heterochromatic segment at the end of one arm of the X chromosome and a small euchromatic segment at the end of the shorter arm of the Y chromosome

with a submedian centromere (Y_2). On the basis of these findings it may be possible to assume that this localization of the heterochromatic and euchromatic segments in the sex chromosomes determines the meiotic pairing of the Y chromosomes with the individual arms of the X chromosome. This finding may also contribute to the elucidation of the earlier known fact that both Y chromosomes neither pair with one another nor form a common chromocentre in the interphase (ŽUK 1969b).

The behaviour of Y chromosomes during the cell cycle

Squash preparations of root-tips stained with acetorceine were used for studying the behaviour of the Y chromosomes during the mitotic cycle in the dividing nuclei of the meristematic zone.

At the beginning of the prophase these chromosomes are partially decondensed; we observed several positively heteropycnotic bodies connected with threads of similar staining properties as those of the threads of the remaining condensing chromosomes (Pl. XVII: 1). At the end of the prophase, the complete condensation of the Y chromosomes occurs earlier than that of the other chromosomes (Pl. XVII: 2). The Y chromosomes can be well-identified during the telophase because a high degree of condensation persists in these chromosomes (affecting either the whole or almost the whole chromosome). The degree of condensation is always higher (and thus the chromosomes more distinct) than at the beginning of the prophase (Pl. XVII: 3). Two main types of structures could be observed on the inner surface of daughter cell nuclei:

a) those of a morphology consistent with that of the Y chromosomes, but more diffusely stained than in the metaphase (Pl. XVII: 4) (sometimes with a constriction — Pl. XVII: 5);

b) structures composed of several chromocentres connected with thin threads (Pl. XVII: 6).

Non-dividing nuclei contained either persisting chromosomes (with a median, submedian or subterminal centromere) (Pl. XVII: 3, see also Pl. XVIII), elongate club-shaped bodies without distinct constriction (Pl. XVII: 7), various types of "partly despiralized chromosomes" (see below and Pl. XVIII) or only chromocentres of morphology similar to that of the nuclei of various tissues in differentiated organs (VÁŇA 1972) (Pl. XVII: 8). In most of the individual interphase nuclei we observed the simultaneous presence of various types of these structures (e.g. one persisting chromosome and one chromocentre etc.). The nuclei contained mostly two these structures, less frequently one. If there were more than two structures present, these were either chromocentres or several chromocentres and one structure of a different type.

Apart from the nuclei containing these morphological types of chromatin structures we observed less frequently also nuclei lacking these large and distinct chromatin bodies; these occurred mainly in the region of the meristematic zone of the roots.

In the nuclei of the daughter cells which were still paired, as well as in the nuclei which appeared to be at the beginning of the interphase (the nucleus was small with a relatively large indistinctly outlined nucleolus the persisting bodies remained even after pretreatment with oxiquinoline always in the unichromatid state and, moreover, in the squashes the cells with these nuclei remained frequently paired) and also in the non-dividing nuclei of the root-tips, we found in addition to typical chromocentres of the sex chro-

matin (Pl. XVII: 8) and to chromosomes persisting in the metaphase form (Pl. XVII: 4, 5; Pl. XVIII: 1, 3 4) structures suggestive of a "transitional form" such as described in an earlier paper (VÁŇA 1972). Some of these were "partly despiralized chromosomes" which, sometimes, can be observed also in telophase nuclei of the meristematic zone. These "partly despiralized chromosomes" can be described as chromosomes in the metaphase form,

Tab. 1. — The incidence of the interphase nuclei with various numbers and types of persisting chromosomes in the root meristematic nuclei of the male plants of *Rumex acetosa*. Material from 10 roots (327 nuclei investigated). The letterings designate centromere position in the persisting chromosome: m — median, sm — submedian, st — subterminal

Number of the persisting chromosomes	Type of the persisting chromosomes	Percentage of nuclei
1	m	26.91
	sm	47.09
	st	13.46
2	m and m	2.76
	m and sm	4.58
	m and st	3.06
	sm and sm	1.84
	sm and st	0.30
	st and st	—
	total	12.54

in which only small segments despiralize at one or several sites without significantly changing the general appearance of the chromosome (Pl. XVIII: 2, 3, 5). The localization of the segments at the onset of despiralization is mostly consistent with that of the identified heterochromatic segments in the metaphase Y chromosomes after pretreatment (compare Pl. XIX: 2 and Pl. XVIII: 2, 3). It will be seen from the photographs of the nuclei at the onset of the interphase (Pl. XVIII: 1—3) that despiralization does not occur simultaneously in both Y chromosomes. It appears that despiralization of the Y chromosome with a median centromere (Y_1) in the nuclei of the root-tips starts a little earlier than that of the Y chromosome with a submedian centromere (Y_2) (Pl. XVII: 5; Pl. XVIII: 2, 3). Nuclei in which despiralization of the Y_2 chromosome started first were less frequent (Pl. XVIII: 1). It should be remembered, however, that this is a nucleus of the elongation zone of the root, i.e. of that part of the root in which cell division is almost nonexistent. In these parts despiralization of the Y chromosomes does not seem to occur always in the same part of the chromosome (see below). It is interesting to compare this suggestion with ŻUK's statement that one of the Y chromosomes of *R. thyrsiflorus* starts DNA replication earlier than the other Y chromosome and that there may be a difference in time in the cycle of both Y chromosomes (ŻUK 1969a).

The assumption that despiralization of a Y chromosome with a median centromere starts slightly earlier than that of a Y chromosome with a submedian centromere is supported also by the results of studies on nuclei with persisting chromosomes in the elongation zone of the root (Tab. 1). If there

persists only one of the Y chromosomes this is mostly the one with a submedian or subterminal centromere and not the one with a median centromere. It appears that the persisting chromosome with the subterminal centromere originated from the Y chromosome with the submedian centromere by despiralization of the euchromatic segment in the distal part of the short arm (compare Pl. XIX: 2 and Pl. XX: 3 and Pl. XVIII: 2). In accord with these assumptions is also the incidence of individual types of nuclei with two persisting chromosomes (Tab. 1). Although the incidence of nuclei with two persisting chromosomes is very infrequent, we observed nuclei with combinations of two persisting chromosomes suggesting that the Y chromosome with a median centromere started to despiralize first. In the nuclei marked "st; m" and "m; m" the one persisting chromosome with a median constriction had always shorter arms than the second persisting chromosome. As indicated by the schematic diagram on Pl. XX: 4, the persisting chromosome with a median constriction may be a partly despiralized Y chromosome with a median centromere.

On the basis of the results of studies on partly despiralized Y chromosomes in the telophase and interphase it is possible to ascribe several transitional forms to each Y chromosome. Some of these transitional forms could be arranged in a row illustrating the assumed changes in morphology of the persisting bodies at the transition of metaphase chromosomes to oval or lens-shaped chromocentres typical of sex chromatin (Pl. XX: 4):

a) In the Y chromosome with a median centromere (Pl. XX : 4A—1) small despiralized segments were observed first in both arms. These separated ovoid chromocentres in the distal part of both arms were connected with the middle part of the chromosome by a thin thread (Pl. XX : 4A—2; Pl. XVIII : 3). Later, this thread elongated and, hence, the connection of the end chromocentres with the central part of the chromosome became obscure (Pl. XX : 4A—3′) or a small chromocentre could be seen between both parts (Pl. XX : 4A—3″). This was followed by some kind of rounding of the segments in the central part of the chromosome (Pl. XX : : 4A—4). At this stage it was often impossible to discern the connection of this part with the chromocentre; it is uncertain whether these segments despiralize or merge with the central part. This stage appeared to be followed by a constriction into the chromocentre — we observed two more or less rounded chromocentres connected with a slightly thinner segment (Pl. XX : : 4A—5; Pl. XVII : 5; Pl. XVIII : 2) and, finally, an oval to lens- or loaf-shaped chromocentre (Pl. XX : 4A—6; Pl. XVII : 8) which resembled in both shape and size a typical sex chromatin of differentiated tissues.

b) In Y chromosome with a submedian centromere (Pl. XX; 4B—1) the despiralization of the short segment in the distal part of the shorter arm occurs first (Pl. XX : 4B—2; Pl. XVIII : : 2). Later, despiralization affects, apparently, a major part of this arm and the "persisting chromosome with the subterminal centromere" originates (Pl. XVII : 5; Pl. XVIII : 4). Sometimes, a thin fibre extends from the end of its short arm (Pl. XX : 4B—3′). Despiralization may also occur in another segment of the short arm and a formation consisting of two approximately identical oval chromocentres connected with the second completely coiled arm originates (Pl. XX : 4B—3″). It is possible that the persisting chromosome with a subterminal centromere originated from this structure by the constriction and mergence of both chromocentres because the diameter of the short arm was often larger than that of the long arm (Pl. XX : 4B—3‴). In some rare instances, some kind of contraction and an irregular shape of the longer arm could be observed (Pl. XX : 4B—4). This may have been a transition to another, frequently observed, formation consisting of two connected oval chromocentres of distinctly different size (Pl. XX : : 4B—5; Pl. XVIII : 1, 7). Later, both chromocentres merged and a typical sex chromatin originated (Pl. XX : 4B—6).

All these transitional forms were found also in the nuclei of differentiated tissues of stems and leaves in mature plants. Moreover, we found also different types of transitional forms in these tissues; these were observed frequently in the tissues of stems and leaves, less frequently in the tissue of

the root tips. In some of them it was possible to determine the Y chromosome from which they originated (Pl. XX: 5). An explanation of differences in incidence and the morphology of the persisting bodies in tissues of completely differentiated (i.e. no longer growing) organs on the one side and of differentiating (i.e. intensively growing) organs on the other hand (VÁŇA 1972) may be offered by the assumption that the time of delyaed despiralization of the Y chromosomes depends on whether cell division in the nucleus will continue and if so, after what time. For example, in nuclei of daughter cells of the meristematic root zone, in which further cell division may be expected to start soon, the occurrence of partly despiralized Y chromosomes is quite frequent (Pl. XVII: 6). By contrast, we observed only an occasional persisting chromosome with clearly visible chromatids, in the tissues of differentiated organs which had ceased to divide (Pl. XVIII: 5). Our assumption is supported by the fact that the highest number of nuclei with persisting chromosomes or with transitional forms occurs in the elongation zone of the root and in parenchyma of young leaves (VÁŇA 1972) i.e. in tissues in which intensive division had occurred very recently, but in which, at the time of observation, mitotic activity had almost ceased. A detailed study of the transition of the Y chromosomes to the chromocentres of the sex chromatin in differentiated organs would, apparently, be most difficult to perform. It is possible that this transition is different from that in the meristematic zone of the root, being, to some extent, due to the different physiological state of the nuclei of these parts of the plant.

In conclusion, there are two problems to be elucidated: a) the occurrence of persisting chromosomes with a subterminal centromere; b) the persistence of complete Y chromosomes into the interphase, although these chromosomes are not completely heterochromatic.

The results of studies on the behaviour of Y chromosomes during the cell cycle and on the localization of heterochromatin in the chromosomes of *Rumex acetosa* indicate that only Y chromosomes of the male plant with a median or submedian centromere can be persisting chromosomes. By contrast, autosomes with a subterminal centromere contain very little heterochromatin (Pl. XIX: 2). These findings indicate that persisting chromosomes with a subterminal constriction originate from chromosomes with a median or submedian centromere by partial despiralization of one arm. This has also been supported by some data given earlier in the text, i.e. that despiralization of the distal euchromatic segment of the shorter arm occurs at the end of cell division in a Y chromosome with a submedian centromere (Y_2) and that the "persisting chromosome with a subterminal constriction" is derived from it. It has, in fact, been observed quite frequently that a thin thread extends from the short arm of this chromosome. This, however, is not a common feature and we found distinct persisting chromosomes with a subterminal constriction in both differentiated and meristematic tissues (compare Pl. XX: 4; Pl. XVII: 8; Pl. XVIII: 4, 5 and Pl. V: 6 in VÁŇA 1972). The assumption that no other but two Y chromosomes persist has been supported indirectly by the fact that only two chromosomes have been found to persist in a single nucleus.

HEITZ (1928) first attempted to explain the persistence of metaphase chromosomes to the interphase. In describing the heterochromatic parts of the chromosomes he maintained that typical of these parts is the shortening of the interkinese in that prophase processes start ahead

of time, while telophase processes are retarded. Changes occurring during the transition of telophase chromosomes to the interphase were studied in the 30s of this century mainly by Belgian and French cytologists (rev. in LAFONTAINE 1968). Their studies resulted in the finding that dechromatization of the euchromatic segments of chromosomes occurs during this phase of the cell cycle and that these are "lost" to the nucleoplasm. This does not affect continuity of the chromosomes but only the visibility of the euchromatic segments. Later this theory was modified in agreement with RIS' hypothesis (1945) explaining the difference between heterochromatin and euchromatin on the basis of the degree of spiralization of the chromosomal thread in these types of chromatin (DELAY 1948). The influence of heterochromatin contents in the chromosome (or in the chromosomal segment) on its spiralization was studied by EBERLE (1957) who concluded that the amount of heterochromatin in the chromosome (or in the chromosomal segment) determines the degree, the initial and final value of its contraction: "...Vielmehr ist das Heterochromatin nicht durch einen besonderen Kontraktionszustand gekennzeichnet, sondern es bewirkt seiner Menge gemäss, in Abhängigkeit vom jeweiligen Kernzustand, einen bestimmten Spiralisationszustand eines Chromosoms bzw. eines Chromosomenabschnittes. In die Spiralen einbezogen werden hierbei beide Chromatine."

Both Y chromosomes of *Rumex acetosa* contain small segments of euchromatin among larger segments of heterochromatin and hence, this high content of heterochromatin associated with late despiralization can be considered to be the cause of the presence of metaphase Y chromosomes in the interphase.

BARLOW et VOSA (1969) studied the behaviour of the heterochromatic B chromosomes during the cell cycle in *Puschkinia libanotica*. Although these chromosomes are fully heterochromatic, the authors did not observe them in the non-dividing nuclei, but only segments of these chromosomes. At the end of the prophase the complete condensation of the B chromosomes occurs earlier than that of the other chromosomes. This type of behaviour of the B chromosomes was described earlier in *Narcissus juncifolius* by FERNANDES (1939 — cited according to BARLOW et VOSA 1969), and it is the same behaviour as described for the Y chromosomes of *R. acetosa* in this paper.

BARLOW et VOSA (1969) tried to elucidate the phenomenon by differentiating two types of heterochromatin. This theory is a modification of earlier HEITZ's theory of α- and β-heterochromatin (HEITZ 1934) One type of heterochromatin remains in the condensed state during the whole cell cycle; the other is despiralized during interphase, but has precocious spiralization in the prophase.

The presence of these two types of heterochromatin in the Y chromosomes of *R. acetosa* can also explain the difficulties in the study of the precise localization of heterochromatic segments in these chromosomes. It is possible that these two types of heterochromatin behave differentially to the various pretreatment agents employed for the differentiation of heterochromatin in the metaphase chromosomes. It is interesting that the positively heteropycnotic X chromosome in mammalian females is partly despiralized in some phases of the cell cycle (MILES 1964, MILES et O'NEILL 1970). We may conclude that both the mentioned types of heterochromatin may be present in the chromosomes that are described as fully heterochromatic.

It is obvious that both hypotheses mentioned above describe the same phenomenon, i.e. the precocious prophasic spiralization of some segments of chromatin among larger segments of heterochromatin.

Note. — Recently KURITA et KUROKI published a series of papers on the chromosomes and heterochromatin of *Rumex acetosa* (e.g. Jap. J. Genet. 45 : 255, 1970; Bot. Mag. Tokyo 84 : : 18–23, 1971). The present study was completed in late 1970 and it was therefore not possible to take these reports into consideration.

Souhrn

Umístění heterochromatinových úseků v chromozómech *Rumex acetosa* L. bylo studováno třemi různými cytologickými metodami: předpůsobením nízkou teplotou, předpůsobením dusičnanem rtuťnatým a působením směsí kyseliny chlorovodíkové a kyseliny octové za zvýšené teploty. Nejlepší výsledky byly získány při použití poslední z uvedených metod. Naproti tomu výsledky získané po předpůsobení nízkou teplotou nasvědčují tomu, že tato metoda není vhodná ke studiu heterochromatinových úseků u tohoto druhu. Obecně byla zjištěna heterochromatinová povaha krátkého ramene j-autozómu a jednoho ramene v-autozómu. Ostatní heterochromatinové úseky v autozómech a X chromozómu jsou menší a nebyly nalezeny ve všech preparátech. V obou Y chromozómech samčích rostlin bylo zjištěno několik různých typů umístění heterochromatinových úseků. Kromě jednoho až tří menších euchromatinových úseků jsou tyto chromozómy celé heterochromatické.

Na roztlakových preparátech kořenových špiček bylo sledováno chování obou Y chromozómů během cyklu buněčného dělení. V některých jádrech tyto chromozómy koncem buněčného dělení prodělávají částečnou despiralizaci a přechod do chromocenter pohlavního chromatinu; jindy je jejich despiralizace zpožděna a potom si zachovávají metafázovou formu až do interfáze, kde jsou potom pozorovány jako perzistující chromozómy. Na základě studia chování těchto chromozómů v telofázi a na počátku interfáze bylo vytvořeno schéma znázorňující změny morfologie Y chromozómů, k nimž dochází během přechodu do chromocenter pohlavního chromatinu.

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See also plates XVII.—XX. in the appendix.

Correction

In the author's previous paper [VÁŇA V. (1972): Studies on the sex chromatin in the various tissues of the vegetative organs of *Rumex acetosa* L. — Preslia, Praha, 44 : 100—111] the legends to plates in the appendix are interchanged. The text under Plate V. refers to Plate VI., and vice versa.

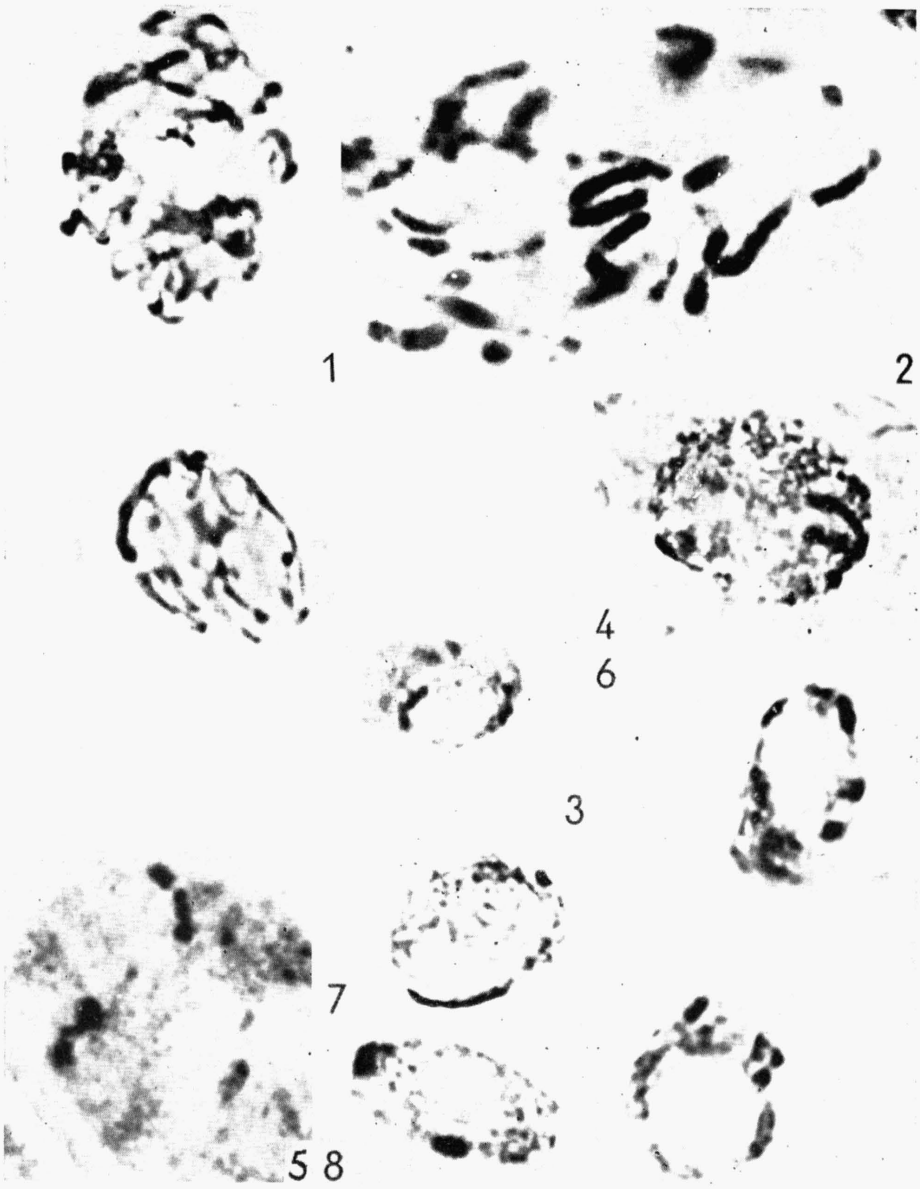

Plate XVII. — The behaviour of Y chromosomes during the mitotic cycle in the meristematic zone of the roots of the male plant of *Rumex acetosa*. All squashes, stained with acetorcein. Magnification approx. 3900×. — 1: early prophase, 2: late prophase — note completely condensed Y chromosomes in the centre, 3: telophase (left) and interphase (right) nucleus; note similarity between the positively heteropycnotic chromosome in the left nucleus and the persisting chromosome in the right nucleus, 4–6: daughter cell nuclei, 7–8: nondividing nuclei.

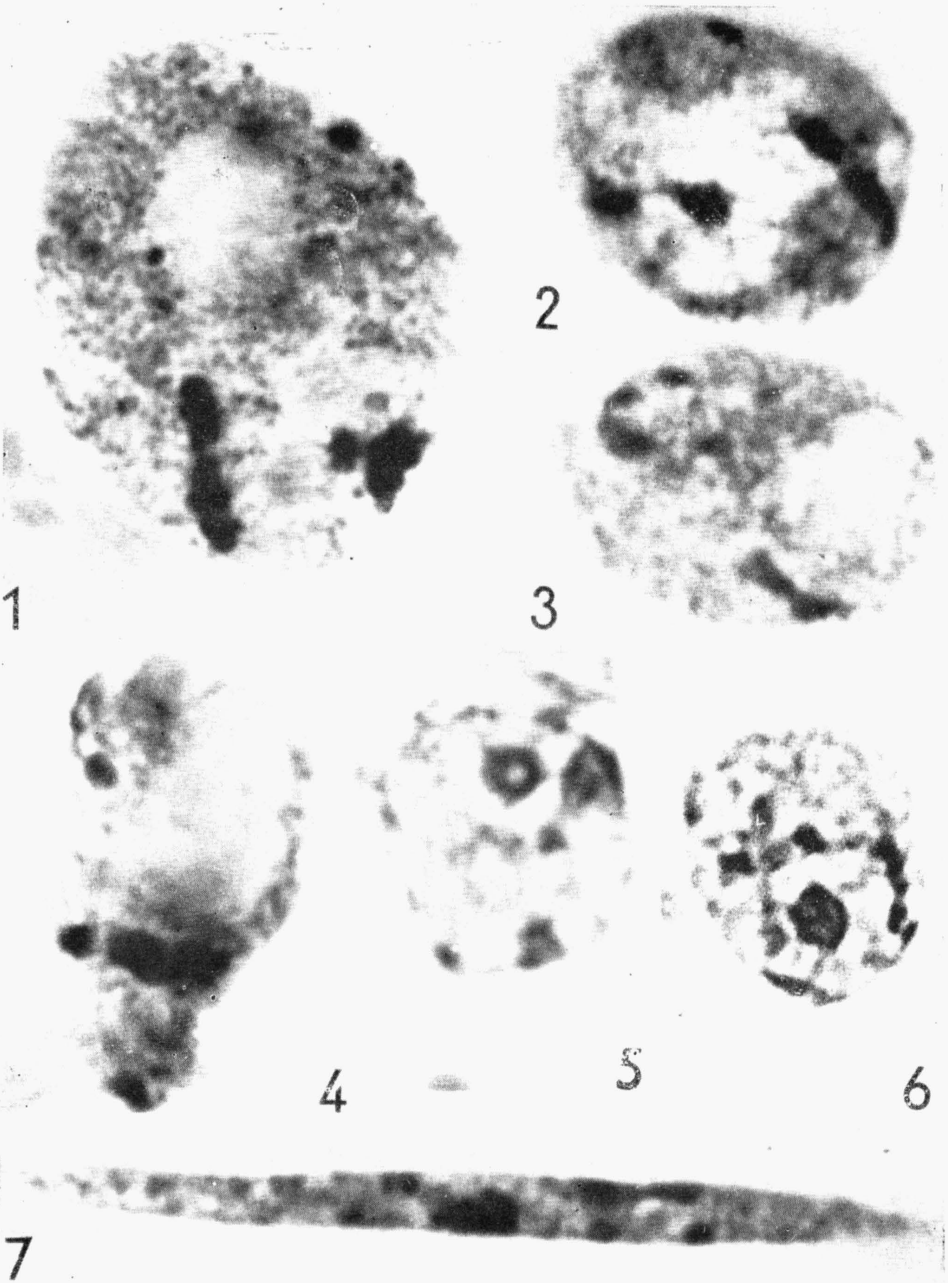

Plate XVIII. — Persisting chromosomes and "partly despiralized chromosomes" in the non-dividing nuclei of various tissues of the male plant of *Rumex acetosa*. Magnification 4500× (6), 4000× (others). — 1—4: root tip squashes stained with acetorcein, 5—6: nuclei of the pith of the stem, stained with Guard's modified method, 7: nucleus from the vascular tissue of the stem, stained with Feulgen reaction.

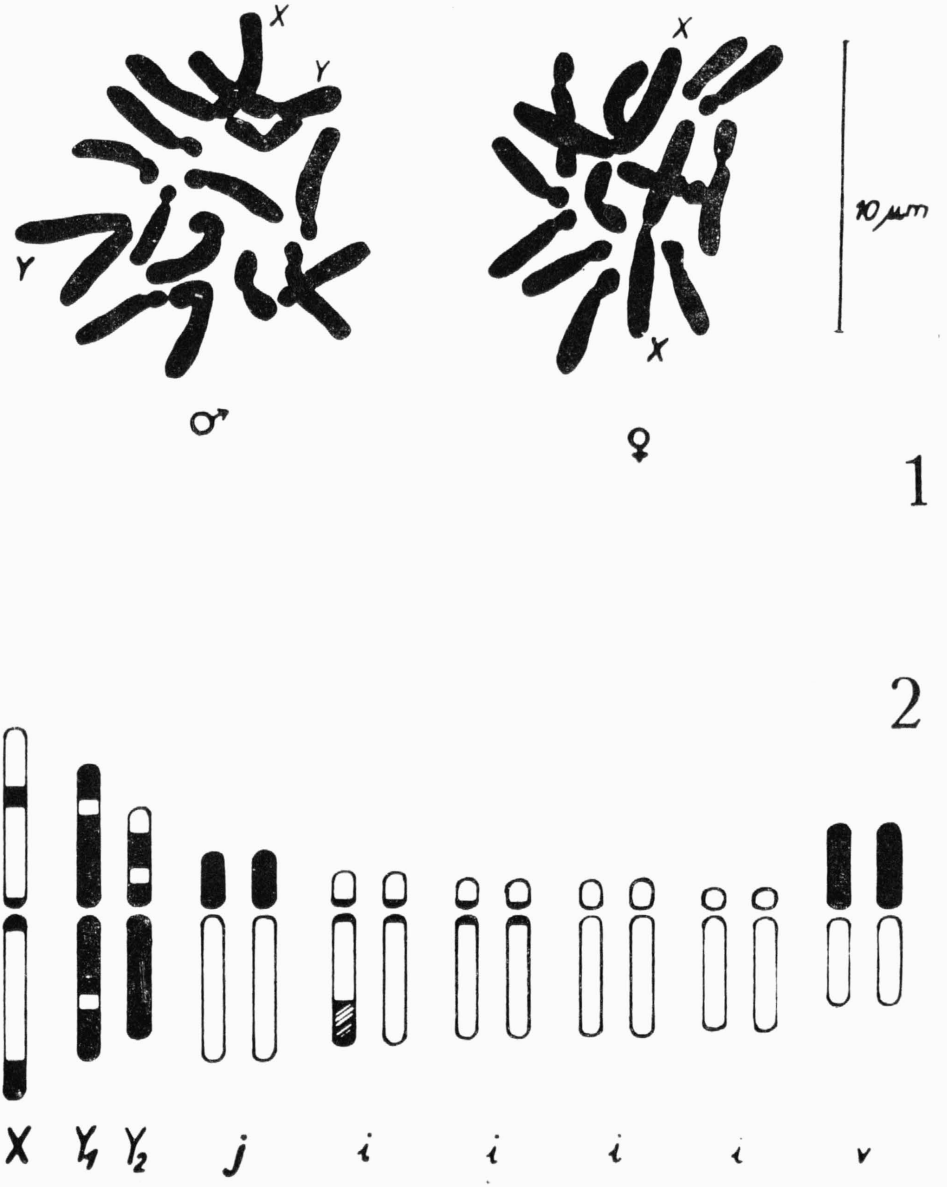

Plate XIX. — 1: The karyotype of the male (left) and the female (right) plant of *Rumex acetosa*. — 2: The localization of heterochromatic (black) and euchromatic segments in the chromosomes of the male plant of *Rumex acetosa*.

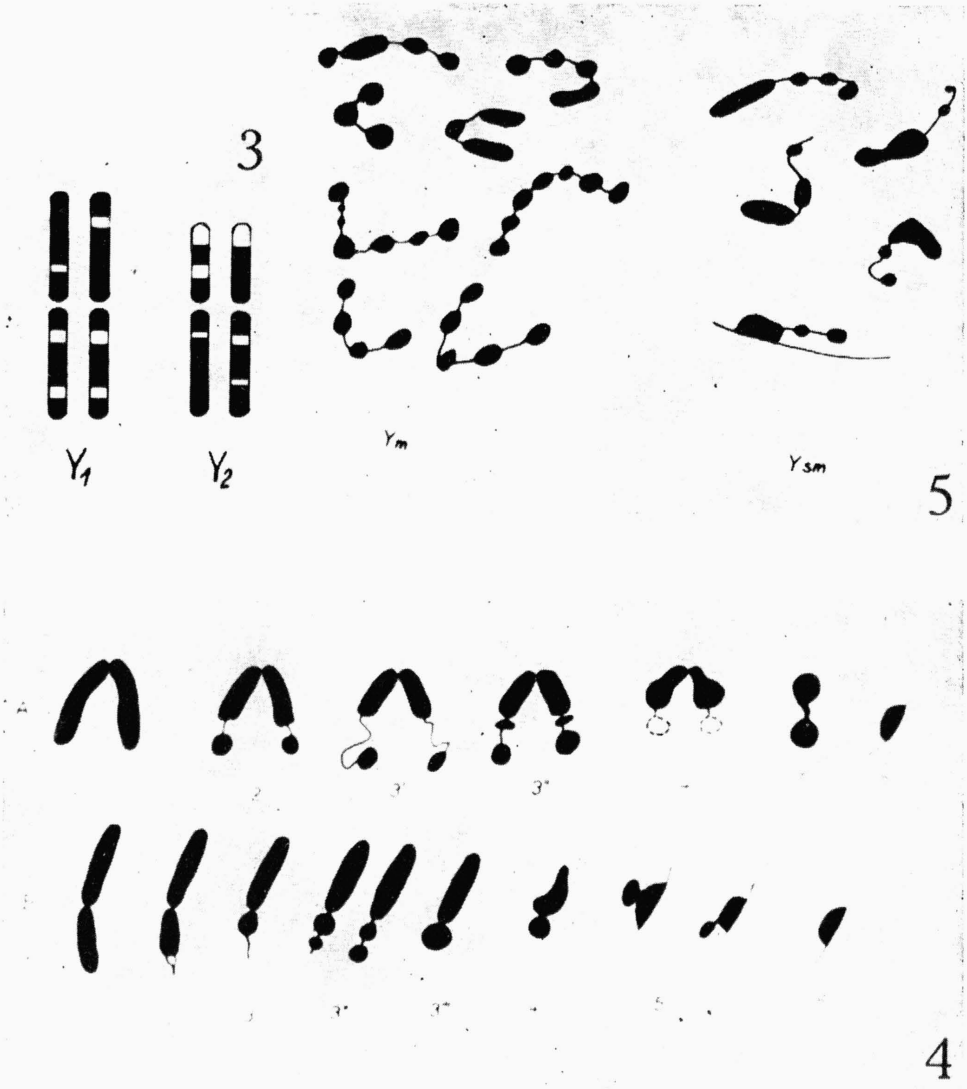

Plate XX. — 3: The other types of localization of heterochromatin in the Y chromosomes. — 4: Schematic drawing of the assumed changes in morphology of the Y chromosomes at their transition to chromocentres typical of sex chromatin; A: Y chromosome with a median centromere, B: Y chromosome with a submedian centromere. — 5: Transitional forms from the nuclei of the male plants of *Rumex acetosa*. The letterings Y_m and Y_{sm} designate the Y chromosomes from which they originated.

V. Váňa: The Localization of Heterochromatic Segments in the Chromosomes of *Rumex acetosa* L.