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Sexual Dimorphism in the Size of Nuclei with Dioecious Plants

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Abstract. Measurements of the size of the nuclei of dioecious plants showed that the nuclei of male and female plants differ in agreement with the larger quantity of chromatin. The male plants of *Rumex acetosella* and *Melandrium album* had larger nuclei, their Y chromosome being larger than the X chromosome, the same is true for *Rumex acetosa* where the Y chromosome is smaller but there are two in the set. *Ginkgo biloba* had larger female nuclei because the Y chromosome was smaller than the X. The curves obtained by grouping all the nuclei of both sexes never had two peaks with regard to the small differences between the classes of maximum frequency.

Up to the present caryological research of the dimorphism of dioecious plants was limited practically to studies of the chromosome set and the description of the sex chromosomes in connection with the study of the sexual mechanism. In this work an attempt has been made to find other criteria of this differentiation, especially in the interkinetic nucleus to which little interest has been shown previously from this point of view. The quantity of chromatin is known to affect the size of the nucleus and of the cell. As a typical example of this relationship the size of the nuclei and of cells of variously ploid material can be given. It can, therefore, be assumed that also in cases where two types of gametes with different X and Y chromosomes are formed, or where Y is missing, differences appear in the size of the gametes. This assumption has been confirmed by H. B. GOODRICH (1916), who measured the spermatozoids of Ascaris incurva, where a curve with two peaks appeared after the grouping of values. Similar results were also obtained with other zoological and botanical material. Experiments with measurements of gametes of pollen grains (Y. SINOTO 1929) permitted the assumption that differences appear not only with different numbers of chromosomes but also when one set is larger than another in which the heteromorphic homologues has a smaller mass. On the basis of this data it was interesting to determine

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whether these differences manifested themselves in the size of the nuclei of cells of differentiated tissues as well.

Material and Methods

The plant material was *Rumex acetosella* L., *Rumex acetosa* L., *Melandrium album* (MILL.) GARCKE and *Ginkgo biloba* L. The material came from cultures grown in the genetical garden of the Charles University in Praha and from wild growing plants from the vicinity of Plzeň and Průhonice. For studies of *Ginkgo biloba* the samples were taken from trees in the botanical garden of the Charles University in Praha.

With this material the caryograms and the size of the nuclei were studied. The caryograms were made in order to determine the exact dimensions of the individual chromosomes, especially of the sex chromosomes, and to gain thus a detailed picture of the chromosome set of the plants investigated.

The nuclei were measured in permanent preparations. The objects were fixed by FAA and the preparations were stained by a modified Guard's method (PAZOURKOVÁ 1964). Caryological analysis of the plants was carried out by the usual rapid methods and by a modified staining method with Schiff's reagent according to the following procedure:

1) Fixing with alcohol-acetic acid fixing fluid in 3 : 1 ratio for 10—30 min.
2) Maceration and simultaneous hydrolysis in a mixture of conc. HCl and 96% alcohol (1 : 1) for 10 min.
3) Washing in distilled water for 10 min.
4) Material placed into Schiff's reagent for 1 to 1/2 hr.
5) After staining (in bulk) the material is transferred to a slide, a drop of levulose sirup is added and a cover slip placed on top. The object is thoroughly squashed with rubber and observed.

When coloration of the material is too weak, it is gently heated over a flame. After heating the chromatin is intensively stained but the slight plasma staining does not interfere with the determination of the number of chromosomes.

With *Rumex acetosella* the nuclei were measured in the roots, leaves and the stem, with *Rumex acetosa* in the roots of germinated seeds and in the leaves and with *Melandrium album* and *Ginkgo biloba* in the leaves. The dimensions of the nuclei were evaluated from the means of two values measured perpendicularly in the plane of the field of view of the microscope. Since the third dimension was not measured, some inaccuracies cannot be excluded. For this reason the nuclei were measured in all organs only with *Rumex acetosella*, with the other plants, however, only the nuclei in the leave cells were measured where they have almost a spherical shape. This removes one source of possible errors. The difference in this evaluation consists, therefore, only in the comparison of longitudinal instead of spacial units, possible errors due to the omission of the third dimension being eliminated by the different orientation of the nuclei in the cells. This method of evaluation was fully satisfactory, because it concerned the relative comparison of the observed differences and not the determination of the absolute size of the nucleus.

Results

The *Rumex acetosella* L. plants studied had a diploid number of chromosomes $2n = 14$. According to the localization of the centromer the male plants had the following types of chromosomes in the chromosome set: $4V + 2L + 4i + + 2T + XY$. The caryotype of the male plants contained morphologically analogous chromosomes: $4V + 2L + 4i + 2T + XX$ (Fig. 1).

Fig. 1 Caryograms of male and female *Rumex acetosella* L. plants.

The results of measurements of the nuclei are summarized in Table 1 and Fig. 2.

The nuclei in the somatic cells of the male plants are significantly larger ($t_{771} = 7{\cdot}297$). Only the nuclei in the preparations of leaves cut parallel to the leave surface are at the limit of significance ($t_{98} = 1{\cdot}932$).

Table 1

Comparison of the size of nuclei in the somatic cells of *Rumex acetosella* L. (The values are given in μm)

	Female plants			Male plants		
	x	s	s_x	x	s	s_x
Root	13·34	2·77	0·277	14·80	2·66	0·266
Stalk	9·29	1·40	0·140	10·11	1·55	0·155
Leave across	11·66	1·89	0·188	13·53	2·29	0·174
Leave cut parallelly to the surface	12·37	3·17	0·449	13·66	3·161	0·447
Whole plant	11·56	2·71	0·145	13·01	2·84	0·138

The chromosome set of *Rumex acetosa* L. differs in male and female plants by the number of chromosomes. Male plants in the diploid state contain 15 and female plants 14 chromosomes. The species used did not have morphologically very different autosomes. Only the first autosomal pair with medially placed centromer and the heterochromosomes differed expressively. The caryotype of the male plants was of the type: $10i + 2V + YY + X$, that of the female plants of type: $10i + 2V + XX$ (Fig. 3).

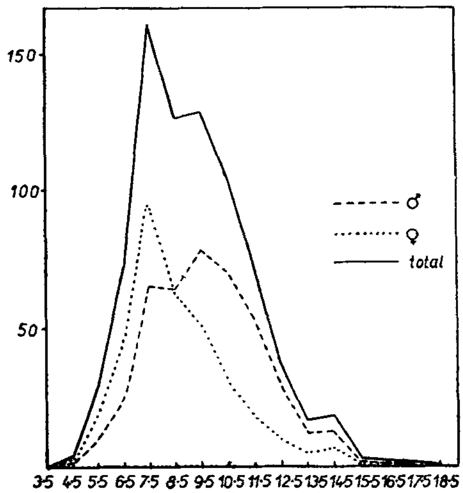

Fig. 2. Frequency polygons of the size of nuclei in somatic cells of *Rumex acetosella* L. (1 part = 1·35μm).

With this material besides the nuclei in the leaves also the nuclei in the differentiation zone of the roots germinated from seeds in Petri dishes were measured.

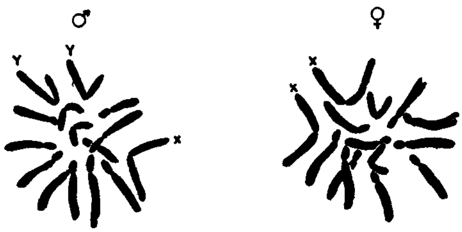

Fig. 3. Caryograms of male and female *Rumex acetosa* L. plants.

There are no significant differences in the average size of the nuclei of the root cell from the individual plants, as can be seen from the values given in Tab. II.

Table 2

Average size of nuclei in the roots of *Rumex acetosa* L. (in μm)

Root No.	1	2	3	4	5	6	7	8	9	10
Size of nuclei	10·2	9·9	9·7	10·1	10·2	10·4	10·8	10·4	10·3	10·0

When all the values measured from all roots are summarized, a polygon characterized by a clear one-peak curve with a comparatively high maximum (Fig. 4) is obtained.

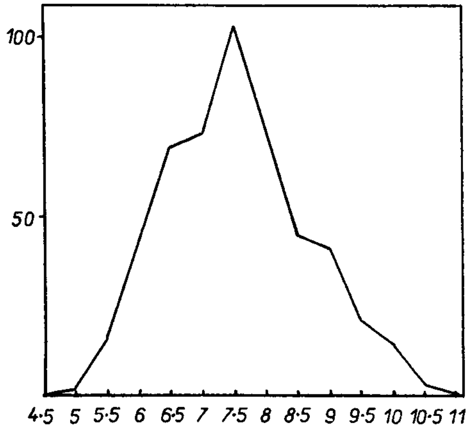

Fig. 4. Frequency polygons of the size of nuclei in the differentiation zone of germinated roots of *Rumex acetosa* L. (1 part = 1·35 μm).

Table 3

Average size of nuclei in the somatic cells of *Rumex acetosa* L. (in μm)

Female plants			Male plants		
$\bar{x}$	s	$s_{\bar{x}}$	$\bar{x}$	s	$s_{\bar{x}}$
5·314	0·755	0·034	5·665	0·805	0·036

$$t_{(998)} = 7{\cdot}11$$

Table 4

Average size of nuclei in the somatic cells of *Melandrium album* (Mill.) Garcke (in μm)

Female plants			Male plants		
$\bar{x}$	s	$s_{\bar{x}}$	$\bar{x}$	s	$s_{\bar{x}}$
5·96	0·86	0·054	4·51	0·86	0·054

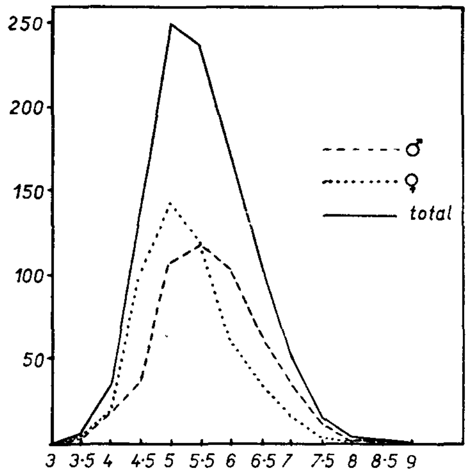

Fig. 5. Frequency polygons of the size of nuclei in somatic cells *Rumex acetosa* L. (1 part = 1·35 μm).

Fig. 6. Caryograms of male and female *Melandrium album* (Mill.) Garcke plants.

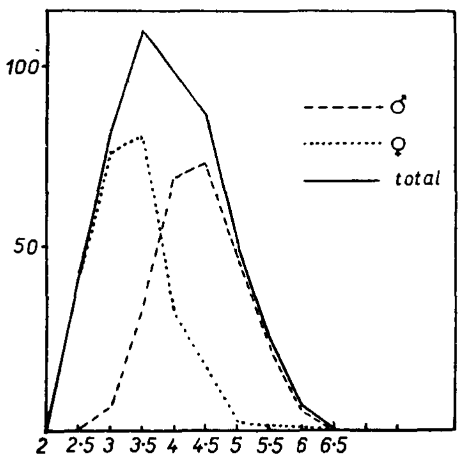

Fig. 7. Frequency polygons of the size of nuclei in somatic cells of *Melandrium album* (Mill.) Garcke.

A different situation prevails, however, with the size of the nuclei of the cell from the mesophyll of male and female plants. The difference between the sexes is highly significant, as can be seen in Tab. 3 and Fig. 5.

Another plant investigated was *Melandrium album* (MILL.) GARCKE, which has 24 chromosomes in the somatic cells. The material had the caryotype 12L to J + 10V + XY in male plants and 12L to J + 10V + XX in female plants (Fig. 6).

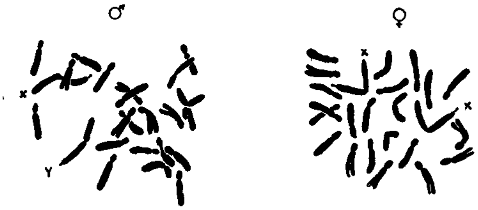

Fig. 8. Caryograms of male and female *Ginkgo biloba* L. plants.

For the determination of differences in the size of the nuclei in differentiated tissues the nuclei of the cells of the leave mesophyll were measured. The results are given in Tab. 4 and Fig. 7.

The differences in the size of the nuclei of male and female plants are highly significant also in this case (t_{998} = 6·9).

With *Ginkgo biloba* L. the number of chromosomes is the same as in the preceding species, i.e. 2n = 24. According to the morphological shape, the diploid set of male plants contained 20 subtelocentrical chromosomes (i), 2 metacentrical chromosomes (V) + XY. The caryotype of the female plants was 20i + 2V + XX. (Fig. 8.)

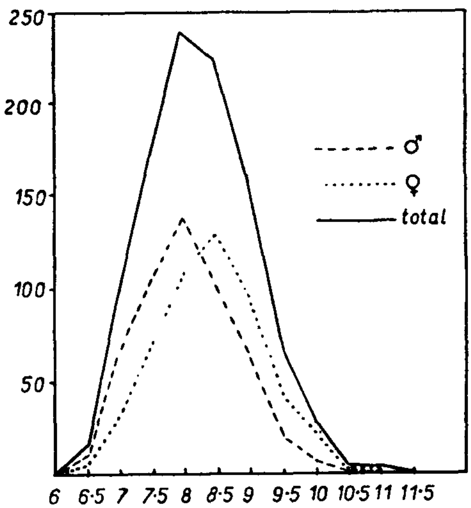

Fig. 9. Frequency polygons of the size of nuclei in somatic cells *Ginkgo biloba* L.

Table 5

Average size of nuclei in the somatic cells of *Ginkgo biloba* L. (in μm)

Female plants			Male plants		
$\bar{x}$	s	s_x	$\bar{x}$	s	s_x
11·36	1·09	0·05	10·89	1·01	0·05

In order to investigate the possible sexual dimorphism in the size of the nuclei, the cell nuclei from differentiated leaves were measured again. The results are summarized in Tab. 5 and Fig. 9. The differences in size of the nuclei from male and female plants are highly significant ($t_{998} = 7{\cdot}03$).

Discussion

Rumex acetosella L. is a diploid species with the basic chromosome number x = 7. According to the morphological character the whole group differs cytologically from the other groups of the family *Rumex*. It has small chromosomes and a different mechanism of the determination of sex. The diploid species have 12 autosomes and two X chromosomes in female plants and 12 autosomes and X + Y chromosomes in male plants, with a medium sized X and a large Y chromosome. The whole sexual mechanism of the family *Rumex* is being studied by Löve (1957). According to the occurrence of polyploids, from detailed studies and the comparison of the sex chromosomes and their pairing the author concludes that the sexual mechanism in this group follows the typical *Melandrium* pattern with very dominant male determinants in the Y chromosomes.

The nuclei of *Rumex acetosella* are larger in the male plant. The polygon illustrating the variability of the male nuclei shows a curve with two peaks and besides a smaller peak in class 14·5. The polygon of the female nuclei has one peak, whose maximum coincides with one peak of the polygon of the male nuclei in the class 7·5, besides the same peak above 14·5. A difference is seen, however, here also in class 9·5 where the polygon of the female nuclei has the main peak. By adding these polygons, a curve with two peaks arises which could be considered characteristic for a system composed of cells belonging to two sexes. With regard to the character of the polygon of both sexes it can be seen that two peaks of the curve originate from the unhomogeneous material represented by nuclei from various organs and not from the difference between the sexes. The statistical differences, on the other hand, are highly significant in all organs as well as in total evaluation, with a single exception. The cell nuclei of the male plants are larger than those in female plants, which probably corresponds to the larger Y chromosome in the set of the male sex.

The cytology of *Rumex acetosa* L. has been studied more intensively than that of the other types of the family Rumex, because of its interesting configuration of the sex chromosome. In the first meiotic division of PMC Kihara and Ono (1925) found six autosomal chromosome pairs and one triple chromosome which is a complex of sex chromosomes connected by their ends. The central chromosome, which is largest, was identified as the X chromosome, both terminal chromosomes being only a little smaller and of about equal size are the Y chromosomes. There are 14 chromosomes in the somatic cells of female plants. Two of these chromosomes are noticeably larger than the other (XX). In the cells of male plants there are 15 chromosomes: one is large and unpaired (X) and the two other chromosomes, which are paired, differ from the others (YY) by their size and morphology.

The mechanism of sex determination is based, similarly as with *Drosophila*, on the equilibrium between the X chromosomes and the autosomes. Ono (1935) and Yamamoto (1938) observed according to the triploid intersexes that the Y chromosomes do not carry the male determinant, these are localized in some autosomes. The female determinant genes are localized in the X chromosomes and the sex is therefore determined by the equilibrium between the genes determining female sex and the genes determining the male sex, which are contained in the autosomes.

In *Rumex acetosa* a highly significant difference in the size of the nuclei of the mesophyll of the male and the female plant was also observed. The polygons of the nuclei of the individual sexes have a single peak of practically the same width, only the peaks are somewhat shifted and differ by one class. Therefore, the polygon characterizing the variability of the nuclei of both sexes summarily has also only one peak. Still, the nuclei of the rootes were also measured in this material, since it appeared interesting whether there are any large differences in the nuclei of the cells just beginning to differentiate. The single peak polygon formed on account of this measurement shows that here also the difference between the classes with the highest frequencies is so small that a curve with two peaks cannot arise.

It can be summarized that the male nuclei are significantly larger in *Rumex acetosa* as well as in *Rumex acetosella* which could with this sex correspond again to three sex chromosomes, as compared to two X chromosomes in the set of the female cells. However, the differences are so small that it is possible to differentiate male and female individuals according to the size of the nuclei.

With *Melandrium album* male plants the pair of sex chromosomes are unequal, the Y chromosome being larger.

Melandrium represents the classical simple type of sex mechanism. The experiments carried out by Warmke and Blakeslee (1939, 1940), Westergaard (1938, 1940) and Ono (1939, 1940) showed that with *Melandrium* the Y chromosome contains very significant male determinants. Male sex develops already if a single Y chromosome is present in the whole multiplied set (e.g. 4A + XXXXY).

In *Melandrium album* the polygons of the nuclei of both sexes differ considerably and are completely shifted mutually except for the negligible frequency in the highest classes in female plants. However, no clear sharp peaks appear in any case, therefore the total curve does not have two peaks although in class 4·0 it has a tendency to form a double peak. This difference between the sexes again corresponds to a larger Y chromosome as in the case of *Rumex acetosella*.

In the case of *Ginkgo biloba* cytological investigation is somewhat more difficult and for this reason the American authors NEWCOMER (1954) and LEE (1954) independently undertook the identification of the heterochromosomes only recently, i.e. more than 40 years after the determination of the number of the chromosomes. However, just as other authors did not agree in the number and shape of the autosomes, these authors also disagreed in the determination of the sex chromosomes. LEE found a difference in one

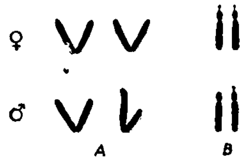

Fig. 10. Sex chromosomes of *Ginkgo biloba* according to NEWCOMER (A) and LEE (B).

pair of small subtelocentrical chromosomes: in male plants one chromosome has a satellite, which is missing in the other pair chromosome, whereas in female plants both homologous chromosomes have a satellite. NEWCOMER found a difference in the pair of the largest chromosomes. In his opinion in the female plants the chromosomes of the largest pair have a medially placed centromer, in male plants this pair is formed by one isobrachial chromosome (V) and by one chromosome in which the centromer is shifted closer to the end of one branch, the length being the same (Fig. 10).

According to my observations one branch of this apparently Y chromosome is considerably shorter, whereas the other keeps the same length. Although in this case a variability of the morphology of the chromosomes is possible, it can still be assumed in analogy to other dioecious plants that this difference in the largest pair is connected with the determination of sex. For this reason I assume, as NEWCOMER but in contrast to LEE, that the sex chromosomes are the two largest chromosomes, however, in contrast to his opinion the Y chromosome is shorter.

The peaks of the polygons of the nuclei in different sexes of *Ginkgo biloba* differ by one class. For this reason the total polygon again does not have two peaks and is similar to that of *Rumex acetosa*. However, the difference in size of the nuclei between the male and female sex in this case is in favour of the female sex in contrast to the preceding plant. The fact that the nuclei in the leaves of female plants are larger is probably connected with the findings reported by GREGUSS (1929). When comparing the assimilation areas of the leaves he found that the leave area of female plants is larger by 34% than that of the male plants. The differences in the weight of fresh leaves, however, were still more marked, the difference being 54% in favour of the female sex. The weight was investigated with 200 leaves of male and the same number of female plants.

When the results of the measurements of nuclei are summarized it can be seen that in all the investigated cases differences were observed between the nuclei in plants of different sex which were correlated to the larger quantity of the chromosome mass. The male plants of *Rumex acetosella* and *Melandrium album* whose Y chromosome is larger than the X chromosome, and of *Rumex acetosa* whose Y chromosome is smaller but there are two in the set, have larger nuclei. *Ginkgo biloba* had larger female nuclei, the Y chromosome being smaller than the X chromosome. The curves obtained by grouping of the values of all nuclei of both sexes, however, never have two peaks with regard to the small differences between the classes with the maximum frequencies.

The results show that sexual dimorphism manifests itself cytologically in different sex chromosomes as well as in the significant size of the interkinetic nuclei.

References

GOODRICH, H. B.: The germ cells in *Ascaris* incurva. — Journ. exp. Zool. 21, 1916 (Ref. according to SCHRADER Fr.: Die Geschlechtschromosomen, Berlin 1928).

GREGUSS, P.: Sexual-dimorphismus der Blätter von *Bryonia dioica* und *Ginkgo biloba*. — Math. und Naturwiss. Anz. d. Ung. Akad. d. Wiss. **46** : 625—631, 1929

HONČARIV, R.: Příspěvek k poznání diferenciace pohlaví u dvoudomých rostlin. [Contribution to knowledge of the differentiation of sex in dioecious plants.] — Preslia **28** : 216—220, 1956.

KIHARA, H., ONO, T.: The sex-chromosomes of *Rumex acetosa*. Ztschr. f. indukt. Abstamm. u. Vererbungslehre **39** : 1—7, 1925.

LEE, C. L.: Sex Chromosomes in *Ginkgo biloba*. — Amer. Journ. Bot. **41** : 545—549, 1954.

LÖVE, A.: Sex determination in *Rumex*. — Proc. genet. Soc. Canada **2** : 31—36, 1957.

NĚMEC, B. et cons.: Botanická mikrotechnika. [Plant microtechnique.] — Praha 1961.

ONO, T.: Chromosomen und Sexualität von *Rumex acetosa*. — Sci. Rep. Tohoku imp. Univ. Tokyo, ser. 4 (biol.) **10** : 41—210, 1935.

ONO, T.: Polyploidy and sex determination in *Melandrium*. I. Colchicine-induced polyploids of *Melandrium album*. — Bot. Mag. Tokyo **53** : 549—556, 1939.

ONO, T.: Polyploidy and sex determination in *Melandrium*. II. The effect of polyploidy upon sex in *Melandrium album*. — Bot. Mag. Tokyo **54** : 225—230, 1940.

PAZOURKOVÁ, Z.: Sex chromatin in *Rumex acetosa* L. — Preslia **36** : 422—424, 1964.

PAZOURKOVÁ, Z., PAZOUREK, J.: Rychlé metody botanické mikrotechniky. [Rapid methods in plant microtechnique.] — Praha 1960.

SINOTO, Y.: Studies in some dioecious plants. — Cytologia **1** : 109—191, 1929.

WARMKE, H. E., BLAKESLEE, A. F.: Effect of polyploidy upon the sex mechanism in dioecious plants. — Genetics **24** : 88—89, 1939.

WARMKE, H. E., BLAKESLEE, A. F.: The establishment of a 4n dioecious race in *Melandrium*. — Amer. Jour. Bot. **27** : 751—762, 1940.

WESTERGAARD, M.: Induced tetraploidy in *Melandrium album*. — Nature **142** : 917, 1938.

WESTERGAARD, M.: Studies on cytology and sex determination in polyploid forms of *Melandrium album*. — Dansk Bot. Arkiv (10) **5** : 1—131, 1 0.

YAMAMOTO, Y.: Karyogenetische Untersuchungen bei der Gattung *Rumex* VI. — Mem. Coll. Agric. Kyoto imp. Univ. **43** (Gen. Ser. 8) : 1—59, 1938.

Z. PAZOURKOVÁ, Katedra mikrobiologie a genetiky přírodovědecké fakulty KU v Praze: **Sexuální dimorfismus ve velikosti jader u dvoudomých rostlin.** — Biol. Plant. **7** : 86—96, 1966.

Při proměřování velikosti jader dvoudomých rostlin jsem zjistila, že jádra rostlin samčích a samičích se od sebe liší v souhlase s větším množstvím chromatinové hmoty. Větší jádra měly samčí rostliny *Rumex acetosella* a *Melandrium album*, u nichž je Y chromozóm větší než X a dále u *Rumex acetosa*, kde je sice Y chromozóm menší, ale jsou v sádce dva. Větší jádra samičí byla u *Ginkgo biloba*, kde jsem zjistila menší Y chromozóm než X. Křivky, vzniklé grupováním všech jader obou pohlaví nebyly však vzhledem k malým rozdílům mezi maximálně frekventovanými třídami nikdy dvouvrcholové.

З. ПАЗОУРКОВА, кафедра микробиологии и генетики естественного факультета Карлова университета, Прага: **Сексуальный диморфизм в размерах ядер двудомных растений.** — Biol. Plant. 7 : 86—96, 1966.

На основании измерений размеров ядер двудомных растений установлено, что ядра женских и мужских растений взаимно отличаются в соответствии с большим количеством хроматинового вещества. Большими ядрами обладали мужские растения *Rumex acetosella* и *Melandrium album*, у которых хромосома Y больше по сравнению с хромосомой X, и дальше *Rumex acetosa*, где хотя и хромосома Y меньше, но она присутствует в наборе в числе двух. Женские ядра больше у *Ginkgo biloba*, где установлена мною хромосома Y меньше X. Кривые, возникшие группированием всех ядер обоих полов, однако, в связи с малыми различиями между максимально фреквентированными классами никогда не обладали двумя пиками.