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IN RELATED SPECIES

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INTRODUCTION

At the present time the existence of a parallelism between nuclear mass and morphology and external structure of organisms can be demonstrated only for polyploids. There is no such clear relationship, however, among the different species. A tetraploid species is not necessarily larger than a diploid species; moreover, instances are known where analogous changes in the chromatin mass have exactly opposite results in two different species.

Such a lack of straight parallelism between nuclear mass and morphology and external organization may be reasonably explained by secondary influences of certain genes modifying the development in such a way that the original relations become obscured. This may be observed even in some polyploids, for instance, in *Oenothera gigas nanella*, which, instead of having the giant stature typical for tetraploids, is a dwarf, owing to a specific influence of a particular gene.

In order to arrive at a clearer understanding of this problem, one must study, therefore, earlier stages of development, prior to any differentiation or mutual interaction of tissues. Such conditions are realized best in the primary meristem of a plant, where one might expect to observe the original relation between the nucleus and development, unobscured by differentiation. But because of lack of differentiation only a few characters can be studied in meristematic cells. Among these primordial characters the size of the cell may be considered the most important; for it can be accurately measured and, moreover, it undoubtedly reflects the fundamental property of the cell, the ability of synthesizing a definite amount of protoplasmic substance.

The cell size was selected, therefore, for the present investigation in the hope that it may give a clue to some understanding of the significance of specific differences in nuclear organization.

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MATERIAL AND METHOD

Primary meristem of root tips was chosen as material for the present investigation. Only primary roots of very young seedlings were used, since it was found that they show less variation in cell size than the adult roots. The seeds of different *Crepis* species were planted in sand and germinated under conditions of moisture and temperature held as constant as was possible under the circumstances. Immediately after the cotyledons of the seedlings had spread, the root tips were fixed in S. Navashin's fixative (chromic acid, acetic acid, and formalin); special care was taken to perform all the following procedures in a uniform manner in order to prevent errors due to unequal shrinkage of different lots of material, etc. The sections were uniformly 10 microns thick; they were stained in iron haematoxylin and mounted in Canada balsam.

Thirteen *Crepis* species belonging to different sections of the genus were selected for the investigation; in their total chromosome length they ranged from 42 to 112.1 relative units.

After several preliminary trials, cells of dermatogen were selected for measurements. They appeared to be more suitable than others since they showed less variation in different species than the cells of the periblem; this was probably due to their superficial position in the root and corresponding possibility to grow more freely as compared with cells surrounded, and therefore compressed, from all sides by other cells. It should be further expected that the physiological conditions, such as water and oxygen content, would be more uniform in the cells of dermatogen because of their exposed position. Certain morphological peculiarities, moreover, as well as complete lack of intercellular spaces in the dermatogen, facilitated the obtaining of reliable results of measurements; this will be shown in detail later.

Fig. 1. Portions of the dermatogen of the thirteen *Crepis* species drawn to the same scale. The typical dimorphism of cells expressed in the occurrence of cells of the two kinds (primary cells, P, and secondary cells, S) may be seen, as well as the differences in cell areas of different species.

Finally, it was found, that the sizes of the cells stand in a certain relation to the number of cells present in the whole root, probably owing to differences in mutual pressure and of conditions of nutrition; this source of error was less pronounced in the cells of the dermatogen.

Several tests showed that, owing to the nature of the cell shapes, only direct measurements of the areas on cross-sections could be applied. Measurements of linear, tangential, and radial dimensions, on the contrary, appeared to be impracticable. This can easily be seen from an examination of figure 1, representing the typical cells of the dermatogen of all thirteen species investigated.

The following method of measuring cell areas was devised; adjacent cells along the circumference of the cross-section of the root were drawn to the same scale by means of the camera lucida. The magnification applied was equal to 1400 diameters. A sample consisting of ten adjacent cells was used for obtaining the average area of a cell. Each root gave from four to six samples, according to the number of cells present. The drawn samples of ten cells each were then cut out with seissors and weighed; a set of weights measured directly in square microns was prepared from the same paper as that which was used for the drawings.

This simple, although laborious method of making measurements proved to be far better than any other possible way, including the use of a planimeter. In order to check the accuracy of the measurements, the same sample of cells was drawn and weighed repeatedly; the error of measurements was found to be negligible for it never exceeded 1 per cent of the measured value. Naturally, before using it, the package of paper was tested as to its uniformity; it was found that the weight varied from sheet to sheet to a negligible degree. The uniformity of the paper was tested, nevertheless, repeatedly during the progress of the work.

It was of the greatest importance to determine, as a preliminary step, the amount of variation of cell areas along the length of the root in cross-sections belonging to different levels. Several samples from different levels were measured; it was found that the cell area rapidly increased toward the base of the root.

This region of growth, however, occupied only the short conical part of the root; but, as soon as the root assumed nearly cylindrical shape, the growth ceased immediately and the cell areas remained constant, so that it appeared immaterial from what part of the root the cell samples were taken, provided they did not belong to the conical

apex of the root. (The cylindrical part of the root usually begins at the level where only one cell layer of the root cap is present. All measurements were confined to this part of the root.) Table 1, representing some data of measurements of cells belonging to different levels of the cylindrical part of the root clearly demonstrates this.

TABLE 1
AVERAGE CELL AREAS BELONGING TO DIFFERENT LEVELS OF THE CYLINDRICAL PART
OF THE ROOT. THE LEVELS ARE 30 MICRONS APART.
DATA OBTAINED ON *Crepis capillaris*

Levels	1	2	3	4
Samples: 1	215	230	215	210
2	220	225	215	220
3	225	220	220	235
4	210	230	225	225
5	190	165	170	190
Average	212	214	209	216

It soon was found, however, that simple average areas obtained from samples could not be used for comparative purposes, owing to the fact that the cells of dermatogen were found to be of two different kinds, the larger primary and the smaller secondary cells (see fig. 1). The two kinds of cells greatly differed in size, the average ratio being equal to 1.5; the presence of secondary cells thus considerably reduced the average figures obtained simply by dividing the total area of a sample by ten (= the number of cells in a sample). From the figures given in table 2 it is plainly evident that the areas of the two kinds of cells do not even overlap but form a typical bimodal distribution. This cell dimorphism was not peculiar to the dermatogen; on the contrary, it occurred in any part of the root where regular cyclic rows of cells were present. Obviously, it was an inevitable result of growth and cell multiplication.

The cells of the two kinds did not alternate regularly; consequently their relative proportions could vary in different individual samples. Furthermore, different species and occasionally different individuals of the same species had different average proportions of the cells of the two kinds. All these circumstances made it necessary to devise some method of correcting the errors of measurements resulting from intra- and interspecific variation in the proportions of primary and secondary cells. As has been shown above, the typical

TABLE 2

COMPARISON BETWEEN PRIMARY AND SECONDARY CELLS. THE FIGURES REPRESENT THE AVERAGE AREAS OF CELLS OF THE TWO KINDS FOR EACH SAMPLE. DATA ON *Crepis pulchra*

Areas	Primary	Secondary
Samples: 1	286	167
2	250	163
3	243	150
4	220	150
5	275	188
6	267	163
7	292	200
8	307	167
9	300	150
10	250	175
11	267	188
12	242	188
13	283	150
14	300	200
15	295	175
16	270	170
17	229	150
18	320	180
19	225	163
20	225	150
21	260	190
22	280	190
23	270	200
24	290	220
25	283	200
26	250	170
27	292	175
28	275	200
29	270	190
30	267	175
31	250	163
Mean	271.3 ± 3.0	181.3 ± 2.3

ratio of the average areas of primary and secondary cells closely approximated 1.5. The necessary correction, however, could not be obtained by simple arithmetical computation since it had been found that the average sizes of the cells changed at a higher rate than one might expect assuming the two kinds of cells to be independent one from another. Table 3 presents the corresponding data.

Since the computation of the correlation coefficient did not seem to be profitable for determination of the nature of relation between both variables, owing to the low number of classes, a graphical method was employed and a straight line was fitted by the method of least squares.

Fig. 2. Graph representing the method of correcting the errors of measurements of cell areas, due to variation in the proportion of primary cells in cell samples. X = proportion of primary cells; Y = corresponding relative average area of one cell. Circles represent the observed values, disks, the corrected values. The horizontal line C represents the theoretical correction level, corresponding to the conditions prior to any cell divisions and, correspondingly, to differentiation of the two kinds of cells.

A nearly linear correlation between the proportion of primary cells and the average area of the cells was found; so that the desired correction could be readily determined. It seemed to be feasible to devise the correction in such a way as to bring the area of the cell up to the value which it would assume in case no secondary cells were to be formed, i.e., if no cell divisions took place. Figure 2 gives a diagrammatic representation of the method employed.

TABLE 3

RELATION BETWEEN THE PROPORTION OF PRIMARY CELLS AND THE AVERAGE AREA OF A CELL. DATA OBTAINED ON 11 DIFFERENT SPECIES, REPRESENTED BY 350 SAMPLES OF 10 CELLS EACH. THE FIGURES REPRESENT RELATIVE VALUES OBTAINED BY TAKING THE AREA CORRESPONDING TO THE PROPORTION $0.5 = 1.0$

Proportion of primary cells	0.5	0.6	0.7	0.8	0.9	1.0
Relative areas:						
Observed.....	1.00	1.07	1.12	1.19	1.20	
Expected.....	1.00	1.04	1.08	1.12	1.16	1.20

TABLE 4

CORRECTION COEFFICIENTS COMPUTED FOR VARIOUS PROPORTIONS OF PRIMARY CELLS

Proportions of primary cells	0.5	0.6	0.7	0.8	0.9	1.0
Coefficients.....	1.299	1.226	1.160	1.102	1.048	1.000

TABLE 5

COMPARISON BETWEEN CORRECTED AND UNCORRECTED AVERAGE CELL AREAS IN DIFFERENT *Crepis* SPECIES

Average areas	Proportions of primary cells			Coefficients of variation
	0.5	0.6	0.7	
<i>C. pulchra</i> :				
Corrected.....	292	293		.3
Uncorrected.....	225	240		6.7
<i>C. tectorum</i> :				
Corrected.....	282	289		2.4
Uncorrected.....	217	236		8.8
<i>C. neglecta</i> :				
Corrected.....	194	191		1.5
Uncorrected.....	149	156		4.7
<i>C. capillaris</i> :				
Corrected.....		240	240	0.0
Uncorrected.....		196	207	5.6
<i>C. parviflora</i> :				
Corrected.....		174	180	3.4
Uncorrected.....		134	147	9.7
Average coefficients of variation:				
Corrected.....				1.5
Uncorrected.....				7.1

On the basis of the above data, constant correction coefficients were computed, each one corresponding to a certain proportion of primary cells in one given sample; through simple multiplication by the corresponding coefficient and dividing by ten the average area of a given sample was transformed into a corrected value for one cell. The values thus obtained represented the maximal cell areas which would arise when all modifying influences, including cell divisions, are eliminated. Table 4 gives these coefficients together with corresponding proportions of primary cells.

In order to check the results of the correction applied, uncorrected measurements were compared with the corrected ones in regard to degree of variability. Table 5 gives the corresponding data for several *Crepis* species. From this table it is evident that the use of this correction greatly reduces the variation in measured areas, thus eliminating the influence of variation in the proportions of primary cells.

The whole method of measuring cross-radial areas of cells then assumed the following form. First, samples of ten cells each were drawn, cut, and weighed in terms of square microns; to every figure thus obtained was added a note regarding the corresponding proportion of primary cells. Second, the corresponding coefficient was set in the computing machine and all the figures obtained by direct measurement were corrected through multiplication by this coefficient and division by ten; first for samples containing five primary cells, then for those containing six, and so on.

The corrected areas obtained were treated as individual observations in calculating the mean values and other constants. The number of samples measured was never less than 25; as will be seen later, this number was sufficient to obtain fairly consistent and reliable results. Nevertheless, a much greater number of samples was measured in the majority of cases; altogether over 1100 samples were measured representing more than 11,000 individual cells.

After the true corrected values for cell areas were obtained, it remained to find the length of the cells along the axis of the root in order to determine the actual volume. This apparently simple task, however, proved to be an extremely difficult one, owing to the very high and variable rate of cell division and, correspondingly, to an extreme variation in cell size. In view of this difficulty it was decided simply to calculate the volumes through multiplication of the areas by their square root. This method seemed to be justified, especially when relative values were needed. One might suppose, however, that

the primary cells could be proportionally shorter than the secondary ones, the respective volumes being thus about the same. Although, a priori, such an assumption seemed to be highly improbable, yet it was checked by direct comparison of both kinds of cells on longitudinal sections. It was found that there is no detectable difference in relative length between primary and secondary cells, the two kinds showing the same range of variation. Inequalities in cell length in different species were always clearly pronounced, provided the species in question differed in cell areas. Although there was considerable difference, the results of measurements could not be evaluated because of extreme variation in proportion of cells of different length.

As measures of the chromatin mass, the values given by Mann-Lesley (1925) and expressed in relative chromosome lengths were used, with the exception of *C. senecioides* which was not included in her work.

THE RESULTS OF MEASUREMENTS OF CELL VOLUMES IN THIRTEEN CREPIS SPECIES

Applying the method described above the following mean values were obtained for cell areas and calculated volumes in the thirteen species investigated (see table 6). From this table it may be seen that the cell areas and correspondingly the calculated cell volumes differ widely in different species. Among the thirteen species one may distinguish groups characterized by similarity of cell size. Thus *C. dioscoridis*, *C. pulchra*, and *C. rubra* all have very similar large cells; *C. foetida*, *C. bursifolia*, *C. capillaris*, and *C. alpina* have medium-sized cells; *C. parviflora*, *C. neglecta*, and *C. setosa* form a group with small cells; *C. senecioides* stands apart with its very small cells.

RELATION BETWEEN CHROMATIN MASS AND CELL VOLUME

The length of somatic chromosomes as they appear in metaphase, was used as a measure of the chromatin mass. It is obvious that the length of the chromosomes may be applied as a measure of chromatin volume only when the width of the chromosomes is constant. As a matter of fact, however, this is not the case; the different species have in many instances chromosomes of very different widths. The measurement of chromosome width appears now to be extremely difficult owing to its exceedingly low value and especially to variation resulting from

the degree of splitting of each chromosome, the latter being always composed of two daughter strands. It is sufficient at present to understand that chromosome length alone* cannot be applied without due reservations.

TABLE 6
CELL AREAS, CALCULATED CELL VOLUMES, AND RELATIVE TOTAL LENGTH OF
CHROMOSOMES IN THIRTEEN SPECIES

Species	Cell area in square microns	Calculated volume in cubic microns	Corrected average of total length of chromosomes*
<i>C. senecioides</i>	156.8 $\pm$ 2.8	1963 $\pm$ 35	42.0
<i>C. parviflora</i>	180.3 $\pm$ 2.2	2421 $\pm$ 30	69.9
<i>C. neglecta</i>	192.0 $\pm$ 2.9	2660 $\pm$ 40	61.7†
<i>C. setosa</i>	201.0 $\pm$ 3.0	2850 $\pm$ 43	63.2
<i>C. aspera</i>	234.6 $\pm$ 2.6	3693 $\pm$ 38	82.6
<i>C. foetida</i>	248.3 $\pm$ 2.7	3913 $\pm$ 43	93.7
<i>C. bursifolia</i>	249.0 $\pm$ 3.6	3929 $\pm$ 57	78.5
<i>C. capillaris</i>	250.9 $\pm$ 5.3	3974 $\pm$ 84	61.4†
<i>C. alpina</i>	256.7 $\pm$ 7.1	4113 $\pm$ 114	87.3
<i>C. tectorum</i>	275.5 $\pm$ 2.2	4573 $\pm$ 37	88.7
<i>C. dioscoridis</i>	290.2 $\pm$ 4.0	4944 $\pm$ 68	109.4
<i>C. pulchra</i>	296.3 $\pm$ 2.4	5100 $\pm$ 41	112.1
<i>C. rubra</i>	296.8 $\pm$ 3.9	5113 $\pm$ 67	102.9

* From Mann (1925) table 3, p. 302 (except *senecioides*).

† These species have thicker chromosomes, cf. p. 223.

TABLE 7
CELL AREAS AND CALCULATED VOLUMES OF THE MEMBERS OF A POLYPLOID
SERIES IN *C. capillaris*

Chromatin mass	Cell areas	Cell volumes
Haploid (one plant, several roots).....	147.7	1789 $\pm$ 22
Diploid (several plants, one root of each).....	250.9	3960 $\pm$ 84
Triploid (several plants, one root of each).....	329.0	5968 $\pm$ 82
Tetraploid (three plants, one root of each).....	425.4	8976 $\pm$ 100

Prior to comparing different species in the matter of the relation between their chromatin masses and the respective cell volumes, a study of intraspecific variability of chromatin masses was made with the expectation that these conditions would be clearer, i.e., unobscured by eventual specific developmental peculiarities or errors due to inaccurate chromosome measurements. For this purpose a polyploid series in *C. capillaris* was used, consisting of haploid, diploid, triploid, and tetraploid individuals. The figures obtained from measurements of cells of the representatives of this polyploid series are given in table 7.

Essentially the same relations between chromatin mass and cell volume were found in the polyploid series of the two other species: *C. tectorum* (2n, 3n, and 4n were investigated) and *C. parviflora* (2n and 3n). The calculated cell volume here was also found to be practically proportional to the chromatin mass.

Fig. 3. Graphic representation of the relation between chromatin mass and cell volume in thirteen *Crepis* species. The length of the bars, indicating different species, corresponds to the respective probable error. The cell volume is expressed in cubic microns; the chromatin mass, in relative units of chromosome length.

As was stated above, these data on the polyploid series were secured chiefly in order to check the method employed; the correctness of which, as may be seen, they have established. As for the accurate determination of the degree of correlation between chromatin mass and cell volume in the polyploid series, the actual computing of the correlation coefficient did not seem to be practicable on account of the few classes of variables.

Different species could now be compared safely in the matter of the relation between their chromatin masses to the respective cell volumes.

The usual method of graphic representation was here employed. Figure 3 gives the corresponding correlation diagram for all thirteen species investigated. This diagram clearly shows that there is a very strong positive correlation between the chromatin mass and the cell volume, save for a few exceptions. These exceptional species deviate in the graph from the expected positions to a significant degree; but, as will be shown in the discussion, this circumstance has its natural explanation.

Computation of the coefficient of correlation gave the value, $r = .90 \pm .04$. Computation of the correlation ratio did not seem to be practicable owing to the low number of classes; it appears from the graph shown in figure 3, that the correlation may be regarded as linear.

DISCUSSION AND CONCLUSIONS

That there is direct relation between the sizes of cells and the nuclei they contain, has been well known since the time of Strasburger (1893). Later, on the basis of numerous observations, R. Hertwig (1903, 1908) developed the idea of the "nucleoplasmic ratio." This theory contends that the quotient, nucleus/cytoplasm, is a constant of fundamental importance for the life activity of the cell. Further investigations have shown, however, that the nucleoplasmic ratio varies very much in different tissues (Kleinberger, 1917); it varies under external influences as well, especially those of temperature (R. Hertwig, 1903; O. Hartmann, 1919). The most striking instances of change of the nucleoplasmic ratio is furnished by sexual cells, the male cells containing practically no cytoplasm, the female cells developing a vast amount of cytoplasmic material. From these and many similar data it is evident that the nucleoplasmic ratio could hold true only for cells prior to any differentiation, specialization, or modifying influences of the surrounding medium.

All the above mentioned data were obtained from studies of resting nuclei, their sizes being regarded as a measure of the amount of nuclear material. It may be observed, however, that the resting nuclei may greatly differ in the quantity and quality of materials they contain, in connection with innumerable changes they undergo in different stages of the cell life, cell division, specialization, and differentiation of cells, etc. Thus the data obtained from observation on "resting" nuclei may be regarded as scarcely reliable; at least they should not be accepted without due reservations.

The comparison of nuclei on the basis of their actual chromatin content, which can be measured in a definite stage of development, gives much more conclusive results. The actual counts and especially the measurement of chromosomes at the metaphase give directly the relative amount of chromatin, as it emerges at this critical moment of karyokinesis.

The first to demonstrate the effect of change of chromatin amount was Gerassimoff (1902), who experimentally produced a diploid gametophyte of *Spirogyra*. Gerassimoff found that the diploid cells greatly exceed in size the normal haploid cells. Thus it was demonstrated that an increase in the actual amount of nuclear substance stands in causal relation to the cell size and, consequently, to the general amount of protoplasm.

Gerassimoff's results were immediately confirmed by Boveri (1905) on animal material. From his famous experiments on *Echinodermata* eggs this investigator concluded that even the addition of a single supernumerary chromosome causes an enlargement of the cell. From his measurements he found that not the volume but the surface of the cell is proportional to the chromatin mass it contains.

Following Gerassimoff and Boveri many investigators became interested in this problem. Tischler on *Musa* (1910), the Marchals on mosses (1906, 1907, 1909, 1911), Winkler on *Solanum* (1916), and especially Gates on *Oenothera* (1909) have found that an increase in chromatin material causes also an increase in the masses of nuclei and of the cytoplasm. The figures obtained from measurements by some of these investigators showed, however, that there was no straight proportionality between chromatin mass and cell size. The cell size was, furthermore, strikingly variable in different tissues of the same organism; this is, of course, to be expected as a natural result of differentiation. It seems strange, therefore, that none of these workers measured the cells of the primary meristem of the root. Nemec (1910), who was the first to find tetraploid cells in normal tissues, worked with root tips; he did not make any measurements.

To cite more recent data, Taylor's work on *Acer* (1920), Blakeslee's on *Datura* (1922), and especially F. Wettstein's investigations on experimental polyploidy in mosses (1924) should be mentioned. Most recently Clausen and Goodspeed (1925) have shown that even under hybrid conditions the cell size tends to correspond to the chromatin mass.

All these and many other similar data proved beyond question that there is a definite relation between chromatin mass and cell size. In the great majority of these cases the differences in chromatin mass were due to polyploidy, i.e., to the addition of entire chromosome sets.

As a matter of fact, however, natural species show differences in chromatin mass owing to variation in chromosome size, the chromosome number being often the same. At present, data concerning the relation between cell size and chromatin mass as determined by the sizes of the chromosomes, are entirely lacking, save for a few observations which contradict one another. Such specific differences as inequalities in chromosome size seem to be more important than variations resulting from polyploidy, since the latter could produce only secondary new forms from pre-existing species which had arisen by different processes. In other words, a new species could not arise through polyploidy, unless sufficient specific changes in the organization of the chromosomes themselves should take place. The visible results of these phylogenetic changes probably represent alterations in both the relative and absolute sizes of chromosomes (M. Navashin, 1925, 1926; L. Delaunay, 1926).

Tischler was probably the first worker who became interested in this problem. Comparing a giant strain of *Phragmites* ("var. *Pseudodonax*") with a normal one (1918), he found it to possess much larger meiotic chromosomes, but the size of pollen mother cells was unaffected by this difference in chromatin content.

Delaunay reached essentially the same conclusion, while measuring meristematic cells (dermatogen of the root tips) in two *Muscari* species (1926). This investigator deduced from his measurements that the cell volumes in the two species investigated were identical, in spite of significant differences in chromosome sizes. He found the number of meristematic cells, not their sizes, to be connected with the chromatin mass.

Most recently Jaretsky (1928), studying various Polygonaceae, came to an opposite conclusion; he has found that there is direct connection between chromosome size and cell size. But he offers no measurements in proof of his statement.

It does not seem difficult to explain the contradictory results obtained by the above mentioned investigators. First, it may be noted that Tischler might have dealt merely with that type of size difference which was not due to actual variation in chromatin mass, but was

rather connected with physiological conditions, such as those known to occur in different tissues of the same organism. The figures presented by Tischler do not give a clear idea as to the nature of chromosome differences in the two *Phragmites* strains studied by him.

In Delaunay's case there is not the slightest doubt that the two species investigated (*Muscari longipes* and *M. tenuiflorum*) actually differ in chromatin amount, the respective total chromosome length being in the ratio 1.35:1.00. From the data of this writer, one may easily see, however, that his cell measurements could have been subject to errors, owing to the same general causes as those described in detail in the present paper. Thus Delaunay merely applied average values of the linear dimensions of cells; he did not select cells, but probably used random samples of cells (90 cells of each species) for determination of the radial and the tangential linear dimensions. The axial length of cells was determined simply by dividing the total length of the meristematic zone by the number of cells present in a longitudinal section. Referring now to what was found by the present writer, it may easily be seen that, if Delaunay's two species differed in the proportion of primary cells, his conclusions regarding the radial and tangential dimensions could have no significance; not to mention the impossibility of obtaining a reliable linear measure of such a complex shape. To illustrate this, suppose that *M. longipes* has 0.5 of primary cells and *M. tenuiflorum*, 0.7. If the relations between the two kinds of cells are similar to those found in *Crepis* (which is highly probable, since in several of the Liliaceae the present writer found exactly the same conditions as in *Crepis*), it would, of course, mean that the average areas of cells obtained from random sampling might happen to be equal, the real mean values being in the ratio 1.12:1.00, which would give the ratio for volumes approximating 1.2:1.0; and this latter ratio is not so far from the ratio of chromatin masses in the two species. Some details mentioned by Delaunay directly suggest the probability of the existence of a difference in the proportions of primary cells in the two species, although he does not even mention the occurrence of cells of the two kinds. Thus, Delaunay has found that *M. longipes* has narrower but longer cells as contrasted with *M. tenuiflorum*. It seems to be very probable, therefore, that the former species has actually fewer primary cells, since the width of cells is above all affected by the proportion of additional (secondary) cells. As for the axial dimensions of cells, there is no simple way to eliminate the extremely great error arising from variation in the rate

of cell division. In Delaunay's case it would be expected that *M. longipes*, having more cells would be apt to have shorter cells, owing to the fact that the larger number of cells is merely the result of a higher rate of cell divisions. Consequently Delaunay's divergent conclusions can by no means be regarded as established. With regard to his conclusion concerning the connection between chromosome length and the number of meristematic cells, the present writer can not confirm them on his material. Although in some cases a parallelism between chromosome length and the number of meristematic cells could be observed, in other cases both characteristics seemed to vary independently. For example, *C. pulchra* has a very short meristematic zone and very long chromosomes, while *C. tectorum* possesses much shorter chromosomes but about twice as many meristematic cells.

The data of the present investigation, however, indicate that there is a strict proportionality between chromosome length and cell volume in related species; this proportionality is not less in this instance than in the polyploid series. Some deviations from the expected relations, however, seem to contradict the generality of this conclusion. Thus *C. capillaris* appears to have larger cells than might be expected from the chromosome measurements; however, *C. neglecta* with chromosomes almost as long as those of *C. capillaris*, possesses much smaller cells. But this apparent disagreement has its simple explanation. As has been stated, in comparing the chromatin masses in different species only the total length of the chromosomes was considered, without reference to chromosome width. It is therefore apparent that in case the chromosomes differ in width, the length no longer can be used as a relative measure of volume. This is precisely the situation in *C. capillaris* and *C. neglecta*, as a glance at the chromosomes of the two species is sufficient to show, the former having considerably thicker chromosomes than the latter (cf. Hollingshead and Babcock, 1930, fig. 11, p. 19). The same difference exists also in several other species.

These differences in relative chromosome length and width raise a very important question as to the relation between the general chromosome shape and the actual amount of the chromatin content. As may easily be seen, the shape of the colloidal body of a chromosome is determined to a large extent by the properties of the surrounding medium, especially by its acidity, osmotic pressure, etc. Very considerable changes in the shape may, therefore, occur when the conditions of the cytoplasm become changed. This was actually demonstrated by

Kuwada and Sakamura (1926), who succeeded in changing the size of the chromosomes by varying the hydrogen ion concentration. It is natural to suppose, however, that different species may differ in the acidity of their protoplasts, so that in some of them the chromosomes may be more contracted (owing to higher acidity), others, on the contrary, may be more elongated (owing to lower acidity). These alterations may go even farther than simple changes of shape due to variation in surface tension; for the amount of water contained in the chromosome body may vary considerably, thus changing the actual mass of the chromosome itself. That the cause of variation in relative width and length of the chromosomes is not inherent in the chromosomes, may be deduced from the fact that chromosomes belonging to the same plate never show differences in widths. This very plausible conclusion leads Jaretsky (*loc. cit.*) to explain certain variations in chromosome shape through differences in the acidity of the cytoplasm.

It is now perfectly clear that a comparison of chromosome masses of two different species cannot safely be made unless the chromosomes are observed under precisely identical conditions of surrounding medium. Such conditions can exist only in one case: viz., when the chromosomes of the two species are located in the same cell. There is an easy way to accomplish this—by bringing the specific chromosomes together by means of hybridization. The present writer has made a large number of comparative observations on F_1 interspecific hybrids (1929), and it was found that the parental chromosomes never differ in width in hybrids, even though they previously displayed marked differences in the pure species. It could further be observed that this equality in width was accompanied by corresponding changes in chromosome length; thus, for instance, in a hybrid, *C. capillaris* $\times$ *C. neglecta*, the *neglecta* chromosomes appeared as thick as those of *capillaris* but this widening of the chromosomes was accompanied by considerable shortening. Table 8 gives the results of chromosome measurements of some pure *Crepis* species and of hybrids between them.

TABLE 8

THE TOTAL LENGTH OF HAPLOID CHROMOSOME COMPLEXES MEASURED IN CELLS OF PURE SPECIES AS COMPARED WITH THAT OBTAINED FROM MEASUREMENTS IN CELLS OF CORRESPONDING INTERSPECIFIC HYBRIDS. ROOT TIPS

Total haploid chromosome length of	From pure species	From <i>capillaris</i> $\times$ <i>neglecta</i> hybrid	From <i>capillaris</i> $\times$ <i>tectorum</i> hybrid
<i>C. capillaris</i>	107.94 $\pm$.40	125.85 $\pm$.69	109.43 $\pm$.47
<i>C. tectorum</i>	155.55 $\pm$.88		147.57 $\pm$.50
<i>C. neglecta</i>	97.02 $\pm$.51	83.33 $\pm$.37	

It appears, therefore, that the length-width ratio of a chromosome is merely a matter of the chemico-physical condition of the cytoplasm; and the situation would not change at all, if one assumes that the properties of the chromosome itself are responsible (to a certain extent at least) for these conditions. Further discussion of this problem will appear elsewhere.

It is clear that comparative measurements of chromosome volume are relatively easy when hybrids are used. It may give reliable results also as far as comparison of pairs of species is concerned. But the conclusions may not be applied without reservations to a larger number of species, since it is obvious, that the conditions in a hybrid cell are determined by the cooperation of the two specific chromosome sets. And, if in certain cases actual change of the absolute chromosome mass should take place, the same species might appear to have different chromosome volumes, depending upon the other species with which it had been crossed. Nevertheless, judging from the available data, it does not seem to be probable that the chromosome changes often go beyond mere shortening and proportional thickening; the total volume being unaffected.

Through comparative study of hybrid chromosomes, it was found that *C. capillaris* has actually much more bulky chromosomes than *C. parviflora*, *C. neglecta*, and even *C. setosa*. Therefore the respective positions of these species in the diagram of figure 3 should be correspondingly changed, thus bringing them much closer to the actual regression line.

As has been shown above, the connection between chromatin mass and cell volume is not less pronounced in interspecific variation, than in intraspecific changes due to polyploidy. This justifies the assumption, with a fair degree of probability, that the same conditions which make the polyploid cells larger, are acting in the case of specific alteration of chromatin mass. Although there is no doubt that members of polyploid series actually differ in the amount of genes present, it might be premature at present to conclude that just this difference in the amount of homologous genes is alone responsible for the variation in the production of protoplasmic material; yet such a conclusion is certainly indicated. In regard to specific differences in chromatin amount, such a conclusion must be made with still more reservations, in spite of its plausibility.

From his studies on *Polygonaceae* Jaretsky (*loc. cit.*) has made much more categorical assertions, namely, that the species having

larger chromosomes must be regarded as polyploids, the large chromosomes being in reality compound structures, each composed of two or even more small chromosomes. This idea is not new, nor is it warranted by a sufficient number of facts; furthermore, this author reverts to the old idea of loss mutations in order to explain the differences between various species. It is, however, perfectly obvious that regarding loss mutations as a cause of evolution, one does not avoid the absurd consequences of such an idea by appealing to polyploidy, irrespective of its kind.

It may be of interest to discuss briefly another problem arising from the results of the present investigation. From the accurate determination of relative proportions of primary and secondary cells in the dermatogen of the root, it was found that these proportions are remarkably constant for a given root; it appeared further to be very constant for a given species. Table 9 gives some corresponding data.

TABLE 9

PROPORTIONS OF PRIMARY CELLS IN THE DERMATOGEN IN DIFFERENT *Crepis* SPECIES

Species	Proportion of primary cells
<i>C. parviflora</i>	.563 $\pm$.012
<i>C. foetida</i>	.568 $\pm$.010
<i>C. senecioides</i>	.567 $\pm$.014
<i>C. bursifolia</i>	.570 $\pm$.011
<i>C. tectorum</i>	.575 $\pm$.010
<i>C. pulchra</i>	.578 $\pm$.008
<i>C. aspera</i>	.572 $\pm$.009
	mean value .571 $\pm$.001
<i>C. capillaris</i>	.665 $\pm$.014

Besides the proportions given in the preceding table, some others were determined, for example, the occurrence of proportions 0.62, 0.71, and 0.75 (possibly also 0.60) was established, also that in two species the proportion of primary cells sometimes dropped below 0.5; and the most common proportion closely approximated 0.571. Different roots of the same species eventually exhibited different proportions; but never did a proportion occur which was intermediate between those enumerated above. The variation in the proportions of primary cells thus proved to be discontinuous.

It is easy to observe, that, since the proportion of primary cells is merely a direct result of the proportion of preceding cell divisions, the

latter must be strictly limited by a definite number, typically constant for each case. The relations between the proportion of dividing cells and the proportion of primary cells in the resulting tissue are shown in table 10.

TABLE 10
THEORETICAL RELATION BETWEEN THE NUMBER OF CELL DIVISIONS AND THE
RESULTING PROPORTIONS OF PRIMARY CELLS

Relative numbers of dividing cells	Relative numbers of resulting cells of the two kinds	Proportion of primary cells
No divisions.....		1.000
1 out of 10	10 prim. + 1 secon.	0.909
1 out of 5	5 prim. + 1 secon.	0.833
1 out of 4.....	4 prim. + 1 secon.	0.800
1 out of 3	3 prim. + 1 secon.	0.750
2 out of 5.....	5 prim. + 2 secon.	0.714
1 out of 2	2 prim. + 1 secon.	0.667
3 out of 5	5 prim. + 3 secon.	0.625
2 out of 3.....	3 prim. + 2 secon.	0.600
3 out of 4	4 prim. + 3 secon.	0.571
All dividing		0.500

The whole process of differentiation of the two kinds of cells (see fig. 1) may be explained in the following way: after the last cell layer of the meristem is formed through divisions in the radial direction, a series of cell divisions in a perpendicular (tangential) direction takes place; each dividing cell forms two cells, one of which is identical with the original (primary) cell, the other being different in shape and size owing to its position opposite the cell wall of the underlying cell of the row underneath. Thus two kinds of cells arise, the primary, equal in number to the cells of the layer below and characterized by pointed inner ends, which occupy the spaces between the cells of that layer; and the secondary, which are located between the primary cells and opposite the cells of the layer below.

The above table gives only a few of the most simple numerical relations which would arise as the result of various proportions of dividing cells; an indefinitely large number of different combinations might be imagined. As a matter of fact, however, only very few of the possible combinations occur in reality; namely, only the following relative numbers of dividing cells occur: 1 out of 3, 2 out of 5, 1 out of 2, 3 out of 5, 2 out of 3, and 3 out of 4, the latter proportion being the most common (it results in the proportion of primary cells being 0.571).

It seems evident, therefore, that the rate of cell division follows an extremely simple rule: only the simplest numerical ratios between dividing and non-dividing cells are realized. Further discussion of this matter not being pertinent to this paper, it is merely stated that the described relations are undoubtedly consequences of some most fundamental physical property of the cell. The study of these relations might possibly lead to some understanding of the most direct causes of cell division and cell differentiation. Further investigation is in progress.

SUMMARY

Many closely related species are known to differ greatly in their chromatin content. There is no strict parallelism between the specific chromatin amount and external morphology. This phenomenon remains as yet unexplained.

In view of the fact that in adult organisms the original relation between chromatin mass and the mode of development could be obscured by some influences of certain genes, it was decided to compare the meristematic cells of different species. The size of the cells of the dermatogen of the root was chosen as material for the investigation, on the supposition that the amount of protoplasmic material produced reflects the most fundamental property of the cell, viz., its ability to synthesize a definite amount of organic matter.

A special method of measuring cell volumes was devised; and all sources of error were eliminated, as far as possible.

It was found for the thirteen species of *Crepis* investigated, that the volume of the cell was proportional to the amount of its chromatin. Thus it was established that the specific alterations in chromatin amount have the same primary consequences as the intraspecific variation in the number of the homologous chromosome sets arising through polyploidy. This close analogy suggests the possibility that the specific differences in chromatin amount are connected with the amount of homologous genes, as in the polyploid series.

Some data of the present investigation clearly show that the proportions of cell divisions in primary meristem are strictly confined to a series of simple fractions; viz., $\frac{1}{3}$, $\frac{2}{5}$, $\frac{1}{2}$, $\frac{3}{5}$, $\frac{2}{3}$, and $\frac{3}{4}$. This indicates that the cell divisions and, consequently, the resulting simplest cell patterns are controlled by some elementary physical process.

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