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LILLIAN HOLLINGSHEAD

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Gates (1924) stated that "it is unknown at the present time how widespread polyploidy in somatic tissues may be." Several writers have since that time reported cases of somatic polyploidy and it was thought worth while to put on record instances of this condition recently discovered in the Genetics Laboratory of the University of California.

In the course of an examination of root tips of various *Crepis* species and species hybrids, two plants which were partly tetraploid have been found.[1] These are not the first cases of chromosomal chimeras reported in *Crepis*, Lesley (1925) and Nawaschin (1926) having previously recorded the phenomenon. Lesley's report is a note stating that in an F_1 between *C. biennis* ($n=20$) and *C. foetida* ($n=5$) a few neighboring cells were found having about twice 25 chromosomes, whereas most of the cells contained the expected 25. Nawaschin reported the occurrence of a tetraploid area in the form of a narrow sector in a diploid root of *C. Dioscoridis* ($n=4$).

The first of the two cases to be described was that of a chimeral root of a derivative from a cross between *Crepis biennis* ($n=20$) and *C. setosa* ($n=4$) which had 24 chromosomes in most of the somatic cells. In this root, however, a large number of cells was found which obviously had many more than the normal 24 chromosomes, several approximated 48, and two cells gave clear accounts of 48 chromosomes. The normal and tetraploid chromosome complexes are shown in figure A. By an examination of successive sections it was determined that the tetraploid cells were confined to a definite region which extended from at least very close to the root cap to a point where no division figures could be found. Apparently the longitudinal outlines of the tetraploid area were fairly regular, since only one case occurred in which diploid and tetraploid cells were found in different sections occupying the same position relative to the circumference, and this was on the line of demarcation between the two areas.

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[1] Since writing the above a root of *C. Hakelei* ($n=8$) containing several neighboring cells with about twice the normal chromosome number and one of *C. montana* ($n=6$) with one tetraploid cell have been found.

Figure B was made up from an examination of the whole root and shows that the tetraploid area occupied the major portion of the root and that its cross-section was very irregular in shape. The dotted lines indicate the portions of the boundary between the $2n$ and $4n$ areas which could not be accurately determined. It is uncertain whether the tetraploid area extended into the central cylinder or not. The tetraploid area at *a* is two cell layers deep, at *b* it is only one, but opposite *a* at *c* it occupies most or all of the cortex. While there is considerable variation in cell size within both areas the average size of the tetraploid cells is larger than that of the diploid.

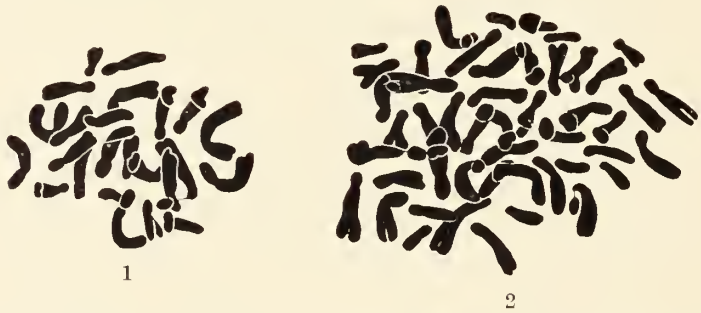

Fig. A. 1, Diploid chromosome complex of *biennis-setosa* hybrid derivative ($2n = 24$). 2, Tetraploid complex ($2n = 48$) from chimeral root of the same plant.

The shape of the tetraploid area is of some interest from the point of view of development as presumably the doubling of chromosomes took place in one of the initial cells from which the root developed. The area does not show the comparative regularity in shape exhibited by Nawaschin's tetraploid sector. On the other hand, tetraploidy here is confined to one definite area, which was not the case with Lesley's (1925) tomato chimeras, where isolated areas of tetraploid cells were observed. The condition is similar to that which Langlet (1927) found in two roots of *Thalictrum* but differs from that reported by Winge (1927) in *Tragopogon* hybrids where the tetraploid parts of two roots by reason of their larger cells rendered the cross-sections of the roots eccentric. The plant bearing this chimeral root was of normal appearance in the rosette stage but unfortunately died before flowering.

The second case showing a chimeral condition was a plant of *Crepis Bureniana* ($n = 4$). Of the thirty roots of this plant which were examined two were tetraploid, having 16 chromosomes in all the

plates observed. Plate 54, figures *a* and *b*, shows the chromosome complexes with surrounding areas from the outer cortex in comparable regions of the diploid and tetraploid roots. Each of the chromosomes of the diploid complex could be recognized in the tetraploid and the longest one of the set could be identified four times in the best tetraploid plate. Undoubtedly there has been a doubling of the diploid set. An examination of plate 54 shows that the average size of cells and nuclei is larger in the tetraploid root.

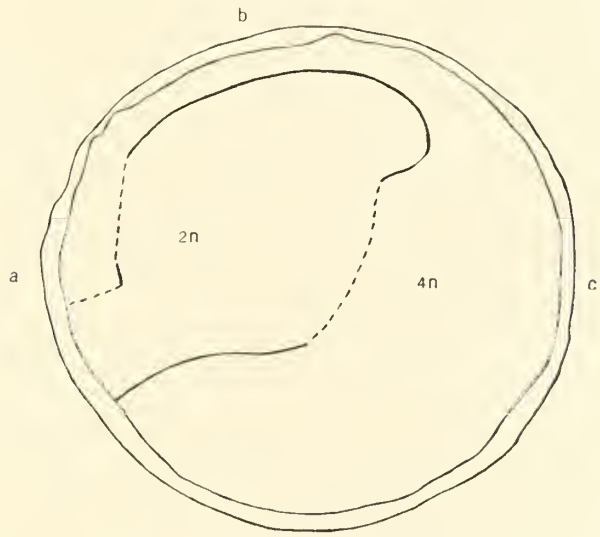

Fig. B. A cross-section of root of *biennis-setosa* hybrid derivative showing the extent of the tetraploid area. The outer ring is the extension of the root cap and contains no dividing cells.

The occurrence of two tetraploid roots in the same plant may be taken as evidence that part of the central cylinder from which branches arise had become tetraploid. It was thought possible that the plant above ground might have been affected similarly, and if so, the pollen produced on a tetraploid branch would be larger than that on a diploid. Examination of pollen from various branches, however, showed no noticeable size differences, so it was concluded that tetraploidy was probably confined to the roots.

In this same plant a root was examined which contained a number of large cells. Most of them were clearly multinucleate, from two to four nuclei having been counted in single cells (pl. 55). Plate 55 *b* shows one of the smallest of these with 2 nuclei, and 2 nuclei may be observed in one of the cells in *a*. In the larger cells nuclei were to be found in successive sections. These cells were scattered throughout

the cortex, mostly near the periphery, and in one region a group of them seems to be responsible for the somewhat misshapen appearance of the root in cross-section (pl. 55 *a,c*). The cells vary in size, sometimes extending through three or four sections 7μ thick. They are more or less vacuolated, depending on their size, but even the smallest ones could be distinguished almost at a glance by their less densely staining cytoplasm.

The normal chromosome complex of 8 was to be seen in several plates of normal cells. In one very large vacuolated cell a large number of chromosomes was observed, apparently in metaphase (pl. 55 *d*). Note the V-shaped arrangement of the metaphase plate. The chromosomes could be seen in 3 successive sections of 7μ thickness.

Nawaschin (1926) has reported a giant cell with over 500 chromosomes in a root tip of *Crepis tectorum* and takes up favorably the theory that it has arisen by successive chromosome divisions without accompanying cell divisions. The cell he shows is not greatly unlike some of those seen in this material. Here, however, there seems to be some evidence that the large cells are the result of fusion of several smaller ones. The V-shape of the one plate observed in a giant cell indicates that it may be a combination of two plates and that a nuclear fusion has taken place. Plate 55 *c* gives the impression that two large cells are in the process of fusion and indeed the cell wall has practically disappeared. It seems likely that such cell fusion might be associated with an abnormal or pathological condition of the root, further evidence of which was to be seen in small black areas probably representing degenerated cells (pl. 55 *b*).

The origin of tetraploidy in diploid tissue has been discussed by various investigators. In some cases it has been associated with specific outside influences. The effect of narcotics in inducing tetraploidy has been investigated by Nemec (1903) and Sakamura (1920). Blakeslee and Belling (1924) found tetraploid shoots in *Datura* plants subjected to cold. Lesley (1926) found tetraploid areas in tomato plants affected by mosaic and thought it might be possible that local changes due to this disease might affect mitotic processes. Cases of polyploid cells have been attributed to abnormal processes related to degeneration as in the investing cells of the ovaries of *Anasa tristis* (Wilson, 1906). The doubling of chromosome numbers in Winkler's (1916) well-known chimeras has been attributed to the effect of wounding. Jorgensen and Crane (1927) have recently secured tetraploidy in *Solanum* by the use of Winkler's method. Winge (1927) finds that

most of the cells of the "crown galls" on sugar beets which can be induced by inoculation with *Bacterium tumefaciens* have the tetraploid chromosome number.

Nawaschin did not venture any suggestion as to causal agencies in connection with his tetraploid sector in *Crepis Dioscoridis*. Lesley believed that it was unlikely that cold played any part as a causal factor in tomato chimeras, as only the roots seemed to be affected. It has been suggested by Mr. C. W. Haney that watering greenhouse plants with cold water would provide the necessary conditions if sudden lowering of temperature has anything to do with the production of tetraploid root cells. The plants described here were entirely normal as far as could be observed and tetraploidy could not be ascribed to any special factor in the environment.

Winkler had suggested that certain tissues may regularly become polyploid and Breslawetz (1926) has reported tetraploidy as the universal condition in the dermatogen of the root tips of *Cannabis sativa*.[2] De Litardiere (1923) found tetraploid and octoploid cells in the dermatogen of *Spinacia oleracea*. In both these cases it would seem that the transforming of diploid into tetraploid cells must have occurred many times in the same root.

The possibility of fragmentation giving rise to these increased numbers is easily excluded in most cases. The two most favorably received theories to account for doubling are: (1) the fusion of nuclei from two cells; (2) the division of the chromosome complex without cytoplasmic division. Breslawetz has brought forward evidence that nuclear fusion gave rise to the tetraploid cells which made up the dermatogen of the roots in *Cannabis sativa*. As no diploid complexes were to be seen in that region of the root one would conclude that fusion of diploid to form tetraploid nuclei had taken place before any normal diploid divisions occurred, or at least at an early stage in the development of the root. If so, one wonders why evidences of nuclear fusion were still to be found in well developed roots. On the other hand, the paired condition of the chromosomes in some of the tetraploid cells in *Spinacia oleracea* convinced de Litardiere that these cells had just completed a chromosome division without separation of the resulting daughter chromosomes.

The occurrence of multinucleate cells in a root of the *Crepis Bureniana* plant which was partly tetraploid has been noted above. The significance of this phenomenon in the origin of the tetraploid

[2] De Litardiere (1924) found rare diploid cells in the periblem and in one case a tetraploid cell in the plerome of roots of this species.

roots is questionable. The presence of several nuclei in one cell and the abnormal appearance of these large cells would incline one to belittle the possibility that tetraploidy here had originated by cell and nuclear fusion. However, it has been pointed out that the one chromosome complex observed in a giant cell indicated that nuclear fusion had taken place. We cannot, therefore, dismiss the possibility that a fusion of nuclei from two cells gave rise to a cell which was tetraploid and thence to tetraploid roots. It seems more likely, however, that the occurrence of tetraploidy and of a root with giant multinucleate cells in the same plant was merely a coincidence.

Whatever the method of origin, the frequent occurrence of tetraploidy in somatic tissues throws some light on two much discussed questions. First, there is that of the mode of origin of diploid gametes. Rosenberg (1926–27), Karpechenko (1927), and others have described processes in the reduction divisions of apogamous species and interspecific hybrids by which diploid gametes are formed. The increasing frequency with which tetraploidy has been recorded in root tips makes it seem likely that it would be found in other tissues were they examined as consistently. Its occurrence in the cells of the germinal line would lead to the formation of gametes with twice the normal chromosome number. This has been shown to occur in *Datura* where tetraploid shoots were found. Presumably a smaller area might be affected and only a portion of the gametes formed on one shoot might be diploid.

In the second place, the frequent occurrence of somatic tetraploidy has a bearing on the origin of tetraploids and tetraploid hybrids. *Primula kewensis* arose as a bud sport probably from an F_1 hybrid of *P. verticillata* and *P. floribunda*. It has the sum of the diploid chromosome numbers of the parent species and Clausen and Goodspeed (1925) have suggested that it is a true tetraploid hybrid, the bud sport having arisen by a doubling of somatic chromosomes. A similar explanation with the doubling occurring immediately subsequent to fertilization was suggested by these investigators to explain the origin of a tetraploid hybrid between *Nicotiana tabacum* and *N. glutinosa*. Rosenberg (1926) has recently proposed an explanation for the origin of the tetraploid *Aegilops—Triticum* hybrid of Tschermak and Bleier (1926) which depends on the chance meeting of diploid gametes formed by a "semi-heterotypic" division. In the light of the foregoing facts it seems much simpler to suppose that a doubling of the chromosomes took place in the fertilized egg, or in some cell of the

young embryo which gave rise to the growing point of the stem. Nawaschin was led to favor this theory of the origin of tetraploids by the frequency of $4n$ plants in *Crepis tectorum*. He calculated the frequency of diploid gametes from the number of triploid plants obtained in over 4,000 individuals, and on this basis determined the number of tetraploids which should occur by chance meeting of those gametes. He found the expected number of tetraploids to be much less than that actually occurring. He concluded, therefore, that tetraploids arose through the doubling of chromosomes in the fertilized egg cells.

In view of the increasing number of cases in which tetraploidy has arisen in normal diploid tissue, one is justified in concluding that it may play a part in the origin of polyploid species and interspecific hybrids.

I acknowledge with pleasure my indebtedness to Dr. J. L. Collins and Professor E. B. Babcock for the material used in this study.

SUMMARY

Tetraploidy was observed in the roots of two different plants. One, a *C. biennis* × *C. setosa* hybrid derivative, had one root partly tetraploid. The other, a plant of *C. Bureniana*, had two roots wholly tetraploid.

No external factors could be associated with the tetraploidy.

Giant multinucleate vacuolated cells occurred in another root of the same *C. Bureniana* plant. Evidence of cell and nuclear fusion was observed. It is doubtful whether this phenomenon has any significance in relation to the origin of the tetraploid roots.

Tetraploidy arising in somatic tissue probably plays a part in the origin of polyploid species and interspecific hybrids.

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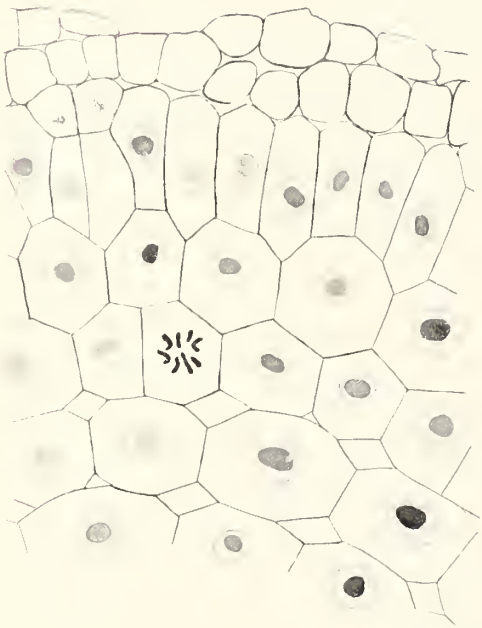
PLATE 54

Comparable areas of *a*, diploid, *b*, tetraploid roots of a *Crepis Bureniana* plant.



a

b

PLATE 55

Photomicrographs of root of the *Crepis Bureniana* plant showing giant multinucleate cells.

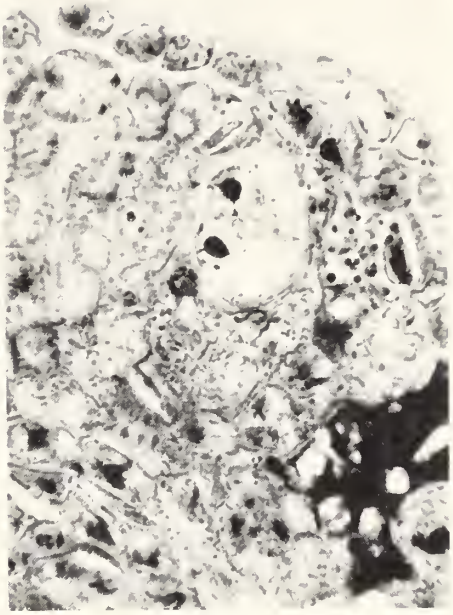
a. A cross-section showing a group of giant cells.

b. One of the smaller giant cells with two nuclei.

c. Two giant cells apparently fusing.

d. A large cell containing a V-shaped metaphase plate with many chromosomes.

a



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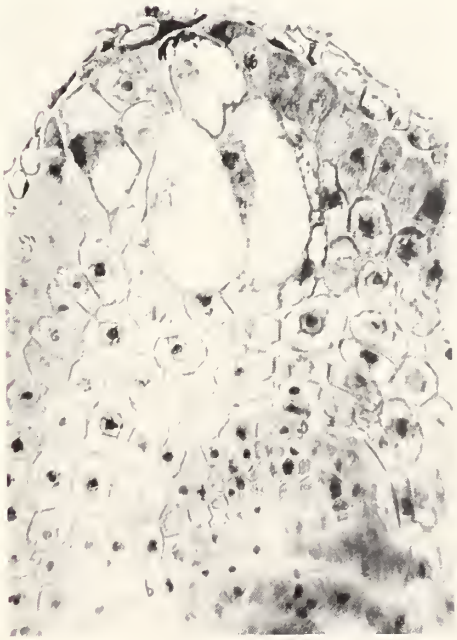
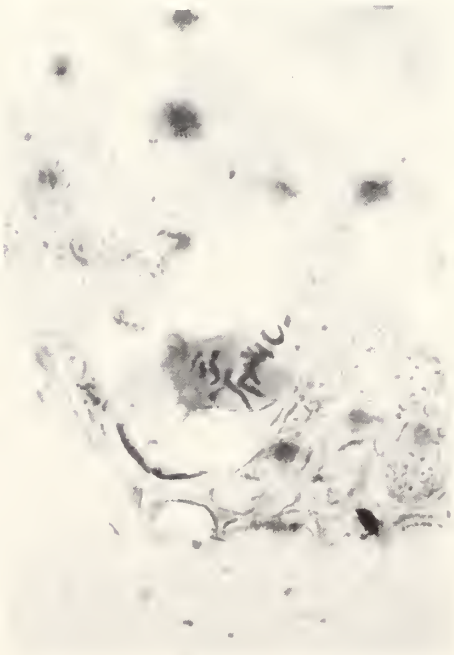


c



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