A COMPARISON OF THE WOOD STRUCTURE OF *OENOTHERA STENOMERES* AND ITS TETRAPLOID MUTATION *GIGAS*[1]

W. W. TUPPER AND H. H. BARTLETT

University of Michigan, Ann Arbor, Michigan

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The object of this paper is to present data bearing upon the nature of the changes in particular cells and tissues which follow mutative changes in the germ-plasm. Thanks to the cytological investigations of GATES, LUTZ, GEERTS and STOMPS,[2] we are beginning to correlate certain mutations with definite modifications in the nuclear mechanism, of which one of the simplest is the doubling of the chromosome number. In Oenothera there are three species, *Oe. Lamarckiana, Oe. stenomeres,* and *Oe. pratincola,* which have undergone such a modification, resulting in the mutations which are named *gigas.* The original 4 x mutation from *Oe. Lamarckiana,* namely *Oe. gigas* de Vries, has been examined histologically by GATES (1909), who found that its nuclei were twice as large as those of the parent species. The ratios representing the relative volume of certain homologous cells of the two forms were roughly 1 : 1.5 for the tapetum and pollen mother cells, 1 : 2 for the petal epidermis, 1 : 3 for the stigma cells, and 1 : 3.75 for the cells of the anther walls. Not only was the increase in cell volume of different tissues far from uniform, but even in a single tissue the increase of the cells in one dimension was out of proportion to the increase in other dimensions. The pollen grains of *Oenothera gigas* have four germination points, and are therefore quadrangular, whereas those of *Oe. Lamarckiana* have three and are triangular. As GATES says, it is clear that *Oe. gigas* is built of larger cells than those of *Oe. Lamarckiana,* and that in some cases the cells have a different shape.

Since this interesting line of inquiry has not been followed up since

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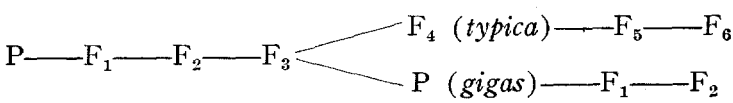
[2] Literature summarized by GATES "The mutation factor in evolution, with particular reference to Oenothera." London: Macmillan and Co. 1915.

the publication of GATES's paper, the authors have undertaken a thorough anatomical comparison of *Oe. stenomeres* and its 4x mutation *gigas*. This paper deals only with the wood anatomy. The vascular bundles of the higher plants are ordinarily considered to be the most conservative structures of the organism, from an evolutionary standpoint, and since statistical comparisons of the wood elements are being rather largely used as a basis for determining relationships among woody plants, a quantitative study of the changes which may come about as the result of a single mutation should be of timely interest to the comparative morphologist and paleontologist. Furthermore, the data should eventually have a bearing on the problem of determining whether or not the mutation has characters of organization which are not a necessary consequence of the modifications of the individual cells of which it is built.

The species and mutation which provided the material for our work were recently described by BARTLETT (1914, 1915). The mutation appeared in a culture of *Oe. stenomeres* which had been self-pollinated for three generations. It has been carried through two generations, by self-pollination, since that time. Typical *Oe. stenomeres*, belonging to the same progeny as the original mutation, has likewise been maintained in the cultures by self-pollination. The pedigree is summarized as follows:



In 1915 plants of the F_6 generation of *Oe. stenomeres* were grown side by side with the F_2 generation of mut. *gigas*. Adjoining plants, in a comparable stage of development, provided the wood for our studies. It was taken from both plants at the base of the main stem just above the circle of branches which spring from the axils of the upper rosette leaves, and is therefore strictly comparable in the two cases.

The secondary wood in both *Oe. stenomeres* and its mut. *gigas* is composed of similar elements, similarly arranged, with the exception that in the former the medullary rays are very much taller. It is very simple in structure, the intraxylar islands of phloem, described by GROSSE (1895) and RAMALEY (1896) in related species, being entirely absent. Vessels occurring singly or in groups; if single, oval in cross-section with the long axis radial; if in groups, polygonal in cross-section; the groups disposed in a radial direction. Walls densely pitted, end walls porous. Tracheids (wood fibres) arranged with marked regularity in radial rows, rather square in cross-section. Wood parenchyma moderately abundant, distributed throughout the year's growth, most con-

spicuous and best developed around the vessels. Medullary rays typically uniseriate, very numerous, seldom separated from each other by more than five or six rows of tracheids, composed of rectangular, radially elongated cells. The secondary rays arise at various distances from the pith.

In macerated wood of each of the two forms measurements were made of the length and diameter of 100 vessels. The 100 lengths gave a mean value of 0.30 mm in the case of *Oe. stenomeres* and 0.46 mm in the case of mut. *gigas*. In table 1 the lengths are classified, showing that the mean length in each case is likewise the length of greatest frequency, or very nearly so. Thus it appears that the vessels of the mutation are fully 50 percent longer than those of the parent species. In table 2 the diameters are similarly classified. The mean values are .056 mm for *Oe. stenomeres* and .089 mm for mut. *gigas*, an increase of 59 percent. This means an increase for the mut. *gigas* over the parent species of 152 percent in area of cross-section of the vessels.

TABLE 1

Vessel lengths of Oe. stenomeres and its mut. gigas.

Length in mm	Number of vessels	
	Oe. stenomeres	mut. *gigas*
.16—.19	5	
.20—.23	12	1
.24—.27	24	5
.28—.31	18	3
.32—.35	19	9
.36—.39	16	12
.40—.43	1	14
.44—.47	1	15
.48—.51	3	13
.52—.55		12
.56—.59		5
.60—.63		4
.64—.67		4
.68—.71	1	
.72—.75		2
.76—.79		
.80—.83		1

As in the case of the vessels, the length and diameter of 100 tracheids of each kind of wood were measured. The results are given in tables 3 and 4. The mean lengths were 0.42 mm and 0.64 mm, for parent species and mutation, respectively; the mean widths were 0.020 mm and 0.029 mm. In each case, just as we found for the length of the vessels, the

TABLE 2

Diameter of the vessels in Oe. stenomeres and its mut. gigas.

Diameter in mm	Number of vessels	
	Oe. stenomeres	mut. *gigas*
.020—.029	5	
.030—.039	12	2
.040—.049	13	7
.050—.059	**21**	9
.060—.069	14	8
.070—.079	**14**	11
.080—.089	10	**14**
.090—.099	8	**17**
.100—.109	0	12
.110—.119	1	7
.120—.129	2	5
.130—.139		5
.140—.149		1
.150—.159		1
.160—.169		
.170—.179		1

dimension was almost exactly 50 percent greater in the mutation than in the parent form. If we calculate the volume of the tracheids either as cylinders or as quadrangular prisms the relative volume of the cells of parent form and mutation is almost precisely 1 : 3.

TABLE 3

Tracheid lengths of Oe. stenomeres and its mut. gigas.

Length in mm	Number of tracheids	
	Oe. stenomeres	mut. *gigas*
.24—.27	4	
.28—.31	9	
.32—.35	9	
.36—.39	19	
.40—.43	**22**	
.44—.47	11	2
.48—.51	10	4
.52—.55	9	9
.56—.59	2	11
.60—.63	1	10
.64—.67	2	14
.68—.71		**17**
.72—.75		11
.76—.79		11
.80—.83	1	5
.84—.87		2
.88—.91		3
.92—.95	1	
.96—.99		1

TABLE 4

Tracheid width of Oe. stenomeres and its mut. gigas.

Width in mm	Number of tracheids	
	Oe. stenomeres	mut. *gigas*
.012—.015	4	
.016—.019	**41**	
.020—.023	**41**	9
.024—.027	13	22
.028—.031	2	**34**
.032—.035		24
.036—.039		5
.040—.043		5
.044—.047		1

The cells of the medullary ray were measured in cross and tangential sections of the wood, rather than in the macerated material. The transverse dimension (width of the ray) was determined in each case by the measurement of a typical cell from each of 50 rays. The results are shown in table 5. The mean width was 0.0115 mm in *Oe. stenomeres,* 0.0205 mm in mut. *gigas,* the mean in each case coinciding with the width of greatest frequency. The increase in width of the ray of mut. *gigas* over that of the parent species is 78 percent. The radial dimension of

TABLE 5

Transverse dimension of ray cells (width of medullary ray).

Width in mm	Number of cells	
	Oe. stenomeres	mut. *gigas*
.004—.007	4	
.008—.011	**18**	
.012—.015	**20**	4
.016—.019	8	10
.020—.023		**27**
.024—.027		6
.028—.031		3
.032—.035		1

the ray cells was determined by counting the number of cells in one-fifth of a millimeter in 50 rays of each form. The result is therefore more accurate than in the preceding case, but it must be borne in mind that the data of table 6 are not derived from the measurements of individual cells and therefore do not show a well defined greatest frequency. It seems from superficial examination, however, that the cells of each ray are much more uniform than the same number of cells from

various rays. The mean radial dimension is 0.027 mm in *Oe. stenomeres* (mean of 369 cells) and 0.0365 mm (mean of 272 cells) in mut. *gigas*, showing an increase of 35 percent for the mutation. The vertical di-

TABLE 6

Radial dimension of ray cells.

mm	Number of cells	
	Oe. stenomeres	mut. *gigas*
.020—.023	72	
.024—.027	**134**	15
.028—.031	**129**	54
.032—.035	18	**54**
.036—.039	11	**53**
.040—.043	5	50
.044—.047		27
.048—.051		12
.052—.055		4
.056—.059		
.060—.063		
.064—.067		3

mension of the ray cells was obtained by measuring the height of 50 rays in tangential section, and counting the number of cells in each. Again, as in the case of the radial dimension, the tabulated data (table 7) are not based upon individual cell measurements. Here, however, there was a strikingly greater uniformity of cell height in the same ray, than among cells of different rays. The means were 0.031 mm for *Oe. stenomeres* (average of 1839 cells) and 0.048 mm for mut. *gigas* (average of 825 cells), showing the increase for the latter to be 55 percent.

TABLE 7

Height of the ray cells in Oe. stenomeres and its mut. gigas.

Height in mm	Number of cells	
	Oe. stenomeres	mut. *gigas.*
.020—.023	96	
.024—.027	145	
.028—.031	**807**	
.032—.035	**599**	13
.036—.039	190	64
.040—.043	6	138
.044—.047		**179**
.048—.051	2	**195**
.052—.055		177
.056—.059		47
.060—.063		12

Summarized, the mean dimensions of the ray cells are as follows:

Dimension	*Oe. stenomeres*	mut. *gigas*
Breadth	0.0115	0.0205
Length	0.027	0.0365
Height	0.031	0.048

From these data it is obvious that mut. *gigas* not only shows a decided increase in the size of the ray cells, but also an equally decided change in their shape. The ratios of the three dimensions are 1 : 2.35 : 2.70 for *Oe. stenomeres* and 1 : 1.78 : 2.34 for mut. *gigas*. The volumes of the ray cells, calculated as rectangular prisms, are .0000096 mm^3 and .0000359 mm^3, respectively, showing that the mutation to the *gigas* form involves a change of 274 percent in volume.

In both forms the height of the 50 rays was measured both in mm and in number of cells. The number of measurements, although not great, brings out the interesting fact that in *Oe. stenomeres* there are rays of three kinds, (1) small rays, most frequently 5-14 cells high, which are

TABLE 8

Height of the ray in Oe. stenomeres and its mut. gigas.

	Number of rays.			Number of rays	
Height in mm	*Oe. stenomeres*	mut. *gigas*	Height in No. of cells	*Oe. stenomeres*	mut. *gigas*
0.0 — 0.2	2	6	2 — 4	2	6
0.2 — 0.4	13	11	5 — 9	**8**	**12**
0.4 — 0.6	5	8	10 — 14	**8**	**7**
0.6 — 0.8	6	3	15 — 19	4	4
0.8 — 1.00	4	3	20 — 24	3	6
1.00 — 1.20	7	4	25 — 29	**7**	**10**
1.20 — 1.40	1	6	30 — 34	3	4
1.40 — 1.60	1	4	35 — 39	3	1
1.60 — 1.80	2	1	40 — 49	0	
1.80 — 2.00		3	50 — 59	2	
2.00 — 2.20	2		60 — 69	0	
2.20 — 2.40			70 — 79	1	
2.40 — 2.60	1		80 — 89	3	
2.60 — 2.80			90 — 99	1	
2.80 — 3.00	1		100 — 109	1	
3.00 — 3.20	1		110 — 119	1	
3.20 — 3.40			120 — 129	1	
3.40 — 3.60			130 — 139	2	
3.60 — 3.80	1				
3.80 — 4.00	1				
4.00 — 4.20	1				
4.20 — 4.40	1				

presumably the secondary rays, (2) medium rays, most frequently 25-30 cells high, which are presumably the primary rays, and (3) tall rays ranging up to 139 cells high, which, from the number of cells composing them, seem capable of being interpreted as a number of primary rays fused top to bottom into a multiple ray. All the tall rays observed might have been composed of 2, 3, 4 or 5 primary rays fused. Most of the tall rays were biseriate throughout small portions of their length, which, in view of the fact that a typical ray is uniseriate, might be interpreted as an argument for regarding them as multiple rays.

Mut. *gigas* showed the same two classes of small and medium rays as *Oe. stenomeres,* with the same heights in cells, as far as the data are adequate to show. Absolutely no tall multiple rays were found, however, although they were searched for in all the sections. Aside from differences in the dimensions of cells, this is the only difference between the woods of the two forms. The data for the ray heights are summarized in table 8.

SUMMARY

The change from the 2x to the 4x chromosome number in *Oe. stenomeres* is concomitant with

(1) An increase of 50 percent in the length of the vessels, and of 150 percent in the area of the cross-section.

(2) An increase of 50 percent in the length and diameter of the tracheids, corresponding to an increase in volume of 200 percent.

(3) An increase in all three dimensions of the ray cells, but not a proportionate increase, resulting in a cell of a different shape with an increase of 274 percent in volume.

(4) A breaking up of the tall multiple medullary rays into their constituent simple rays.

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