AN ANALYSIS OF THE CHANGES INVOLVED IN A CASE OF PROGRESSIVE MUTATION¹

CARL D. LA RUE AND H. H. BARTLETT

University of Michigan, Ann Arbor, Michigan

[Received June 14, 1917]

The relation of cell size to the size and organization of organisms has been investigated by many workers. The earliest and still one of the most important contributions to the subject was that of SANIO (1872), who studied the size of the cells in the pine. He found that at the base of the trunk the length of the tracheids increases in the first few annual rings to a maximum, which then remains constant. This length, which is constant at only one height, increases upward for a distance, reaches a maximum, and then decreases towards the top of the tree. The cells of the branches are smaller than those of the trunk but vary according to their position, following the same rule as those of the trunk.

It was SACHS who first appreciated the desirability of determining to what extent the size of an organ is correlated with the size of the cells composing it. In 1892 he set the following as the annual prize problem for the UNIVERSITY OF WÜRZBURG: "Es sind zahlreiche Messungen anzustellen, welche Auskunft darüber geben, ob und inwiefern Beziehungen zwischen dem Volumen der Zellen und dem Volumen der Pflanzenorgane bestehen." The prize was won by AMELUNG, who published his data in 1893. His chief conclusion was that in morphologically similar parts of plants the mean cell size remained so nearly constant that the difference in size of organs could be ascribed, without sensible error, wholly to the number of cells composing them. In this conclusion SACHS (1893) concurred, making the further deduction from AMELUNG's data that under normal conditions every plant or animal species attains a definite specific mean size, and that regardless of how much this specific mean size may vary from species to species, homologous cells are always of approximately the same size. In drawing these conclusions it is obvious that SACHS and AMELUNG disregarded fluctuating

¹ Papers from the Department of Botany of the UNIVERSITY OF MICHIGAN, No. 157.

variations as small as those which SANIO had found occurring within an individual.

STRASBURGER (1893) stated as a result of his work that just as the stronger or weaker development of an organ is without commensurate influence on the size of its constituent cells in their fully extended condition, so it is also without influence on the cells in their embryonic condition. He was struck by the fact that although individuals of the same species differed in number but not in size of cells, species of the same genus showed not inconsiderable deviations from one another. He added as an example *Lycopodium Selago*, *L. clavatum*, and *L. complanatum* and pointed out the large cell size of the Ranunculaceae, differing appreciably from other Dicotyledones and agreeing with the Monocotyledones.

As more critical work than that of SACHS and AMELUNG was done, it became obvious that these investigators had perhaps generalized more freely than their data warranted. GAUCHERY (1899) studied various cases of environmental nanism and found that even when other cell characters are unmodified, the diameter of the vessels is usually less in the dwarfs. The epidermal cells are ordinarily the same in dwarfs as in plants of the normal size (e.g., *Datura Stramonium*) but a dwarf of buckwheat proved an exception in having smaller epidermal cells. SCHNEGG (1902) published cell measurements of several species of *Gunnera* which are of interest chiefly because they so signally fail to substantiate the conclusions which their author draws from them. The genus *Gunnera* is remarkable for including species of ordinary size, together with others which are among the most gigantic of non-woody Dicotyledones. SCHNEGG extended AMELUNG's thesis to cover large and small species within the same genus, but his data, except with regard to the epidermis of the leaf, do not bear out his conclusion.

It would be fair to sum up the older work as follows: In general, robust plants differ from dwarf ones primarily in the number of cells of which they are composed, rather than in the size of the constituent cells, although environmental or internal factors that tend to accelerate growth also tend to increase slightly the size of the cells.

The most recent work has shown the necessity of taking nuclear characteristics into consideration in dealing with the relation of cell size to the organization of the individual. It is now known that accessions to the number of chromosomes in the nucleus bring about an increase in cell size, since the size of the cell may be considered as a function of the size of its nucleus. (See literature cited in the preceding paper

by TUPPER and BARTLETT (1918)). Consequently, in the cases where the difference between two species is primarily due to a difference in chromosome number, SACHS's conception is obviously inapplicable.

SIERP (1914) has made the most recent and exhaustive study of the relation of cell size to nanism, and has given especial attention to hereditary dwarfs. He makes a clear distinction between merely depauperate plants and true constitutional dwarfs, pointing out that his predecessor GAUCHERY had not, as he thought, dealt with the latter class of plants. He found that all depauperate plants had smaller cells than normal plants, but by no means smaller in proportion to the reduction of the plant size from the normal. The fact was established that the cells of a depauperate plant are not smaller than those of any juvenile plant of the same species, thus indicating that the difference is due to nutritional factors similar to those that are presumably concerned in the variation of wood cells in different parts of the pine. He compares his results with those of SANIO (1872), pointing out that the mean cell size increases from internode to internode, from a minimum at the base of the stem, where the cells are no larger than in depauperate plants, to a maximum at about the middle of the stem, and decreases to a minimum again at the apex of the stem. With regard to constitutional dwarfs, he found three conditions: (1) The cells are smaller than in the typical form (e.g., *Solanum*, *Zea*, *Pisum*, *Clarkia*). (2) The cells are of the same, or about the same, size (e.g., *Mirabilis*, *Lathyrus*). (3) The cells of the dwarf are larger (e.g., *Nigella*).

We may leave the third case out of consideration, as presumably one of cell gigantism due to chromosomatic modification (although in *Primula* there are cell giants that have the same number of chromosomes as the typical forms; may there not, however, be an increase of chromatin even in such cases?). The other two cases concern us more in the present paper. It has been possible to use the rich material presented by our *Oenothera* mutations to study cell conditions in constitutional dwarfs of known ancestry, grown under reasonably uniform conditions. It must be said in criticism of SIERP's admirable paper that he was unable to study dwarfs of known derivation, and that therefore he had no proof that the dwarfs with smaller cells than typical plants had actually sprung from strains presenting the same cell conditions as the non-dwarfs studied by him. In the two species whose dwarfs did not show a reduced cell size, the normal plants and dwarfs were undoubtedly of the same lineage. *Mirabilis Jalapa* is the only commonly cultivated species of the genus, and is relatively constant, aside from color varieties.

Lathyrus odoratus is likewise so well circumscribed that it crosses with no other species; it has given rise to its cultivated varieties, including the dwarfs, by mutation (BATESON 1913). In the cases of *Zea*, *Solanum* (group of *S. tuberosum*), *Pisum*, and *Clarkia*, the dwarfs are of unknown lineage, being derived from polymorphic groups within which there may well be considerable variation with regard to cell size. In the case of *Zea*, particularly, there is evidence (KUWADA 1911, 1915) that the chromosome number varies considerably from form to form, so that we should expect, *a priori*, a correlated variation in cell size. Aside from the occurrence of slight variations analogous to those of depauperate plants, there is little adequate evidence that hereditary dwarfs with the same chromosome number as the parent species ever have smaller cells.

In this paper we bring forward remarkably consistent evidence that hereditary nanism, in cases where the reduction in size is not associated with a changed chromosome number, is due to a diminished capacity for cell division. The cells of typical plants and dwarfs are potentially of equal size but differ in number and distribution. The series of forms investigated by us is remarkable in that the first three members show successive reduction stages from the typical individual of normal size to an extreme dwarf. We interpret these stages as exemplifying retrogressive evolution. The fourth member, however, shows a reversal of the direction of evolution. In the plant as a whole, the path of retrogression is partially retraced, and in one organ, the capsule, the original point of departure is passed, resulting in a net progression at the end of the evolutionary process. We are especially concerned in this paper with an analysis of the progressive mutation, as the title of the paper indicates, but the retrogressive mutations receive, incidentally, as complete a study.

Before presenting the data, our final conclusion may be stated briefly as follows: In a series of mutations showing both reduction and increase in the size of the entire plant and its organs, the size characters prove to involve only number and arrangement of cells, and not at all the size of the cells, which remains the same throughout the whole series.

In previous publications (BARTLETT 1915; LA RUE and BARTLETT 1917), we have described the origin by mutation from *Oenothera Reynoldsii* of three types of dwarfs, known as mut. *semialta*, mut. *debilis*, and mut. *bilonga*. They arise in the order in which they are listed, *Oenothera Reynoldsii* giving rise to mut. *semialta*, mut. *semialta* to mut. *debilis*, and mut. *debilis* in turn to mut. *bilonga*. The forms have also

all originated from *Oenothera Reynoldsii* directly. Mut. *semialta* shows considerable reduction from *Oenothera Reynoldsii* forma *typica* in both stature and leaf size, and may be characterized as a half-dwarf. Mut. *debilis* is a true dwarf with about a quarter the height of f. *typica*. It is not so greatly reduced as some of those derived from other species of *Oenothera*, notably *Oe. pratincola*, but is about as small a dwarf as any that are sufficiently fertile to be maintained in cultures. Mut. *bilonga* shows a progressive trend, returning to the stature of mut. *semialta*. It is therefore a half-dwarf. In the case of the capsules, there is a very remarkable progression, resulting in a capsule twice as long as that of the other half-dwarf, mut. *semialta*, and much longer than that of f. *typica* which forms the starting point of the evolutionary series.

Years ago SACHS (1893) made some interesting speculations in regard to the changes in the number of cells in an organism, the repetition of which may serve to indicate why we have interpreted increases in cell number as progressive evolution, and decreases as retrogressive. If one were to select from a genus of many species one typical of the genus, and of medium size, it would be possible to conceive of the changes that might follow either from increasing or decreasing the number of cells. In the former case the possibilities of variation would diminish as the number of cells diminished; simplification of structure and decrease of differentiation would inevitably follow if the process were carried far enough. In the latter case, however, it would be impossible to predict the differentiations and specializations that might result with increase in number of cells. The possibilities of evolution in the one case are limited, in the other case unlimited. We do not wish to be understood as denying that specialization may not sometimes accompany decrease in cell number, for it is well known that many small organisms are highly complicated and many larger ones are relatively simple. Nevertheless, we believe that the proposition is in general true, and that in the absence of other criteria, increase in cell number leads to greater complexity and may therefore be interpreted as evidence of progressive evolution.

Within the family Onagraceae we think no one would be inclined to doubt that *Circaea* is a more reduced type than *Oenothera* or *Epilobium*. It has presumably been evolved by reduction from the more generalized types like *Oenothera*. It has a meristic difference from other members of the family (two carpels instead of four). The same meristic variation occurs in *Oenothera pycnocarpa* Atkinson and Bartlett as a somatic mutation. Its most marked reduction, however, has been in the number

of cells comprising the capsule. The reduction has gone so far that the seeds are reduced to two in *Circaea Lutetiana*, and to one in the weakest and most reduced species of all, *Circaea alpina*. With the reduction of the fruit the mechanism for dehiscence has been lost, a simplification resulting as a natural sequence from reduction in the number of the cells. It is hard to see how further evolution can take place in the direction that has led to the genus *Circaea*. *C. alpina* is reduced almost to lowest terms, and unless a reversal of the direction of evolution occurs, the group must remain a terminal one.

The case of the genus *Gaura* is similar, although not so extreme. Here the meristic reduction has not occurred, the carpels remaining four in number as in the generalized types of the family. The reduction of the carpels has taken place, however, resulting in indehiscent fruits with only one or two seeds developing. In a tribe very closely allied to the Gaureae, and presumably descended from the latter, the meristic reduction to a 2-celled ovary has taken place. Moreover the same tribe, the truly remarkable Gongylocarpeae (J. DONNELL SMITH and ROSE 1913) shows us a new evolutionary trend, marked as we should have anticipated, by an increase in number of cells. The annual or biennial *Gaura*-like type has become perennial. The indehiscent fruits remain attached and become imbedded in the wood of the growing stem, giving rise, in the genus *Burragea*, to one of the most bizarre and unusual types in the plant kingdom. Here we find a good illustration of the truth of SACHS's remark that the possibilities of increase in cell number are hardly to be foretold.

We have therefore interpreted the change from dwarf to half-dwarf stature which occurs in the production of mut. *bilonga* from mut. *debilis* as progressive evolution. We view the increase in the length of the capsule of mut. *bilonga* as even more positive evidence of evolutionary progress. Authors have sometimes expressed the view that real progress would be indicated by Mendelian dominance of the more highly evolved type. There is no considerable body of positive evidence to support this view, which is based on the observation that recessives are very frequently weaklings, obviously defective, or unfitted to persist in the struggle for existence. Because of the existence of such a view, however, it is essential to point out the fact that the mutations forming the subject of this paper do not show Mendelian inheritance (LA RUE and BARTLETT 1917). If the view should turn out to be justified, it is interesting that mut. *bilonga* more nearly approximates the behavior of a dominant than any other *Oenothera* mutation except *Oe. rubricalyx*.

Gates. Crossed with one another, the mutations and the parent form all give matroclinic progenies except when mut. *bilonga* is one of the forms involved. In that case, mut. *bilonga*, crossed with its immediate parent, mut. *debilis*, gives a matroclinic progeny when mut. *bilonga* enters the cross as the egg parent, and a dimorphic progeny consisting of both parent forms when it enters as the sperm parent.

We have no numerical data with regard to plant height of the mutations. Such data have, however, been kept for the dimensions of the leaves, which show exactly the same tendencies as the heights. Table 1 shows measurements of leaf length and leaf width in these forms. By comparing it with table 2, in which the capsule measurements of all the forms are given, it may be seen that the reduction in leaf size is nearly proportional to that of capsule length in mut. *semialta*. In mut. *debilis*, however, the reduction in capsule length is considerably less, proportionately, than that in leaf length and leaf width. The discrepancy between the percentage of reduction in stature and that in capsule size is still greater, especially in mut. *debilis*. These facts suggest that the factors determining fruit length may not be affected in the mutative process which gives rise to mut. *debilis* and mut. *semialta* but that the

TABLE I

Length and width of leaves in Oenothera Reynoldsii and some of its mutations.

Length in mm	<i>typica</i>	<i>semi- alta</i>	<i>debilis</i>	<i>bilonga</i>	Width in mm	<i>typica</i>	<i>semi- alta</i>	<i>debilis</i>	<i>bilonga</i>
40-49	.	.	I	.	12-13	.	.	19	5
50-59	.	.	12	.	14-15	.	.	75	8
60-69	.	.	60	16	16-17	.	.	72	32
70-79	.	2	101	69	18-19	.	.	36	52
80-89	.	21	37	110	20-21	.	.	8	79
90-99	.	49	I	30	22-23	.	3	2	28
100-109	.	59	.	I	24-25	.	15		15
110-119	6	73	.		26-27	I	30		7
120-129	5	35			28-29	0	42		I
130-139	2	14			30-31	6	51		
140-149	19	3			32-33	4	48		
150-159	46				34-35	4	39		
160-169	122				36-37	5	21		
170-179	88				38-39	10	19		
180-189	14				40-41	34	3		
190-199	I				42-43	66			
200-209					44-45	71			
210-219					46-47	42			
220-229					48-49	17			
230-239					50-51	I			

TABLE 2

Length of capsule in Oenothera Reynoldsii and some of its mutations.

Length in mm	f. <i>typica</i>	mut. <i>semialta</i>	mut. <i>debilis</i>	mut. <i>bilonga</i>
14-15	.	.	7	
16-17	.	.	38	
18-19	.	.	86	
20-21	.	6	105	
22-23	.	43	104	
24-25	.	97	84	
26-27	.	122	51	
28-29	.	94	31	
30-31	4	57	12	
32-33	21	21	6	
34-35	65	13	3	
36-37	165	2	.	
38-39	260	1	.	
40-41	223	1	.	
42-43	82	.	.	1
44-45	10	.	.	0
46-47	.	.	.	4
48-49	.	.	.	8
50-51	.	.	.	49
52-53	.	.	.	87
54-55	.	.	.	122
56-57	.	.	.	130
58-59	.	.	.	141
60-61	.	.	.	110
62-63	.	.	.	71
64-65	.	.	.	36
66-67	.	.	.	18
68-69	.	.	.	5
70-71	.	.	.	3
72-73	.	.	.	1

slight reduction may be due to lessened vegetative vigor. This idea applies especially well to mut. *debilis*, which is a very weak form. When mut. *debilis* gives rise to mut. *bilonga* there is a doubling, not only of height and leaf size, but of capsule length as well. The resulting capsules, although borne by a half-dwarf, are the largest among the evening primroses, far exceeding in length those of any of the eastern American species with which experimenters are familiar. An inflorescence in fruit is shown at natural size in fig. 1.

It should be mentioned that table 1 is made up of measurements taken in 1916 from material grown in Michigan while those of table 2 were taken in 1915 from material grown in Maryland. This fact does not, however, affect the ratios between the four types since all capsules are compared with others of the same locality and season, and the same is true of the leaves.

FIGURE 1.—*Oenothera Reynoldsii* mut. *bilonga*. Fruiting inflorescence, exactly natural size. The longest capsule is 64 mm long. The pedigree of this particular individual through six generations was: *typica*→*typica*→*typica*→*debilis*→*bilonga*→*bilonga*. (A member of culture 23; see LA RUE and BARTLETT 1917, p. 123.)

All of the forms show a considerable amount of sterility, as is common in *Oenotheras*. In muts. *semialta* and *debilis* the degree of sterility is greatest and these are the forms with the shortest capsules. In view of this fact a correlation of percentage of sterility and length of fruit seemed probable. Another explanation was possible, namely that capsule length might depend upon the total number of ovules rather than upon the number which develop. Mut. *bilonga* offered a more interesting case since the evidence given by it was expected to show whether or not the type represents a true progressive mutation. DAVIS (1915), in discussing these forms, suggested that a large increase in the total number of ovules in mut. *bilonga* over that in mut. *debilis* and the others would indicate a "true progressive advance," but should the increase in length be attended by an increase in the number of *fertile ovules* only it would indicate merely a reversion to an ancestral type having a higher degree of ovule fertility.

To determine the facts concerning the sterility of the ovules in these forms, counts of the sterile and of the fertile ovules were made in each of the four types. Capsules were selected for these counts of modal length as nearly as possible. All of these plants were growing in the same field and were of about the same maturity. The capsules were of mature size though the seeds were not fully ripened in any of them. The fruits were all gathered on the same day and were preserved in alcohol until the counts could be made.

The ovules were removed from the capsules, placed on a slide, and counted under a binocular microscope. After the ovules had been apparently removed from the capsules, it was found that many flat, aborted ovules still remained clinging to the placentae. It was therefore necessary to examine the placentae in all cases and count the ovules found there. This process was more troublesome than that of counting the other ovules and most of the errors made in counting were made here. Some errors were unavoidable as the interiors of the capsules are very mucilaginous and the very small, flat, aborted ovules cling together and to the larger fertile ones very tightly. The ovules were separated by fine needles and each was turned over to avoid overlooking any small attached ones. By using great care in this way it was possible to avoid any considerable percentage of error. In any case the errors should have been about the same for each of the forms and the large number of ovules in each capsule tends to make them less significant. Only those ovules were considered sterile which were entirely flat and obviously contained no embryos. Others, though small, were found to contain embryos and were classed as fertile.

Table 3 shows that mut. *semialta* and mut. *debilis* have about the same total number of ovules as forma *typica*. On the other hand the number of sterile ovules is greatly increased. The decrease in fruit length here must be due at least in part to an increase in ovule sterility. This increase in sterility may be due to decreased vegetative vigor and thus to decreased nutrition. This explanation seems more probable in the

TABLE 3
Number of ovules in the capsules of *Oenothera Reynoldsii* and its mutations.

Type	No. of capsule	Length in mm	Normal ovules	Sterile ovules	Total ovules
<i>f. typica</i>	1	39	404	383	787
	2	39	530	283	813
	3	38	446	389	835
	4	40	574	283	857
	5	39	537	208	745
	6	39	444	203	647
	7	38	516	289	805
	8	39	587	270	857
	9	39	537	293	830
	Mean	39	508	289	797
mut. <i>semialta</i>	1	26	116	620	736
	2	27	364	454	818
	3	28	163	496	659
	4	26	138	610	748
	5	27	195	593	788
	6	27	102	790	892
	7	27	253	484	737
	8	26	175	608	783
	9	26	141	508	649
	10	26	120	608	818
	Mean	27	177	586	763
mut. <i>debilis</i>	1	26	133	691	824
	2	22	17	505	522
	3	25	64	766	830
	4	18	158	319	477
	5	16	79	319	398
	Mean	21	90	520	610
mut. <i>bilonga</i>	1	52	669	591	1260
	2	55	688	484	1172
	3	53	604	571	1175
	4	52	660	293	953
	5	56	464	469	933
	6	58	524	726	1250
	7	54	821	526	1347
	8	53	830	405	1235
	9	58	742	418	1160
	10	53	654	439	1093
	Mean	54	666	492	1158

light of some recent work of DAVIS (1916) in which he tried the effect on fertility of cutting down the nutrition of plants of *Oenotheras* by removing leaves. He found that the reduction of nutrition had a very decided effect on the fertility of his plants.

Mut. *bilonga* is not normally so sterile as mut. *debilis* which produces it. Table 3 shows it to have about the same percentage of sterility as f. *typica*. The total number of ovules, on the contrary, is greatly in excess of that in f. *typica* so that the increase in length of fruit is correlated with an increased number of ovules rather than with an increase in fertility. It appears that the formation of this mutation has introduced a higher ovule number and that the type represents a real case of progressive mutation.

A study of the cells of the capsules seemed desirable for the purpose of determining whether or not the changes in capsule length were due to or accompanied by giant or dwarf conditions of the cells. KEEBLE (1912) described cell gigantism in *Primula* unaccompanied by an alteration in the chromosome number and the case of *Nigella* mentioned by SIERP (1914) may be similar. It seemed possible that such a condition of cell gigantism might account for, or at least accompany, the difference in capsule length between mut. *debilis* and mut. *bilonga*. Tables 4 and 5 show the results of measurements of spiral tracheids and fiber tracheids from capsules of each type. For this work modal capsules were selected, macerated, and the wood elements measured with an eye-piece micrometer. A mechanical stage was used to avoid duplication of measurements.

TABLE 4

Length of spiral tracheids from capsules of Oenothera Reynoldsii and some of its mutations.

Length in mm	f. <i>typica</i>	mut. <i>semialta</i>	mut. <i>debilis</i>	mut. <i>bilonga</i>
.0332-.0664	2	7	7	5
.0664-.0996	22	34	19	19
.0996-.1328	43	55	37	45
.1328-.1660	36	36	28	26
.1660-.1992	30	26	23	21
.1992-.2324	26	10	20	18
.2324-.2656	9	10	4	14
.2656-.2988	10	5	5	10
.2988-.3320	6	6	1	10
.3320-.3652	4	2	1	6
.3652-.3984	5	2	0	6
.3984-.4310	0	2	0	4
.4310-.4684	0	1	0	0

TABLE 5

Length of fiber tracheids from capsules of Oenothera Reynoldsii and some of its mutations.

Length in mm	f. <i>typica</i>	mut. <i>semialta</i>	mut. <i>debilis</i>	mut. <i>bilonga</i>
.1992-.2324	.	.	2	1
.2324-.2656	.	.	7	8
.2656-.2988	1	.	8	10
.2988-.3320	6	7	13	11
.3320-.3652	13	21	16	13
.3652-.3984	21	27	27	19
.3984-.4310	42	36	31	25
.4310-.4684	54	42	34	38
.4684-.4980	74	44	41	46
.4980-.5146	78	50	60	73
.5146-.5478	76	44	42	63
.5478-.5810	70	38	28	63
.5810-.6142	60	34	24	55
.6142-.6474	30	30	18	52
.6474-.6806	28	24	14	37
.6806-.7138	19	23	13	36
.7138-.7470	13	9	12	20
.7470-.7802	5	8	4	20
.7802-.8134	3	3	3	18
.8134-.8466	1	1	.	9
.8466-.8798	4	3	.	9
.8798-.9130	.	.	.	7
.9130-.9462	.	.	.	4
.9462-.9794	.	.	.	3
.9794-1.0126	.	.	.	2
1.0126-1.0458	.	.	.	0
1.0458-1.0790	.	.	.	2

The results given in tables 4 and 5 show that both types of tracheids have the same modal length in all four forms. The range of fluctuation is greatest in mut. *bilonga*, and the environmental effect of diminished nutrition is obvious in the case of mut. *debilis*, through the greater number of short cells; but on the whole the conclusion is obvious that the cells of all four forms are so similar in size as not to account in any degree for the differences of capsule length.

In order to trace the possible influence of cell size on plant height, mature normal stems of each type were selected and samples of wood were taken from the base of the stem. These samples were macerated and studied in the same way as the capsules but in this case only one type of cell was measured. Table 6 gives the result of this study. In no case was the wood of a type taken from the same plant as the capsule yet the results are the same. This is the more interesting because the wood of the capsule is primary, and that of the lower stem almost all secondary. From this evidence it appears that mutative pro-

TABLE 6

Length of fiber tracheids of wood at base of stems of Oenothera Reynoldsii and its mutations.

Length in mm	f. <i>typica</i>	mut. <i>semialta</i>	mut. <i>debilis</i>	mut. <i>bilonga</i>
.1328-.1660	.	.	.	1
.1660-.1992	.	1	.	0
.1992-.2324	.	2	.	1
.2324-.2656	.	2	5	0
.2656-.2988	1	4	8	2
.2988-.3320	3	5	12	8
.3320-.3652	7	9	19	15
.3652-.3984	7	27	33	25
.3984-.4310	16	33	56	30
.4310-.4684	31	43	65	40
.4684-.4980	32	47	66	44
.4980-.5146	58	57	75	49
.5146-.5478	40	27	49	13
.5478-.5810	36	21	27	11
.5810-.6142	23	9	14	4
.6142-.6474	13	1	7	0
.6474-.6806	6	1	5	1
.6806-.7138	5			
.7138-.7470	3			
.7470-.7802	3			

cesses which produce forms entirely distinct from the parent in general characters and genetic behavior may still not be sufficiently deep-seated to affect such conservative tissues as those of the xylem.

The object of using wood from the base of the stem was, of course, to eliminate errors from variation due to environmental factors within the plant. As already pointed out, SANIO (1872) and later authors have shown the existence of considerable place effects on cell size within the plant. Since SIERP (1914) has emphasized the juvenile characteristics, as to size, of cells at the base of the stem of large plants, and has shown that all the cells of dwarfs approximate the juvenile condition, one would naturally select the base of the stem, in studying a series of forms, as the region most comparable. In *Oenothera*, however, it is probable that place effects within the plant are, for wood tissues, negligible. The evening primroses are more or less well-marked biennials. While in the rosette state, before the stem is sent up, they become very strong and robust, and store an abundance of reserve food for the subsequent rapid growth. In *Oe. Reynoldsii* f. *typica* the rosette stage has a tendency to be transitory, but it is developed sufficiently so that it might be expected to produce a distinct modification of the juvenile character of the cells of the growing point. The dwarfs are almost pure annuals,

mut. *debilis* and mut. *bilonga* often failing to persist for even a short time in a rosette stage. The wood specimens studied were therefore not as strictly comparable as one might infer from their position in the stem. The identical modes of all the variation curves consequently afford all the more striking confirmation of our general conclusion that the retrogressive and progressive evolution in the mutation series of *Oe. Reynoldsii* is tied up with changes in the number and arrangement of cells, rather than with their size.

Samples of wood of such of the hybrids between the various forms as have been secured were also studied. The authors' studies on these hybrids are described in the paper cited elsewhere in this article. It was found that these forms, with one exception, show matroclinic inheritance. Since all the forms have the same type of wood cells, it was not to be expected that these would shed any light on the problem of matroclinic inheritance in the hybrids. Nevertheless it is highly desirable to know just what their behavior is. The studies of the hybrids were made in the same manner as those of the uncrossed types. It is noted in the paper on matroclinic inheritance that some of the possible crosses have not been secured. These are mut. *semialta* $\times$ mut. *debilis* and mut. *bilonga* $\times$ mut. *debilis*. In addition to these missing forms the hybrid mut. *debilis* $\times$ f. *typica* is lacking in the wood studies because all the plants of this form were very weak so that good specimens could not be obtained. Table 7 shows the results, which, in the main, are what might be expected from the data given on the parents. The one notable exception will be discussed later. It will be noticed that in the cross mut. *debilis* $\times$ mut. *bilonga* data are given on two forms. This cross gives twin hybrids, one of which resembles the ovule parent and the other the pollen parent. All of the crosses showed wood cells like those of the parents. An effect on the cells akin to heterosis (the vigor of heterozygosis) was the most that might have been expected if the crosses had been different from the parents. As we have shown (LA RUE and BARTLETT 1917) no such effect was discernible externally, and none could be discerned in the cells. This is just what might have been anticipated from the hypothesis of non-equivalent gametes, which we have elsewhere applied to the mutations in question.

The cross mut. *debilis* $\times$ mut. *semialta* departs from the others in its behavior and gives a bimodal curve. The first set of results aroused a suspicion of an accidental mixture of the material. So a second sample was macerated from the same plant. All possible care was taken to secure a thorough mixing of the cells but the result was the same. In

TABLE 7
Length of fiber tracheids of wood at base of stems of the mutation crosses of *Oenothera Reynoldsii*.

Length in mm	typica × semialta	typica × debilis	typica × bilonga	semialta × typica	semialta × bilonga	bilonga × typica	bilonga × semialta	debilis × semialta (lot 1)	debilis × semialta (lot 2)	debilis × bilonga (debilis twin)	debilis × bilonga (bilonga twin)
.1992-.2324			1		2				1		
.2324-.2656			3	4	7				1	2	2
.2656-.2988		2	5	5	13	8	5	6	6	3	6
.2988-.3320		7	19	8	16	22	15	11	12	6	4
.3320-.3652	4	20	24	20	32	25	20	19	20	23	8
.3652-.3984	12	34	37	37	34	26	38	42	46	31	23
.3984-.4310	20	52	40	44	46	47	46	55	97	69	32
.4310-.4684	34	54	60	50	69	61	53	38	83	82	45
.4684-.4980	60	67	60	66	69	61	58	40	89	93	73
.4980-.5146	41	32	30	30	32	39	28	14	32	35	49
.5146-.5478	37	27	17	14	13	13	16	6	17	26	22
.5478-.5810	17	10	17	6	7	9	6	4	4	14	14
.5810-.6142	12	2	6	6	6	2	3	0	1	2	10
.6142-.6474	9	1	6	3	3	3	4	1		1	3
.6474-.6806		0	4	2	1	2					
.6806-.7138		1			2						
.7138-.7470											

measurements involving a population of any sort a bimodal curve is interpreted as an indication of a mixture of types. In a single plant it may be explained in a similar way. If a mutation should occur in the form of a chimaera such a mixing would occur. In this case it would affect only a part of the plant, the remainder having the same curve of variability as the other types. Unfortunately the wood samples were taken before the seeds were ripe so that no seeds of this plant were secured. One can only speculate on the possible behavior of a mutation which affects only the internal structure. If there had been external evidence of its existence it would have been detected and saved, for the culture in which it occurred was repeatedly inspected by both the writers. It might have been possible to determine from the existing material whether the chimaera was of a sectorial or a mosaic character but, since the study must have ended there, it was not thought worth the trouble.

SUMMARY AND CONCLUSIONS

(1) The series of mutations derived from *Oenothera Reynoldsii* shows both retrogressive and progressive evolution with regard to plant height, retrogressive from f. *typica* through mut. *semialta* to mut. *debilis*, progressive from mut. *debilis* to mut. *bilonga*. The changes are all concerned with the number and arrangement of cells, not with the size of the individual cells.

(2) The length of the wood elements is the same in the various matroclinic mutation crosses as in the self-pollinated strains. The crosses do not show, either in external or internal characters, any vigor due to heterozygosis. No such effect would be anticipated in view of the considerations developed in a former paper, in regard to non-equivalent gametes.

(3) The mutation series shows a relatively small reduction in capsule length from f. *typica* through mut. *semialta* to mut. *debilis*. This reduction is interpreted as probably a physiological manifestation, the less vigorous forms having the capacity to mature fewer ovules into seeds, and therefore producing shorter mature capsules. The number of ovules is nearly the same in the three forms, but the sterility increases with the degree of dwarfness of the form. The wood elements of the capsules of all three forms are of the same size.

(4) The process which results in the formation of mut. *bilonga* involves an increase over any of the other forms in the number of ovules, but not an increase in fertility over f. *typica*. It is therefore interpreted as a case of progressive evolution. The increase in number of ovules

is accompanied by a doubling in the length of the capsule; and this doubling is due to an increase in number of cells rather than to an increase in the size of the cells. The capsule of mut. *bilonga* is the largest known among the small-flowered *Oenotheras* of the *biennis* alliance.

(5) Aside from the possibility that one bimodal curve may have indicated the occurrence of a sectorial chimaera with smaller cells, there is no evidence that the retrogressive and progressive evolutionary changes which have taken place in *Oe. Reynoldsii*, affecting plant height, capsule length, and number of ovules, are accompanied by significant alterations in such conservative tissues as the xylem.

LITERATURE CITED

- AMELUNG, E., 1895 Über mittlere Zellengrösse. *Flora* **77**: 176-207.
 BARTLETT, H. H., 1915 Mutation *en masse*. *Amer. Nat.* **49**: 129-139.
 BATESON, Wm., 1913 Problems of genetics. pp. 258. Yale University Press.
 DAVIS, B. M., 1915 Additional evidence of mutation in *Oenothera*? *Amer. Nat.* **49**: 702-706.
 1916 Malnutrition as a factor in pollen and seed sterility of *Oenothera*. (Read at New York meeting of the Botanical Society of America.)
 GAUCHERY, P., 1899 Recherches sur le nanisme végétal. *Ann. Sci. Nat.* 8^e ser. **9**: 61-156.
 KEEBLE, F., 1912 Gigantism in *Primula sinensis*. *Jour. Genetics* **2**: 163-188.
 KÜWADA, Y., 1911 Maiosis in the pollen mother cells of *Zea Mays* L. *Bot. Mag. Tokyo* **25**: 163-181.
 1915 *Bot. Mag. Tokyo* **29**: (Cited by ISHIKAWA, M. A list of the number of chromosomes. *Bot. Mag. Tokyo* **30**: 404-448.)
 LA RUE, C. D., and BARTLETT, H. H., 1917 Matroclinic inheritance in mutation crosses of *Oenothera Reynoldsii*. *Amer. Jour. Bot.* **4**: 119-144.
 SACHS, J., 1893 Physiologische Notizen. VI. Ueber einige Beziehungen der spezifischen Grösse der Pflanzen zu ihrer Organisation. *Flora* **77**: 49-81.
 SANIO, KARL, 1872 Über die Grösse der Holzzellen bei der gemeinen Kiefer. *Jahrb. f. wiss. Bot.* **8**: 401-420.
 SCHNEGG, H., 1902 Beiträge zur Kenntniss der Gattung *Gunnera*. *Flora* **90**: 161-208.
 SIERP, HERMAN, 1914 Über die Beziehungen zwischen Individuengrösse, Organgrösse und Zellengrösse, mit besonderer Berücksichtigung des erblichen Zwergwuchses. *Jahr. f. wiss. Bot.* **53**: 55-124.
 SMITH, J. DONNELL, and ROSE, J. N., 1913 A monograph of the Hauyaeae and Gongylocarpeae, tribes of the Onagraceae. *Cont. U. S. Nat. Herb.* **16**: 287-298.
 STRASBURGER, E., 1893 Über die Wirkungssphäre der Kerne und die Zellengrösse. *Hist. Beitr.* **5**: 95-124.