

CELL MEASUREMENT AS AN AID IN THE ANALYSIS OF QUANTITATIVE VARIATION*

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The inheritance of quantitative characters is now commonly interpreted on a basis of multiple Mendelian factors. It is not too much to say, however, that relatively little has been done to identify these factors. Enough of them have ordinarily been postulated to account mathematically for the results in any given case, but a biological analysis has seldom been attempted. One must except the work of Emerson,¹ who has found that the height of a bean plant is due to at least three, and probably more, independent factors, namely, the number of internodes, the length of the individual internodes, and the habit of growth, whether determinate or indeterminate. It has seemed desirable to us to proceed still further with the analysis and we have taken up first the problem of internode length.

Variation in the length of an internode may be correlated either with variation in the number or size of the constituent cells. *A priori*, therefore, there are at least two factors whose relative importance it should be possible to measure in any given case. If, in addition, it should be possible to demonstrate the hereditary behavior of either factor, or of both, and to determine the fluctuations of both due to environment, the whole problem of the inheritance of quantitative characters would become much more concrete and would be brought correspondingly nearer to a solution.

Before proceeding to the analysis of genetic variations, we undertook to test the proposed method by applying it to a variation brought about by environmental agencies. The etiolated epicotyl of *Phaseolus multiflorus* Willd. was chosen, because it is much longer than the normal epicotyl grown in light. Not only do the results demonstrate the feasibility of resolving internode length into less complex characters, but they are also of considerable interest *per se*, adding, as they do, to

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¹ Emerson, R. A. A genetic study of plant height in *Phaseolus vulgaris*. Nebr. Agr. Exp. Sta. Res. Bull. 7: 1-73. 1916.

the none too ample quantitative data concerning the effect of light on the growth and division of cells.

The classical work on etiolation is that of Gregor Kraus,² who made a large number of cell measurements in both normal and etiolated stems. He concluded that the greater length of etiolated internodes was due almost entirely to the greater length of the cells, but that a certain part of the increase over the normal internodes had to be ascribed to an increase in the number of cells. Kraus made thousands of measurements, and, as a whole, his work was painstakingly done and of substantial value. He did not, however, make enough measurements in individual cases to establish true means, or to determine ranges of variation, and he likewise failed to distinguish, in measurements of epidermis, between primary and secondary cells. Moreover, as we shall show further on, his work must share with that of others the criticism that it was probably based upon material that was not strictly comparable. The comparability of individual plants grown under different conditions can only be assured by determining the range of fluctuating variation of a sufficient number of plants grown under each condition.

The entire subject of etiolation was reviewed in 1903 by MacDougal,³ to whose memoir the interested reader should turn for references to the extensive literature. With regard to the epidermal cells of etiolated stems his conclusion (*l. c.* p. 247) is as follows: "Epidermal cells were found to be as long as the normal in all instances, except in *Menispermum (canadense)*, in which species alone the superficial measurements were less than in normal stems. The epidermal cells showed an increase in all dimensions in a great number of instances in which a multiplication of these elements had also ensued. Among the earlier investigators various contentions arose as to whether the excessive elongation of stems was accompanied by increase in size, or by increase in number, of the epidermal cells, the conclusions of the various workers being based upon the small number of species examined. It is to be seen, however, that no general law has been discovered by which the action of the epidermis in darkness may be predicated." Except for the explicit case of *Menispermum*, Mac-

² Kraus, Gregor. Ueber die Ursachen der Formänderungen etiolierender Pflanzen. Jahrb. Wiss. Bot. 7: 209-260. 1869-'70.

³ MacDougal, D. T. The influence of light and darkness upon growth and development. Mem. N. Y. Bot. Gard. 2: 1-319. 1903.

Dougal's work (he gives few measurements, but the figures are drawn to scale) seems to bear out Kraus's conclusion that the elongation of an etiolated stem is due to increase in both number and size of cells. Kraus's data for *Phaseolus*, however, were in conflict with his own general conclusion. He found that etiolated internodes of *Phaseolus vulgaris* L., although elongated, had fewer cells than normal internodes, seemingly indicating that the entire increase in length was due to cell size. Our work leads us to believe that in this case there was an unusually extreme error due to failure to make cell measurements from plants of comparable position in the range of fluctuating variation.

In our experiments seeds of the scarlet runner bean (*Phaseolus multiflorus* Willd.) were grown in complete darkness and in light. The length of the epicotyls of 80 etiolated and 92 normal plants was measured; the symmetrical frequency distributions gave the following constants:

	Range of Variation	M	σ	CV
Grown in darkness.....	168-517 mm.	305	71.4	23.4
Grown in light.....	30-141 mm.	85	16.9	19.9

Thus, the etiolated stems were 3.6 times as long as those grown in the light, and had only a slightly greater coefficient of variation.

An extremely long normal epicotyl (141 mm.) was taken for cell measurements. For our purposes it was more favorable than one of modal length, in that the epidermis was surely made up of almost the maximum number of cells for a normal epicotyl. A rough check on this statement is afforded by multiplying the length of the normal epicotyl (141 mm.) by the factor 3.6, the ratio between the mean length of normal and etiolated stems. The result is 508 mm., in sufficiently close agreement with the actual maximum length (517 mm.) for an epicotyl grown in darkness. If, therefore, an etiolated epicotyl of less extreme position in the variation curve should contain more cells, it would prove conclusively that the effect of light is to retard cell division as well as to diminish cell size, and that in *Phaseolus*, as in other plants, the etiolated internodes have more cells than the normal. The etiolated epicotyl chosen for cell measurements was 372 mm. long; *i. e.*, it was exceeded in length by 20 percent of the variates, and corresponded to a normal epicotyl 133 mm. long.

Each epicotyl was divided into 10 equal segments, and in each segment 100 primary epidermal cells, taken at random, were measured. Thus for each epicotyl 1,000 primary cells were measured, uniformly distributed along its length. Many of these primary cells were divided by transverse walls into secondary cells, which were also measured, bringing the number of measurements up to 2 or 3 thousand for each epicotyl. Kraus had observed in his work that the ends of many epidermal cells were not even approximately perpendicular to the longitudinal axis, but were pointed. He wrote: "It is a common phenomenon that the cells, at any rate those of the epidermis, do not remain parenchymatous during elongation in etiolation, but like the elongating wood-forming cambial cells, become completely prosenchymatous, the long, sharp ends dove-tailing in between one another." This condition occurs in the normal as well as in the etiolated epidermis, but a further fact is that many cells do have perpendicular end walls, and appear, as we have already indicated, to have been formed at a late stage in the development of the internode. With regard to the latter point, MacDougal says of epidermal cells of etiolated stems in general: "The epidermis, in common with many other tissues, does not advance beyond a certain primary stage of development, and retains the power of growth and division in the cells during a much longer period than in the normal plant; consequently it can respond to stresses and other factors, which may cause it to undergo increase in size, alterations in form, or multiplication of the cells by division." In the epidermis of *Phaseolus multiflorus* it proved to be a simple matter to distinguish by the character of the end walls between the primary cells, formed by division of the primary meristem, and secondary cells, formed by subsequent divisions. The prosenchymatous shape of the former is obvious, even after they have divided into secondary cells. The latter have at least one transverse end wall, as in any typical parenchymatous cell. (See figs. 1 and 2, reduced to scale from camera lucida drawings.) Measurements were made of cells taken at random, but were so recorded so as to distinguish undivided primary from secondary cells. All the secondary cells derived from a single primary were measured, and their lengths added to obtain the total length of the epidermis derived from it.

It is obvious that in any discussion of cell length as a factor in internode length, one must distinguish carefully between primary and secondary cells. Our tables give statistical data (I) for primary

cells (undivided, divided, and taken at random), (2) for cells taken at random, whether undivided primary cells or secondary cells, and (3)

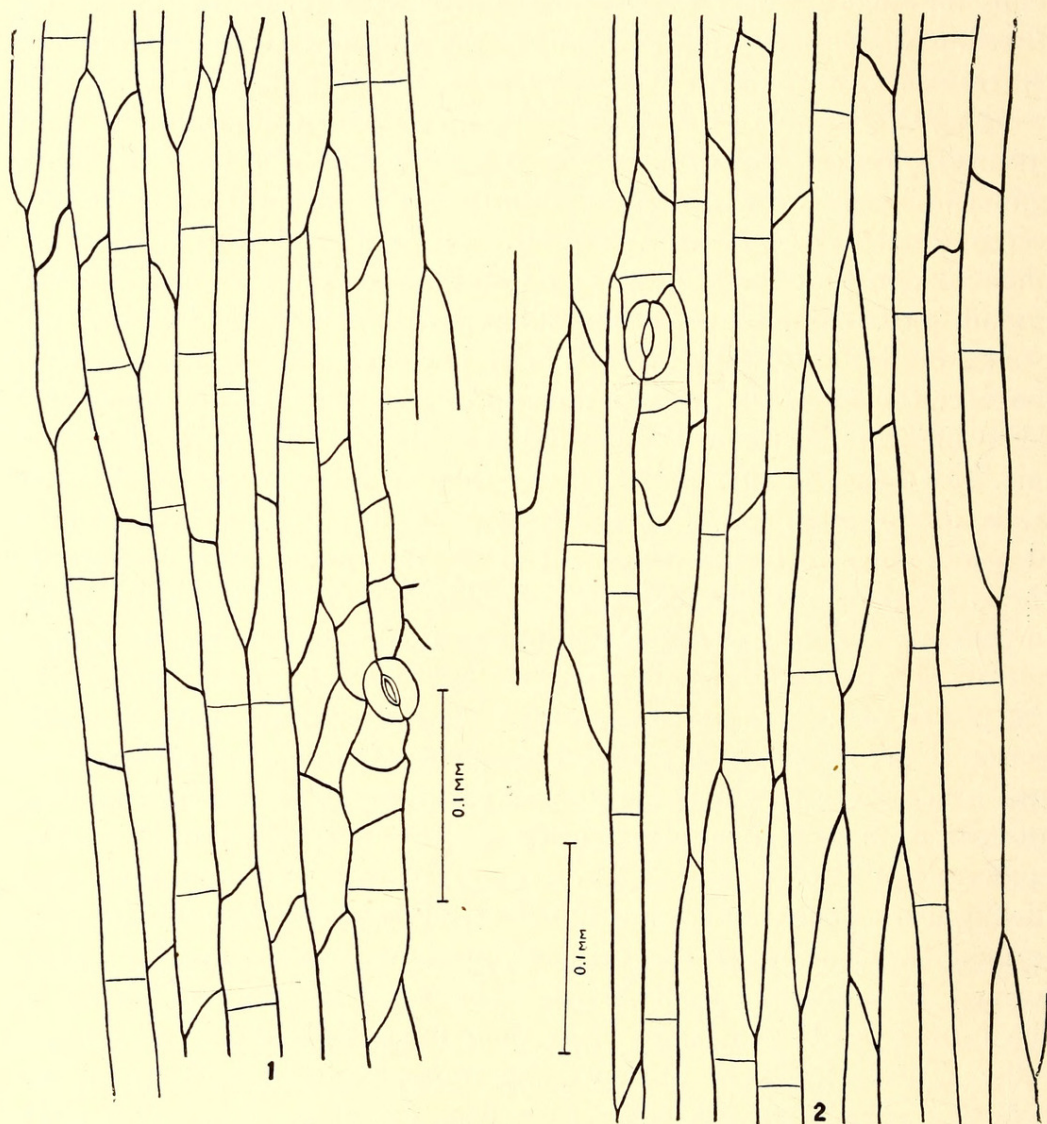


FIG. 1. Epidermis from epicotyl of *Phaseolus multiflorus*, grown in the light. (Material chosen from a section in which the primary cells were of the same mean length as those of the whole internode.)

FIG. 2. Epidermis from epicotyl of *Phaseolus multiflorus*, grown in the dark. (Material chosen from a section in which the primary cells were of the same mean length as those of the whole internode.)

for secondary cells alone. The measurements of undivided primaries proved to be remarkably interesting, for they tend to indicate that in

Phaseolus there is a physiological limitation to the length which can be attained by a cell without undergoing division.

Plant physiologists recognize, of course, that there is presumably for each kind of cell a specific size at which division takes place. The evidence, in the case of primary meristem, is based upon such experiments as those of Newcombe⁴ in which growing tips were incased in gypsum, in order to prevent growth by mechanical means. Under such conditions the primary meristem ceases division, and does not resume it until the release of the pressure permits the growth of the cells to the specific size at which division takes place. The somewhat differentiated epidermal cells present a different condition. The cells are in a state of extension, accompanied by increase in volume of the vacuole, but not, as far as known, by any increase in the amount of protoplasm. Although it is quite in accord with expectation to find that cells in course of extension, providing they retain the meristematic function, should have a specific size for division, it is nevertheless a distinct gain to have additional data bearing upon the subject.

TABLE I

Length in Mm. of Primary Epidermal Cells (including both Divided and Undivided Cells) of Epicotyl of Phaseolus multiflorus Grown in Light. (The Sections are Numbered from the Basal Tenth (No. 1) upwards)

Class	Section										Entire Internode
	1	2	3	4	5	6	7	8	9	10	
.030-.057.....	9	12	7	1	0	1	1	0	0	2	33
.060-.087.....	47	45	37	13	2	11	3	6	5	9	178
.090-.117.....	37	25	33	24	18	15	13	29	15	21	230
.120-.147.....	7	17	22	26	23	26	24	20	33	26	224
.150-.177.....		1	1	21	22	20	27	17	23	23	155
.180-.207.....				12	12	15	18	10	15	15	97
.210-.237.....				2	12	6	8	11	4	3	46
.240-.267.....				1	5	5	4	1	3	1	20
.270-.297.....					2	1	2	2	1		8
.300-.327.....					3			3	0		6
.330-.357.....					1			0	1		2
.360-.387.....								1			1

Tables I and VI give the frequency distributions for the primary cells, disregarding secondary divisions. That is to say, the measurements were taken from end to end of the sharp-pointed outline of the

⁴ Newcombe, F. C. The influence of mechanical resistance on the development and life-period of cells. Bot. Gaz. 19: 149-157, 191-199, 229-236. 1894.

TABLE II

Length in Mm. of Undivided Primary Cells of Epicotyl of Plant Grown in the Light

Class	Section										Entire Internode
	1	2	3	4	5	6	7	8	9	10	
.030-.057.....	9	12	7	1	0	1	1	0	0	2	33
.060-.087.....	47	45	37	13	2	11	3	6	5	9	178
.090-.117.....	37	25	31	22	17	14	9	28	13	18	214
.120-.147.....	7	17	21	19	12	11	11	6	14	5	123
.150-.177.....		1	1	10	6	4	4	0	5		31
.180-.207.....				1		2		0			3
.210-.237.....								1			1

TABLE III

Lengths in Mm. of Divided Primary Epidermal Cells of Epicotyl of Plant Grown in Light

Class	Section										Entire Internode
	1	2	3	4	5	6	7	8	9	10	
.090-.117.....			2	2	1	1	4	1	2	3	16
.120-.147.....			1	7	11	15	13	14	19	21	101
.150-.177.....				11	16	16	23	17	18	23	124
.180-.207.....				11	12	13	18	10	15	15	94
.210-.237.....				2	12	6	8	10	4	3	45
.240-.267.....				1	5	5	4	1	3	1	20
.270-.297.....					2	1	2	2	1		8
.300-.327.....					3			3	0		6
.330-.357.....					1			0	1		2
.360-.387.....								1			1

TABLE IV

Length in Mm. of Epidermal Cells Taken at Random, Including Undivided Primary Cells and Secondary Cells, from Epicotyl of Plant Grown in Light

Class	Section										Entire Internode
	1*	2*	3	4	5	6	7	8	9	10	
.003-.027.....	0	0	0	0	0	0	2	0	0	0	2
.030-.057.....	9	12	10	8	9	7	10	9	10	10	94
.060-.087.....	47	45	36	36	38	40	42	40	49	48	421
.090-.117.....	37	25	32	30	33	32	35	40	27	37	328
.120-.147.....	7	17	21	15	14	16	10	11	12	5	128
.150-.177.....		1	1	10	6	3	1		2		24
.180-.207.....				1		2					3

* These sections contained no divided primary cells.

group of cells derived from the original primary cell, in case the latter had undergone division. In many cases, of course, the shorter primary cells were undivided. Some mistakes in the differentiation of primary from secondary cells were doubtless made, but not enough to vitiate

TABLE V
Length in Mm. of Secondary Cells of Epicotyl of Plant Grown in the Light

Class	Section										Entire Internode
	1	2	3	4	5	6	7	8	9	10	
.003-.027.....	0	0	0	0	0	0	2	0	0	0	2
.030-.057.....			3	8	10	9	9	15	11	12	77
.060-.087.....			3	30	41	47	52	44	57	56	360
.090-.117.....				25	35	35	30	33	25	31	214
.120-.147.....				4	11	9	7	8	7	1	47
.150-.177.....				1	3						4

the results. In Tables II, III, VII and VIII the divided and undivided primary cells are separately enumerated. In Tables IV and IX are given the measurements of undivided primary and secondary cells, taken at random. The data of these two tables are therefore comparable with those of Kraus (l. c.). Tables V and X concern the secondary cells only. Tables XI and XII give a convenient summary of the data in Tables I to X.

TABLE VI
Length in Mm. of Primary Epidermal Cells of Epicotyl of Plant Grown in Dark. The Cells were Taken at Random, without Regard to Whether or Not they had Undergone Secondary Division

Class	Section										Entire Internode
	1	2	3	4	5	6	7	8	9	10	
.060-.117.....	8	1	5	4	1	1	4	4	7	12	47
.120-.177.....	42	9	16	15	8	14	17	11	13	23	168
.180-.237.....	34	24	22	28	41	21	21	19	16	22	248
.240-.297.....	11	30	30	30	18	27	29	28	26	14	243
.300-.357.....	5	23	16	11	18	15	17	12	18	11	146
.360-.417.....		8	8	7	7	13	6	8	12	9	78
.420-.477.....		4	2	1	5	8	4	8	6	7	45
.480-.537.....		1	1	2	2	1	2	8	2	2	21
.540-.597.....				2				2			4

Beginning with section 1, and reading downward in all the columns in Table XI, it is seen that under both conditions of growth the epidermal cells, whether primary, secondary, or taken at random, show a

gradual increase in length from the base upward, to a maximum. After the maximum mean length is reached, there is a decrease toward the upper end of the internode, but not a decrease to the minimum which occurs at the base. The gradual increase in cell size may possibly be partially accounted for by increase in the amount of water available during the elongation of the internode. When the seedling is very young the root system is simple, and relatively inadequate. Moreover, the differentiation of conductive tissue is not complete. Consequently the cells cease expanding earlier than do subsequently formed cells, which have the advantage of a copious water supply and a more highly developed conductive system. In attributing to water supply a possible influence upon the size of epidermal cells, we do not commit ourselves to any theory as to the cause of extension. Whether extension is due to turgor pressure or not, the process could not take place at all without sufficient water to keep the protoplasm in contact with the cell wall, and that amount would hardly be measurably different for a moderately rigid cell wall whether the turgor pressure were great or small.

TABLE VII

Length in Mm. of Undivided Primary Epidermal Cells of Epicotyl of Plant Grown in Dark

Class	Section										Entire Internode
	1	2	3	4	5	6	7	8	9	10	
.060-.117.....	7	1	2	4	1	1	4	3	6	11	40
.120-.177.....	30	3	4	10	3	9	12	6	4	19	100
.180-.237.....	4			3	1	4	6	4	2	6	30
.240-.297.....							1	3		2	6

TABLE VIII

Length in Mm. of Divided Primary Epidermal Cells of Epicotyl of Plant Grown in Dark

Class	Section										Entire Internode
	1	2	3	4	5	6	7	8	9	10	
.060-.117.....	1	0	3	0	0	0	0	1	1	1	7
.120-.177.....	12	6	12	5	5	5	5	5	9	4	68
.180-.237.....	30	24	22	25	40	17	15	15	14	16	218
.240-.297.....	11	30	30	30	18	27	28	25	26	12	237
.300-.357.....	5	23	16	11	18	15	17	12	18	11	146
.360-.417.....		8	8	7	7	13	6	8	12	9	78
.420-.477.....		4	2	1	5	8	4	8	6	7	45
.480-.537.....		1	1	2	2	1	2	8	2	2	21
.540-.597.....				2				2			4

Whatever the cause of the variation within the internode, it is obvious that tissues for cell measurement should be taken from strictly comparable regions in the plant. It would seem to be a safe rule that in studies of the stem the tissue should come from the middle of the internode, unless a sufficiently large number of cells are measured so that an equal number can be taken from each aliquot part of the internode.

TABLE IX

Length in Mm. of Epidermal Cells Taken at Random Including Undivided Primary Cells and Secondary Cells from Epicotyl of Plant Grown in the Dark

Class	Section										Entire Internode
	1	2	3	4	5	6	7	8	9	10	
.030-.057.....	2	0	3	0	1	0	0	2	3	3	14
.060-.087.....	22	22	24	8	8	11	5	6	12	13	131
.090-.117.....	41	32	37	25	48	31	21	23	19	36	313
.120-.147.....	21	30	27	37	29	33	39	25	33	17	219
.150-.177.....	12	13	9	25	10	17	22	16	15	22	161
.180-.207.....	1	3		5	4	7	9	5	13	8	55
.210-.237.....	1					1	3	11	3	1	30
.240-.267.....							1	7	2		10
.270-.297.....								3			3
.300-.327.....								2			2

TABLE X

Length in Mm. of Secondary Epidermal Cells of Epicotyl of Plant Grown in the Dark

Class	Section										Entire Internode
	1	2	3	4	5	6	7	8	9	10	
.030-.057.....	2	3	3	9	1	0	0	2	3	2	25
.060-.087.....	31	24	25	32	8	11	6	5	10	10	162
.090-.117.....	44	37	37	42	49	32	23	22	16	33	325
.120-.147.....	18	27	26	21	29	30	42	24	33	18	268
.150-.177.....	5	9	9	4	10	17	18	20	18	25	135
.180-.207.....				2	3	7	8	4	14	8	46
.210-.237.....						3	3	11	4	2	23
.240-.267.....							1	6	2	2	11
.270-.297.....								4			4
.300-.327.....								2			2

Kraus assumed that the quotient of the length of the internode divided by the mean length of the cells would bear a simple relationship to the number of cells making up the length of the internode. Since there is considerable dovetailing of the cells between one another, the quotient does not, of course, indicate the number of cells in a

TABLE XI

Mean Length in Mm. of Epidermal Cells of Epicotyl of *Phaseolus multiflorus*, based upon Data Given in Tables I-X

Section	Plant Grown in Light					Plant Grown in Darkness				
	All Primary Cells	Undi- vided Primary Cells	Divided Primary Cells	Undi- vided Primary and Sec- ondary Cells	Second- ary Cells	All Primary Cells	Undi- vided Primary Cells	Divided Primary Cells	Undi- vided Primary and Sec- ondary Cells	Second- ary Cells
I	.087	.087	(None)	.074	(None)	.187	.175	.216	.109	.102
2	.090	.090	(None)	.087	(None)	.273	.135	.274	.114	.109
3	.096	.096	.090	.092	.058	.250	.147	.258	.106	.108
4	.135	.115	.163	.097	.087	.225	.146	.276	.130	.100
5	.169	.122	.190	.096	.088	.267	.150	.250	.117	.119
6	.145	.112	.179	.094	.088	.280	.163	.292	.126	.130
7	.162	.124	.178	.085	.084	.259	.199	.288	.138	.135
8	.155	.093	.189	.087	.085	.291	.176	.316	.153	.158
9	.153	.117	.170	.086	.083	.264	.129	.283	.135	.141
10	.144	.097	.163	.083	.081	.242	.148	.300	.153	.163
Entire internode	.135	.102	.189	.085	.090	.258	.149	.282	.126	.124

TABLE XII

Statistical Constants Based upon Data Given in Tables I-X

Constants	Plant Grown in Light					Plant Grown in Dark				
	Primary Cells	Undi- vided Primary Cells	Divided Primary Cells	Undi- vided Primary and Sec- ondary Cells	Second- ary Cells	Primary Cells	Undi- vided Primary Cells	Divided Primary Cells	Undi- vided Primary Cells and Secondary Cells	Second- ary Cells
M (mm.)	.135	.102	.189	.085	.090	.258	.149	.282	.128	.124
σ (mm.)	.053	.033	.040	.033	.026	.097	.025	.083	.040	.043
C. V.	39.2	32.4	26.0	38.8	28.9	37.6	16.9	29.4	31.2	34.7

vertical series, from node to node, but for the comparison of very similar material, such as ours, it probably affords quite as useful a measure of the cell number factor. It will be remembered that the normal internode chosen for cell measurements came at the extreme upper limit of the range of variation, and that the etiolated internode corresponded to a much shorter normal one, the two lengths being in the ratio 141 to 103. (The value 103 is obtained by dividing the actual length of the etiolated internode, 372 mm., by the factor 3.6,

thus correcting for the total effect of light.) As already explained, the choice of an extremely long normal internode obviated any possibility of failure to detect the cell number factor in the elongation due to etiolation, in case any such factor existed. The available evidence indicates that under relatively constant environmental conditions, variation in internode length is correlated with the number rather than with the size of cells. (*Teste* Kraus; results with long and short internodes of *Philadelphus*.) If it had been grown under normal conditions, therefore, the etiolated internode from which our measurements were made might have been expected to produce 27 percent fewer cells than the normal one with which it was compared, yet in the dark it actually produced 38 percent more, if we base the comparison upon primary cells, including both undivided and divided, or 78 percent more, if we base the comparison upon undivided primary and secondary cells, taken at random. There can remain no doubt, therefore, that the effect of light, directly or indirectly, is to retard cell division.

If a correction is made for the difference in the position of the two plants in the range of variation, the number of primary meristematic divisions in darkness shows an increase of 88 percent over the number in the light, accounting for 34 percent of the total increase in length, leaving 66 percent to be accounted for by increased extension of the cell or group of cells derived from each division. In case primary and secondary cells are not distinguished, it appears that the number of cells in etiolated internodes is greater by 142 percent than in the normal ones, and that 55 percent of the increase in length is due to the cell number factor, and only 45 percent to the cell size factor. Since Kraus's conclusions were based upon cells taken at random, as in the latter case, the discrepancy between his results for *Phaseolus vulgaris* and ours for *P. multiflorus* requires a further word of explanation. He found the entire increase in length to be due to the cell size factor, but that his material was not strictly comparable is indicated by the fact that he did not determine the fluctuating variation of the plants which he used. Consequently he could neither select comparable internodes in the first place, nor correct for their deviation from comparability. In our work we have determined the range of variation for both normal and etiolated seedlings, and have assumed that, within the limit of experimental error, the cell number factor and the cell size factor have the same relative weight in bringing about the elonga-

tion of all etiolated internodes grown under like conditions. Although it is unlikely that this assumption is wrong, it requires proof; we have not yet undertaken the labor of making enough measurements to place it beyond criticism. It must be admitted, however, that relative position in the frequency distribution affords a logical basis for the determination of comparability.

In order to establish a relationship between length of the primary cells and their division into secondary cells, the divided and undivided primary cells were separately enumerated, with the striking results shown in Tables II, III, VII, and VIII. In both normal and etiolated internodes there is a high correlation between length and condition with regard to division, the shorter cells in each case being the undivided ones, which are but little more than half as long as the divided ones. There is a pronounced tendency for the short cells at the base of the internode to remain undivided, and this is particularly so in the case of the markedly shorter cells of the normal internode.

In comparing the data, one is impelled to speculate as to whether light directly retards division of the primary cells, or acts indirectly by retarding the extension of the cells, so that relatively few of them attain the specific size for division. The latter supposition is the more simple. Bearing it in mind, we observe that in the plant grown in the light, 59 percent of the cells are undivided, 41 percent divided. In the dark 15 percent are undivided, 85 percent divided. From the frequency distributions in Tables I and VI we find that all below 59 percent of the illuminated cells or 15 percent of the etiolated cells would include all below a length of about 0.140 mm. Some divided cells are shorter, and some undivided ones are longer, the two classes approximately balancing one another.

Turning to Tables II and VII, we find that the length 0.240 mm. is exceeded only by a trivial number of undivided cells, whether primary or secondary. Conversely (Tables III and VIII) we find that in only a trivial number of divided cells does the sum of the lengths of the derived secondary cells fall below 0.120 mm. The conclusion appears reasonably justified that the range of length within which division generally takes place is 0.120 to 0.240 mm., and that the greater part of the divisions occur at a length not far from 0.140 mm.

The expression "specific size," used without qualification, refers, of course, to specific volume, and since our measurements concern only one dimension, length, they are insufficient to determine a specific

size for division, in any strict sense. Observation, unsupported by measurements in the case of the present material, but borne out by studies of epidermal cells in the case of the genus *Oenothera*,⁵ leads to the conclusion that cells which attain an excessive length are usually very slender, and vice versa. On this ground it is possible to explain the great range of variation in the length attained by different cells before division. It is quite correct, however, to speak of specific mean length for division, which is simply the mean of the lengths at which division takes place in a large number of cells, and this is the constant which we have determined with some approximation to accuracy as 0.140 mm. in the stem epidermis of *Phaseolus multiflorus*.

It appears that the specific mean length for division is the same in both light and darkness. The mean length of undivided primary cells is indeed greater in the etiolated internode, but this fact is readily explained. In the light only 76 out of 1,000 primary cells taken at random were both undivided and longer than 0.140 mm.: 583 were undivided. In the dark, however, there were only 176 undivided primaries in 1,000, and a relatively larger number of them, 103, exceeded the putative specific mean length, 0.140 mm. The extension of a considerable number of cells to somewhat beyond the specific mean length would be expected to bring about the division of most of the cells in the lower part of the range within which division takes place, and to leave only the cells which, because of slenderness or some other cause, come within the extreme upper part of the range. The small number of undivided primaries in the material grown in the dark suggests that most of those that remain must have passed the specific mean length. It is therefore not surprising that their mean length is 0.149 mm., whereas in every other instance the mean length of undivided cells, whether primaries or secondaries, was found to be well below 0.140 mm.

The simplest assumption with regard to the effect of light is that it retards extension of the cells, and that as an indirect result there are fewer secondary divisions, since relatively fewer primary cells enter the range of length within which division takes place. With regard to the cell divisions in the primary meristem, it is clear that more of them take place in the dark than in the light, but there is no evidence with regard to the cause.

⁵ Tupper, W. W. and Bartlett, H. H. The relation of mutational characters to cell size. *Genetics* 3: 93-106. 1918.

SUMMARY

The mathematical formulation of the results of size inheritance according to the multiple factor hypothesis should be paralleled by a biological analysis, the object of which is the identification of the several factors concerned. In such a biological analysis, it will inevitably be found that quantitative variations may be correlated with variation in either the number or the size of the constituent cells of the organisms or organs involved. Still other variations involve both cell number and cell size.

In the investigation of quantitative variations of a hereditary nature, it seems likely that the study by the histological method of reactions to the environment and of the obscure reaction known as "vigor of heterozygosis" will afford a means of correcting for these disturbing factors.

In connection with genetical studies in *Phaseolus*, we have made some studies of fluctuating variation due to the effect of light, one of the most disturbing factors concerned in size inheritance. The results are of considerable interest in themselves, and may be summarized as follows:

1. In *Phaseolus multiflorus*, growth in darkness results in the elongation of the internodes to 3.6 times the length of normal internodes grown in the light.
2. This increase in length is accounted for to the extent of 34 percent by increase in the number of divisions taking place in the primary meristem, the remainder of the increase being due to increase in length of the cell or group of cells derived from each primary division.
3. It is possible to recognize the group of secondary cells formed by division of a primary cell during its extension, since the outline of the primary cell is pointed at the ends, whereas the subsequently formed cross walls are approximately perpendicular.
4. There appears to be a specific mean length for division of the primary epidermal cells, with a value of about 0.140 mm., which is independent of light or darkness.
5. In consequence of the fact that the length for division is attained in a larger number of primary cells in the etiolated than in the normal internode, it is necessary, in appraising the relative importance of the cell number and cell size factors, to discriminate carefully between primary and secondary epidermal cells.



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