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HEREDITY, VARIATION AND EVOLUTION IN PROTOZOA. II.

HEREDITY AND VARIATION OF SIZE AND FORM IN PARAMECIUM, WITH STUDIES OF GROWTH, ENVIRONMENTAL ACTION AND SELECTION.[1]

BY H. S. JENNINGS.

(*Read April 24, 1908.*)

TABLE OF CONTENTS.
(See pages 544–546.)

I. INTRODUCTORY.

The first of this series of studies[2] gave a general introduction to the investigations, and dealt with the fate of new or acquired characters in protozoa, showing that these are as a rule not inherited and that there is no difference in principle on this point between protozoa and metazoa. The present paper takes up heredity and variation in size and form in *Paramecium*.

Our present questions are then mainly as follows: In what respects do the individuals of *Paramecium* resemble each other? In what

[1] From the Laboratory of Experimental Zoölogy, Johns Hopkins University, Baltimore, Md.

[2] *Journal of Experimental Zoölogy*, Vol. 5, 1908, pp. 577–632.

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respects do they differ? What are the causes of the resemblances or differences, as the case may be?

The attempt is made to treat these questions broadly, determining experimentally the different classes of causes concerned, without prejudice as to their relative importance. External and internal factors are therefore equally considered, the purpose of the investigation being to give as complete an analysis of the phenomena of resemblances and differences as possible. Our problem, then, requires an analysis from this point of view of all things which may result in producing, increasing or decreasing the similarities and differences between individuals—reproduction, growth, conjugation, the effects of environment, of selection, and the like.

The investigation will be best introduced by proposing at once what is really the central problem—that concerning *heredity*. Is size inherited in *Paramecium?*

How would heredity of size be shown? If certain individuals differ in size, and the progeny of these individuals, under identical conditions, show corresponding differences, this is what would commonly be called heredity of size. "Heredity is a certain degree of correlation between the abmodality of parent and offspring" (Davenport, 1899, p. 35). Do large individuals of *Paramecium* produce, under the same conditions, larger progeny than do small ones? Is it possible to obtain by selection large and small races of *Paramecia?*

To study this question, we must first examine the variations in size commonly found in *Paramecium.*

II. PRELIMINARY STUDY OF VARIATION IN PARAMECIUM.

We owe our present knowledge of variation in *Paramecium* mainly to Pearl and his co-workers (see Pearl, 1907; Pearl and Dunbar, 1905). A more extensive work by Pearl on variation in *Paramecium* has been mentioned as in prospect; I learn from personal communication, however, that this is not to appear. I shall therefore publish my own results more fully than I should otherwise have done. Certain points in connection with variation in *Paramecium* have been dealt with by Simpson (1902) and Pearson

(1902); also by McClendon (1908). But we have at present nothing like a thorough analysis of the matter, based on extensive data.

1. General Methods of Work; Statistical Treatment and Its Uses.

Before we can study experimentally the nature and causes of the existing variations, we must, of course, know their extent, character and distribution. To this end I have made a statistical study, constructed frequency polygons, and determined the more important constants of variation and correlation. This has, of course, not been done because of belief in any occult virtue in mathematical treatment. Statistical methods have been used in this preliminary survey merely because they form the most natural and direct way of discovering and displaying the problems on which we wish to work; I doubt whether the most determined critic of the use of such treatment in biology could suggest any other way for our material. But I am fully convinced that "crucial evidence is always individual in the last analysis" (Whitman); that the preliminary statistical examination of the facts requires development as soon as possible into precise experimental knowledge. It is valuable to know just how many men out of a thousand will die in a given period, but it is infinitely more valuable to know which ones will die if the conditions are not changed, and why; and the latter knowledge includes the former. I have therefore advanced at once from the descriptive statistical work to experimental treatment. A curve or polygon of variation (such as Diagram 1) or a correlation table (such as Table I.) is to be looked upon as a mass of problems. The place occupied in the polygon or table by any individual is due to certain causes, and it is these causes that we seek.

In seeking these causes by experimental methods, statistical treatment is again found to be of the greatest value for detecting and registering the effects of single factors, under complex conditions. This method may be compared to a microscope; it enables us to detect and deal with causes and effects which we could not handle without it. I am convinced that it is a great mistake to hold that the only or the main use of statistical treatment is for "dealing

with the sphere of indefinitely numerous small causes—amenable only to the calculus of chance, and *not to any analysis of the individual instance.*" Such treatment is a most valuable instrument for precisely such analysis as will bring out the effects of individual factors when we are unable to experimentally disengage them completely from others; it aids us most essentially in the "analysis of the individual instance." Of this I hope the present paper may furnish illustrations. As Johannsen (1906, p. 98) has well expressed it, the mathematical treatment must, to give valuable results, be "based upon an accomplished sorting of the special facts and a biological setting out of the premises which are to be treated." Davenport (1899) states that "the statistical laws of heredity deal not with the relations between one descendant and its parent or parents, but only with the mean progeny of mean parents." The object of the present work is precisely to discover so far as possible the relation between one descendant and its parent (or other relatives); for this, statistical methods show themselves most useful.

2. A Typical Culture.

We will then first examine a typical culture of *Paramecium,* made in the usual way with pond water and decaying vegetation, in a circular glass vessel about nine inches across and three inches deep. This culture we will call *Culture 1.*

Inspection showed that *Paramecia* of markedly different size were found in this culture, so that it seemed a favorable one for a study of inheritance in size. Cursory examination seemed to indicate the existence of two sets of individuals, those of one set being nearly double the length of the others.

Of this culture a large number were killed on April 10, 1907, and four hundred specimens, taken at random, were measured as to length and breadth.

3. Methods of Measuring and Recording.

The animals were killed with Worcester's fluid, which is known to cause practically no distortion when properly used. Worcester's fluid consists of ten per cent. formalin saturated with corrosive sublimate. In using it, a large number of the infusoria must be brought into one or two drops of

water, then these must be overwhelmed with a considerable quantity of the fluid. If the infusoria are in a larger quantity of water, the killing takes place more slowly, the animals have time to contract, and distortion results.

The measurements were made on the slide, the organisms being either still in the killing fluid or in ten per cent. formalin. Transference to the latter has no effect on the form of the fixed animals. Most of the measurements were made directly with an ocular micrometer. In the case of cultures of large individuals, however, the form was projected on paper with the camera, in the way described by Pearl (1907), the extremities of length and breadth marked with the pencil, then these were measured with a scale made by projection of the ocular micrometer.

Such combinations of lenses were used that one division of the micrometer scale was equal to 4 microns (or in a few cases, which will be expressly noted, to $3\frac{1}{3}$ microns). The measurements were thus recorded in units, each of which was equal to 4 microns, so that the recorded units are multiplied by four to give results in microns. When the measurements fell between two lines of the micrometer, the line nearest the actual measure was that recorded; if the measurement fell just half way between two lines, the higher line was recorded. Thus, the recorded unit 45 included all measurements beginning with $44\frac{1}{2}$, and *less than* $45\frac{1}{2}$. In the tables, the measurements, given in microns, are therefore grouped about such values that each group includes values from two microns below to two microns above the one recorded. Thus, in Table 1, the length 180 includes all the specimens measuring from 178 up to (but not including) 182.

It will be well to summarize here, once for all, the method of treating the data obtained in the measurements. For most of the tables the constants computed (and recorded below the tables) were the following: the mean, standard deviation, and coefficient of variation, for length and for breadth; the mean index or ratio of breadth to length; and the coefficient of correlation. The computation of the constants was based on the well-known formulæ that have been brought together by Davenport (1904) and others. I used as a rule the actual methods set forth so clearly by Yule (1897). The computations were made by the aid of seven-place logarithms and of Crelle's and Barlow's tables. Two independent computations, at considerable intervals of time, were made in each case. While I cannot hope that errors in computation are excluded, I believe that such as may exist do not in any way affect the conclusions to be drawn.

Certain points of detail should be mentioned. While, as will appear, most of the tables do not give symmetrical curves, I have used only the simple statistical methods applicable in strictness to such curves; the methods are quite sufficient as a basis for the comparisons we wish to make.

In computing the standard deviation, Sheppard's correction of the second moment was used throughout. That is, if we employ the method of Yule (1897),

$$\sigma = \sqrt{\Sigma(f\xi^2) - d^2} - .08333,$$

or using the signs employed by Davenport (1904)

$$\sigma = \sqrt{\frac{\Sigma(V - V_0)^2}{n} - r_1^2} - \frac{1}{12}.$$

TABLE I.

"Wild" Culture 1. Correlation Table for Length and Breadth of a Random Sample
(See Diagrams 1 and 2, polygons *A* and *a*.)

Length in Microns.

Breadth in Microns.	84	88	92	96	100	104	108	112	116	120	124	128	132	136	140	144	148	152	156	160	164	168	172	176	180	184	188	192	196	200	204	208	212	216	220	224	228	232	236	240	Totals.
28					1	4		5	3	5	2	3	4																												27
32	1	1			1	3	3	6	9	19	14	17	9	8	4	2																									97
36									3	2	6	5	9	7	7	3								1	1								1								45
40									1		1		3		5	2					2				1		2		1												18
44										1			2	1	2			1		1	1	2	1	2	4	1	3	7	9	5	3	6	4	1	1	1					59
48								1								1					2	1	3	3	1	4	2	7	5	5	4	8	11	5	1		1				65
52																						1			2	1	1	2	3	5	4	4	7	6	7	3	2	2			50
56																					1				1	1	2	1	1	3	1	5	1	2	2	4	1	3		1	30
60																						1										1		1		1	1	1	1		7
64																					1								1												2
Totals.	1	1	0	0	2	7	3	12	16	27	23	25	27	16	18	8	0	1	0	1	7	5	4	6	10	7	10	17	20	18	12	24	24	15	11	9	5	6	1	1	400

Mean Length, 165.840μ.
Mean Breadth, 48.860μ.
Mean Length of Left Hand Group, 125.420μ: of Right Hand Group, 200.972μ.
Mean Breadth of Left Hand Group, 33.396μ: of Right Hand Group, 49.216μ.

The *mean index* given below the tables is the mean of the quotient $\frac{\text{breadth}}{\text{length}}$: it shows essentially what percentage the breadth is of the length. This mean was found, without computing the index for each individual, by the following formula:

$$i = \frac{A_B}{A_L}(1 + C_L^2 - rC_BC_L).$$

Where i is the mean index, A_B is the mean breadth, A_L the mean length, C_B the coefficient of variation for breadth, C_L the same for length, and r is the coefficient of correlation between length and breadth.

I am greatly indebted to Dr. Raymond Pearl for assistance in the mathematical treatment of the data.

The results of the measurements of a random sample of 400 of Culture 1 are given in Table I.

It is evident on inspection of this table that the individuals fall into two well-marked groups, one set varying in length from 84 to 144 microns, the other set varying from 164 to 240 microns, while between these groups, in the region from 144 to 164 microns, only two specimens are found. The mean length for the entire sample falls at 165.840 microns, almost precisely in the region where no specimens are found. The smaller set have their mean length at 125.420 microns: the larger set at 200.972 microns.

These results are shown as frequency polygons in the lower portions of Diagrams 1 and 2.

4. Method of Constructing the Polygons.

In making the polygons for length, three units of measurement (12 microns) were grouped together to make a single unit of the abscissa of the polygon. This was done in order to destroy any irregularities due to unconscious prejudice on the part of the measurer for certain numbers. Thus, in measuring a large number of individuals, it may be found, for example, that few are recorded at 51, while at 50 there are many; or the reverse may occur. This is due only to the fact that in doubtful cases falling between these numbers the measurer unconsciously gives the preference regularly to one of them. The error thus introduced is extremely small (it can hardly be more than one micron in any case), but if the polygon is made without grouping together adjacent classes, there appear extreme irregularities in its outline, irregularities that are quite without significance. When three units are thrown together, any marked irregularities remaining in the polygons are almost certainly due to peculiarities in the material itself. It is of course possible that small peculiarities really existing may be hidden in

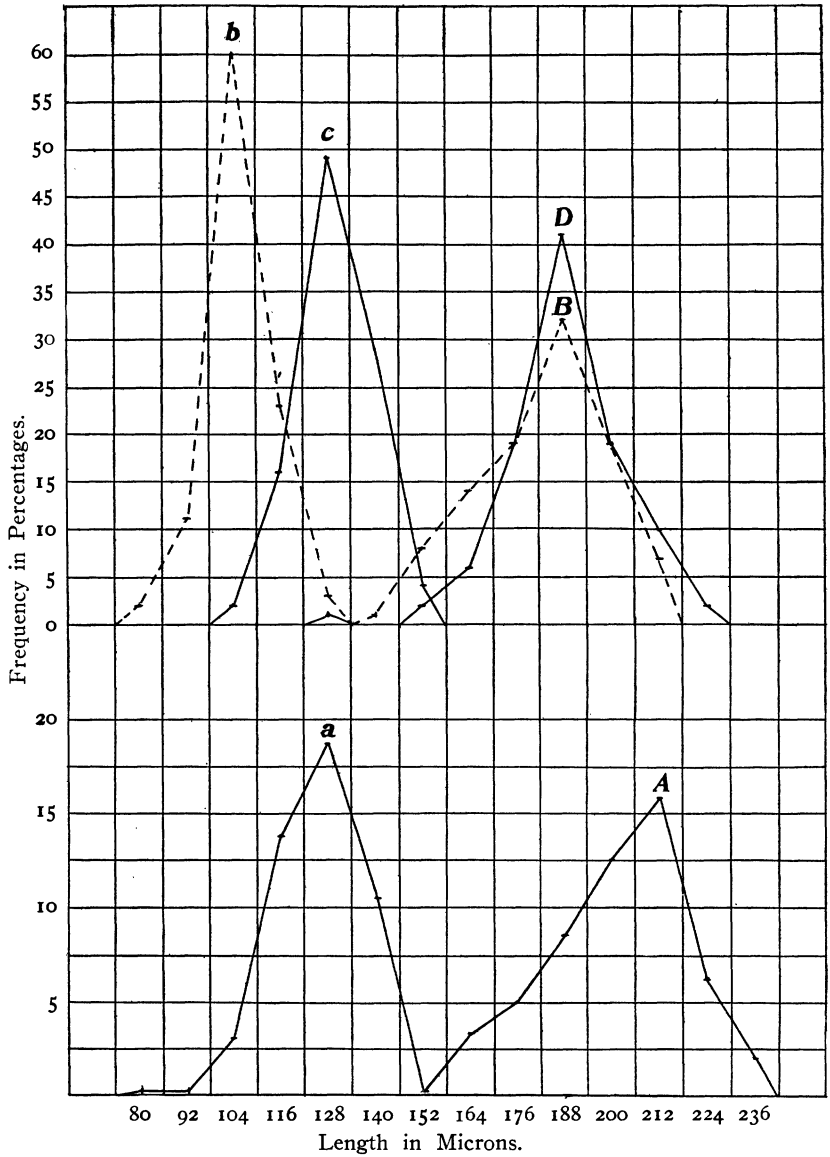

DIAGRAM 1. Polygons of variation for length in Culture 1 and its descendants. *A* and *a* form together the polygon for 400 specimens taken at random from the original culture 1, on April 10, 1907. *B*, polygon for 100 descendants of ten of the larger individuals of Culture 1. *D*, polygon for 100 descendants of the single large individual *D*, from culture 1. *b*, polygon for 100 descendants of fifty smaller individuals from culture 1. *c*, polygon for 100 descendants of the single small individual *c*, from culture 1.

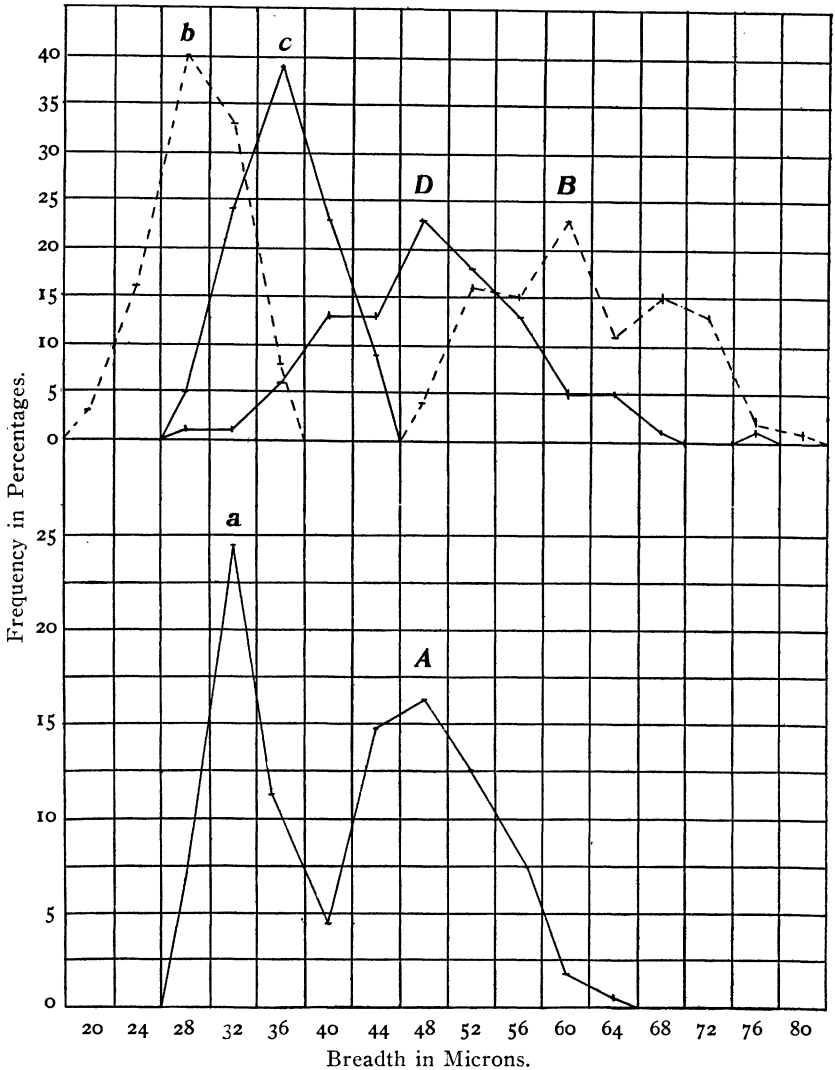

DIAGRAM 2. Polygons of variation for breadth in culture 1, and in its descendants from selected specimens. The letters have the same significance as in Diagram 1.

this way, but it was thought wiser to be conservative in this matter. Thus the space between two perpendicular lines of the polygons includes three of the groups of the correlation table, and is marked at its base with the middle value of the three groups which it includes.

In making the polygons for breadth, it was found that there was little evidence of error due to unconscious preference for certain numbers in making this measurement. This is probably due to the comparatively small numbers of units in the breadth measurement, and to the fact that it is possible to hold both limits of the measurement on the scale sharply in the eye at once, while this is hardly possible in measuring length. In the polygons for breadth, therefore, one unit of the polygon was made to correspond to one unit of measurement (four microns).

In all the polygons the numbers to the left indicate percentages of the entire number, so that all the polygons are of equal area, whatever the number of specimens on which they are based. The only exception to this is in the case of the double polygons *a* and *A*, of Diagram 1, resulting from plotting the random sample of Table I. Since this sample falls into two groups, the entire (double) polygon was made of twice the area of the other polygons. Each half polygon therefore becomes approximately equal to any one of the single polygons of the other diagrams, thus permitting ready comparison.

The numbers at the foot of the diagrams are the dimensions in microns. Each number corresponds to the value of the center of the column beneath which it stands.

5. Two Groups of Paramecia.

Thus the *Paramecia* in our natural culture I fall into two groups which are almost completely separated, so far as length is concerned, but which overlap a certain amount in breadth. Characteristic outlines of varied members of the two groups, drawn to the same scale, are shown in Fig. 1.

Are these two groups permanent differentiations, such as might be called distinct species, or are the differences possibly due merely to temporary dimorphism of some sort? To answer this question individuals of the two sizes were isolated and allowed to multiply separately, in cultures made of boiled hay. After varying periods of time 100 individuals, taken at random, were measured from each of these pure cultures, and the frequency polygon derived from these was compared with the two (nearly distinct) polygons from the original culture. The following cultures were made and measured:

1. Fifty of the smaller individuals were selected from the original culture, placed together, and allowed to multiply for twelve days (from April 10 to April 22). The measurements of 100 of this

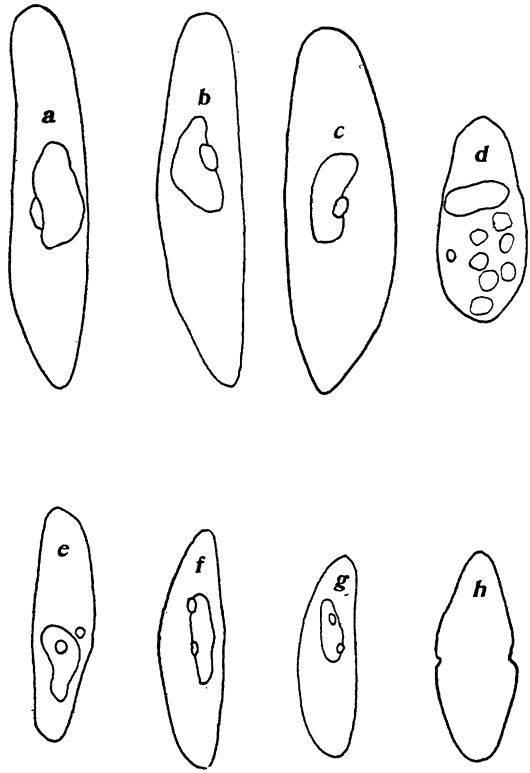

FIG. 1. Outline of characteristic specimens from the original wild culture 1, April 10, 1907. The upper row shows examples of the larger "*caudatum* form"; the lower row examples of the smaller "*aurelia* form." *d*, Young of the caudatum form; *h*, dividing specimen of the *aurelia* form. All × 235.

culture are shown in curve *b* (broken line), Diagrams 1 and 2; their dimensions are given in the correlation Table II. It is evident that this group corresponds in a general way with the smaller group of the original culture, though its mean length and breadth are somewhat lower (96.280 × 29.080 microns instead of 125.42 × 33.396), and it shows a little less variation.

2. Ten of the larger individuals selected from the original culture were likewise allowed to multiply in the same vessel for twelve days, then 100 were measured. The results are shown in curve *B*, Diagrams 1 and 2, and in the correlation Table III. It is evident

TABLE II.

Correlation Table for Length and Breadth of a Random Sample from Descendants of 50 of the Smaller Individuals from Culture 1, allowed to Multiply for 12 Days. (*See polygons b, Diagrams 1 and 2.*)

Breadth in Microns.	Length in Microns.													
	80	84	88	92	96	100	104	108	112	116	120	124	128	
20					1		2							3
24					3	3	4	5		1				16
28					6	8	10	8	5	1	1		1	40
32	1	1	1			3	9	8	4	3	2	1		33
36									1	3	3		1	8
	1	1	1	0	10	14	25	21	10	8	6	1	2	100

Length—Mean, 96.280 ± .552μ Breadth—Mean, 29.080 ± .212μ
St. Dev., 8.160 ± .388μ St. Dev. 3.320 ± .168μ
Coef. Var., 7.678 ± .368 Coef. Var. 12.100 ± .585

Mean Index, or Ratio of Breadth to Length, 27.428 per cent.; Coef. of Cor., .3768 ± .0579.

that the progeny of these ten correspond to the larger set (*A*) of the original culture, though with slight differences in the means and in the amount of variation.

3. A single smaller individual, *c*, was selected from the original culture. As near as could be measured when alive, this individual

TABLE III.

Correlation Table for Length and Breadth of a Random Sample from Descendants of 10 of the Larger Individuals from Culture 1, allowed to multiply for 12 Days. (See polygons B, Diagrams 1 and 2.)

Breadth in Microns.	Length in Microns.																			
	144	148	152	156	160	164	168	172	176	180	184	188	192	196	200	204	208	212	
48		1		1	1			1											4
52			1	3	1	1	1	1	3		1	1	1	1				1	16
56	1		1	1	4		1	1			1	1	3		1				15
60					2	1	1	2		2	1	8	1	2	2	1			23
64								2	1	1	1	1	2	1	1		1		11
68							1			1	2		3	3	1	1	2	1	15
72									1	2	2	1	2	2		1		2	13
76														1	1				2
80										1									1
	1	1	2	5	8	2	4	7	5	7	8	12	12	10	6	3	3	4	100

Length—Mean, 182.760 ± 1.096μ Breadth—Mean, 61.360 ± .496μ
St. Dev., 16.264 ± .776μ St. Dev., 7.376 ± .332μ
Coef. Var., 8.899 ± .428 Coef. Var., 11.912 ± .576

Mean Index or Ratio of Breadth to Length, 33.652 per cent.; Coef. Cor., .5288 ± .0486.

was 120 microns in length. It was allowed to propagate in a culture free from all other *Paramecia,* from April 9 to June 11 (thus a little more than two months). Now a random sample gave the polygons shown at *c,* Diagrams 1 and 2; the measurements are given in Table IV. This group corresponds very closely to the smaller group *a* of

TABLE IV.

Correlation Table for Length and Breadth of a Random Sample Descended from the single small Individual c, taken from Culture 1 and allowed to Multiply 63 Days. (See Polygons *c,* Diagrams 1 and 2.)

Breadth in Microns. \ Length in Microns.	104	108	112	116	120	124	128	132	136	140	144	148	152	156	
28	1			2	1	1									5
32				2	6	5	5	3	3						24
36	1				3	8	8	7	5	1	4	1		1	39
40				1	1	2	5	3	3	4	3	1			23
44						1		1	2	2	2	1			9
	2	0	0	5	11	17	18	14	13	7	9	3	0	1	100

Length—Mean, 130.120 ± .628μ Breadth—Mean, 36.280 ± .260μ
St. Dev., 9.284 ± .443μ St. Dev., 3.880 ± .184μ
Coef. Var., 7.134 ± .342 Coef. Var., 10.700 ± .516

Mean Index or Ratio of Breadth to Length, 27.913 per cent.; Coef. Cor., .5208 ± .0492.

the original culture, though with slight differences in breadth.

4. A single very large specimen, *D,* approximately 250 microns in length, was isolated from the original culture on April 12 and allowed to propagate freely till June 11 (two months): 100 specimens taken at random then gave the measurements shown in the polygon *D,* Diagrams 1 and 2, and Table V.

Examination of the polygons and tables shows that the two forms retain their essential characteristics when isolated and propagated. The results shown in the diagrams are typical of many others. I have kept distinct strains of each of these groups for periods (at the present time) of more than eighteen months, and measurements made at frequent intervals during that time show that they have always remained quite distinct.

Thus it is clear that these colorless *Paramecia* fall into two distinct groups, which are at least relatively permanent. As is well known, two species of colorless *Paramecia* have long been distin-

TABLE V.

Correlation Table for Length and Breadth of a Random Sample Descended from the Single Large Individual D, taken from Culture 1, and allowed to Multiply 60 Days. (See polygons D, Diagrams 1 and 2.)

Length in Microns.

Breadth in Microns.	128	132	136	140	144	148	152	156	160	164	168	172	176	180	184	188	192	196	200	204	208	212	216	220	224	228	
28	1																										1
32													1														1
36											1		2		1	2											6
40											2			3	4	3		1									13
44												1			3	4	2	1	1			1					13
48							1	1					4	1	3	5	1	2	1	1		2	1				23
52										1	1		3		2	2	3	2		2	1					1	18
56									1			1		2			2	2	1	1		2		1			13
60															1	2	1						1				5
64													1						1	1	1	1					5
68																				1							1
72																											0
76																		1									1
	1	0	0	0	0	0	1	1	1	1	4	2	11	6	14	18	9	9	4	6	2	6	2	1	0	1	100

Length—Mean, 188.360 ± .980μ Breadth—Mean, 49.000 ± .548μ
St. Dev., 14.532 ± .692μ St. Dev., 8.144 ± .388μ
Coef. Var., 7.715 ± .370 Coef. Var., 16.618 ± .814

Mean Index, 26.029 per cent.; Coef. Cor., .4188 ± .0556.

guished under the names *Paramecium aurelia* Müller and *Paramecium caudatum* Ehr. The two groups we have found correspond to the descriptions heretofore given of the two species, the smaller set representing *Paramecium aurelia,* the larger *Paramecium caudatum.* Besides the differences in size certain other characteristics have been held to distinguish the two species, and these distinguishing characteristics are evident in our two groups. *Paramecium aurelia* is described as having two micronuclei and *P. caudatum* but one; this is true for our larger and smaller groups respectively. *Paramecium aurelia* is said to be more rounded behind, while *P. caudatum* is pointed. In spite of many variations in form within each group, it is clear that our smaller group corresponds in this respect also with *P. aurelia,* the larger one with *P. caudatum.*

Calkins (1906) has brought forward evidence tending to show that the supposed distinction into permanently differentiated forms is not well based, so that there are not two species, the different sizes being merely variants of one. Calkins based his doubts as to the

really specific distinctness of *P. aurelia* and *P. caudatum* on the fact that in one of his pedigree cultures of *P. caudatum* the number of micronuclei changed from one to two, remained at two for many generations, and finally changed back again to one.

The results here published tend to indicate that the distinction into two groups is not without some sort of foundation. But it will be best to reserve the discussion of species until we have more data at hand. We may temporarily speak of the smaller set as the *aurelia* group, the larger one as the *caudatum* group. In a later part of the paper the question of distinguishing species will be taken up in detail, in the light of full data.

6. Are Differences in Size Hereditary within Each of the Two Groups?

We have found that among the variations of *Paramecium* in size are two groups, limited by internal causes, so that even under the same external conditions they differ in size; these two groups have heretofore been considered two species. But within each of these groups we find likewise many variations in size, so distributed, however, as to produce a curve with a single apex (Diagrams 1 and 2, etc.). These variations are at times very considerable, as will be evident from an examination of the polygons shown in Diagrams 3 and 6 (pages 413, 470), or the tables numbered VII. (page 412) and XX. (page 466). The next question to be considered is: Are the differences in size within such a group hereditary? That is, do the differences in size depend upon internal conditions, of such a character that the differences will persist in the progeny, even when the external conditions remain the same?

The experimental answer to this question is to be obtained by isolating individuals of different size belonging to one of the two groups (either "*aurelia*" or "*caudatum*"), allowing these to multiply and determining whether the progeny show differences in size corresponding to those in the parents. Can we by selection and propagation produce within the limits of a single group races of different mean size?

Experiments designed to answer this question were undertaken in the following way. As representing the *caudatum* group I

selected the cultures descended from the individual *D*; while the progeny of *c* represented the *aurelia* group. Now, from each of these groups the largest and smallest individuals were isolated and allowed to multiply, under uniform conditions. Thus, the selected large and small individuals of a given group were all *progeny of a single individual,* forming thus a "pure line"; this fact is of great importance, as the sequel will show.

A large number of experiments gave throughout negative results. The progeny of large and of small individuals (within a given pure line) *showed no characteristic differences in size.* Large specimens of the *caudatum* form produced progeny on the whole no larger than those produced by small specimens of the same form, and the same was true in the *aurelia* group. In many experiments a single large and a single small specimen were isolated, and their progeny compared; in other cases a number of large specimens were placed together in one vessel, a number of small ones in another, and their progeny compared after lapse of a considerable period. Since the results of these experiments were throughout negative, I will give the details of but a single illustrative experiment:

On July 27 ten large and ten small specimens were selected from a lot of the *caudatum* group, all being descendants of a single individual *D*. The ten large specimens measured, as nearly as could be determined while alive, approximately 250 microns each, and were thick in proportion to the length. The ten small specimens were about 150 microns long, and were thin. The two sets were placed in equal quantities of the same culture fluid.

At the end of three days the large set had produced many individuals. Fifty of these taken at random gave a mean length of 189.040 microns, a mean breadth of 60.560 microns.

The smaller individuals did not increase rapidly and five of them died before dividing, so that all the progeny came from six individuals. The six increased in size before dividing. At the end of three days there were twenty-one individuals. The mean length was 205.140 microns, the mean breadth 56.570 microns.

Thus the smaller specimens had produced progeny that were a little longer, but not quite so broad, as those resulting from the larger set. The existing differences are clearly without significance.

In other cases there was more variation in size among the different sets of progeny of *D,* particularly if the measurements were made after but few fissions had occurred. But sometimes the progeny of the large specimens were smaller, sometimes larger, than those of the small specimens. On the whole, both large and small specimens produced progeny of about the mean size for the group, under the given conditions.

Thus it is apparent that the differences in size shown within such a polygon as *D,* Diagram 1, are not due mainly to hereditary internal factors. Before we can determine with certainty whether any such factors are involved, we must make an analysis of the variation polygon, determining so far as possible the different factors, external and internal, which go to make it up.

7. Proposed Analysis of the Polygons of Variation.

Our present task is then to determine, so far as possible, what factors produce such polygons of variation as are shown in Diagram 1; to define what the individuals of different sizes and proportions really are, and to what their particular characteristics are due.

There are several sets of problems to be considered; these we may classify as follows:

1. What are the causes and the significance of the variations shown in a single variation polygon, such as *D,* Diagram 1? Why, in a group of *Paramecia* grown under the same conditions, and perhaps all descended from the same ancestor, do certain indviduals show the mean length, while others are larger and others smaller? Each size must have its determining factors.

2. In different polygons from *Paramecia* of the same general group and even when all are progeny of the same individual, the *mean* size differs much. Thus, in Diagram 6 (page 470) the mean length for polygon 8 is 146.108 microns; for polygon 11 it is 191.360 microns, though both represent descendants of the individual *D,* of the *caudatum* group. What are the causes of such variations in mean size among different sets of individuals?

3. In different sets of individuals belonging to the same general group, or descended from the same individual, the amount and range

of variation differs much. This is readily evident to the eye on comparing the polygon 8 of Diagram 6 and its correlation table, XIX. (page 466), with polygon 9 (Diagram 6) and its table, XX. In the former the length ranges only from 120 to 176 microns, and the coefficient of variation is 7.003, while in the latter the range of length is from 120 to 220, and the coefficient of variation is 12.767. What is the cause of these great differences in the variation of different groups?

4. In different sets belonging to the same general group the *correlation* between length and breadth differs greatly. Thus, in Table XX. (page 466) the correlation is high and positive, a difference in one dimension being accompanied, with much regularity, by a corresponding difference in the other. In Table XXXI. (appendix), on the other hand, there is almost no correlation, while in Tables XXIX. and XXXII. the correlation is marked, but negative —an increase in length being associated with a decrease in breadth, and vice versa. What are the causes and significance of these differences in correlation found in different sets?

In dealing with these questions, there are three main sets of possible factors to be examined, as follows:

1. *Hereditary Factors.*—Some of the factors concerned may be internal and largely independent of the environment—so that the differences in size are hereditary. The existence and nature of such factors form our main problem, but they can be dealt with only after the other factors are investigated.

2. *Growth.*—Some of the variations in size, and in proportions, may be due to different stages of growth, so that this matter must be carefully examined.

3. *Environmental Influences.*—It appears probable that the differences in the means, the differences in the range and amount of variation, and in the correlation, may depend partly on the nature of the environment.

We shall take up in detail these three sets of factors, beginning with growth.

III. GROWTH IN PARAMECIUM.

One significant fact was noted in the breeding experiments described in a previous section. Whenever a large and small specimen (belonging to a given group) were isolated at the same time, *the large specimen as a rule divided first.* Often at the end of forty-eight hours the large specimen had produced eight or sixteen progeny, while the small specimen had either not divided at all, or had produced but a single pair.

This suggests that the differences in size may be largely matters of growth; that the small specimens may be young ones, and that the variations shown in the frequency polygons may be largely growth differences. It is clear that a study of growth in *Paramecium* is imperative before intelligent work can be done with variation. The subject of growth in the Protozoa is an interesting one in itself, so that this study will be made as thorough as possible for its own sake, as well as for the light it throws on variation.

Growth was studied by three different methods: (1) By observation of abnormal specimens bearing localized appendages, noting the changes in position during growth; (2) by following the changes of form and size in living specimens; (3) by a statistical examination of the dimensions of individuals of known age.

The observations on growth in abnormal specimens have been described in my first communication (Jennings, 1908). By observations on the living specimen it is not possible to obtain precise measurements. It will be best therefore to begin our account with the statistical examination, taking up the observations on the living specimens by way of control.

Effects of Growth on a Variation Polygon.

If our suspicion that growth differences make up an important part of the observed variations in size of *Paramecium* is justified, then cultures rapidly multiplying and growing should be more variable than those that are stationary. To test whether this is true, two lots were removed from a rather old culture of descendants of *D,* in which inspection showed that the individuals were not multiplying rapidly. One of these lots was killed at once, while the other

was placed in fresh culture fluid. Twenty-four hours later this second set was found to be multiplying rapidly; a portion of it was then killed. The measurements of the two lots are given in Tables VI. and VII., while the facts are graphically represented in the

TABLE VI.

Correlation Table for Lengths and Breadths of a Random Sample from a Culture of Descendants of D, in which Multiplication was not in Progress. For comparison with Table VII. (See also Diagram 3.) (Row 3, Table XVIII.)

Breadth in Microns.	Length in Microns.																	
	148	152	156	160	164	168	172	176	180	184	188	192	196	200	204	208	212	
32	1				2		1	1	3	1								9
36				2		6	5	1	2	2	2							20
40		1		1		3	8	4	4	4	3	1	3	3				35
44				1		1	2	2	2	3	3	4	1	3				22
48							1	1	3	2	1	3	4	3	1	4	2	25
52					1			1	1			2	1		2	3	4	15
56										1				2		1		4
60						1						1	1		2			5
	1	1	0	4	3	11	17	10	15	13	9	11	10	11	5	8	6	135

Length—Mean, 185.008 ± .836μ Breadth—Mean, 43.556 ± .392μ
St. Dev., 14.420 ± .592μ St. Dev., 6.748 ± .276μ
Coef. Var., 7.794 ± .324 Coef. Var., 15.490 ± .651

Mean Index, 23.517 per cent.; Coef. Cor., .5955 ± .0375.

TABLE VII.

Correlation Table for Lengths and Breadths of a Random Sample of Descendants of D, at a Time when Rapid Multiplication was in Progress. For comparison with Table VI. (See also Diagram 3.) (Row 4, Table XVIII.)

Breadth in Microns.	Length in Microns.																									
	104	108	112	116	120	124	128	132	136	140	144	148	152	156	160	164	168	172	176	180	184	188	192	196	200	204	208	212	216	220	
32													2																		2
36										1	1	1	4	1	4	1	1	2		1											17
40								1		1	1	1	2	2	2		3	3	5	2	3	1	1	1							29
44	1					1	1	1	1	1	1	1	1	4	2			3	1	5	6	2	6	1	2						41
48			2	1						1	1	2				1	1	1		2	4	5	4	4	4	3	1			37
52			1	1				1				1						1	2	1	5	4	1	5	5	2				31
56	1																	1	2	3	1	3	2	3	2	4	1			23
60														1			1			1	2					2	3	1	1	12
64																	1		1											2
68																														0
72																					1									1
	2	0	3	2	0	1	1	3	1	4	4	6	9	8	8	2	7	11	11	15	22	15	14	14	13	11	5	1	1	1	19

Length—Mean, 176.124 ± 1.128μ Breadth—Mean, 47.364 ± .344μ
St. Dev., 23.360 ± .797μ St. Dev., 7.132 ± .244μ
Coef. Var., 13.262 ± .461, Coef. Var., 15.057 ± .526

Mean Index, 27.153 per cent.; Coef. Cor., .3945 ± .0408.

polygons of Diagram 3. It is evident that the variability has become much greater in the rapidly growing culture. The range of variation of length in the stationary culture is from 148 to 212 microns; in the growing culture it is from 104 to 220 microns, so that in the latter the range has almost doubled in extent. The coefficient of variation in length has likewise almost doubled, changing from 7.794 when the culture was stationary to 13.262 when it was growing. For breadth the range of variability has likewise increased considerably, though the coefficient of variability shows little change. The correlation between length and breadth has become considerably less in the rapidly multiplying culture, decreasing from .5955 to .3945. The mean length has slightly decreased, the mean breadth slightly increased, in the growing culture.

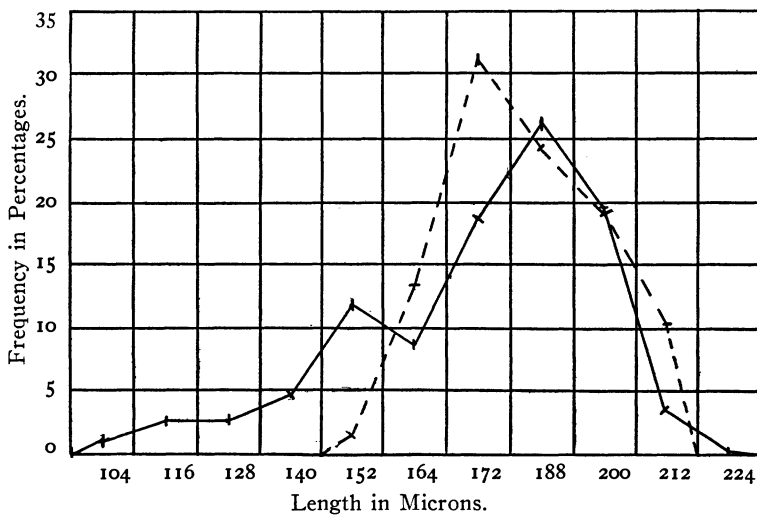

DIAGRAM 3. Polygons of variation in length for (*a*) a culture of descendants of *D* that is rapidly multiplying and (*b*) one that is not. The continuous line represents the rapidly multiplying culture of Table VII.; the broken line the stationary culture of Table VI.

From this example it is clear that growth and multiplication may, and probably do, play a large part in determining the character and distribution of the variations, as well as in determining the mean dimensions and their correlations. We shall now attempt to deter-

mine as accurately as possible what this part is by a systematic study of growth.

Material and Methods of Work.

In order to exclude possible differences due to different ancestry, the study of growth was made with the progeny of a single individual for each of the two groups. Of the *caudatum* group a single individual *D* was isolated April 12. This individual was a large one, measuring approximately 250 microns in length. From it many cultures were made under various conditions, and all the results on growth in this group were reached with progeny of this individual *D,* save in cases where the contrary is expressly stated. In the same way the results for the *aurelia* group were reached with the progeny of a single individual *c,* unless otherwise noted.

The method of work in the statistical study of growth was as follows: Numbers of dividing *Paramecia* of known descent were isolated and kept for varying periods, so that the age of the individuals was known to within a few minutes or even less. The individuals were then killed at different ages by the use of Worcester's fluid, and measured. In this way the usual size at various ages was determined, and those variations in size that are due only to varying age of the individuals were excluded. By pursuing this method, an approximate curve of growth is obtained and the part played by growth in the observed variations elucidated; much light is in this way cast on many obscure matters.

To persons who have worked with *Paramecium* it is unnecessary to point out the extremely laborious and time-consuming character of the operations required. Dividing specimens must be sought for with the microscope, among hundreds of their rapidly moving fellows; they must be taken up with the capillary tube, isolated, placed in culture fluid, and the time of capture noted. They must then, after lapse of the proper interval, be killed and measured; this is the smallest part of the work. To thus deal with individuals of known age by the hundred involves an incredible amount of exhausting labor, so that if the mathematical student finds in any stage the numbers employed not always as large as would be ideally desirable, he will realize that there is good reason for this. But it is hoped that the numbers used are amply sufficient, on the whole, for the purposes designed; the results are drawn from the measurement of over 1,500 specimens of known age; together with control cultures of mixed ages in still larger number.

Especially in the study of individuals that are very young (up to the age of half an hour or so), there is very great difficulty in dealing with large numbers owing to the fact that the time required for picking them out is very large in proportion to the amount of time they are to be kept, so that but few can be dealt with at once. Another great difficulty lies in the fact that to be strictly comparable, the sets of different ages must be chosen *on the same day from the same culture;* otherwise differences due to cultural conditions show themselves, confusing our results. No culture remains the same for two successive days, and the differences quickly show

themselves in the statistical results. The condition just mentioned cannot be absolutely fulfilled, but much effort was directed toward filling it as completely as possible, and where it could not be fulfilled, strict account of that fact was taken.

The fixing and measurement of the specimens was done by the methods already described (p. 396).

1. Description of Different Stages of Growth.

First Stage: the Young Before Separation is Complete.

In the earliest stage recognizable, the young *Paramecium* forms half of a dividing specimen. Before the constriction appears the macronucleus has become band-like, and the mother infusorian is shorter and thicker than the specimens not preparing to divide (see Fig. 2, *a*). The oral groove and other differentiated parts have become less marked. At the first appearance of the constriction the anterior and posterior halves still retain something of their characteristic form, and the body of the mother has extended a little (Fig. 2, *b*). The constriction does not pass squarely across the body, but is a little oblique, being farther back on the oral side (Fig. 2, *c, d, e*). As a result, when the two halves are measured separately, they will seem to differ in length, according to the place where the measurement is taken. Thus, if *d,* Fig. 2, is measured from the ends to the constriction along the oral side, the anterior half measures 96 microns, the posterior half 84 microns, while if the measurements are taken along the aboral side these proportions are exactly reversed. Measurements taken from one of the lateral sides give the same length for the two halves. The *Paramecia* may lie in various positions and this obliqueness of the constricting groove is not always evident. Misled by this fact, I took great pains to measure the precise length of each half in a large number of cases, finding considerable differences, though without any marked preponderance of either half. But I am now convinced that in early stages of fission the most accurate measurements of the young are to be obtained by considering each to be one half the length of the two together.

The breadth of the two halves frequently differs a little, the posterior half being at times slightly broader than the anterior half.

As the constriction deepens, the two halves lengthen (Fig. 2, *b* to *f*; *g* to *l,* etc.). This lengthening progresses with the advancing

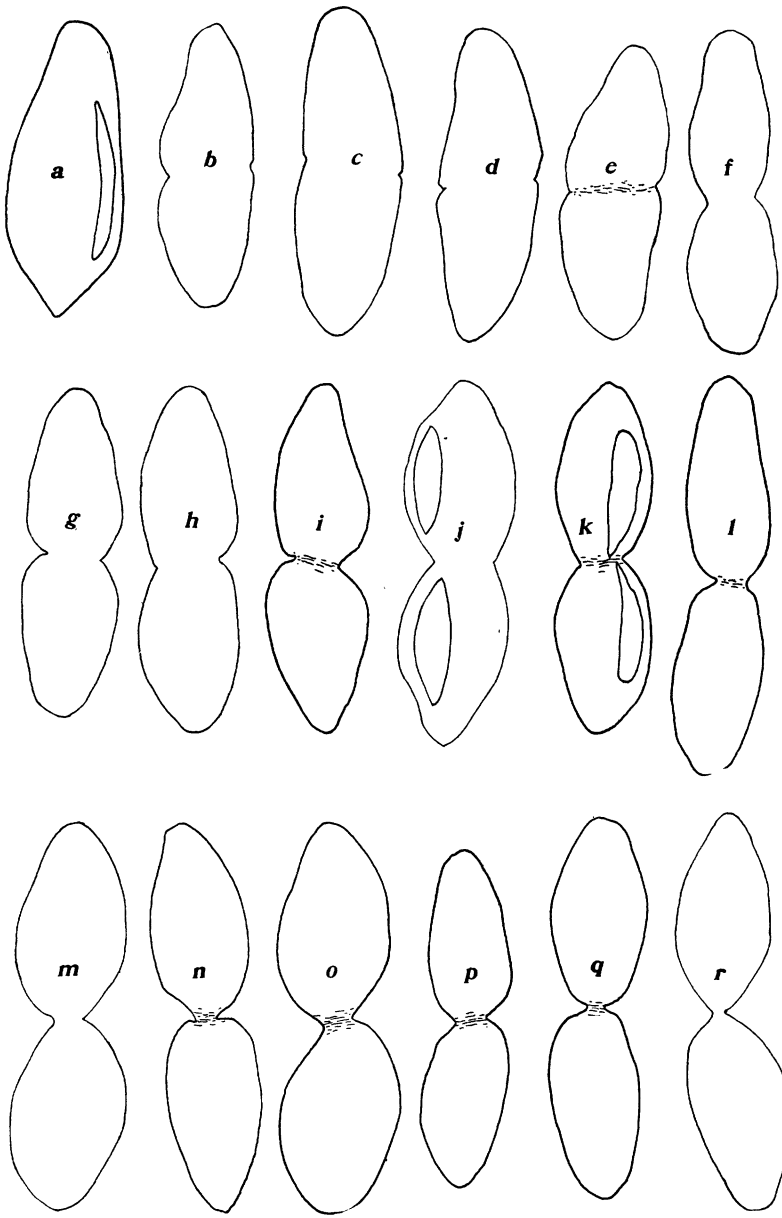

FIG. 2. Dividing specimens of the *caudatum* form, descended from the individual *D*. Note the increase in length and decrease in breadth as the constriction deepens. Anterior ends above. All × 235.

constriction until the two halves separate. This lengthening is clearly evident in the figures and in the correlation table giving depth of constriction with length of body (Table XI., page 441). As Table XI. shows, there is a period at the beginning, before the constriction reaches a depth of about 10 microns, when there is little relation between the length of the body and depth of constriction, showing that in this period the halves have not yet begun to lengthen. We may therefore take the length of the young at this period as that characteristic for the young individuals in their earliest recognizable condition, before growth has begun. By dealing with these alone we are able to compare the variability of the young with that of the adults, or with random samples including all ages. In the further treatment, therefore, the measurements of the unseparated young are divided into two classes: (*a*) those before lengthening has begun; (*b*) those after lengthening has begun.

(*a*) *The Unseparated Halves before Lengthening Has Begun.*—Studies were made of the young of three lots of the *caudatum* group (descendants of the individual *D*), and of two lots of the *aurelia* group (descendants of the individual *c*). Each "lot" included individuals taken on the same day from the same small culture. In most of the lots there were examined: (1) The unseparated young before growth had begun; (2) the unseparated young after growth had begun; (3) a random sample, including all sorts of individuals found in the culture. The results of these measurements are given in Table VIII., page 418.

(1) The *caudatum* Form (Descendants of *D*).—The most thorough study was made of lot 1, of the *caudatum* group; the results there reached are typical, and perhaps more reliable than any others, owing to the large numbers examined. We shall therefore make the results on this lot the basis of our discussion, afterward bringing out points of difference and resemblance shown in the other lots.

From this lot 1, I measured 313 dividing specimens, which, of course, included 626 unseparated young; a random sample of 200 individuals not dividing was likewise measured. A correlation table for the 313 dividing specimens, giving *the depth of the constriction* below the general body surface and the length is given on page 441

TABLE VIII.

Mean Dimensions and Constants of Variation for Youngest Stages, in Com-

is for convenience of reference in the text. The column headed which fuller data are given on the lot in question. A "Lot" consists of Table X., page 428.

Row.	A. *Progeny of D (Caudatum Form).*	Number of Individuals.	Table.	Length.		
				Mean.	Standard Deviation.	Coefficient of Variation.
1	Lot 1. Young halves, where depth of constriction is 4μ or less	262	9	87.848±.278	4.716±.197	5.368±.224
2	Lot 1. Halves, where depth of constriction is more than 4μ.....	364	(62)	93.033±.355	7.104±.251	7.636±.271
3	Lot 1. Random sample.....	200	14	199.960±.740	15.528±.524	7.765±.263
4	Lot 2. Halves, where depth of constriction is less than ¼ breadth.....	80	(43)	82.600±.468	4.394±.332	5.320±.402
5	Lot 2. *All* halves of dividing specimens.....	124	(42)	85.774±.593	6.924±.420	8.072±.492
6	Random sample.....	200	30	184.100±.776	16.264±.548	8.834±.300
7	Lot 3. Halves, depth of constriction less than ¼ breadth.....	84	(44)	83.810±.498	4.782±.352	5.706±.421
8	Lot 3. Adults 24 hours old.	300	41	168.532±.419	10.768±.296	6.389±.175
	B. *Progeny of c (aurelia form).*					
9	Lot 4. Halves, where depth of constriction is less than ¼ breadth.....	132	(47)	51.868±.325	3.912±.190	7.541±.445
10	Lot 4. Halves, lengthening begun (constriction more than ¼ breadth).....	106	(63)	60.692±.527	5.684±.372	9.365±.613
11	Lot 4. Random sample.....	225	49	114.163±.784	17.443±.555	15.279±.497
12	Lot 5. Halves, where constriction is less than ¼ breadth.....	76	48	56.666±.425	3.889±.302	6.862±.533
13	Lot 5. Random sample.....	100	50	114.033±.820	12.140±.580	10.646±.513
14	Lots 4 and 5. All halves where constriction is less than ¼ breadth (combination of rows 9 and 12).....	208	—	53.622±.300	4.535±.212	8.459±.398

(Table XI.). In 131 of these specimens the constriction had sunk less than one unit of the micrometer (4 microns) below the surface, while in the other 182 the depth of the constriction was greater. We may take the 131 specimens in which constriction had barely begun

TABLE VIII.—*Continued.*

parison with Random Samples and Adults. (The column headed "Row" "Table" gives the number of a table found elsewhere in the paper, in specimens all taken from the same culture on the same day.) Compare

Breadth.			Ratio of Breadth to Length, or Mean Index Per Cent.	Coefficient of Correlation.
Mean.	Standard Deviation.	Coefficient of Variation.		
55.480±.297	5.040±.210	9.082±.382	63.136	.6546±.0337
49.540±.215	4.296±.152	8.671±.309	53.592	—.0938±.0496
50.220±.308	6.468±.218	12.877±.441	25.114	.6064±.0302
50.700±.364	3.532±.260	6.769±.513	61.530	.1048±.1055
50.388±.307	3.584±.217	7.112±.433	59.166	—.1136±.0840
46.020±.251	5.256±.177	11.421±.390	25.084	.4282±.0389
65.716±.706	6.784±.499	10.322±.768	78.563	.2215±.0999
40.320±.230	5.892±.162	14.615±.411	23.899	.5496±.0272
34.850±.287	3.453±.203	9.911±.587	67.246	.6502±.0479
34.590±.383	4.147±.273	11.989±.797	57.296	.3100±.0837
34.207±.241	5.363±.171	15.683±.511	30.177	.6757±.0244
45.263±.597	5.463±.423	12.071±.947	79.806	.6744±.0597
47.300±.437	6.490±.310	13.720±.667	41.455	.8152±.0226
38.653±.437	6.607±.310	17.089±.822	71.835	.7476±.0292

as types of the earliest stage of fission, and their 262 halves as young *Paramecia* in the earliest stage. The lengths and breadths of these 262 halves are given in Table IX. The constants derived from the measurements of these, as well as from the measurements of the 364

TABLE IX.

Correlation Table for Length and Breadth of 262 Unseparated Halves of Dividing Specimens, in which the Depth of Constriction was less than four microns. All descendants of the single individual *D*, and taken from the same culture on the same day.

Length in Microns.

Breadth in Microns.	78	80	82	84	86	88	90	92	94	96	98	100	102	
44		2		2										4
48	2	6	12	6	8	2		2						38
52		2	4	16	12	4	12	2	2					54
56		6	4	8	20	14	30	4	4	2	2			94
60			4	2	2	10	16	2	2	4	2			44
64					2		4	6	2	4	4			22
68										2		2		4
72													2	2
	2	16	24	34	44	30	62	16	10	12	8	2	2	262

Length—Mean, $87.848 \pm 278\mu$ Breadth—Mean, $55.480 \pm .297\mu$

St. Dev., $4.716 \pm .197\mu$ St. Dev., $5.040 \pm .210\mu$

Coef. Var., $5.368 \pm .224$ Coef. Var., $9.082 \pm .382$

Mean Index, 63.136 per cent.; Coef. Cor., $.6546 \pm .0337$.

halves in which lengthening had begun, and of the random sample, are given in the first three rows of Table VIII.[3]

We will for the present limit the discussion to the relations shown by comparing the youngest stages (row 1) with the random sample (row 3) which consists mainly of adults. The following important facts are shown:

1. The mean length of the youngest stages of the new individuals is considerably *less* than one half of the mean length of the individuals that are not dividing. The mean length of the young is 87.848 microns, while that of the individuals not dividing is 199.960 microns, or 24.264 microns more than twice the mean length of the young individuals. This remarkable relation will be taken up later, in discussing the measurements of dividing specimens (page 443).

2. The mean breadth of the youngest stages is slightly greater than that of adults not dividing—55.480 microns, in place of 50,220 microns.

[3] In Tables VIII. and IX. the measurements were made and the constants were first computed, for the entire dividing specimens. The constants for the halves were of course readily obtained from these; they are the same, save that the mean and standard deviation for length are halved, and the mean index is doubled. The computation of the probable errors was based on the *number of dividing specimens,* not on the number of halves.

3. The mean index, or ratio of breadth to length, is considerably more than twice as great in the young as in the adults; in the former it is 63.136 per cent.; in the latter 25.114 per cent.

4. The variability in length is less in the earliest stages of the young than in the individuals that are not dividing. In the former the coefficient that measures the variability is but 5.368, while in the latter it is 7.765.

5. The variability in breadth is likewise much less in the youngest stages—the coefficient being 9.082 in place of 12.877.

6. The correlation between length and breadth is nearly the same in the youngest stage as in the random sample, being .6546 in the former, .6064 in the latter.

From the other lots smaller numbers were examined. These gave on the whole similar results, though with certain significant differences. The facts are as follows:

From lot 2 (descendants of *D*), 124 halves were obtained. On account of the small number, I threw together all in which the depth of the constriction was less than one fourth the breadth, and considered these the earliest stage (the depth of constriction and length are given for the entire dividing specimens in Table XLII., appendix). There were thus obtained eighty young individuals (dimensions for the entire dividing specimens in Table XLIII., appendix). It is evident that this lot includes individuals varying more in age and growth than in lot 1, since in lot 2 we have included those having a much greater depth of constriction. The results are shown, in comparison with a random sample of the same lot, in rows 4 and 6 of Table VIII. The facts are in the main parallel with those for lot 1. As compared with the random sample, the mean length of the young is less than one half, the mean breadth a little greater, the mean ratio of breadth to length more than double, the coefficients of variation for length and breadth much less. A striking difference between this set and the young of lot 1 is that in the present case the correlation between length and breadth has decreased to such an extent that the coefficient computed (.1048) is without significance, being less than its probable error (.1055). This is due, as we shall clearly see later, to the fact that we have included in the

young of row 4 individuals older (constriction deeper) than in those of row 1.

From a third lot of descendants of *D*, 154 halves were obtained; in 84 of these the constriction was less than one fourth the breadth. Unfortunately no random sample of this culture was preserved. But 300 individuals just twenty-four hours old were taken from it for other purposes, and the young halves may be compared with these (rows 7 and 8, Table VIII.).[4] It should be noted, however, that the adults of row 8 had been kept for twenty-four hours in a rather small quantity of water, where food was relatively scarce, so that they were smaller than would have been the case if they had lived throughout under the same conditions as the dividing specimens.

In general, the same relations are shown here as in the other lots. A striking peculiarity is the great breadth of the young halves (65.716 microns), as compared with that of the adults (40.320 microns), so that the ratio of breadth to length (the "mean index") is more than three times as great in the young as in the adults (78.563 per cent. in the former, 23.899 per cent. in the latter). Owing to the inclusion of older halves, in which lengthening has begun, the correlation between length and breadth is again low ($.2215 \pm .0999$).

(2) The *aurelia* Form (Descendants of *c*).—Two lots of dividing specimens of the *aurelia* form were examined, the first including 132 halves in which lengthening had hardly begun, the second 76. The constants for these, in comparison with random samples of those not dividing, are given in rows 9 to 14 of Table VIII. These show the same relations that we have already seen in the *caudatum* group, with one exception. In the smaller collection (lot 5), the mean breadth of the halves was a little *less,* instead of greater, than that of the random sample. In this culture the animals were extraordinarily broad, the mean ratio of breadth to length in the random sample being 41.455 per cent., in place of the usual ratio of about 30 per cent. This was due to the fact that these animals had been placed twenty-four hours before in a rich nutrient solution and had

[4] The dimensions of the entire dividing specimens of which row 7 are the halves are given in Table XLIV. of the Appendix; the dimensions of the 300 just twenty-four hours old are given in Table XLI.

become very plump. The point of interest is that the breadth of the young individuals in the earliest stages tends toward a constant dimension, becoming greater when the adults are thin, less when the adults are plump. Outlines of dividing specimens, and of those not dividing, from this culture, are shown in Fig. 3, *a* to *f*; the great difference in breadth is noticeable.

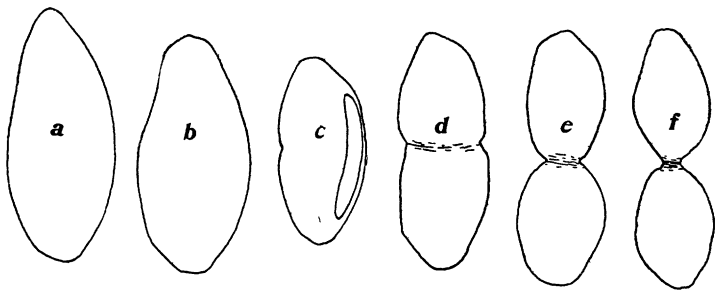

FIG. 3. Outlines of specimens of the *aurelia* form (descendants of *c*), from Lot 5, Table VIII. *c* to *f*, Successive stages of fission. Note the greater breadth of the specimens not dividing (*a* and *b*). Same magnification as Fig. 2. (235 diameters.)

In row 14, Table VIII., are given the constants for all the young halves examined of the *aurelia* group; that is, for the sum of rows 9 and 12. The coefficients of variation are, as might be expected, increased by adding these two dissimilar groups. The fact that the correlation between length and breadth is likewise increased, as compared with what we find in either group taken alone, might not, perhaps, be anticipated. These changes in variation and correlation are environmental effects, to be studied later.

(*b*) *The Unseparated Halves after Lengthening Has Begun.*— As we have already seen, the length of the halves increases as the constriction deepens (see the correlation tables for length with depth of constriction, Nos. XI. (page 441), XLV., XLVI.; compare also the outlines of dividing specimens, Figs. 2 and 3). The coefficient of correlation between depth of constriction and length is, for the 626 halves of Table XI., .6882; with each increase of 10 microns in depth of constriction the length increases 4.30 microns. If we include only the individuals in which lengthening has clearly begun (thus omitting the uppermost row of Table XI.), we find that for

these 364 halves the correlation between depth of constriction and length is greater, amounting to .7818; while the increase in length with each 10 microns of increase in depth of the constriction is 5.598 microns.

While the length thus increases, the breadth decreases. This is evident on inspection of Table XII. The correlation between depth of constriction and breadth of body is therefore negative; its coefficient, in the case of Table XII., is —.5232. With each increase of 10 microns in the depth of constriction the breadth of body decreases 2.630 microns. If again we take into consideration only the 364 halves in which lengthening has decidedly begun, omitting thus the uppermost row of Table XII., we find that the correlation decreases to —.3316, and the decrease in breadth for an increase of 10 microns in depth of constriction is but 1.252 microns. This appears to indicate that a large part of the decrease in breadth occurs in the first stages of constriction.

If we compare with the means of the 262 halves in which lengthening has not begun, the means of the 364 in which lengthening has begun (Table VIII., rows 1 and 2), we find that the length has increased from 87.848 to 93.033 microns, while the breadth has decreased from 55.480 to 49.540 microns. If we examine the means at successively older stages, we find, of course, greater differences. Thus, when the constriction has reached a depth of 36 microns, the 10 specimens in that stage show the mean length increased to 101.200 microns, while the mean breadth is but 46.400 microns. Similar relations are to be observed if we compare the means of the younger and older sets of each lot shown in Table VIII.

Since, while the length is increasing, the breadth is decreasing, the growth tends to decrease the correlation between length and breadth or even to make it negative. Thus, while in the stage before lengthening has begun (row 1, Table VIII.) the correlation is .6546, in the 364 specimens of the same lot, *after* lengthening has begun the correlation has decreased to —.0938 (row 2, Table VIII.). In a second lot, containing 124 halves, when we throw all the halves together the coefficient of correlation between length and breadth becomes —.1136 (row 5, Table VIII.). In the *aurelia* form, 106 halves after lengthening has begun give a positive correlation between

length and breadth of .3100 (row 10, Table VIII.). Why there should sometimes be a slight positive correlation, sometimes a negative one, at this stage, will be discussed in the section where we deal with the various factors determining correlation.

A variation polygon for the youngest stage of lot 1 of Table VIII. is shown in Diagram 4, p. 440, at *a*.

The changes above set forth from statistical data were in a number of cases observed in living individuals. These observations give a number of additional points of importance, so that they will be described. The facts, as illustrated mainly by a typical specimen of the *aurelia* form, are as follows:

Some time before fission the body thickens and becomes shorter, taking the form shown at *a*, Fig. 2, or *c*, Fig. 3. The form and dimensions differ very noticeably from those of the specimens not preparing to divide. How long before the appearance of the constriction these preparatory changes in form begin it is not possible to say, because it is not possible to distinguish with certainty whether a given specimen is to divide or not until we can see the constriction, and this is at a relatively advanced stage of the process. At the time the constriction first appears the anterior and posterior halves still differ in form, though they are losing their characteristic features.

As the constriction deepens the two halves become longer (Fig. 2, *b* to *f*, Fig. 3, *c* to *d*). A specimen of the *aurelia* form (descendant of *c*) was at about the stage shown at *d*, Fig. 3, at 12.05; each half measured very nearly 80 microns in length.

Ten minutes later (at 12.15) the connecting portion had become smaller, while the two halves had lengthened, so that each measured about 85 microns in length. The anterior half was more pointed and slightly more slender than the posterior half (*f*, Fig. 3); this is regularly the case.

Six minutes later (at 12.21) the posterior half measured about 90 microns, the anterior half 94. The connecting band was now extremely slender.

Five minutes later (at 12.26) the two halves separated. The anterior half was still clearly distinguishable from the posterior one by its pointed, somewhat pear-like form. It measured 100×44

microns, while the posterior half was shorter, but thicker, measuring 96 × 52 microns. The succeeding changes of form will be described in the next section.

Thus from the condition shown at *d,* Fig. 3, to the completion of fission a period of twenty-one minutes elapsed. From the earliest appearance of the constriction the time till separation is usually a little more than one half hour.

Second Stage: the Young Immediately after Fission up to the Age of Ninety Minutes.

Observation of Living Specimens.—Immediately after separation of the two halves, growth occurs rapidly, and the shape changes, both halves becoming more pointed at both ends. In the specimens of the *aurelia* form under description at the close of the last section, the posterior half had two minutes after fission increased in size from 96 × 52 microns to 104 × 48 microns. Eight minutes after separation both halves measured 112 microns in length, so that they had during that period increased respectively 12 and 16 microns in length. The difference between anterior and posterior individuals was still marked.

Now followed a period of slower growth. At 12.53, twenty-seven minutes after division, each half measured approximately 120 microns in length. They had taken nearly the characteristic adult form and it was no longer possible to distinguish the anterior product from the posterior one.

At 2 P. M. (one hour and thirty-four minutes after separation) the length was about 135 microns and the progeny were similar to the adult specimens of the *aurelia* form.

Thus, at the time of separation the two individuals have somewhat more than half the adult length; they grow rapidly at first, then slowly, and in an hour and a half have reached nearly the adult size. (As later statistical studies show, growth continues for a long time still.)

Observation on the growth of living specimens of the *caudatum* form gave a parallel series of phenomena (see Fig. 4). Thus, in a descendant of *D,* the length of each half at the time of separation

was about 120 microns; width 48 microns. Five minutes later the length had increased to 132 microns, while the width was still 48 microns. Nine minutes later the length of the anterior product was 148 microns; that of the posterior product 144 microns. The width had decreased a little; it was now about 44 microns.

After thus increasing in fourteen minutes by nearly one fourth the original length, growth became less rapid. Forty minutes later (fifty-four minutes after separation) the length was about 156 microns. During two succeeding hours no increase in length could be detected. The form was that of the normal adult, though the adult size was not yet reached.

We may summarize as follows: Some time before fission (perhaps a half hour) the body shortens and thickens, so that each half is at first less than half the adult length. As the constriction deepens the two halves grow longer, till at the time of separation they are somewhat more than half the adult length. For five to twenty minutes after separation growth in length is very rapid, while the thickness remains stationary or decreases. Then follows a period of several hours of slower growth, till the adult size is reached.

This somewhat indefinite account, based on the observation of living specimens, will now be supplemented by a statistical investigation of a large number of individuals at various ages. The main results of this statistical investigation are brought together in Table X.

(*c*) *Age 0 to 5 Minutes* (*Table XXIX.*).—A large number of dividing specimens, all descendants of the individual *D* (*caudatum* form), were removed from a rapidly multiplying culture and kept for from 0 to 5 minutes in a watch-glass of culture fluid, then killed and measured. The method of work was to spend five minutes in picking out dividing specimens with the capillary tube and placing them in the watch-glass; at the end of the five minutes the lot was killed. Then other lots were prepared in the same way. In each lot killed, therefore, there occurred specimens that were in the early stages of fission; others that had separated at the moment of removal and were hence just five minutes old; and all stages intermediate between these two. All together, 62 unseparated pairs and 59 separated individuals were secured in this way. The latter set consists of individuals from 0 to 5 minutes old (reckoning from the moment

TABLE X.

Dimensions and Constants of Variation for Paramecia of Various Ages, in taken from the same culture on the same day. The lots where identical column headed "Row" is for convenience of reference. The column elsewhere, in which fuller data are given on the lot in question.)

Row.	A. *Progeny of D (Caudatum Form).*	Number of Individuals.	Table.	Length.			
				Mean in Microns.	Standard Deviation in Microns.	Coefficient of Variation.	Range of Variation in Microns.
1	Lot 1. Youngest unseparated halves, constriction beginning	262	9	87.848± .278	4.716± .197	5.368± .224	78–102
2	Lot 1. Halves, lengthening begun	364	(62)	93.033± .355	7.104± .251	7.636± .271	80–112
3	Lot 1. Random sample	200	14	199.960± .740	15.528± .524	7.765± .263	148–240
4	Lot 2. From beginning of constriction to 5 minutes after separation	183	—	92.940± .718	14.400± .508	15.494± .559	72–132
5	Lot 2. 0 to 5 minutes after separation	59	29	107.660±1.296	14.780± .916	13.729± .868	76–132
6	Lot 2. Random sample	200	30	184.100± .776	16.264± .548	8.834± .300	140–216
7	Lot 6. Age 0 to 19 minutes	24	31	128.000±1.908	13.856±1.348	10.825±1.066	108–152
8	Lot 6. Age 18 to 28 minutes	49	33	143.348± .624	6.480± .440	4.521± .309	132–160
9	Lot 6. Age 35 to 45 minutes	25	35	149.920±1.012	7.512± .716	5.010± .479	132–160
10	Lot 6. Age 75 to 90 minutes	42	36	161.524±1.004	9.648± .712	5.974± .441	140–180
11	Lot 6. Age 0 to 90 minutes (sum of rows 7–10)	140	—	147.544± .824	14.464± .584	9.803± .399	128–180
12	Lot 6. Random sample	100	51	184.680± .848	12.596± .600	6.821± .327	156–224
13	Lot 7. Age 0 to 19 minutes	39	32	134.256±1.663	15.394±1.176	11.468± .857	108–160
14	Lots 6 and 7. All 0 to 19 (sum of rows 7 and 13)	63	—	131.872±1.288	15.176± .912	11.507± .701	108–160
15	Lots 6 and 8. Age 18 to 28 minutes (sum of row 7, and of 57 of another lot)	106	34	143.82 ± .544	8.296± .384	5.769± .268	112–168
16	Lot 9. Age 3 to 4 hours	93	37	149.636± .688	9.856± .488	6.587± .327	132–176
17	Lot 9. Age 4.20 to 5 hours	95	38	186.736± .652	9.416± .460	5.043± .247	164–216
18	Lot 9. Age 3 to 5 hours (sum of rows 16 and 17)	188	—	168.384±1.028	20.904± .727	12.415± .438	132–216
19	Lot 9. Random sample.	195	7	176.124±1.128	23.360± .797	13.262± .461	104–220
20	Lot 10. Age 12 hours	73	39	188.988± .996	12.612± .704	6.672± .374	136–216
21	Lot 10. Age 12 hours (same as row 20, but omitting 2 smallest)	71	39	190.424± .752	9.388± .531	4.930± .280	164–216
22	Lot 10. Age 18 hours	105	40	199.048± .380	11.844± .552	5.949± .278	168–228
23	Lot 3. Age 24 hours	300	41	168.532± .419	10.768± .629	6.389± .175	140–200
24	Lot 3. Early fission, depth of constriction less than ¼ breadth	42	44	167.620± .996	9.564± .704	5.706± .421	152–192
25	Lot 1. Early fission, constriction 4μ or less	131	13	175.696± .556	9.432± .393	5.368± .224	156–240

TABLE X.—*Continued.*

Comparison with Random Samples. (Each "Lot" consists of specimens with those of Table VIII. are numbered the same as in Table VIII. The headed "Table" gives the number of a table found in the appendix or

Breadth.				Mean Index, Ratio of Breadth to Length. Per Cent.	Coefficient of Correlation.
Mean in Microns.	Standard Deviation in Microns.	Coefficinet of Variation.	Range of Variation in Microns.		
55.480±.297	5.040±.210	9.082±.382	44–72	63.136	.6546±.0337
49.540±.215	4.296±.152	8.671±.309	40–68	53.592	—.0938±.0496
50.220±.308	6.468±.218	12.877±.441	36–72	25.114	.6064±.0302
48.852±.210	4.216±.149	8.633±.307	36–64	54.080	—.3625±.0433
46.372±.332	3.804±.236	8.200±.524	36–56	44.037	—.3138±.0792
46.020±.251	5.256±.177	11.421±.390	36–60	25.084	.4282±.0389
60.168±.788	5.712±.556	9.495±.933	52–76	47.573	—.0337±.1375
54.284±.364	3.788±.260	6.976±.478	48–64	37.921	.1937±.0927
55.840±.636	4.724±.452	8.461±.813	48–64	37.296	.2799±.1243
54.192±.600	5.752±.424	10.617±.790	40–68	33.558	.5232±.0756
55.544±.308	5.416±.220	9.748±.397	40–76	38.038	—.0844±.0566
64.880±.580	8.624±.412	13.292±.645	44–88	35.131	.6469±.0392
46.768±.408	3.792±.288	8.108±.623	36–52	35.616	—.2546±.1010
51.872±.680	7.980±.480	15.382±.946	36–76	40.028	—.2476±.0798
50.832±.320	4.900±.228	9.640±.451	36–64	35.438	.1319±.0644
51.568±.322	4.752±.236	9.212±.459	40–64	34.546	.3201±.0628
60.168±.360	5.224±.256	8.679±.428	52–76	32.225	.5557±.0478
55.916±.324	6.588±.229	11.785±.416	40–76	33.372	.7132±.0242
47.364±.344	7.132±.244	15.057±.526	32–72	27.153	.3945±.0408
62.796±.464	5.872±.328	9.350±.526	48–80	33.275	.4868±.0602
63.156±.443	5.536±.313	8.763±.500	48–80	33.197	.3474±.0704
56.496±.292	4.428±.108	7.837±.367	48–68	28.427	.4304±.0536
40.320±.230	5.892±.162	14.615±.411	28–56	23.899	.5496±.0272
65.716±.706	6.784±.499	10.322±.768	48–80	39.286	.2215±.0999
55.480±.207	5.040+.210	9.082±.382	44–72	31.568	.6546±.0337

TABLE X.—*Continued.*

Row.	A. *Progeny of D (Caudatum Form).*	Number of Individuals.	Table.	Length.			
				Mean in Microns.	Standard Deviation in Microns.	Coefficient of Variation.	Range of Variation in Microns.
26	Lot 1. Fission, all stages but earliest	182	62	186.066± .710	14.208±.502	7.636±.271	160–224
27	Lot 1. Random sample	200	14	199.960± .740	15.528±.524	7.765±.263	148–240
28	Lot 1. Largest specimens of random sample, all more than 196 long	134	—	208.268± .566	9.720±.400		196–240
29	Lot 1. Combination of early fission with largest of random sample (sum of rows 25 and 28)	264	—	192.108	18.904		
30	Lot 2. Early stages of fission	40	44	165.200± .936	8.788±.664	5.320±.402	152–192
31	Lot 2. All stages of fission	62	42	171.548±1.188	13.848±.840	8.072±.492	144–212
32	Lot 2. Random sample	200	30	184.100± .776	16.264±.548	8.834±.300	140–216
	B. *Progeny of c (aurelia form).*						
33	Lot 4. Early fission, depth of constriction less than ¼ breadth	66	47	103.737± .650	7.823±.379	7.541±.445	83.3–126.7
34	Lot 4. Later stages of fission	53	63	121.383±1.053	11.367±.743	9.365±.613	100–156.7
35	Lot 4. Random sample	225	49	114.163± .784	17.443±.555	15.279±.497	73.3–160
36	Lot 5. Early fission	38	48	113.333± .850	7.778±.603	6.862±.533	93.3–126.7
37	Lot 5. Random sample	100	50	114.033± .820	12.140±.580	10.643±.513	86.7–146.7
38	Lots 4 and 5. All in early fission (sum of rows 33 and 36).	104	—	107.243± .600	9.070±.423	8.459±.398	83.3–126.7

of separation of the two halves). The measurements of these 59 young specimens are given in Table XXIX., while the polygon of variation for length appears at *b*, Diagram 4. For control, Table XXX. gives the measurements of a random sample of the culture from which these young specimens were selected. The constants deduced from the measurements of the young and of the random sample are shown in Table X., rows 4 to 6.

The following are the important facts which result from the examination of the young, in comparison with the adults (rows 5 and 6, Table X.).

1. The mean length of the young (0 to 5 minutes old) is considerably more than half that of the culture as a whole, being 107.660 microns as compared with 184.100 microns. Of course, the culture

TABLE X.—*Continued.*

Breadth.				Mean Index, Ratio of Breadth to Length. Per Cent.	Coefficient of Correlation.
Mean in Microns.	Standard Deviation in Microns.	Coefficient of Variation.	Range of Variation in Microns.		
49.540±.215	4.296±.152	8.671±.309	40–68	26.796	—.0938±.0496
50.220±.308	6.468±.218	12.877±.441	36–72	25.114	.6064±.0302
52.360±.348	5.964±.246		40–72		.4681±.0455
53.908	5.752				.0350±.0415
50.700±.364	3.432±.260	6.769±.513	48–80	30.765	.1048±.1055
50.388±.308	3.584±.216	7.111±.433	40–60	29.583	—.1136±.0840
46.020±.251	5.256±.177	11.421±.390	36–60	25.084	.4282±.0389
34.850±.287	3.453±.203	9.911±.587	26.7–43.3	33.623	.6502±.0479
34.590±.383	4.147±.273	11.989±.797	26.7–46.7	28.648	.3100±.0837
34.207±.241	5.363±.171	15.683±.511	20–50	30.177	.6757±.0244
45.263±.597	5.463±.423	12.071±.947	33.3–56.7	39.903	.6744±.0597
47.300±.437	6.490±.310	13.720±.667	36.7–66.7	41.455	.8152±.0226
38.653±.437	6.607±.310	17.089±.029	26.7–56.7		.7476±.0292

as a whole contains a large number of young specimens, so that the mean of the adults would be greater than that of the random sample.

2. The mean breadth of the young is almost exactly the same as that of the culture as a whole.

3. The relative variation in length is much greater for the young than for the culture as a whole, the coefficient being 13.729 for the former as compared with 8. 834 for the latter. Moreover, the coefficient of variation is almost three times as great as in the very youngest stages before separation (Table X., row 1), or in the first stages of fission (Table X., rows 25, 30, 33, 36).

This great variability of the young at this age indicates that they are growing rapidly in length; those five minutes old are considerably longer than those that have just separated, so that when all are taken

together the variation is great in proportion to the mean length. While the statistical data are themselves open to other interpretations, observation of the changes in living individuals, as described earlier, shows that this explanation is the correct one.

The *absolute* variation of the young, as shown by the standard deviation, is less, as might be expected, than that of the culture as a whole, though the difference is not great.

4. The variation in breadth, both absolute and relative, is less in the young than in the culture as a whole. The fact that it is still considerable perhaps indicates that changes in breadth are taking place during growth. To this we shall return immediately.

5. The correlation between length and breadth is negative in the young, while in the culture as a whole it is positive. In the former the coefficient is $-.3138$; in the latter it is $+.4282$.

The fact that the correlation is negative in young specimens (greater length associated with less breadth) indicates that while the animals are growing in length they are becoming more slender. With an increase of 10 microns in length the decrease in breadth is .757 micron. If we group together the unseparated halves (124 in number) with the separated ones (59), we find that the negative correlation between length and breadth is still greater, becoming $-.3625$ (see row 4, Table X.).

6. The mean ratio of breadth to length ("mean index") is much greater in the young than in the random sample. In the former the breadth is 44.037 per cent. of the length; in the latter but 25.084 per cent. If we include the unseparated halves with those under five minutes old, the breadth is 54.080 per cent. of the length (row 4, Table X.), while in the unseparated halves alone it is 59.166 per cent., and in the earliest stages of the unseparated halves it is 61.530 per cent. (see Table VIII., rows 4 and 5). There is thus a steady reduction of the ratio of breadth to length; to this is due the negative correlation of the two, when those of different ages are thrown together.

(*d*) *Age 0 to 19 Minutes* (*Tables XXXI. and XXXII.*).—From another culture composed of descendants of the individual *D*, specimens were taken on June 14 and kept to several different ages. The

various ages and measurements are given, with those of a random sample of the culture in lot 6, Table X.

The first set taken consisted of but 24 specimens, aged from 0 to 19 minutes. Though the number is small it is worth while to work out the constants for comparison with other stages in this same culture; it must be remembered that it is extremely difficult to get large numbers at any one time of individuals so young. The measurements are given in Table XXXI., while the constants are shown in row 7, Table X. For comparison with these a second lot of the same age, but containing 39 specimens, was taken from the same culture two weeks later. The measurements are given in Table XXXII.; the constants in row 13, Table X. The constants for the two sets taken together (63 specimens aged 0 to 19 minutes) are given in row 14, Table X.

Comparing these with the specimens but 0 to 5 minutes old, we find that the mean length has increased by 36 to 40 microns. The breadth is about the same in one of the lots (row 13, Table X.), but is much greater in the other (row 7). This difference is due to environmental effects. The coefficient of variability in length shows a decided decrease, indicating that growth is relatively more rapid during the first five minutes than later. The correlation between length and breadth is, as might be expected, negative in the sets 0 to 19 minutes old, as it was in the set still younger.

A number of specimens were killed at precisely known ages, and the measurements taken. Thus, from lot 7 (row 13, Table X.) a typical pair of young at the moment of separation measured 110×52 microns. At the age of one minute the two members of a pair measured each 124×52 microns; at two minutes another pair were each 120×52 microns. At three minutes one member of a pair measured 120×48 microns, the other 124×44. At five minutes the lengths of the two resulting from a certain fission were respectively 124×48 and 112×44 microns. Five specimens kept till they were precisely nineteen minutes old measured respectively 160×48 microns; 160×44; 152×36; 152×40; 156×44. The mean dimensions were thus 156×42.4 microns.

Outlines of individuals from 0 to 19 minutes old, showing the

relative sizes, are given in Fig. 4. These may be compared with the adults of this race, *a* to *c*, Fig. 1.

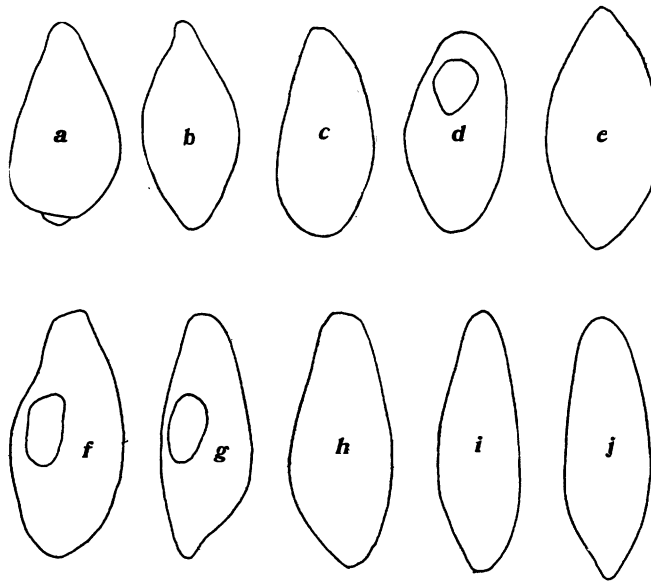

FIG. 4. Young *Paramecia*, descendants of *D* (*caudatum* form), from immediately after separation to the age of 19 minutes. *a* has just separated; *b*, *c* and *d* are two to three minutes old; *i* and *j* are 19 minutes old; the others are intermediate. These should be compared with the adults *a* to *c* of Fig. 1 (page 403), which are drawn to the same scale. All $\times 235$.

(*e*) *Age 18 to 28 Minutes* (*Tables XXXIII. and XXXIV.*).—The first lot of this age (row 8, Table X.) contained 49 specimens (Table XXXIII.) and came on the same day from the same lot as the first lot of 24 of the preceding stage, so that the two are strictly comparable. The mean length has increased in the period of about thirteen minutes by nearly 16 microns, while the mean breadth has decreased 7 to 8 microns. The ratio of breadth to length has decreased almost 10 per cent. The correlation between length and breadth is in the present lot positive though small (.1937). If we should throw together the two lots (rows 7 and 8, Table X.), the correlation would, of course, be decidedly negative.

A second lot of 57 specimens aged 18 to 28 minutes was taken from the same culture about two weeks later. If we throw the two

lots together (Table XXXIV.) we have 106 specimens at this age (row 15, Table X.); the mean length is 143.82 microns, the mean breadth 50.832 microns, while the mean ratio of length to breadth is 35.438 per cent.

The polygon for variation in length at this age is shown at *c*, Diagram 4, p. 440.

(*f*) *Age 35 to 45 Minutes* (*Table XXXV.*).—From the same lot 6 (Table X.) from which came the first sets aged 0 to 19 and 18 to 28 minutes, there were taken on the same day 25 specimens that were allowed to reach the age of 35 to 45 minutes (row 9, Table X.). Growth has now become much slower. These specimens average 17 minutes older than the last set, yet they have increased in length only about 6.5 microns. The breadth remains about the same; the slight increase shown in the figures is probably not significant, since it disappears at the next stage. The mean ratio of breadth to length continues to decrease, reaching now 37.296 per cent. The correlation between length and breadth is more strongly positive than before (.2799), indicating that these dimensions are not changing so decidedly in opposite ways.

The polygon for variation in length at this age is shown at *d*, Diagram 4.

(*g*) *Age 75 to 90 Minutes* (*Table XXXVI.*).—Forty-two specimens of this age were measured, taken on the same day from the same lot from which came the sets last described (lot 6, Table X.). The specimens average about twice the age of those in the last set, the absolute increase being 45 minutes, yet the growth in length has been only about 12 microns, which is about the same as the growth in the first five minutes after separation. The breadth still remains about the same; it is notably less than in the very earliest stages. The ratio of breadth to length continues to decrease, reaching now 33.558 per cent. Meanwhile the correlation between length and breadth has increased greatly, till now, at .5232, it is not much below that of the culture as a whole (.6469).

(*h*) *Age 0 to 90 Minutes.*—From a single culture of *D*, on a single day, we have thus measured 140 young specimens, varying in age from 0 to 90 minutes. The constants for variability and correlation of such a collection are of interest; they are therefore given

in Table X., row 11. The variability, as measured by its coefficient, is less in both length and breadth than in the random sample, or in the collection of young specimens including only those under nineteen minutes in age. There is practically no correlation in the collection taken as a whole between length and breadth. This is because breadth at first decreases while length increases (giving negative correlation); later they increase together (giving positive correlation); the two tendencies about cancel each other in the collection as a whole.

Third Stage: Three to Five Hours Old (Tables XXXVII. and XXXVIII.).

Three days later than the sets shown in lot 6, Table X., and under as nearly the same conditions as possible, I took from the same culture of progeny of *D* two sets of young, keeping the first set till the age was between 3 and 4 hours, the second set till the age was between 4.20 and 5 hours (see lot 9, Table X.). The culture was, however, in a different condition from that of lot 6; it contained a very large number of young and dividing specimens. A random sample of this culture, containing 195 specimens, is shown in Table VII. (page 412), while the constants for this sample are shown in row 19, Table X. The entire left portion of Table VII., up to the length of about 160 microns, or more, evidently consists of young individuals in various stages of growth. This decreases the main length (176.124 microns) and the correlation (.3945), while it greatly increases the variability in length (13.262, as against 6.821 for the random sample of the previous lot).

(*i*) *Age 3 to 4 Hours (Table XXXVII.).*—The effects of different environmental conditions are at once seen on comparing this set of 93 specimens (Table X., row 16) with the set 75 to 90 minutes old, from the previous culture (Table X., row 10). The specimens of the present lot, though $1\frac{1}{2}$ to $2\frac{3}{4}$ hours older than the others, are shorter, the length (149.636 microns) being less by about 16 microns. The breadth is about the same as in the previous set; the correlation between the two is rather low (.3201).

(*j*) *Age 4.20 to 5 Hours (Table XXXVIII.).*—Ninety-five specimens kept for about an hour longer than those in the foregoing

set showed a rapid growth in length and breadth. The length now reaches 186.736 microns, the breadth 60.168; both dimensions are considerably greater than the mean of the random sample. Thus, the animals at this age had reached about the average size of the infusoria in a collection of the same descent taken at random. Table VI. (page 412) shows a sample of this same culture taken twenty-four hours earlier, at a time when little division was occurring; the mean length is very nearly the same as that of the young of the present set. The correlation between length and breadth has considerably increased.

Certain peculiar facts are brought out by considering these two sets together (Table X., row 18). Here we have a collection of 188 young individuals taken at practically the same time from a small watch-glass culture. The variability and correlation depend in a high degree on the length of time we keep these. If they are all kept three to four hours (row 16) or 4.20 to 5 hours (row 17), the variability in length is about 5 to 6, in breadth about 9. But when we keep part of them for the shorter period, part for the longer, the variability rises to about 12.5 for length and 12 for breadth. Again, the correlation between length and breadth is but .3201 and .5557 in the two lots taken separately, but when we take them together the correlation is much greater, rising to .7132. These relations show the important part which may be played by growth in determining observed variability and correlation; their significance will be taken up again in our general sections on these topics.

Fourth Stage: 12 to 18 Hours Old (Table X., Lot 10).

From the same culture of the progeny of *D* from which came the lots last described, but three days later were taken two lots of young, of 73 and 105 specimens, respectively, which were kept, the former to the age of 12 hours, the latter to the age of 18 hours.

(*k*) *Age 12 Hours (Table XXXIX., and rows 20 and 21, Table X.).*—There is a still further increase in both length and breadth, as compared with the specimens 4.20 to 5 hours old (see Table X., rows 20 and 21). Among the 73 specimens of this lot were two of about the same size which were much smaller than the others (see Table XXXIX.). There is little doubt, I believe, that these are the prod-

ucts of a second division; either one of the twelve-hour specimens had divided, or there was accidentally taken with them an older specimen which divided. In either case these two specimens do not belong in the twelve-hour lot, as they are much younger. On this account I have calculated the constants for this twelve-hour lot twice, once including these two small specimens (row 20, Table X.), the second time excluding them (row 21). The variability in length is much reduced—from 6.672 to 4.930—by the omission of these two. At the same time the correlation between length and breadth is likewise reduced from .4868 to .3474.

(1) *Age 18 Hours (Table XL., and row 22, Table X.).*—Growth in length continues, though very slowly; in six hours the increase has been less than during the first five minutes after separation. The animals at this age are decidedly longer than the mean for the culture as a whole, as judged from the random sample of Table VII. (page —), taken three days earlier. The mean breadth of the eighteen-hour specimens, while greater than that of the random sample, has decreased as compared with that of those only twelve hours old.

The variability of these two lots (12 and 18 hours old) of adult size is less than that of the random samples (for examples, rows 3, 6, 12, 19, Table X.).

Fifth Stage: 24 Hours Old (Table XLI., and row 23, Table X.).

A final lot of 300 specimens was selected while dividing and these were kept till they were 24 hours old. These were progeny of *D,* but were taken from the culture somewhat more than a month later than those 0 to 18 hours old. To understand their measurements it is necessary to take into consideration the cultural conditions. These animals were living in an ordinary hay culture, which was getting old, so that they were not dividing rapidly; they were rather slender in form. Now a large number of these was placed in a fresh decoction of hay and left there for 24 hours. They increased in size and began to divide rapidly. Now 150 dividing specimens (producing, of course, 300 young) were taken out and *returned to the original culture fluid.* This was for the purpose of preventing a second division before the end of the period of twenty-

four hours. As a result of this treatment they did not grow so rapidly as did the twelve- and eighteen-hour lots, and are smaller than these. The purpose in studying this group (as well as other groups) was mainly to determine the variability and the correlation between length and breadth. Both are less, as Table X. shows, than is usually the case in random samples.

The specimens 12, 18 and 24 hours old may be taken as types of adult *Paramecia* of this strain (progeny of *D*; *caudatum* form) before the changes leading to fission have begun.

Diagram 4 gives polygons of variation for the different ages, in descendants of *D*, as compared with a random sample; it shows clearly the part played in the observed variations by the presence of different stages of growth.

Sixth Stage: Preparing for Fission.

As Table X. shows, the adults of the progeny of *D* (*caudatum* form) reach a mean length of 168.532 to 199.048 microns (rows 23 and 22) under the cultural conditions employed, while the mean breadth varies from 40.320 (row 23) to 62.796 microns (row 20). But the *maximum* length is (under the same conditions), of course, much greater than the mean. In the random samples we find individuals up to 224 microns in length and 88 in breadth (see, for example, Table LI.); and among those 18 hours old (Table XL.) we find a length of 228 microns.

Now, when we compare these large adults with the specimens actually beginning fission (which are supposedly the oldest of all), certain peculiar facts appear. *The specimens beginning fission are by no means the longest of the lot;* a given culture contains many specimens much longer than those showing the first signs of division. Thus, in the "Lot 1" of Table VIII., we find 131 specimens in the very earliest stages of fission (Table XIII., page 442). The mean length of these is 175.696 microns (row 25, Table X.), and the longest specimen is 204 microns long. But in the random sample of the specimens that are not dividing, from this same lot (taken at the same time) the mean length is 199.960 microns (row 27, Table X.), and certain individuals reach a length of 240 microns (Table XIV., page 443). Of the two hundred specimens of the random

sample, 69, or more than one third, *are longer than the longest of the specimens beginning fission.* Only nine of the entire 200 falls below the mean length of the specimens beginning division.

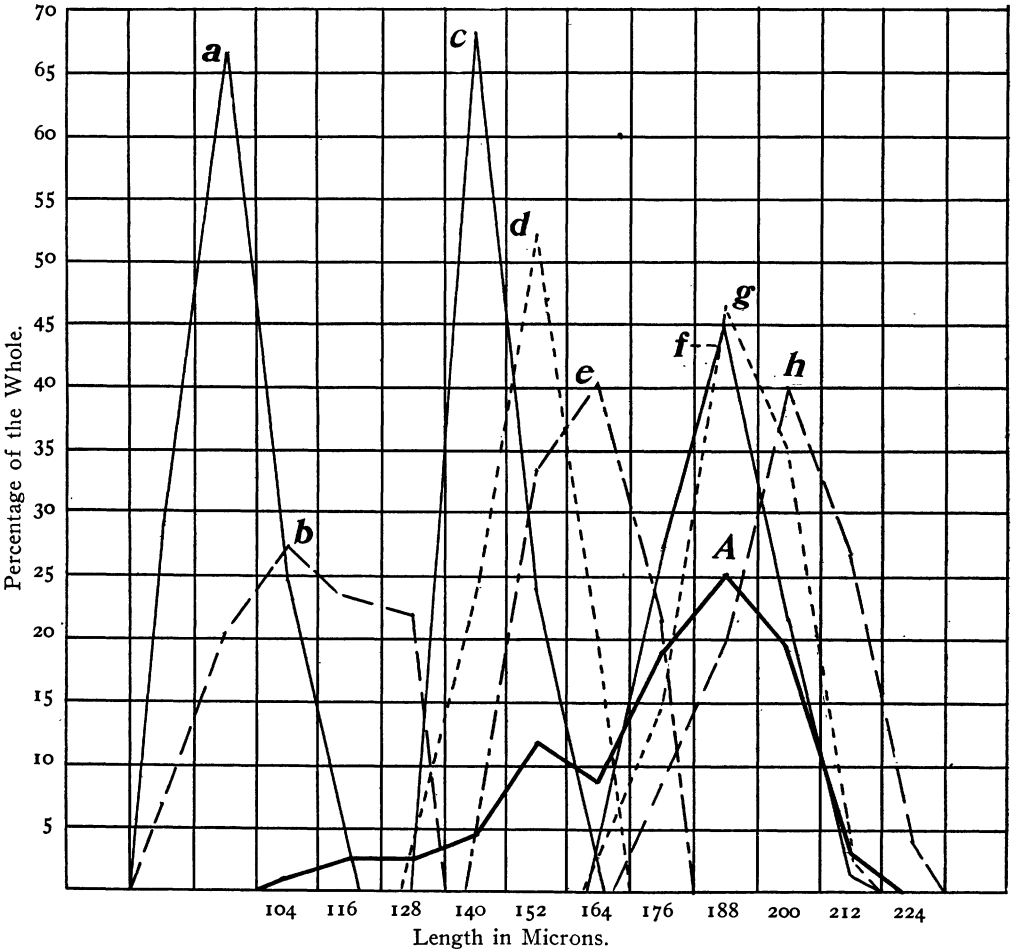

DIAGRAM 4. Polygons of variation in length for descendants of individual *D*, at various ages. *A* (heavy line), Random sample, 195 specimens (row 19, Table X.). *a*, youngest halves, constriction beginning (row 1, Table X.). *b*, age 0 to 5 minutes (row 5, Table X.). *c*, age 18 to 28 minutes (row 8, Table X.). *d*, age 35 to 45 minutes (row 9, Table X.). *e*, age 75 to 90 minutes (row 10, Table X.). *f*, age 4.20 to 5 hours (row 17, Table X.). *g*, age 12 hours (row 21, Table X.). *h*, age 18 hours (row 22, Table X.).

Since then the specimens beginning fission are not the longest of the culture, it is clear that *the length decreases before fission begins.* This is borne out by the form of the specimens beginning fission; though their mean length is less than that of the random sample, their mean breadth is greater (mean breadth 50.220 microns in the random sample, 55.480 in those beginning fission). While then the

TABLE XI.

Correlation Table for Depth of Constriction and Total Length in 313 Dividing Specimens from a Single Culture of Descendants of D.

All taken the same day.

Total Length of Body, in Microns.

Depth of Constriction in Microns.	156	160	164	168	172	176	180	184	188	192	196	200	204	208	212	216	220	224	
4	1	8	12	17	22	15	31	8	5	6	4	1	1						131
8		1	2	7	8	5	7	5	2										37
12			2	1	10	9	3	3				1				1			30
16				2	3	5	4	4	4	2	3	2							29
20			1	1		3	1	3	1	1	1								12
24		1						3	4	5	2		1						16
28					1		1	4	2	5	1	2	2	2	1	1			22
32				1		1				3	1	2	4	3	1	1			17
36									2		2	2	1		1	1		1	10
40											2		1	1		2	1	1	8
44																	1		1
	1	10	17	29	44	38	47	30	20	22	16	10	10	6	3	6	2	2	313

Length—Mean, 181.725 ± .512μ Depth of Constriction—Mean, 13.265μ
St. Dev., 13.446 ± .362μ St. Dev., 2.721μ
Coef. Var., 7.399 ± .201

Coef. of Cor. between Depth of Constriction and Length, .6882 ± .0201; Increase in Length for 1 unit of depth, .860μ; Coef. of Cor. if first row is omitted, .7818 ± .0194.

length decreases preparatory to fission, the breadth increases at the same time. How long before fission this change of dimensions begins I can see no way of determining. The period may perhaps be one or two hours.

Thus, the longest individuals of the culture are the adults that have not begun the changes preparatory to fission. These decrease in length and increase in breadth before fission.

TABLE XII.

Correlation Table for Depth of Constriction and Breadth of Body, in 313 Dividing Specimens from a Single Culture of Descendants of D.

(Same lot shown in Tables XI., XIII. and LXII.)

Breadth in Microns.

Depth of Constriction in Microns.	40	44	48	52	56	60	64	68	72	
4		2	19	27	47	22	11	2	1	131
8		1	13	18	3	2				37
12		3	13	9	3	1		1		30
16	1	2	15	4	4	3				29
20	1	1	3	7						12
24		4	5	5	2					16
28		7	9	3	3					22
32	1	6	7	1	2					17
36	2	2	4	2						10
40		4	1	2	1					8
44			1							1
	5	32	90	78	65	28	11	3	1	313

Breadth—Mean, $52.026 \pm .209\mu$ Depth of Constriction—Mean, 13.265μ
St. Dev., $5.473 \pm .148\mu$ St. Dev., 2.721μ
Coef. Var., $10.544 \pm .287$

Coef. of Cor. between Depth of Constriction and Breadth, $-.5232 \pm .0277$; Decrease in Breadth with Increase of 10μ in Depth, 2.630μ.

Omitting uppermost row: Coef. of Cor., $-.3316 \pm .0445\mu$; Decrease in Breadth with Increase of 10μ in Depth, 1.252μ.

TABLE XIII.

Correlation Table for Length and Breadth of 131 Specimens of Lot 1 in the Earliest Stages of Fission. (Descendants of D, Table X., row 25.)

Length in Microns.

Breadth in Microns.	156	160	164	168	172	176	180	184	188	192	196	200	204	
44		1		1										2
48	1	3	6	3	4	1		1						19
52		1	2	8	6	2	6	1	1					27
56		3	2	4	10	7	15	2	2	1	1			47
60			2	1	1	5	8	1	1	2	1			22
64					1		2	3	1	2	2			11
68										1		1		2
72													1	1
	1	8	12	17	22	15	31	8	5	6	4	1	1	131

Length—Mean, $175.696 \pm .556\mu$ Breadth—Mean, $55.480 \pm .297\mu$
St. Dev., $9.432 \pm .393\mu$ St. Dev., $5.040 \pm .210\mu$
Coef. Var., $5.368 \pm .224$ Coef. Var., $9.082 \pm .382$

Mean Index, 31.568 per cent.; Coef. Cor., $.6546 \pm .0337$.

Seventh Stage: Fission.

Some of the data bearing on the dimensions during fission have been incidentally taken up in the account of the young in the earliest stages, before the two halves have separated.

(*m*) *Beginning Fission.* Descendants of *D* (*caudatum* Form). —Four lots of dividing specimens descended from the individual *D* were studied. These lots were taken at different times; the first included 313 dividing specimens (Tables XI. and XII., and rows 25–29, Table X.); the second 62 (Tables XLII., XLIII. (appendix) and rows 30–32, Table X.); the third 77 (Table XLIV., and rows 23–24, Table X.); the fourth 37. The dimensions of random samples of the same lots are given in Table X.

The large lot containing 313 dividing specimens may be described as typical; the others show the same relations, except as hereafter noted.

TABLE XIV.

Correlation Table for Random Sample of Specimens not Dividing, of Lot 1 (from which came the dividing specimens of Table XIII.). (See Table X., row 27.)

Breadth in Microns. \ Length in Microns.	148	152	156	160	164	168	172	176	180	184	188	192	196	200	204	208	212	216	220	224	228	232	236	240	
36	1							1		2															4
40		1			1	2				1	3	1		2		1									12
44	1		2					1	3	5	4	10	5	4	4	2		2	1						44
48						1		1	1	1	5	3	7	4	2	4	3	3	2		1				38
52									2	2	2	5	6	6	7	7	3	4	2	1					47
56											2	2	1	11	2	3	3	1	4	3	1				33
60												1			2	1	2	4	3	1					14
64														1					1	2	1			1	6
68																									0
72																		1						1	2
	2	1	2	0	1	3	0	3	6	11	16	22	19	28	17	18	11	15	13	7	3	0	0	2	200

Length—Mean, 199.960 ± .740μ Breadth—Mean, 50.220 ± .308μ
St. Dev., 15.528 ± .524μ St. Dev., 6.468 ± .218μ
Coef. Var., 7.765 ± .263 Coef. Var., 12.877 ± .441
Mean Index, 25.114 per cent.; Coef. Cor., .6064 ± .0302.

In the dividing specimens the length of the body increases as the depth of the constriction between the two halves becomes greater; this is well shown in Fig. 2, page 416. In order to include only the earliest stages of fission we shall, of course, have to take the speci-

mens in which constriction is beginning. Among the 313 dividing specimens of lot 1 (Table XI.) there were 131 in which the depth of the constriction below the body surface was less than one unit of the micrometer scale (less than 4 microns). These may be taken as representing the earliest stages of fission. The depth of the constriction is in these specimens less than one twelfth the breadth. Their measurements are given in Table XIII., while the constants deduced from the measurements are shown in row 25, Table X. These should be compared with the measurements and constants for the random sample of the specimens not dividing in this same culture (Table XIV., and row 27, Table X.).

Examination of these tables shows the following remarkable facts:

1. The mean length of the specimens beginning fission (175.696 microns) is much *less* than the mean length of the random sample (199.960 microns)—although the latter must contain many specimens that have not reached adult size.

2. The range of variation in length is much less in the specimens beginning fission than in the culture as a whole. In those beginning division the range is from 156 to 204 microns; in the random sample it is from 148 to 240 microns.

3. The longest specimens beginning fission are 36 microns shorter than the longest of the random sample. In the random sample, 34.5 per cent. of all the specimens are longer than the longest of those beginning fission, while 95.5 per cent. are longer than the mean length of the specimens beginning fission.

4. The variation in length is decidedly less in the specimens beginning fission than in the random sample. In the lot beginning fission the coefficient of variation is but 5.368, while in the random sample it is 7.636.

It may here be noticed that coefficient of variation in the specimens beginning fission is less than that for conjugating specimens, as studied by Pearl (1907). To this matter we shall return later.

5. In the specimens beginning fission the mean breadth (55.480 microns) is greater than the mean breadth of the random sample (50.220 microns).

6. The variation in breadth is much less in the specimens begin-

ning fission than in the others. In the former the coefficient is but 9.082, while in the latter it is 12.877.

7. The mean index, or ratio of breadth to length, is much greater in the specimens beginning fission; in these it is 31.568 per cent., as contrasted with 25.114 per cent. in the random sample.

8. The correlation between length and breadth is high in the specimens beginning fission; it is somewhat greater than in the random sample. In the former it is .6546; in the latter .6064.

Owing to the smaller numbers in the other lots of dividing specimens, I included in the group "beginning fission" all those in which the depth of the constriction below the body surface was less than one fourth the breadth of the animal. Thus, all specimens with constriction 12 microns deep, or less, were included. Of course, these groups contained specimens in decidedly more advanced stages of fission than in the large group we have been considering. The numbers of specimens in early stages of fission thus secured were respectively 40 (Table XLIII.) and 42 (Table XLIV.). The constants for these, in comparison with random samples or adults, are shown in Table X. (rows 24 and 30).

As the tables show, these manifest in most particulars the same relations which we have brought out above for the larger and more precise set containing 131 specimens. The differences between the dividing specimens and the other individuals (as shown by the random samples, etc.) are in the main somewhat less in amount than in our first example. This is because in the smaller lots specimens are included in which lengthening and narrowing had begun, causing the dimensions to approach those of the specimens not dividing.

The most striking difference between our large lot (Table X., row 25) and the smaller ones (Table X., rows 24 and 30) is in the correlation between length and breadth. While in the larger lot the correlation was high, in the smaller ones it is small or quite lacking. This is again due to the inclusion of more advanced stages in the smaller lots; as the length increases the breadth decreases, tending to destroy the correlation.

Descendants of *c* (*aurelia* Form).—Two lots of dividing specimens were examined from the descendants of the small individual *c*. The first contained 119 specimens (Table XLV.); the second 63

specimens (Table XLVI.).[5] Selecting from these, as representing the early stages of fission, all those in which the depth of constriction is less than one fourth the diameter of the body, we obtain from the larger lot 66 specimens (Table XLVII.); from the smaller lot 38 specimens (Table XLVIII.). The constants for these, in comparison with those for random samples, are given in Table X. (lots 4 and 5, rows 33 to 38). The measurements of the random samples are shown in Tables XLIX. and L.

These specimens of the *aurelia* form show the same relations that are found in the *caudatum* form, with one exception. In lot 5 (Table X., row 36) the mean breadth of the specimens beginning fission is *less* than that of the random sample, instead of greater as in all other cases. But this peculiarity is due to environmental conditions. In lot 5 the breadth was very great in proportion to the length, as is shown by the dimensions of the random sample (Table L., and row 37, Table X.). In this lot the breadth was 41.555 per cent. of the length, while in most cases it is near to 30 per cent. This was due to the recent transference of the animals to a nutritive solution; they became very plump. Evidently, when preparing to divide the body tends to return to a constant form; in this case, therefore, it becomes narrower instead of broader.

In the specimens of the *aurelia* form, as in the *caudatum* form, all dimensions are less variable in the specimens beginning fission. This difference in variability, as compared with the random samples, is very great in some cases. Thus, while the coefficients of variation in length for the random samples of lots 4 and 5 are 15.279 and 10.643, for those of the same lots beginning fission they are but 7.541 and 6.862, respectively. Had we included in the lots beginning fission only specimens in which the depth of constriction was still less, the coefficients of variation would have been still smaller.

The constants for all specimens of *c* that are beginning fission, taken together, are shown in row 38, Table X. The standard deviations and coefficients of variation are, of course, greater than for

[5] In making these measurements of descendants of *c*, a higher power of the microscope was used, so that the single unit of measurement was $3\frac{1}{3}$ microns. This caused the tables (in the appendix) to take a somewhat different appearance from those of the descendants of *D*.

each of the two component lots taken separately, since the two lots differed as a result of different environmental conditions.

(*n*) *Later Stages of Fission.*—As the constriction deepens the animal as a whole becomes more elongated, while the breadth decreases slightly. These relations are shown both for the descendants of *D* (*caudatum* form) and the descendants of *c* (*aurelia* form) in Table X. (rows 25 and 26; 30 and 31; 33 and 34). In the large lot 1 of dividing descendants of *D,* comprising 313 specimens (Table XI.) the correlation between length of body and depth of constriction below the surface is .6882. The length increases 8.6 microns with every increase of 10 microns in the depth of constriction. The correlation between breadth and depth of constriction (Table XII.) is —.5232, the breadth decreasing 2.63 microns for each 10 microns increase in depth of constriction. If we include only the specimens in which lengthening has decidedly begun (thus omitting the earliest stages, in the uppermost rows of Tables XI. and XII.), then the correlation between length and depth of constriction is .7818; between breadth and depth of constriction, —.3316. With an increase of 10 microns in depth of constriction the length now increases 11.195 microns, while the breadth decreases 1.252 microns. In this same culture while the mean length of the 131 specimens beginning fission is 175.696 microns, that of the seven specimens having a connecting portion but 4 microns wide is 212.572 microns. Thus, the increase in length before separation takes place is 36.876 microns, or about 21 per cent. of the length at the time fission begins. The breadth has decreased from 55.480 microns at the beginning of fission to 43.428 microns in the seven specimens with the narrowest connections—a decrease of about 21 per cent. The ratio of breadth to length decreases from 31.568 per cent. at the beginning of fission to 20.430 per cent. just before separation.

Corresponding relations are shown in other lots of dividing specimens; some of the data are given in Table X.

2. Summary on Growth in Paramecium with a Growth Curve.

We have thus followed the growth from the time when the individual is but half a constricting specimen to the period when it is again ready to separate into two new individuals. We are ready,

therefore, to outline the main features of the growth of *Paramecium,* and to construct curves which shall give an idea of the processes involved. In spite of an incredible amount of work devoted to collecting the data, certain of the less important features of the growth curves must remain obscure, but the main facts are clear.

The main outlines of the changes due to growth are as follows: From the time the constriction appears in the mother until a few minutes after separation takes place, the length increases rapidly, while the breadth decreases a little. A few minutes after separation the processes become less rapid. The breadth soon reaches its minimum, then begins to increase like the length, though more slowly. Growth in length continues for at least eighteen hours; the time undoubtedly varies with the conditions. The breadth continues to increase for some time, but it undergoes marked fluctuations, due to environmental conditions. In lot 10 (Table X.) it decreased between the ages of 12 and 18 hours; this is probably an environmental effect, not one due to the normal growth processes.

As the time for fission approaches the animals are considerably more than twice as long as the original halves from which they developed. Now as fission comes on they shorten and thicken, all tending to approach a uniform length and thickness. There is thus much less variation in the dimensions at the beginning of fission than in specimens taken at random. Now the constriction appears and the animal begins to narrow and extend in the way already described, finally separating into two parts.

If from our data we construct curves showing these changes, we get such results as are shown in Diagram 5.

Method of Constructing the Curves.—The horizontal scale represents the time in hours, while the vertical scale represents the measurements of the animals in microns. The upper curve shows the length, the lower one breadth, as measured from the base line. Fission is assumed to take place once in twenty-four hours, which is an approximation to a rate commonly occurring. The time between the appearance of the constriction and the actual separation of the two halves is taken as one half hour.

The relative distances of the two curves from the base line shows the relative dimensions of length and breadth. The vertical rise of

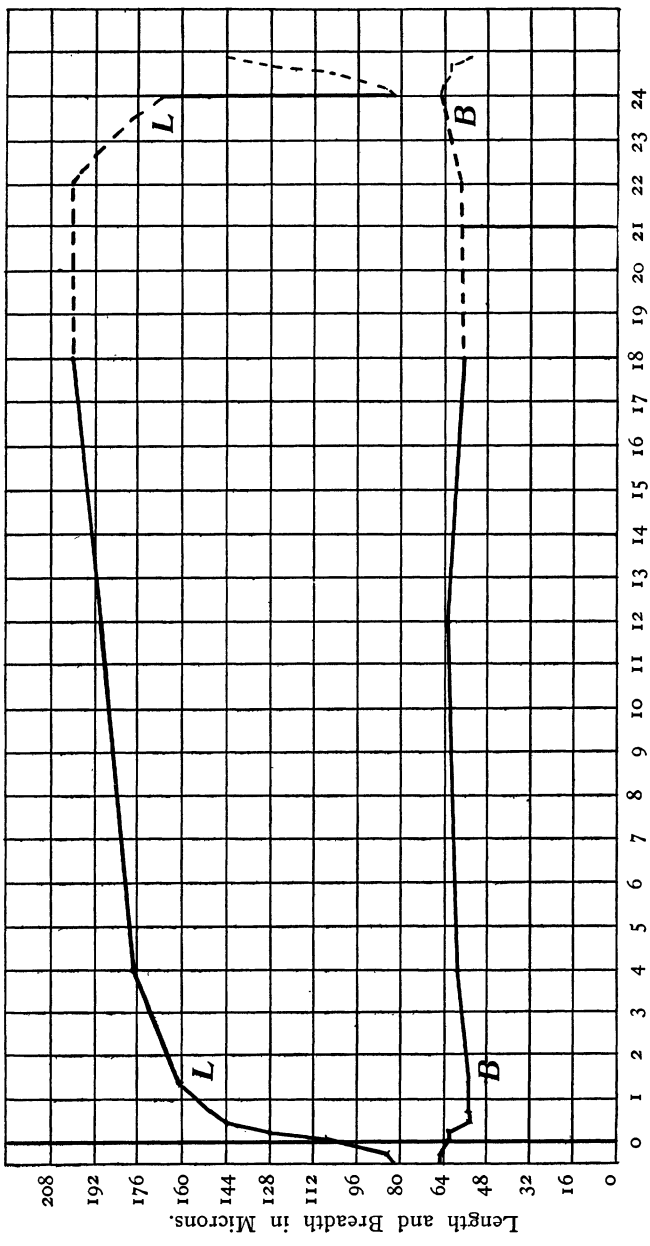

DIAGRAM 5. Curves of growth, for length and breadth in *Paramecium* plotted directly from Table XV., page 451. The upper line marked *L* is the curve of length; the lower line (*B*) that for breadth. The horizontal scale represents the time in hours; the vertical scale gives the measurements in microns. The relative distances of the two curves from the base line *o* shows the proportions of length and breadth at the different ages. The vertical rise of the curve shows the proportion of growth to the original length. The distance from the base line to the curve is 357 times the actual dimensions at the given ages. Fission occurs at *o* and at 24 hours. The curves are not smoothed. The broken parts of the curves are not constructed from precise data.

the curve of length shows the actual proportion of growth to the original length. The distance from the base to the curves is 357 times the actual dimension at the given time.

In order to show changes due to growth alone all the data for such a curve should be measurements from a single uniform culture on a single day; otherwise environmental differences complicate the matter, as we shall see more clearly in the next division of this paper. Now, it is impracticable to obtain from a single culture on a single day measurements of all the required stages. We are compelled therefore to make certain corrections in some of the measurements, to compensate so far as we can for environmental differences. As Table X. shows, the mean dimensions of random samples differ much in (for examples) lots 1 (row 3) and 6 (row 12). It will not do, therefore, to compare *directly* the young of these two lots. Since we have from lot 6 the greatest number of different stages, it is best to make the measurements from this the basis for the curve, correcting others, so far as possible, to compare with this. In lot 2 the mean length (Table X., row 6) is almost exactly the same as for lot 6, so that we may use the measurements of lot 2 without correction, so far as length is concerned. On this account we shall employ lot 2 for the earliest stages, in place of lot 1, though the latter is based on a larger number of specimens.

Since the mean breadth of the sample of lot 6 is 64.880 microns, while that of lot 2 is but 46.020 microns, it is necessary to correct the breadth for lot 2. At first thought it would seem that the proper method of making this correction would be by multiplying the breadths of the different sets of lot 2 by the ratio 64.880/46.020. This would be the proper method of procedure if we were dealing with the same stages of growth in the two lots; the specimens of lot 2 would be made plump, like those of lot 6. But the stage with which we are dealing is that of the beginning of fission. Now, we have already seen that when the specimens not dividing are plump, the breadth does not increase at the approach of fission nearly so much as when the specimens not dividing are thin. Indeed, if the specimens are very plump, there is an actual decrease, instead of an increase, at the approach of fission. Our problem is: What would be the breadth of specimens beginning fission, in which the length is 82.600, and the animals are very plump, as in lot 6? This problem can best be solved by asking what is the ratio of breadth to length in specimens beginning fission, in a very plump culture? In lot 3 (row 7, Table VIII.) we have such a plump culture, and we find that the ratio of breadth to length is, in the earliest stage of fission, 78.563 per cent. We therefore take this as the ratio of breadth to length for the earliest stage of lot 2, from which the corrected breadth is found to be 64.893. If this decreases at the same relative rate as actually occurred in lot 2, then the breadth 15 minutes after the beginning of constriction would be 64.493 microns.

We are compelled to use, further, lots 9 and 10 (Table X.). In lot 9 both length and breadth require correction to make them comparable with the measurements of lot 6. The correction is made by multiplying the

dimensions by the ratio between the length of the random samples of the two lots. In lot 9 we use only the average of the two sets, as given in row 18, Table X.

In lot 10, since we unfortunately have no random sample, we are unable to make a correction.

Owing to the very great difference in the environmental conditions of lot 3 (rows 23 and 24, Table X.) we are unable to use the 24-hour-old specimens of that lot, although we need measurements at that age. The older portions of the curve (beyond 18 hours, at the right) cannot be plotted from exact data, and there are certain features of much importance for which it appears that the collection of such data would be almost impossible. As we have shown, before fission the animals shorten and thicken. How long before fission this begins it is not possible to say; in making the curve the period is arbitrarily taken as two hours.

When we make the corrections above described, we have the following mean dimensions at different ages, as data for the construction of our curve. The *ages* given are the *average* ages for the lots considered; thus the age for row 8, Table X. (18 to 28 minutes) is taken as 23 minutes.

TABLE XV.

Dimensions in Microns of Paramecia (Descendants of D) at Different Ages, Corrected (so far as possible) to Correspond with Those of Lot 6, Table X. Data used in making the Curves of Growth.

Age.	Lot.	Mean Length in Microns.	Mean Breadth in Microns.
Beginning constriction	Row 4, Table VIII.	82.600	64.893
Fifteen minutes after beginning constriction	" 5, " VIII.	85.774	64.493
2½ minutes after separation	" 5, " X.	107.660	59.355
9½ minutes	" 7, " X.	128.000	60.168
23 minutes	" 8, " X.	143.348	54.284
40 minutes	" 9, " X.	149.920	55.840
82½ minutes	" 10, " X.	161.524	54.192
4 hours	" 18, " X.	176.560	58.922
12 hours	" 20, " X.	188.988	62.796
18 hours	" 22, " X.	199.048	56.496
Beginning constriction	" 30, " X.	165.200	64.893

When we lay off on the vertical scale the distances corresponding to the lengths and breadths at the different periods, as given in the above table, and connect these points, we obtain the curves given in Diagram 5.

Characteristics of the Curves.—As the curves show, the length increases with great rapidity for about twenty minutes after fission; continues less rapidly for about an hour, and still less rapidly for four or five hours. Now the increase continues, though very slowly, till a maximum is reached at a length considerably greater than twice the original length; later the length decreases in preparation for

fission; this decrease continues till the length is just twice the original length. Now the constriction appears, so that the animal may be looked on as two; the length, therefore, drops in a straight line to the original length found at the beginning of the curve. The breadth decreases from the beginning till about an hour after fission; then slowly increases; it shows in the course of the twenty-four hours many fluctuations which are doubtless mainly due to differences in the environment—especially to differences in the amount of food taken. In preparation for fission the breadth increases at the same time that the length decreases.

The curve of length is much the more interesting of the two, since it is the one which represents mainly the actual growth. It is of great interest to find that this curve of growth in a single cell is of essentially the same form and character as those which have been obtained for the growth of many higher organisms, composed of many cells. A number of such curves are brought together in the recent interesting paper of Robertson (1908). Inspection shows at once that the curve of growth in *Parmecium* closely resembles that for growth of the rat, as worked out by Donaldson (1906); for growth of man, and for growth in various other organisms.

The curve of growth, as is well known, is a logarithmic curve in the cases where it has been worked out mathematically. While the growth in *Paramecium* has merely been plotted empirically, it is evident that it is essentially a similar logarithmic curve; this could doubtless be worked out from the data given.

The fact that the curve of growth is essentially the same in the unicellular organism as in the animal composed of millions of cells is in some respects surprising. In the brain of the rat, or in its body, the curve of growth is the resultant of the growth of many different groups of cells, some groups growing at one period, some at another; yet the resultant curves are of the same character as when there is growth in but a single cell.

The temporal relations shown in the curves are likewise of much interest. As our diagram shows, that portion of the curve showing the greatest curvature requires in *Paramecium* about four hours from the beginning. In the rat the corresponding part of the curve takes several months, while in man it requires several years. It

seems extraordinary that a process following the same laws should in some cases be measured by hours, in other cases by months, in others by years.

3. Effects of Growth on the Observed Variation.

A random sample of an ordinary culture of *Paramecium* contains specimens falling in all parts of the growth curves represented in Diagram 5. If we measure the various members of such a sample, as was done by Pearl (1907), we shall then find many variations in size, which variations consist to a considerable extent of different growth stages. Not all the observed variations are due to this factor, but its importance is very considerable. This will best be appreciated by running through the columns headed "coefficients of variation" in Table X. If we take samples including specimens falling in the early parts of the growth curve, when the absolute size is small but the changes with growth are very marked, then the coefficients of variation in length are high; thus in rows 4 and 5 they are 15.494 and 13.729, respectively, while in the random sample of the same culture the coefficient is but 8.834 (row 6). On the other hand, if we take specimens restricted to a very small portion of the curve, the coefficient of variation becomes very low; thus in a lot whose age falls between 18 and 28 minutes the variation is but 4.521 (row 8); at the age of 4.20 to 5 hours is 5.043 (row 17), though the variation for a random sample of this same culture is 13.262 (row 19). The effects of growth on variation are shown to the eye in Diagram 4, p. 440.

Variation at Fission.—The effects of growth on the observed variation are likewise seen when we compare random samples with individuals that are at a definite stage in the life history. Thus, if we take specimens at the beginning of fission, when the constriction first appears, we find the coefficient of variation very low, as compared with those of random samples of the same cultures. This is readily seen in the following tabulation of the coefficients of variation for the four cultures of Table X. in which the specimens beginning fission were studied (see next page).

Variation in Conjugants.—Again, the same thing appears when we compare conjugating individuals with random samples of the

same cultures. Conjugation does not occur till a certain stage of growth has been reached, and the conjugants do not include specimens undergoing the changes preparatory to fission. The conjugants would then fall in those portions of the growth curve that are nearly straight; that is, there would be in these little variation due to growth.

TABLE XVI.

Coefficients of Variation.

Lot.	Length.		Breadth.	
	Beginning Fission.	Random Sample.	Beginning Fission.	Random Sample.
1	5.368	7.765	9.082	12.877
2	5.320	8.834	6.769	11.421
4	7.541	15.279	9.911	15.683
5	6.862	10.643	12.071	13.720

Pearl (1907) has already shown that the observed variability of conjugants is less than that of random samples of the same culture. I have made extensive studies of conjugants and find the same thing. Details regarding the relation of conjugation to variation and heredity are to be taken up in a later communication; here I give merely the coefficients of variation for certain cases, as compared with those of random samples.

TABLE XVII.

Coefficients of Variation for Conjugants, as compared with those for random samples of non-conjugants of the same culture.

Lot.	Length.		Breadth.	
	Conjugants.	Non-Conjugants.	Conjugants.	Non-Conjugants.
A, Pearl.	6.668	8.185	9.398	11.112
C, "	7.439	9.123	7.910	10.894
a, Jennings.	7.392	11.578	12.409	19.176
b, "	7.678	11.026	15.766	18.142

On comparing the coefficients of variation in conjugants, as given in Table XVII., with those for specimens beginning fission (Table XVI.), and those for specimens at definite ages (Table X.), it is found that in the conjugants the variation is not so small as it is in specimens at definite growth stages. This shows clearly that nothing is required to explain the low variation of conjugants, save the fact that a certain number of growth stages (the earlier and later ones)

are lacking in these. There is no evidence of an unusually low degree of congenital variation in the conjugants, for the non-conjugating specimens beginning fission show a still lower variability (Table XVI.).

It appears highly probable that if we could examine a large number of individuals, derived from the same parent, cultivated under identically the same conditions, and all in precisely the same stage of growth, we should find coefficients of variation considerably smaller than the smallest we have found, which is 4.521 (row 8, Table X.). Indeed, if we could further exclude all inaccuracies of measurement, it is quite possible that the coefficient of variation would approach closely to zero, if it did not reach it completely. This would, of course, mean that the variations observed among the progeny of a single individual are not congenital, but are all due to growth and environmental action. Further evidence of this will come out later in this paper.

4. Effects of Growth on the Observed Correlation Between Length and Breadth.

As Diagram 5 shows, the curves of length and breadth diverge at the beginning, then run for a considerable distance nearly parallel, then finally approach each other. That is, at first the breadth decreases while the length increases; later they increase together; and still later the breadth increases while the length decreases. If a collection of specimens includes individuals in various different stages of growth (as is usually the case), then these various relations of breadth to length will deeply affect the amount of correlation observed between the two dimensions.

Thus, if we take a collection composed of various ages under one hour, when the length is increasing while the breadth is decreasing, then on the whole greater length will be associated with less breadth, so that the correlation between them will tend to be negative. This is the explanation of the negative correlation shown in Table X., rows 2, 4, 5, 7, 11, 13, 14. Next follows a period (from about the end of the first hour to the fourth) in which the inclusion of individuals of different ages tends to cause a certain degree of positive correlation, since the two dimensions are increasing together. Then

comes a long period in which both dimensions remain nearly the same—the length increasing slowly, while the breadth fluctuates. Different growth stages during this period have little marked effect on the coefficient of correlation between length and breadth; they tend to prevent its reaching 1.000, but this it would not reach for other reasons.

Now, for a certain period before fission (taken as two hours, in our curves), the length decreases while the breadth increases. Greater breadth will then be associated with less length, tending to produce again a negative correlation. If we make a collection of individuals representing various stages in this process, we should, therefore, expect to find the correlation much less than in collections taken (1) either before these processes have begun, or (2) after they are ended. We can realize this, in the main, by taking from a large random sample all the largest specimens (which are, of course, the older ones) and combining these into a single correlation table with specimens from the same culture that are beginning fission (the *oldest* specimens of the culture). I performed this operation for lot 1 of Table X. This collection contains 131 specimens beginning fission (row 25, Table X.), and 134 specimens (not dividing) that are 196 microns, or more, in length (row 28, Table X.); throwing these together, we have a collection of 264 of the oldest specimens in the culture (row 29, Table X.). For the 131 specimens beginning fission the coefficient of correlation is + .6546; for the 134 large specimens it is + .4681. When the two are taken together the correlation disappears. The computation gives us a coefficient of + .0350, but this is less than its probable error (.0415), so that the figures have no significance; no correlation appears.

The effects of the inclusion of various growth stages on the observed correlation shows itself in many other ways, which will become evident to anyone who carefully examines the data of Table X., in connection with our curves of growth (Diagram 5), and the relations brought out in the foregoing paragraphs. Note, for example, the coefficients of correlation for lot 9 (rows 16–18, Table X.). For the specimens 3 to 4 hours old the coefficient is but .3201, and for those 4.20 to 5 hours old it is .5557. When we throw these two lots together, so as to include a much greater proportion of the

growth curve, the correlation rises to .7132. In this larger collection the short specimens are much the narrower, the large specimens much broader—giving high positive correlation. Slight changes in one dimension may not be accompanied by notable changes in the other, while *great* changes in one are always accompanied by changes in the other. This is a relation which we shall meet again.

While thus growth has a very great effect on the correlation to be computed from the measurements of a collection of *Paramecia,* it is important to bear in mind the fact that it is by no means the only factor concerned in correlation. This becomes evident as soon as we take a collection in which the specimens are all in nearly the same stage of growth; the coefficient of correlation is then high. This is perhaps best realized by considering specimens in the beginning of fission. As we have before noticed, in the collection of 131 specimens beginning fission, from lot 1, great pains were taken to include only a single stage in the process. This collection gives a high positive correlation of .6546. This correlation can be due only to the fact that in specimens at a single growth stage the length and breadth tend to bear a certain proportion to each other. The effects of this are clearly seen in many other collections of Table X. Thus, in rows 8, 9 and 15 the specimens all fall in the period when length is increasing while breadth is decreasing; yet there is in each case a small positive correlation. This is due to the fact that the period of growth over which each collection extended was small, so that the negative correlation due to growth was more than counterbalanced by the inherent proportionality of length to breadth. A collection including only specimens that were all in the same stage of growth would undoubtedly (other things begin equal) show a high correlation between length and breadth, no matter what point on the growth curves they represented. This signifies, of course, that in any given stage of growth the relation of length to breadth tends to be the same in all specimens—although in *different* stages of growth this is often not the case. Other factors which modify the correlation will be considered in the later sections of this paper; a summary of all these factors will be presented in a special section.

With this we conclude our study of growth in *Paramecium;*

being prepared to understand the part played by this in the observed variations and correlations, we may pass to other factors affecting these.

IV. THE EFFECTS OF ENVIRONMENTAL CONDITIONS ON DIMENSIONS, VARIATION AND CORRELATION.

The data for the study of growth, just concluded, show incidentally that environmental conditions affect profoundly the dimensions, variation and correlation in *Paramecium*. As we have seen, samples taken from the same culture on two successive days are not strictly comparable for determining matters relating to growth, because of the environmental changes from day to day, inducing marked changes in the organisms. Thus, in a given culture we found that the mean length at the age of 1¼ to 1½ hours was 161.524 microns; three days later specimens more than twice as old, from the same culture, were smaller, measuring but 149.636 microns. We wish now to investigate the causes of such differences.

We shall not attempt at present a systematic investigation of the effects of different chemical and physical agents on size, form and variation, though this is a matter which much needs study. Our present object is rather to examine the effects of altered nutritional conditions and of the commoner "favorable" and "unfavorable" conditions. We shall study the variations from the standpoint of interest in the organism rather than in the agents inducing them, the purpose being to form a conception of the changes which may be looked for in *Paramecium* as a result of common alterations, mainly nutritional, in its cultural conditions. One of the results of this study will be to show that we cannot assign a definite effect to each agent taken in any absolute way. What effect a given agent will have depends on the previous condition of the organisms on which it acts. The same agent produces at one time an increase in size, at another a decrease; at one time it increases the variability; at another it decreases it. A given agent may either increase the positive correlation between length and breadth, or it may decrease it or convert it into a negative correlation. In succeeding days the same agent may produce these diverse effects on the same set of *Paramecia*.

Yet, of course, these results are not produced haphazard; what we wish to study are the laws they follow.

The effects of the environment were studied mainly on the same animals that served for the study of growth. Two strains were used; one consisted of descendants of the individual *D*, of the *caudatum* form, the other of descendants of *c* (*aurelia* form). The results show the extent of the variations producible through environmental action in the progeny of single individuals multiplying by fission. No conjugation occurred in the *D* strain during the time it was under experimentation. On a given date, therefore, the age of the individuals, as measured in generations of the "cycle," was about the same.

Table XVIII. gives a summary of the statistical results in the experiments on the effects of the environment; it will be referred to frequently in the following account (see next page).

1. Progeny of *D* (*caudatum* Form).

The individual *D* was isolated April 12, 1907; it measured, as nearly as could be determined when alive, about 250 microns. It was placed in culture fluid made of boiled hay and the progeny were kept in such cultures for months. Characteristic progeny of *D* are shown in Fig. 1, *a* to *d*.

The experiments with the descendants of *D* may be divided into three series.

First Series.

Old Large Culture.—On June 11 a sample of 100 of the descendants of *D* was killed, from a hay culture that had stood several weeks and was flourishing, though multiplication was not occurring actively. This culture was in a vessel about nine inches across. The measurements of this sample are given in Table V. (page 406), while the constants are found in row 1, Table XVIII.

Effects of Fresh Hay Infusion.—Three days after these measurements were taken, a number of individuals of this culture were removed and placed in a fresh hay infusion, in a watch-glass; in this they were allowed to remain 24 hours. The increased food in the fresh infusion caused them to increase much in breadth (from 49.000 microns to 64.880 microns), and at the same time to begin to

TABLE XVIII.

Effects of Environmental Conditions on Dimensions and Constants of Varia-
same culture at the same time (except in rows 12, 15 and 20). The
appendix or elsewhere, in which fuller data are given for the lot in

Row.	A. *Progeny of D. First Series.*	Number of Individuals.	Table.	Length.			
				Mean in Microns.	Standard Deviation in Microns.	Coefficient of Variation.	Range of Variation in Microns.
1	Random sample of *D*, June 11, 1907	100	5	188.360± .980	14.532± .692	7.715±.370	128–228
2	Same after 24 hours in fresh hay infusion, June 15	100	51	184.680± .848	12.596± .600	6.821±.327	156–224
3	Two days after last; culture fluid not renewed, June 17	135	6	185.008± .836	14.420± .592	7.794±.324	148–212
4	Same, after 24 hours in fresh hay infusion. Rapid multiplication, June 18	195	7	176.124±1.128	23.360± .797	13.262±.461	104–220
5	Same, one week later; bacteria multiplied injuriously, June 25	178	52	201.888±1.147	22.680± .811	11.233±.407	140–256
6	Starvation, same as row 2, but left 11 days in small quantity of fluid, June 25	100	53	149.360± .736	10.896± .520	7.296±.350	128–188
	Second Series.						
7	24 hours in fresh hay infusion; rapid multiplication, July 17	200	30	1 .100 .776	16.264± .548	8.834±.300	140–216
8	Same as last, but starved a week, July 24	150	19	146.108± .563	10.228± .398	7.003±.274	120–176
9	Same as last, but 24 hours in fresh hay infusion, July 25	350	20	163.932± .754	20.928± .533	12.767±.331	120–220
10	Same as last, but kept 1 week without change of fluid, July 31	150	21	174.400± .819	14.876± .579	8.530±.335	132–212
11	Same as last, but kept 48 hours in fresh hay infusion, Aug. 3	150	22	191.360± .943	17.116± .666	8.945±.351	136–240
12	Rows 8, 10 and 11 combined	450	—	180.624± .748	23.537± .529	13.795±.316	120–240
	Third Series.						
13	Slender, old culture, in large jar, September 15	100	54	202.280±1.031	15.284± .729	7.556±.362	160–232
14	Same as last, after 48 hours in fresh hay infusion, September 15	100	55	175.320±1.060	15.708± .749	8.959±.431	124–216
15	Rows 13 and 14, combined	200	—	188.800± .980	20.540±1.092	10.879±.371	124–232
	B. *Progeny of c.*						
16	Random sample of *c*, June 11, 1907	100	4	130.120± .628	9.284± .443	7.134±.342	104–156
17	Random sample of *c*, August 9	100	56	123.666± .813	12.040± .573	9.736±.469	100–160

TABLE XVIII.—*Continued.*

tion in Paramecium. Each row consists of specimens taken from the column headed "Table" gives the number of a table found in the question.

Breadth.				Mean Index, or Ratio of Breadth to Length. Per Cent.	Coefficient of Correlation.
Mean in Microns.	Standard Deviation in Microns.	Coefficient of Variation.	Range of Variation in Microns.		
49.000±.548	8.144±.388	16.618± .814	28–76	26.029	.4188±.0556
64.880±.580	8.624±.412	13.292± .645	44–88	35.131	.6469±.0392
43.556±.392	6.748±.276	15.490± .651	32–60	23.517	.5955±.0375
47.364±.344	7.132±.244	15.057± .526	32–72	27.153	.3945±.0408
56.112±.395	7.808±.279	13.913± .507	36–80	27.850	.6771±.0274
38.080±.356	5.288±.252	13.881± .675	28–52	25.515	.4481±.0539
46.020±.251	5.256±.177	11.421± .390	36–60	25.084	.4282±.0389
31.180±.212	3.881±.151	12.473± .493	20–40	21.337	.3906±.0467
46.684±.488	13.484±.344	28.879± .793	20–80	28.236	.8463±.0102
44.800±.429	7.796±.304	17.397± .698	32–68	25.657	.5704±.0372
54.880±.431	7.824±.305	14.255± .566	36–84	28.639	.7364±.0252
43.600±.377	11.852±.266	27.184± .654	20–84		
49.600±.298	4.412±.210	8.896± .428	40–60	24.593	.4085±.0562
63.160±.472	7.000±.334	11.083± .535	44–80	36.123	.5376±.0480
56.380±.427	8.956±.302	15.884± .549	40–80	30.350	—.2613±.0414
36.280±.260	3.880±.184	10.700± .516	28–44	27.913	.5208±.0492
33.600±.400	5.917±.283	17.608± .865	23.3–50	27.136	.6258±.0410

TABLE XVIII.—*Continued.*

Row.	B. Progeny of *c*. Continued.	Number of Individuals.	Table.	Length			
				Mean in Microns.	Standard Deviation in Microns.	Coefficient of Variation.	Range of Variation in Microns.
18	Same as last, but 24 hours after addition of boiled grass, August 10	225	49	114.163± .784	17.443± .555	15.279±.497	73.3–160
19	Same as row 17, but 24 hours in fresh hay infusion, August 12	100	50	114.033± .820	12.140± .580	10.646±.513	86.7–146.7
20	Rows 17 and 19, together; same animals, half in old fluid, half in new	200	—	118.850± .622	13.037± .440	10.698±.374	86.7–160
21	Conjugating culture, large vessel, September 25	200	57	158.800± .877	18.384± .620	11.578±.396	124–200
22	Same culture, 5 days after, food getting scarce	100	58	129.640± .867	12.848± .613	9.911±.477	100–152
23	Large, old culture, January 23, 1908;	100	59	144.880±1.097	16.264± .776	11.224±.542	100–176
24	Same, two days later, January 25, 1908	50	—	130.640±1.227	12.863± .868	9.846±.670	104–156
25	Another old culture, January 23, 1908	100	—	137.200± .842	12.488± .596	9.102±.438	104–162
26	Same as row 23, but starved 3 weeks, February 14	37	—	102.594±1.161	10.467± .821	10.202±.808	76–128
27	Same as row 23, but cultivated in small watch glass, January 30–February 15, 1908	100	60	100.320± .528	7.828± .373	7.804±.374	76–120

multiply. The measurements of a sample of 100 of these are given in Table LI. (appendix), while the constants are found in row 2, Table XVIII. The increased breadth, with little change in the length, of course, results in an increase of the mean index or ratio of breadth to length; while in row 1 this was but 26.029 per cent., in the present lot it is 35.131 per cent. It is worthy of notice that with the increase in ratio of breadth to length there is an increase in the correlation between length and breadth from .4188 to .6469.

Scarcity of Food.—The watch-glass culture just described (row 2, Table XVIII.) was now allowed to stand for three days (till June 17) without renewing the culture fluid. The animals had multiplied greatly, so that food became scarce; as a result they became thin. The measurements are given in Table VI. (page 412) and the constants in row 3, Table XVIII. While the length remained about the same, the mean thickness of the body decreased from 64.880 to 43.556 microns. The mean ratio of breadth to length fell from 35.131 per

TABLE XVIII.—*Continued.*

Breadth.				Mean Index or Ratio of Breadth to Length. Per Cent.	Coefficient of Correlation.
Mean in Microns.	Standard Deviation in Microns.	Coefficient of Variation.	Range of Variation in Microns.		
34.207±.241	5.363±.171	15.683± .511	20–50	30.177	.6757±.0244
47 300±.437	6.490±.310	13.720± .667	36.7–66.7	41.455	.8152±.0226
40.450±.441	9.247±.312	22.857± .810	23.3–66.7		.1758±.0462
38.560±.353	7.396±.249	19.176± .670	16–60	24.244	.7135±.0234
35.440±.400	5.928±.283	16.730± .820	20–48	27.262	.7576±.0287
54.160±.765	11.346±.541	20.948±1.042	32–84	37.106	.8500±.0187
37.760±.639	6.697±.452	17.736±1.233	28–52	28.975	.4141±.0790
37.960±.413	6.128±.292	16.142± .790	24–56	27.625	.6691±.0373
23.892±.644	5.804±.455	24.291±2.014	16–40	23.067	.8018±.0396
26.480±.266	3.944±.188	14.895± .753	16–36	26.321	.7671±.0278

cent. to 23.517 per cent., and at the same time correlation between the two fell from .6469 to .5955.

Thus, within a week we find enormous fluctuations in breadth, due to changes in the amount of food, while the length remains about the same. The breadth is much more affected by nutritional changes than is the length.

Rapid Multiplication.—To the watch-glass culture just described (row 3) new hay infusion was added. Twenty-four hours later (June 18) multiplication was occurring actively; stages of fission and all the stages of growth were numerous. Measurements of 195 specimens, taken at random at this time (Table VII., page 412, and row 4, Table XVIII.) show a very great increase in the range and amount of the variability in length, while there is little change in the breadth. This is, of course, due to the fact that the culture contains many young; these differ much from the adults in length, but little in breadth. The mean length decreases from 185.008 to 176.124

microns, and the variability in length almost doubles, increasing from 7.794 to 13.262. Owing to the inclusion of many young individuals, in which the length is increasing while the breadth is stationary or decreasing, the correlation between length and breadth decreases to .3945. Inspection of Tables VI. and VII. (page 412) shows at a glance the great effect of nutrition and division on the range and distribution of variations in size and form.

Injurious Bacteria.—A remarkable effect of what may be called "bad" conditions is shown in this series of experiments. The same watch-glass culture shown in row 3, Table XVIII., was allowed to stand for a week, till June 25. Bacteria of a certain character multiplied greatly, and seemed to get the upper hand of the *Paramecia.* The latter became opaque and abnormal in appearance, and some of them died, disintegrating into shapeless masses. It was now observed that many of the specimens still living were very large, and that variation in size was extreme. The distribution of the variations is shown in Table LII.; the constants in row 5, Table XVIII. Though no multiplication is occurring, so that no young are present, the range of variation is from 140 to 256 microns, while in row 3, from which this lot is derived, the range is only from 148 to 212 microns. The mean length has increased to 201.888 microns, one of the greatest mean lengths ever observed in progeny of *D.* The maximum size for descendants of *D* was likewise reached in this culture; in no other case were specimens 256 microns long observed.

Starvation.—In striking contrast with the effects of much nutrition (row 4, Table XVIII.) and of injurious bacteria (row 5) are the results of starvation (Table LIII., and row 6, Table XVIII.). The starving culture consisted of individuals from the same culture as row 1, placed in fresh hay infusion June 14. The constants before they were placed in the hay infusion are given in row 1, Table XVIII., while the immediate effects of the infusion are shown in row 2 of the same table. The same animals were left in this fluid for eleven days, till June 25. They had evidently begun to starve; they were small and thin and almost half of them had died. The dimensions are given in Table LIII., and the constants in row 6, Table XVIII. The length had fallen from 184.680 to 149.360 microns; the breadth from 64.880 to 38.080 microns. The breadth

decreases with lack of food proportionately more than does the length, so that the ratio of length to breadth has fallen from 35.131 per cent. to 25.515 per cent. It is to be noticed, however, that this greater proportionate decrease of breadth takes place in the first days after the withdrawal of abundant food, since after the animals had been only three days without new food the ratio of breadth to length fell to 23.517 per cent. (row 3, Table XVIII.); it did not decrease farther after starvation began.

A comparative inspection of Tables VII. (page 412) and LIII. (appendix) shows to the eye the very great effects of nutrition on size and variation.

Second Series.

After the series of experiments described above, the progeny of *D* were kept in large culture jars of hay and water for about three weeks. Then followed an exceedingly instructive series of experiments on the effects of environmental conditions, the results of which are shown in Tables XIX.–XXII. and in the large Table XVIII., rows 7 to 12. Mere inspection of the correlation tables shows the effects in such a striking way that I have placed the main tables together in the text, instead of relegating them to the appendix.

Fresh Hay Infusion.—On July 16, 1907, specimens from the large cultures were placed in a watch-glass of hay infusion and allowed to remain twenty-four hours. This induced rapid multiplication; while this was occurring a random sample of 200 specimens was measured, with the results shown in Table XXX. (appendix), and in row 7, Table XVIII.

Starvation.—Next these were allowed to starve for a week; then 150 specimens were measured (Table XIX., and row 8, Table XVIII.). The results may be compared with our other starving culture of Table LIII., and row 6, Table XVIII. It will be noticed that for both length and breadth the amount of variation is not great; that the absolute dimensions are small; that the ratio of breadth to length (21.337 per cent.) is the least we have even seen, and that the correlation between length and breadth is very low (.3906).

Effects of Abundant Food on a Starving Culture.—Now this starving culture (Table XIX.) was placed for twenty-four hours in

TABLE XIX.

Correlation Table for Length and Breadth of a Starving Culture of Descendants of D. (Row 8, Table XVIII.)

Length in Microns.

Breadth in Microns.	120	124	128	132	136	140	144	148	152	156	160	164	168	172	176	
20						1										1
24	1	1	1		1	2	2		1	1						10
28	1		6	2	5	12	10	3	9	4	2					54
32		1		4	3	8	1	8	11	9		2		1		48
36					2	3	3	7	1	8	3	1	1		1	30
40			1					3		1	1	1				7
	2	2	8	6	11	26	16	21	22	23	6	4	1	1	1	150

Length—Mean, 146.108 ± .563μ Breadth—Mean, 31.180 ± .212μ
St. Dev., 10.228 ± .398μ St. Dev., 3.881 ± .151μ
Coef. Var., 7.003 ± .274 Coef. Var., 12.473 ± .493

Mean Index or Ratio of Breadth to Length, 21.337 per cent.; Coef. Cor., .3906 ± .0467.

TABLE XX.

Correlation Table for Length and Breadth of Descendants of D when a Starving Culture (Table XIX.) is placed for 24 Hours in Fresh Hay Infusion. (Row 9, Table XVIII.)

Length in Microns.

Breadth in Microns.	120	124	128	132	136	140	144	148	152	156	160	164	168	172	176	180	184	188	192	196	200	204	208	212	216	220	
20		1			1																						2
24	3	1	1	1	5	4	1	2																			18
28	1	1	2	3	3	11	5	4	1	2	1																34
32			1	1	6	3	6	4	3	2		1															27
36			1	1	3	3	1	3	4	5		1															22
40			1		2		2	3	4	3	3	3	2	2	2												27
44				1		1	2	1	4	2	5	6	3	1			1										27
48					1		2		2	5	5	9	4	2	2	3	1		1	1							38
52							1	1	2	2	7	7	4	3	1	1	4	2	3								38
56										1	3	2	1	3	10	6	3	2	6	1	1						39
60									1		1		1	2	4	5	6	3	2	3	2		2				32
64														1	1	8	3	4	2	4						1	24
68																	1		3	1	6		1		1		13
72															1	1		1	1					1			5
76																1			1			1					3
80																								1			1
	4	3	6	7	21	22	20	18	21	22	25	29	15	14	21	25	19	12	19	10	9	1	3	2	1	1	350

Length—Mean, 163.932 ± .754μ Breadth—Mean, 46.684 ± .488μ
St. Dev., 20.928 ± .533μ St. Dev., 13.484 ± .344μ
Coef. Var., 12.767 ± .331 Coef. Var., 28.879± .793

Mean Index, 28.236 per cent.; Coef. Cor., .8463 ± .0102.

TABLE XXI.

Correlation Table for Length and Breadth of Descendants of D, after Remaining One Week in Hay Infusion, Unchanged. (Row 10, Table XVIII.)

Length in Microns.

Breadth in Microns.	132	136	140	144	148	152	156	160	164	168	172	176	180	184	188	192	196	200	204	208	212	
32					1		1	3	2		1	1										9
36	1		2			3	2	4	3	3	1	2	1									22
40						1	4	2	3	3	2	10	2	2	1		1					31
44						2	1	1	2	7	5	5		1	2	1						27
48					1						3	2	3	4	3	1	2	4				23
52					1			1		2		4	2	1	2	2	1	1	2		1	20
56									1			1	2			1	3			1		9
60										1	1		1	2		1						6
64																						0
68												1						1	1			3
	1	0	2	0	3	6	8	11	11	16	13	26	11	10	8	6	7	6	3	1	1	150

Length—Mean, 174.400 ± .819μ Breadth—Mean, 44.800 ± .429μ
St. Dev., 14.876 ± .579μ St. Dev., 7.796 ± .304μ
Coef. Var., 8.530 ± .335 Coef. Var., 17.397 ± .698

Mean Index or Ratio of Breadth to Length, 25.657 per cent.; Coef. Cor., .5704 ± .0372.

TABLE XXII.

Correlation Table for Length and Breadth, after the Culture shown in Table XXI. has remained 48 hours in Fresh Hay Infusion. (Row 11, Table XVIII.)

Length in Microns.

Breadth in Microns.	136	140	144	148	152	156	160	164	168	172	176	180	184	188	192	196	200	204	208	212	216	220	224	228	232	236	240	
36	1				2	1																						4
40				1					1	1																		3
44							1	1		1	1	3	1															8
48			1					1	4	2	3	2	7	3	1	1		1										26
52								1			3	3	2	5	8	3	1	1		2								29
56										1		2	2	4	1	8	1		2	6	1				1			29
60											1		1	1	4	3	4	3	3	2	1	1						24
64												1	2	1	1	3	3	1	3	1	1		1					18
68																1			1	1		1						4
72																			1	1	1	1						4
76																												0
80																												0
84																											1	1
	1	0	1	1	2	1	1	3	5	5	8	11	15	14	15	19	9	6	10	13	4	3	1	0	1	0	1	150

Length—Mean, 191.360 ± .943μ Breadth—Mean, 54.880 ± .431μ
St. Dev., 17.116 ± .666μ St. Dev., 7.824 ± .305μ
Coef. Var., 8.945 ± .351 Coef. Var., 14.255 ± .566

Mean Index, 28.639 per cent.; Coef Cor., 7364 ± .0252.

a fresh hay infusion. At once the culture "spread out" greatly, in a way that will appear on comparing Table XIX., for the starving culture, with Table XX., for those twenty-four hours in nutritive fluid. Many of the animals began to grow at once after they were placed in the nutritive fluid, so that the maximum length increased from 176 to 220 microns, the maximum breadth from 40 to 80 microns (see rows 8 and 9, Table XVIII.). Others had not yet begun to increase when the sample of Table XX. was taken, so that

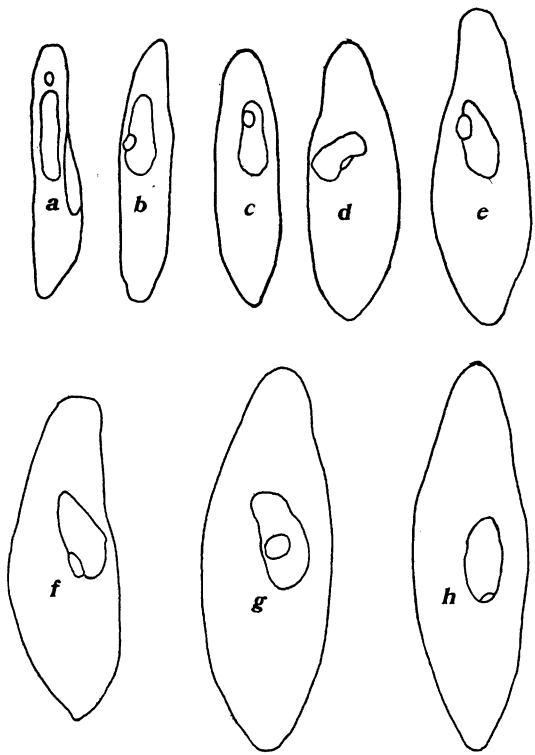

FIG. 5. Characteristic forms and sizes from a culture of descendants of *D* (*caudatum* form), that had been starved for a week (Table XIX.), then was left twenty-four hours in fresh hay infusion (Table XX.). *a* and *b*, Starved specimens. *c*, *d*, *e*, *f*, transitional forms, becoming large and plump in the abundant food; *g*, characteristic large, plump form. *a* to *g* from Table XX. *h*, characteristic form a week later (Table XXI.); animals becoming thinner again, but retaining the increased length. All $\times 235$.

the minimum size remained as before; and between these extremes all intermediate gradations were found. Fig. 5 shows characteristic forms and sizes from this culture, *a* and *b* showing the starving condition, while *c* to *f* show various stages in the transition to the largest size, one of which is shown at *g*.

As a result of these changes, the variability has increased enormously. The coefficient of variation in length has increased in twenty-four hours from 7.003 to 12.767; that for breadth has more than doubled, increasing from 12.473 to 28.879. The mean size has likewise increased greatly, while the ratio of breadth to length has changed from 21.337 per cent. to 28.236 per cent. Perhaps the most striking change is in the correlation between length and breadth. In the starving culture this is but .3906; twenty-four hours later it has become, in the growing culture, .8463—one of the highest coefficients of correlation that I have ever found in *Paramecium*. It is evident that breadth and length are increasing proportionately, on the whole, so that the inclusion of different degrees of increase in size in Table XX. gives a high coefficient of correlation. Furthermore, the fact that fission had not begun in this lot permits the correlation to remain high; if there were many young included, the correlation would, of course, be lowered. With every increase of 10 microns in length the breadth increases 5.452 microns.

Fluid Unchanged for a Week.—Now the same culture was kept for a week in the same fluid. The animals had reached more nearly a condition of equilibrium; the variability, and with it the correlation, had greatly decreased, while the mean length had increased (Table XXI., and row 10, Table XVIII.). It is noticeable here, as in many other cases, that the coefficient of correlation decreases when the ratio of breadth to length decreases.

Forty-eight Hours in New Culture Fluid.—The addition of new hay infusion to the culture just described caused in forty-eight hours a considerable increase in mean length and breadth, while the variation did not change greatly (Table XXII., and row 11, Table XVIII.). Again, as the ratio of breadth to length increases, the correlation between the two likewise increases.

Résumé.—Polygons showing the changes in the animals of this series, from the starving condition of Table XIX. to the well-fed

condition of Table XXII. are given in Diagram 6; these, taken in connection with Fig. 5 and with Tables XIX. to XXII. give a good idea of the changes in dimensions and variation that may be pro-

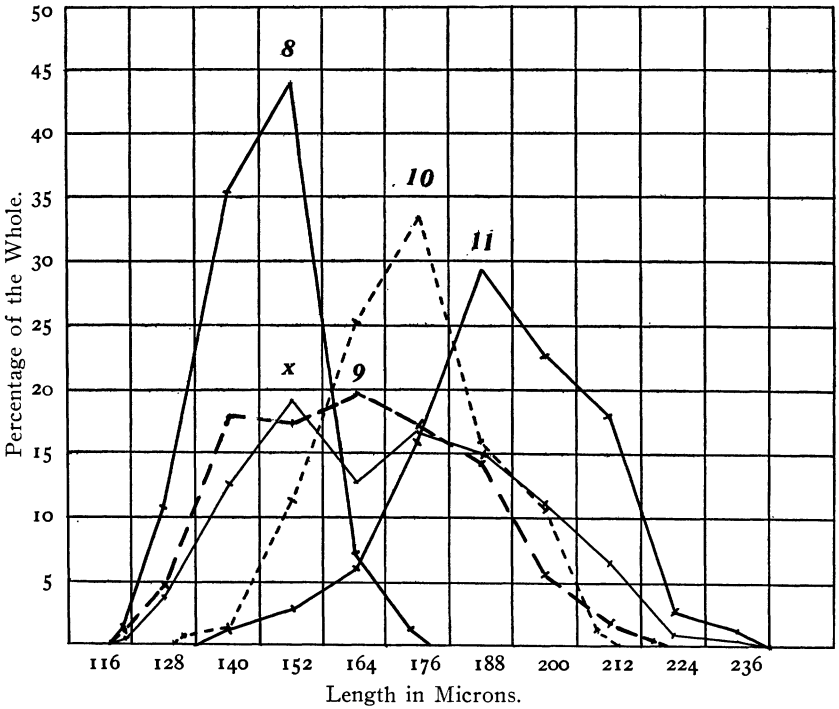

DIAGRAM 6. Polygons of variation in length for a culture of descendants of the individual *D* when subjected successively to varied conditions of nutrition. The numbers above the highest points of the polygons correspond to rows of Table XVIII., in which are given the constants for the different polygons. *8*, culture starved a week. *9* (heavy broken line), same as 8, but after 24 hours in fresh hay infusion. *10*, same after one week in the same fluid, unchanged. *11*, same after 48 hours in fresh hay infusion. *x*, polygon for combination of 8, 10 and 11, showing its resemblance to the polygon for 9 alone.

The correlation tables for these polygons are numbers XIX. to XXII., pages 466, 467.

duced in a short time by changes in the conditions of nutrition. Evidently Table XX., taken twenty-four hours after the starving specimens were placed in the fresh hay infusion, is a transitional

condition, including representatives of the small, starving condition, the well grown condition, and intermediate states; it is a sort of a résumé of the variations due to nutrition. If we add together the tables given by the starving culture (earlier than Table XX.) and the two well-fed cultures (later than Table XX.), we get a collection of 450 individuals, in which the variation in length and breadth is about the same as for Table XX. (see row 12, Table XVIII.). For Table XX. the coefficients of variation for length and breadth are 12.767 and 28.879; the corresponding coefficients for the three lots combined are 13.795 and 27.184.

Although the animals are all descended from the same parent and have lived under the same conditions save for the ten days during which these experiments lasted, we find that in the period just mentioned the polygons of distribution of variations in length have so changed that the one for the end of the ten day period (11, Diagram 6) hardly more than overlaps at one end that for the beginning of the period (8, Diagram 6).

Addition of fresh hay infusion causes in these cases an increase in length, in breadth, in variation, and in the correlation between length and breadth. But whether these results shall follow depends upon the previous condition of the animals. This is illustrated by the fact that there is one exception to the statement just made; the variability in breadth decreased in place of increasing in the transition from Table XXI. to Table XXII. The effect of the previous condition is better seen in the experiments of the third series, to be described next.

Third Series.

A culture of the descendants of *D* was rather ill-fed, though not starving; the animals were long and slender (Fig. 6, *a* and *b*). Half of these were allowed to remain in the old fluid, while half were placed in fresh hay infusion. After forty-eight hours, a random sample of each set was measured. The measurements of the set in the old fluid are given in Table LIV., the constants in row 13, Table XVIII. The results of keeping the animals forty-eight hours in the fresh infusion are shown in Table LV., and in row 14, Table XVIII. The animals grew plump and multiplied; the mean breadth increased

from 49.600 microns to 63.160 microns (characteristic form shown at *c*, Fig. 6). But *the mean length decreased* from 202.280 to 175.320 microns. This is probably due to rapid multiplication; the animals now divide before they reach the length which they had at first. As a result of the increase in breadth and decrease in length, of course,

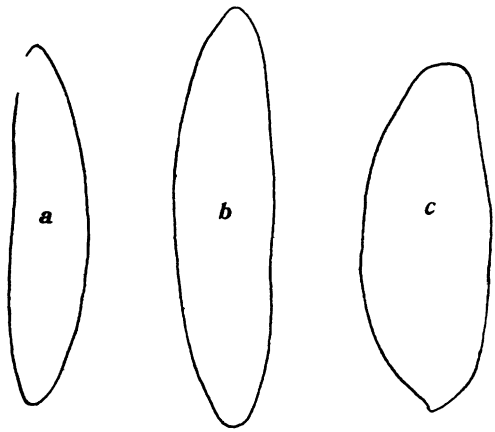

FIG. 6. *a* and *b*, characteristic slender specimens from row 13, Table XVIII. *c*, characteristic short plump specimen from row 14, Table XVIII.; produced by allowing those of row 13 to remain 24 hours in fresh hay infusion. Descendants of *D* (*caudatum* form). All × 235.

the mean ratio of breadth to length increased greatly, from 24.593 per cent. to 36.123 per cent. With the increase of this ratio, the correlation likewise increased, as is usually the case. The variation increased, both in breadth and in length.

These are the results if we consider separately the two samples, taken forty-eight hours apart. But if we throw them together, looking at them merely as a sample of the descendants of *D*, taken at intervals, we get a surprising effect on the correlation between length and breath. The marked positive correlation in the two samples taken separately *disappears and is replaced by a negative correlation.* In the first sample the correlation is + .4085; in the second it is + .5376; in the two together it is — .2613. (The constants for the two together are given in row 15, Table XVIII.) The negative correlation is, of course, due to the fact that the nutritive fluid causes the breadth to increase and the length to decrease, so that, on the

whole, when the two samples are taken together, greater breadth is associated with less length.

2. Progeny of *c* (*aurelia* Form).

With the smaller *Paramecia,* progeny of the small individual *c,* a similar series of experiments was undertaken. The individual *c* came from the same wild culture as *D;* its length, as nearly as could be determined in life, was 120 microns. It was isolated April 8, 1907. Fig. 3 shows some examples of the descendants of *c,* drawn to the same scale as the figures of the descendants of *D.*

Random Sample.—On June 11 one hundred of the progeny of *c* gave the measurements shown in Table IV., page 405, the constants being given in row 16, Table XVIII.

Effect of Adding Boiled Hay.—On August 9 a fairly flourishing culture of the descendants of *c* was examined, with the results shown in Table LVI., and in row 17, Table XVIII. To this culture a quantity of boiled grass was added; this caused rapid multiplication. Twenty-four hours later a sample of 225 specimens was measured, with the results shown in Table XLIX., and row 18, Table XVIII. The added nutrition has caused the mean length to decrease, while the mean breadth remains nearly the same. This is due to the fact that the main effect of the nutrition was to cause rapid multiplication rather than growth in size. The coefficient of variation in length increased greatly, from 9.736 to 15.279, while the variation in breadth remained about the same, though with a slight *decrease.* This peculiar result is mainly due to the fact that the culture after the addition of the grass (row 18) contains many young specimens, which differ from the adults greatly in length, but little in breadth. As usual, we find that an increase in the ratio of breadth to length is accompanied by an increase in the correlation between the two.

Effect of Fresh Hay Infusion.—The next day (August 11) another lot from the culture shown in Table LVI. (row 17, Table XVIII.) was placed in a fresh hay infusion and left twenty-four hours. This nutritive fluid caused the animals to become very plump, while at the same time a moderate amount of fission was induced. The results are shown in Table L., and in row 19, Table

XVIII. As there appears, the mean breadth increased from 33.600 to 47.300 microns. The length, on the other hand, decreased from 123.666 to 114.033 microns. The mean ratio of breadth to length thus increased very greatly, from 27.136 per cent. to 41.455 per cent. The latter is the largest mean index I have ever observed in *Paramecia* not selected with relation to the age of the individuals; it is exceeded only by the mean index of the young halves during fission (see Table X.). With the increase in the mean ratio of breadth to length, there is as usual an increase in the correlation between the two dimensions; this reaches the unusually high value of .8152. The nutritive fluid left the variation in length about the same, but considerably decreased the variation in breadth. This is undoubtedly due to the fact that before the hay infusion was introduced some of the specimens were well fed, some poorly fed, as the chances of the daily life determined; while after the infusion was introduced *all* were well fed, so that there was less variation in breadth than before. Characteristic forms after the infusion was introduced are shown in Fig. 3, *a* to *c* (page 423).

The facts in these cases are nearly parallel with those observed in the third series of experiments on the progeny of *D* (Table XVIII., rows 13–15). If we combine the two samples of *c* (row 20, Table XVIII.), as we did those of *D*, the effect is, as in the case of *D*, to decrease greatly the correlation between length and breadth But in the present case the very high positive correlation of the two samples taken separately is not entirely overcome by combining them, though the correlation falls to .1758. The actual numerical coefficient just given is the resultant of a number of conflicting factors. In the two samples taken separately greater length is associated on the whole with greater breadth, giving high positive correlation, which in passing from Table LVI. to Table L. an increase in breadth is associated with a decrease in length, tending to diminish the correlation. The facts show clearly that the observed statistical correlation does not involve any necessary and constant relation of the one dimension to the other; both dimensions depend on various factors, which sometimes act in the same way on both, sometimes differently.

Combining the two samples of *c* (as in row 20, Table XVIII.), gives, of course, increased variation, illustrating, like most of our

results, the fact that a definite coefficient of variation cannot be considered characteristic of a given species or race. The observed variation depends on many factors.

Conjugating Culture.—The progeny of *c* I divided into two sets, both of which were kept in larger culture vessels and maintained by adding boiled hay at intervals. September 25 one of these cultures was found to be undergoing an epidemic of conjugation (though, of course, all were progeny of a single individual). The details regarding the relation of conjugation to the phenomena we are studying are to be taken up in a later communication, but I will give here the essential facts regarding dimensions and constants of variation, in order that our picture of the changes undergone by the *c* line may be as complete as possible. A random sample of the non-conjugants of this conjugating culture gave the results shown in row 21, Table XVIII., and in Table LVII. The mean length (158.800 microns) was considerably greater than has been observed in any other culture of *c*. Whether this fact has any relation to the occurrence of conjugation, or whether it is merely a matter of the environmental conditions must remain for the present a question.

Scarcity of Food After Conjugation.—This conjugating culture was allowed to stand five days. All conjugation ceased and the food began to get scarce. Now a sample gave the results shown in row 22, Table XVIII., and in Table LVIII. The length had decreased from 158.800 to 129.640 microns. Breadth likewise decreased, though not in so great a proportion as length, so that the ratio of breadth to length increased. As is usual when this ratio increases, the coefficient of correlation likewise increased.

Variation in Different Divisions of the Same Pure Line on the Same Date.—After the observations just described, the two cultures composed of the progeny of *c* were maintained for several months. On January 23, 1908, samples from each were measured, giving the results shown in rows 23 and 25, Table XVIII. As is evident, the two differed considerably. The details do not demand attention, save that in one of these old cultures (row 23, and Table LIX.) the coefficient of correlation between length and breadth was the highest I have ever observed in *Paramecium,* reaching .8500. Both these cultures were flourishing and well fed.

Effects of Lack of Food.—From the culture shown in row 23, Table XVIII., a large number of specimens were removed and placed in a small watch-glass, which was allowed to stand for two days. The food decreased rapidly and the animals became smaller, giving the results shown in row 24, Table XVIII. The mean length had decreased 10.174 per cent.; the mean breadth 33.024 per cent. These were now allowed to stand for three weeks more in the watch-glass, without adding food. At the end of this time they were in the extremes of starvation, and only 37 specimens remained of the many hundreds originally present. These 37 gave the results shown in row 26, Table XVIII. As compared with the original condition of row 23, the mean length had decreased 30.638 per cent., the mean breadth 55.886 per cent. A peculiar fact is that this starving culture shows a very high coefficient of correlation between length and breadth (.8018), while in our other starving cultures this has not been the case (see rows 6 and 8, Table XVIII.).

From the culture of large specimens shown in row 23 another lot was removed January 30 and kept in a small watch-glass, new hay infusion being added at intervals. In spite of this addition of new food material, and the fact that they continued to flourish and multiply, these decreased in length even more than in the starving culture, the mean being 100.320. This is the smallest mean length observed in any lot of the *c* line. The data for this lot are given in row 27, Table XVIII., and in Table LX.

3. Summary on the Effects of Environment.

The facts given above show that the nature of the environment affects greatly the dimensions, proportions and variations of *Paramecium,* and that these effects are produced with great ease and rapidity by such changes as are common in any culture of these infusoria. Some of the more important effects may be summarized as follows:

Effect on Length.—Under the influence of varied nutritional conditions the *length* varies extremely. In the line descended from the individual *D* the mean length varied under different conditions from 146.108 to 202.280 microns—the difference being 38.445 per cent. of the smallest mean length. In the *c* line the variation in mean length

under the influence of the environment was from 100.320 to 158.800 microns, or 58.293 per cent. of the lowest mean. The *extreme* lengths in each line, of course, differed still more; in the *D* line the extreme variation in length was from 104 to 256 microns, or 146.153 per cent. of the least length; in the *c* line it was from 73.3 to 200 microns, or 172.851 per cent. of the minimal length.

Effect on Breadth.—The breadth (the thickness of the body) varies under different environmental conditions more readily and in a higher degree than does the length. In the *D* line the mean breadth varied in different cultures from 31.180 to 64.880 microns, or by 108.08 per cent. of the lowest mean; the *extreme* variation in breadth, under different conditions, was from 20 to 88 microns, or 340 per cent. of the minimal breadth. In the *c* line the mean breadth varied under different conditions from 23.892 to 54.160 microns, or by 126.69 per cent. of the lowest mean; the *extreme* variation in breadth was from 16 to 84 microns, or 425 per cent. of the minimal breadth. The greater variability of the breadth, as compared with the length is seen in the coefficients of variation of the single cultures. The largest coefficient of variation for length is 15.279, while for breadth it is 28.879.

Relation of Length to Nutrition.—In general, increased nutrition increases the length. But the result is not always the same, because increased nutrition has two main effects: to increase directly the size of the adults, and to bring about multiplication. The latter effect, of course, decreases the mean length of the individuals of a culture, since it induces the presence of many specimens that are young, and therefore small. Increase in mean length due to added nutrition is seen in Table XVIII., rows 8 to 9, 10 to 11. *Decrease* in mean length, due to added nutrition is seen in the same table on comparing rows 1 and 2; 3 and 4; 13 and 14; 17 and 18. This decrease is due to the fact that in the nutritive fluid the animals divide before they reach the length of those in the poor fluid.

Decrease of length, due to decrease of nutrition, is seen in Table XVIII., by comparing rows 2 and 6; 7 and 8; 21 and 22; 23 and 24; 23 and 26.

Relation of Breadth to Nutrition.—The relation of breadth to nutrition is simpler than that of length; in all cases increase of nutri-

tion increases the breadth; decrease of nutrition decreases it. The response of breadth to changes in nutrition is immediate and very marked. Within twenty-four hours increased nutrition caused in the *D* line an increase of 49.724 per cent. in breadth (rows 8 and 9, Table XVIII.); in the *c* line it caused in twenty-four hours an increase of 40.778 per cent. (rows 17 and 19, Table XVIII.).

But the decrease of breadth with decrease of nutrition does not vary directly with the time; when plump individuals are left without food, they decrease much more rapidly at first than later. Thus, in the series shown in Table XVIII., rows 2, 3 and 6, the breadth decreased in the first forty-eight hours 21.324 microns, or 32.867 per cent.; in nine days more of lack of food the breadth decreased only 5.476 microns, or 8.440 per cent. more.

Proportion of Breadth to Length.—Since changes in nutritional and other conditions act more readily and more strongly on breadth than on length, and since the same agent may increase the breadth while decreasing the length, the proportion of breadth to length varies greatly under different conditions. The mean index, or ratio of breadth to length, varies in different cultures of the *D* line from 21.337 per cent. to 36.123 per cent.; in the *c* line from 23.067 per cent. to 41.455 per cent. Since the breadth is more dependent on nutritive conditions than is the length, we find the lowest ratio of breadth to length in the starving cultures (rows 8, 26, Table XVIII.); the highest ratio in well-fed cultures (rows 2, 14, 19, Table XVIII.). An increase of nutrition causes uniformly an increase of the ratio of breadth to length; a decrease of nutrition has almost uniformly the reverse effect. A single exception to the relation last mentioned is seen in the change from row 21 to row 22, Table XVIII.; here other causes, connected with conjugation, were probably at work. Whenever the mean breadth increases, the mean ratio of breadth to length likewise increases. (The only exception is the case just mentioned, where conjugation was involved.) It must be understood that this does not mean that in all cases the mean ratio of breadth to length varies directly with the mean breadth; if we compare rows 6 and 7, Table XVIII., for example, we find that this is not the case. But whenever, as a matter of experimental procedure, the mean breadth was caused to increase, the mean ratio of breadth to

length likewise increased. This is due to the two facts mentioned in the first sentence of this paragraph.

Effect of Environment on Variation.—The amount of observed variation, as measured by the coefficient of variation, depends largely on environmental conditions; this is true both for length and for breadth. In the *D* line the coefficient of variation for length varies in different cultures from 6.821 to 13.262;[6] for breadth it varies from 8.896 to 28.879. In the *c* line the coefficient varies for length from 7.134 to 15.279; for breadth from 10.700 to 24.291.

The effects on the coefficient of variation of changes in nutrition vary much in different cases; increased nutrition sometimes increases the coefficient, sometimes decreases it, sometimes produces first one effect, then the other. There are evident physiological reasons for the different effects. In a starving culture the first effect of rich nutrition is to cause many of the individuals to increase in size, while those individuals in which the effects of starvation had gone far do not at first take food and change. Hence there is a great increase in the coefficients of variation; in changing from row 8 to row 9 (Table XVIII.) both coefficients approximately doubled in twenty-four hours. Later, though the animals were kept in the same fluid, the coefficients decreased again—all of the specimens having reached more nearly a condition of equilibrium. If the animals are fairly well fed before the additional nutrition is met, an early effect is to cause rapid multiplication; the consequent presence of both young and old individuals in the culture increases the coefficients of variation, and particularly that for length. An example of this is seen in the change from row 3 to row 4, Table XVIII. A little later, when the multiplication has ceased, the coefficients of variation become small again. The coefficients of variation are likely to be small in starving cultures, owing to the fact that there is little multiplication and the adults have reached a condition of relative equilibrium. By taking into consideration the immediate and the remote effects of a given agent on growth and multiplication, its effects on the coefficients of variation usually become intelligible.

[6] Of course the cultures contain specimens in all stages of growth; as we have previously seen, the coefficient of variation becomes much less when the animals are selected with reference to age.

It is not necessary to emphasize the fact that since different environmental conditions produce different dimensions, the coefficients of observed variation will be much increased by throwing together specimens from different environments, or those taken at different times from the same culture. Examples of this are seen in rows 12, 15 and 20, Table XVIII.

The question may be asked, How can we account for the large coefficients of variation in given lots, taken all from the *same* environment (as in the various "rows" of Table XVIII.)? Surely, it may be said, the age differences among the individuals are not sufficient to account for coefficients of 12, 13, 20, etc., such as we actually find. This is undoubtedly true, and it becomes still more striking when we consider cases like Table XLI. (appendix), where the individuals are all of practically the same age, and all come at one time from the same small watch-glass of hay infusion, yet we find the coefficients of variation to be respectively 6.389 and 14.615. The considerable variation is to be understood only by realizing that even a small mass of fluid constitutes a relatively large and varied environment for *Paramecium*. A watch-glass of hay infusion is a microcosm to this animal. Bacteria gather on the surface, while they may not be found on the bottom or through the middle. The bacterial zoöglœa may become thicker at one edge than at the other, owing to the accidents of the original distribution of the seed bacteria or of the infusoria. Some of the *Paramecia* thus get more food than the others, perhaps at a critical period of growth; they thus get a start, which enables them perhaps to obtain more food than the others, even under uniform conditions. Some of the individuals get crowded away from the bacterial zoöglœa, and remain against a rough spot on the glass instead, where they get no food. In short, even in a few drops of water the conditions are *not* uniform throughout; some of the animals are well nourished, others poorly nourished, and the results show in the variations of their measurements.

The question whether some of the variations in such cases are not congenital and hereditary will be taken up later; we shall find little evidence that this is the case.

It is clear that no particular coefficient of variation can be considered characteristic of a particular race, except as the conditions

are very precisely defined. If all conditions of environment and growth were made absolutely the same, there is reason to believe (as we shall see farther) that for a given line (descended from a single individual) the coefficient of variation would be very close to zero. Its actually observed value in a given lot then depends almost entirely on environmental and growth differences.

Effect of Environment on Correlation.—The observed correlation between length and breadth varies greatly under different environmental conditions. In the *D* line the coefficient which measures correlation varies in different cultures from .3906 to .8463; in the *c* line from .4141 to .8500 (see Table XVIII.). Such differences are easily and quickly produced by environmental changes; thus the two extremes just mentioned for the *D* race were found in samples of the same lot of *Paramecia* taken twenty-four hours apart—one before, the other after, the addition of a nutritive fluid.

The correlation between length and breadth expresses the accuracy with which length and breadth vary proportionately. The actual proportion of one to the other, in a given lot, is, of course, of no consequence; length and breadth might be the same, or one might be 50 per cent. or 1 per cent. of the other; the correlation would still be complete (1.000) provided this same proportion were maintained throughout the particular lot examined. Any factor which causes the proportion of breadth to length to vary in a given lot, of course causes the correlation to fall below 1.000. If in a given lot many different ratios of breadth to length are represented, the correlation is, of course, lowered. In such a lot, any factor which tends to make the proportion of breadth to length more constant, of course, increases the correlation.

Examining the various factors which have the effects just mentioned, we find that the observed correlation depends upon many things.

(*a*) In considering the effects of growth (page 455), we saw that the proportion of breadth to length differs in different stages. Some of the effects of the environment on correlation are due to its effect on multiplication and growth.

(*b*) Certain environmental agents (as increased nutrition) increase the breadth while decreasing the length. Now, if this happens at

the same time and in the same proportion in all the individuals, then at any given moment the coefficient of correlation will, of course, not be altered by it. But if for any reason the changes occur more quickly or strongly in certain individuals than in others (as is usually the case), then, of course, the coefficient of correlation will be decreased. Or, if we throw together individuals taken at different stages of the process, the correlation becomes greatly decreased; it may even become negative. For examples, see rows 15 and 20, Table XVIII.

(*c*) Even if a given agent causes a change in the same direction (*e. g.,* an increase) in both length and breadth, the inclusion of different stages in the process may reduce the correlation (if it is already high). This will occur (1) if the two dimensions are not changed proportionately to each other, and (2) if the change in a given dimension varies at different stages of the process. Both these conditions, as we have seen, are fulfilled in the changes in dimensions induced by the environment. Under almost any environmental change breadth is altered more than the length. Furthermore, when nutrition is decreased, breadth decreases more rapidly at first than later. The inclusion of different stages of the process in a collection therefore results in the inclusion of various different proportions of breadth to length—lowering the correlation.

(*d*) If the correlation is already low, indicating the presence of many different ratios of length to breadth, then varied changes in these ratios may compensate some of the existing differences. causing an increase in the correlation. Whether this shall or shall not occur depends upon the condition of affairs before the changes are made, and on the nature of the changes themselves. A special case of this comes up in the next.

(*e*) When a culture containing thin, poorly fed individuals is given added nutriment, the correlation between length and breadth increases (compare, in Table XVIII., rows 1 and 2; 8 and 9; 10 and 11; 13 and 14; 17 and 18; 17 and 19, etc.). This is because, when fresh nutriment is added, the thinnest, poorest-fed individuals naturally take more food than do the individuals that are already plump and well-fed; they therefore increase most in breadth. As a result, existing differences in breadth are compensated; all the animals take

on that relative proportion of breadth to length that belongs to well-fed specimens.

Thus, we find almost throughout that an increase in the ratio of breadth to length is accompanied by an increase in the coefficient of correlation; a decrease in the ratio of breadth to length by a decrease in the coefficient of correlation. Examining these two constants, in the last two columns of Table XVIII., we find this relation to hold in every case of experimental procedure save one. (In the change from row 3 to row 4 it does not hold; this is due to another factor, to be taken up later.) If without regard to experimental procedure, we merely compare the mean index (or ratio of breadth to length) with the coefficient of correlation, we find the relation a little less general, though still marked; a large mean index is usually accompanied by a high coefficient of correlation.

Since, as we have previously seen, greater breadth is usually accompanied by a higher mean index, it follows that greater breadth is likewise usually accompanied by a higher correlation between breadth and length. This is, on the whole, evident on inspection of Table XVIII., though since other factors are involved, the relation is not without exception. But in general, broader specimens tend to show a more constant proportion of breadth to length than do thin ones.

(*f*) In poorly-fed cultures, as we have just seen, the breadth is apt to be variable in proportion to the length (giving low correlation) because some of the individuals get more food than others. But if all are reduced to an actually starving condition, then this source of variation is removed, and we may again get high correlation between breadth and length. This condition appears to be realized in row 26 of Table XVIII. Here a large culture had been reduced by starvation to a population of but 37, and these give the very high correlation of $.8018 \pm .0396$.

(*g*) When a given agent causes rapid multiplication, so that the sample taken includes many different stages of growth, with their different proportions of breadth to length, the correlation becomes low. This is the reason for the marked decrease in correlation in changing from row 3 to row 4 in Table XVIII.

All together, it is clear that no particular coefficient of correlation

can be taken as characteristic of a particular race of *Paramecia;* certainly not without very precise definition of the conditions. It appears probable that if all conditions of environment, growth, food taken, etc., could be made absolutely the same for individuals derived from the same ancestor, the coefficient of correlation would be close to 1.000.[6a] By varying these conditions any degree of positive correlation, down to zero, and many degrees of negative correlation can be attained.

V. INHERITANCE OF SIZE.

Having examined the effects of growth and of environment on size and form, we are now prepared to investigate how far these are determined by internal factors, handed on from parent to progeny. Without such a preliminary study of growth and environmental action it would be impossible to investigate successfully the heredity of size and form.

We have already seen that not all differences in size are due to growth and environment; in the first culture examined (Table I., page 398) there were at least two sets of individuals of characteristic different sizes, and these differences in size are lasting. Progeny of the two typical individuals *D* and *c,* from these two sets, still retain their characteristic relative sizes after more than a year of culture under all sorts of conditions.

The differences between these two sets are about the same as those which have been described as distinguishing two species, *D* corresponding to the accounts of *Paramecium caudatum, c* to *Paramecium aurelia.* The next problem is to determine whether there are still other races of *Paramecium,* distinguishable on the basis of differences in size, independently of the environment. Can we by selecting individuals of differing sizes isolate races of corresponding sizes? Can we find races of all sorts of sizes intermediate between the largest and smallest adult representatives of such a heterogeneous culture as is shown in Table I.?

The clear grouping of the culture of Table I. into two sets seems to indicate that we have present simply two races or species. My

[6a] Of course if all variation disappeared, as would perhaps be the case, then the concept of correlation would have no further application.

first experiments consisted of attempts to break the two lines derived respectively from *D* and *c* into other races of different sizes by selection and breeding of individuals of different sizes. This led incidentally, as we have seen, to the study of the effects of growth and environment on size; it was found that the observable differences between different members of either race were due to these factors, so that selection of such members did not lead to the establishment of races of different sizes. The results of a large amount of time-consuming work along this line, done before the investigation of growth and environmental action, were throughout negative.[7]

As a result of this work, I was disposed toward the belief that the characteristic sizes of *D* and *c* represent conditions of stability, which have properly been distinguished as two species, and that races of other sizes were not to be found or produced.

But the work thus far has, of course, been based on "pure lines," in the sense in which that expression is used by Johannsen (1903, 1906). The lines *D* and *c* are each derived from a single individual, reproducing asexually, so that no admixture from outside has entered them during the experiments. Now, while it appears difficult or impossible to produce other races *within* these pure lines, there remains, of course, the possibility that still other lines exist in nature. Can we find in a "wild" culture, by proper selection of differing individuals, still other races of differing size? This was the question next investigated.

1. Selection for Different Races in a Wild Culture.

(*a*) *Races Isolated from Cultures Not Conjugating.*

Attempts to separate out other races than those represented by *D* ("*caudatum* form") and *c* ("*aurelia* form") were first made with a wild culture which I called *OI*. This culture developed in decaying vegetation from a marsh. It contained two well marked sets of individuals: (1) very large individuals, corresponding in many respects to the *D* line, but with a mean length on January 3, 1908, of 238.280 microns; these we will designate *E*; (2) smaller

[7] To the experiments on selection within a pure line we return in a later section.

individuals corresponding in many respects to the *c* line, with a mean size on November 14, 1907, of 140.133 microns. These two sets occurred mixed, but each reached its maximum development at the dates mentioned. Isolated samples of the two sets retained their characteristic differences in size, just as happened in the case of *D* and *c*.

But the interesting condition showed itself in the smaller set. Among these were individuals of such different sizes, that in spite of our knowledge of the great differences produced by growth and environment, it seemed worth while to try to isolate and breed them. In a random sample of 60 specimens the length varied from 96 to 176 microns—the smaller sizes being grouped about 120 microns, the larger about 160 microns.

Accordingly, on November 9, 1907, I separated two lots, one containing ten of the smaller specimens, the other ten of the larger ones. These were placed in watch-glasses with equal quantities of the same culture fluid, and kept under identical conditions, where they were allowed to multiply. One week later (November 16) thirty specimens measured from each showed mean dimensions of 125.600 × 36.200 microns for the progeny of the larger ten, 96.400 × 30.00 microns for the progeny of the smaller ten. On November 27, a random sample of 100 from each gave for the progeny of the larger ten, dimensions of 134.320 × 36.280 microns; for the smaller set, 92.240 × 26.920 microns. Thirty-seven days later (January 2, 1908) the two lots still showed their characteristic differences, though cultivated under identical conditions. The mean dimensions of the two sets (from random samples of 100) were now 134.360 × 33.440 microns (for the larger), and 104.208 × 26.583 microns (for the smaller).

Thus, we have clearly two sets, with differences in size persisting from generation to generation (in spite of fluctuations in each due to environmental changes), and both falling, in a general way, in the dimensions previously found for the line *c*. It is evident, therefore, that *D* and *c* did not represent the only existing different lines.

Since the two sets under experimentation had come each from ten individuals which may be of heterogeneous origin, I isolated from each, as soon as it was evident that they were retaining their

differences, a single characteristic individual. This was done on November 13. The specimen from the larget set I called *g*; it measured approximately 130 to 140 microns in length. The specimen from the smaller set I called *i*; its length was about 90 to 95 microns. These two individuals were kept under the same conditions and allowed to multiply.

The small specimen *i* multiplied more rapidly than the large one *g*. On November 16 there were but seven progeny of *g*, while *i* had produced a large number. Two typical specimens of *g* were killed and gave measurements of 160×48 microns and 164×56 microns. Five typical specimens of *i* ranged in size from 92×36 to 128×44 microns, with a mean of 103.2×39.2.

Evidently, therefore, the progeny of *g* and *i* tend to retain the differences in size characteristic of the parents. The two lines were kept for a long time, under the same conditions; at intervals random samples were measured. The measurements at different dates, with the number of specimens on which they are based are given in Table XXIII., p. 488. (The small numbers of specimens employed on certain dates are due to the fact that only a small number existed at that time.)

The great fluctuations in the dimensions of each line will of course surprise no one who has examined that part of this paper which deals with the effects of the environment. These fluctuations are due mainly to differences in nutritional conditions. At intervals it was necessary to add new culture fluid; the dimensions in both lines thereupon rose at once; they then gradually declined till new fluid was added. Details on this matter are not necessary for our present purpose.

The important fact is, that in spite of all fluctuations, the lines *g* and *i* retained throughout the three months in which they were under observation their characteristic relative sizes. Multiplication was probably at the rate of about one fission a day, so that the table represents 90 to 100 generations. We have here two lasting races comparable to the two races from our first culture, which we called *D* and *c*. It is clear that neither *g* nor *i* is identical with *D*, since the latter is much larger; whether either is the same as *c* we shall inquire later.

TABLE XXIII.

Comparative Sizes in Microns of g and i and their Progeny at Different Dates, when Cultivated under the Same Conditions.

Date.	*g* and Its Progeny			*i* and Its Progeny.		
	No. of Specimens.	Mean Length.	Mean Breadth.	No. of Specimens.	Mean Length.	Mean Breadth.
1907						
Nov. 13	1	130–140	35–40?	1	90–95	30–40?
" 16	2	162.000	52.000	5	103.200	39.200
" 18	7	140.000	40.000	12	103.666	35.666
" 23	30	129.333	34.933	30	88.268	30.268
" 26	100	137.120	38.720	100	99.560	28.200
Dec. 7	61	120.590	41.110	96	98.709	34.208
" 16	17	127.059	38.588	23	98.608	29.739
" 30	40	112.600	31.300	64	86.756	22.062
1908						
Jan. 2	100	146.640	40.600	100	106.680	26.400
Feb. 5	57	116.912	36.070	43	93.583	27.500

It will be recalled that in the original culture from which came *g* and *i*, there was a still larger set which we called *E*. Ten of these were selected and cultivated under the same conditions as *g* and *i*. They retained throughout their much larger size (numerical results are given later), so that from this culture we have isolated three lines or races which retain their differences in size under the same external conditions.

At this period, then (January 1, 1908), I had in the laboratory a number of lines or races which had been studied with care. These formed two sets, so far as our knowledge of them up to this point is concerned. The two lines, *D* and *c*, from culture *I*, were clearly distinct even under identical conditions. The three lines, *g*, *i* and *E*, from the second wild culture *OI*, are likewise clearly distinct from each other. But the relation of *g*, *i* and *E* to *D* and *c* is uncertain; we may have on hand five distinct lines, or only four, or three.

To determine whether any of these five lines are identical, it is necessary to cultivate all five under the same conditions. A certain number must be selected from each; these must be brought into the same culture fluid and allowed to multiply in the same environment.

It is extraordinary what difficulties are presented in carrying out this apparently simple plan. The different lines have become adapted to certain diverse nutritive conditions; if now they are brought at once into the same culture fluid, some of them die. In the present case, *g* and *i* had been living in comparatively fresh hay infusion, *D* and *c* in different old hay cultures, *E* in a culture of decaying pond weeds. When all were brought into fresh

hay infusion, *E* died at once, *c* after a day or two; *D* multiplied slowly, then died in the course of a week or so, while *g* and *i* throve and multiplied.

It was therefore necessary to bring the different lines gradually into the new fluid, by mixing some of it with the fluid in which they lived, increasing the proportion of new fluid at intervals. This was found to be a very delicate undertaking. Certain of the lines would thrive for a time, under this procedure, then would begin to degenerate; in this way much time was lost. Finally, however, the different sets were induced to thrive in the same hay infusion.

Procedure Necessary for Making the Conditions Identical for Different Lines.—The procedure followed, in order to be certain that the cultural conditions were the same for all, was as follows: From each race ten typical individuals were selected. These were mixed with gradually increasing amounts of hay infusion, in the way just set forth—while at the same time of course they multiplied in number. After they had all gotten accustomed to the infusion, it was necessary to take measures to assure the identity of the solutions in which the different sets were living. For this it is not sufficient merely to transport the individuals to definite quantities of the same nutritive solution. For up to this point each set has been living in a solution which has received an admixture of the original culture for that set. Now, *these different original cultures contained different kinds of bacteria.* On transferring the infusoria to the hay infusion, they of course carried some of their own bacteria. By repeated changes the number of bacteria introduced could be much reduced. Nevertheless different kinds were brought in in different cases, so that we still have the different lines in cultures of diverse bacteria. From this fact naturally diverse chemical properties may develop in the different cultures, though the basic nutritive solution is the same. These diverse chemical properties would of course modify the organisms, making it impossible to compare them with regard to inherited size. To make the conditions of existence the same, *it is not sufficient to attend merely to the basic fluid; the bacteria in the fluid must also be the same.* This is a principle of wide practical importance in all experimental work with such infusoria. It is not a mere theoretical requirement; death frequently results from the introduction of a certain kind of bacteria into a certain culture, while another culture of identically the same fluid flourishes, because the bacterial infection is different.

This requirement was met in the following way: After the different sets had become acclimatized to the same hay infusion, ten of each were removed with a fine capillary pipette, and washed twice in fresh hay infusion. The second washing of the different sets was done *in the same mass of fluid,*—a small watch-glass full. The different sets might of course each carry with them a few of the bacteria characteristic of their original culture. After all had been washed in the same mass of fluid, this fluid would of course be infected with bacteria from all the different sets. Now, after the washing was finished, a definite quantity of this fluid in which all had been washed *was added to the final culture fluid for each lot.*

Thus each lot of ten is in the same quantity of the same nutritive fluid,

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and infected with the same bacteria as all the others. All are kept in watch-glasses of the same form and size, close together in the same moist chamber. Any characteristic differences in the resulting progeny must then be due to conditions within the animal, and not to differences in the environment. If we reach the same result, not merely in one experiment, but in a series conducted in this manner, we can be sure of our results.

Cultures of the five lines, *D, c, g, i* and *E,* prepared in the way just described, were set in progress January 19, 1908. In order to determine with certainty how much effect possible environmental differences might have on the results (as well as for certain other purposes), two lots each of *D, g* and *i* were used. If the two lots of *g,* for example, show differences as great as those between *g* and *c,* then, of course, we have no ground for considering *g* and *c* inherently different; the environmental differences account for all. These lots were allowed to multiply till February 5. Then a sample of each was killed and measured. Now a new lot of ten of each set was prepared by the methods given above, and the animals were again allowed to multiply till February 15, when samples were again measured.

It will be recalled that *E* is a lot derived from ten specimens of possibly diverse ancestry, from the culture *OI,* with an original mean length of 238.280 microns; that the line *D* has shown in repeated determinations a highest mean length of 202.280 microns (Table XVIII.); that *c, g* and *i* are smaller lines, derived from single individuals; *g* is known to be larger than *i,* but the relation of *c* to these is unknown.

The results of these breeding experiments are given in the following Table XXIV.

The experimental results given in this table show certain things clearly.

1. The method of culture is adequate for bringing out the inherent differences in different lines without confusion due to environmental effects. This is shown by the fact that when two cultures are made from certain single lines, these show themselves after breeding for many generations to be nearly identical, while the different lines give diverse results. In only one case (*D* on February 15) is there a notable difference btween the two samples of a single line, but this is much less than the difference between that line and any other.

TABLE XXIV.

Mean Dimensions in Microns of the Five Lines E, D, c. g and i, when Cultivated under the same Conditions, January 19 to February 5 and February 5 to February 15.

Date.	Dimensions of *E*.		Dimensions of *D*.		Dimensions of *c*.	
	Number Measured.		Number Measured.		Number Measured.	
Feb. 5	43	169.395×52.930	57 19	(1) 169.754×46.877 (2) 169.895×43.579	60	99.667×26.333
Feb. 15	100	200.320×52.400	100 100	(1) 180.240×46.880 (2) 173.240×49.760	100	100.320×26.480
Feb. 27	100	172.040×55.520	100	175.360×47.160		

Date.	Dimensions of *g*.		Dimensions of *i*.	
	Number Measured.		Number Measured.	
Feb. 5	50 57	(1) 114.720×33.920 (2) 116.912×36.070	50 48	(1) 92.000×26.960 (2) 93.583×27.500
Feb. 15	100	125.240×35.440	100	95.440×30.040

2. At least four distinct lines are present, *D*, *c*, *g* and *i*; these maintain their relative different sizes throughout the experiments, which lasted about twenty-five generations.

3. The lines *E* and *D* are nearly or quite the same. On February 5 they show nearly the same measurements, but on February 15 there was a marked difference. To test the meaning of this these two were cultivated twelve days more; then on February 27 they gave again nearly the same measurements. It will doubtless be safest to consider them the same.

We have now, therefore, four different lines or races of *Paramecium*, characterized by persisting relative differences in size. One of these (*D* and *E*) belongs, from its size, to the "*caudatum* group"; the other three are much smaller and fall in the "*aurelia* group." Of these, *g* is the largest, *i* the smallest, while *c* is intermediate. Under a similar change in the environment these all change in a corresponding way, as is shown by the fact that on February 15 all were somewhat larger than on February 5. It may be noted that

the differences in size among these four lines were very evident to the eye on inspection with the low power of the microscope, and that the difference was clearly present at all periods between the dates when the measurements were made. The measurements merely make precise what is evident to the eye without them.

Before attempting to determine whether still other lines can be isolated, and particularly whether it is possible to fill the wide gap between the *caudatum* group and the *aurelia* group, another question must be investigated—a question which strikes at the foundation of our conclusions up to this point. This is the question of the relation of these lines of diverse size to conjugation and the life cycle.

(*b*) *Are the Lines of Different Size Merely Different Stages in the Life Cycle?*

Calkins (1906) and others have set forth the fact that *Paramecium* and other infusoria show different dimensions in different stages of the life cycle—the cycle which begins with conjugation, extends over many generations of reproduction by fission, and ends with another conjugation. The question arises, therefore, whether our lines of diverse dimensions are not merely different stages in the life cycle; whether they would not, if brought to the *same* stage of the cycle, show the same dimensions. This possibility must be investigated before we proceed farther.

The details of the relation of conjugation and the life cycle to variation, inheritance, etc., are to be dealt with in a separate paper of this series. But since the question which stands at the head of this section is an absolutely fundamental one for the proper interpretation of the results of the present paper, it must be dealt with here.

To answer this question, it is evidently necessary to proceed as follows: Cultures showing epidemics of conjugation must be examined for conjugating pairs of diverse sizes. If such are found, the individuals must be isolated and allowed to multiply, in order to determine whether the progeny retain the diverse sizes characteristic of the parents. If from a conjugating culture we can obtain diverse lines standing all in the same relation to conjugation and the life cycle, then evidently our diverse lines represent something more

than different stages in the life cycle. The problem also can be attacked in certain other ways, which will be described.

The relation of diverse sizes to conjugation and the life cycle was studied with special thoroughness in the case of a culture in which there was an epidemic of conjugation January 29, 1908. This culture was found in decaying vegetation from a small pond near Baltimore; I called it culture *M*. Table LXI. (appendix) shows a random sample of this culture, including both conjugants and non-conjugants; of the 238 specimens in the table, 38 were conjugants, 200 non-conjugants.

From this culture *M* a large number of pairs were isolated, for various purposes, and allowed to multiply. Without going here into the details of the experiments, on February 21 I had from this culture eight sets or lines, each descended from a single equal pair or a single ex-conjugant; these lines were designated in my notes *L2*, *G1*, *A1*, *A2*, *I*, *C2*, *F1* and *F2*. (The designations are the same as those given to the original pair or individual from which the lines came.) In addition to these eight "pure lines," I had two cultures derived each from eight pairs of conjugants of approximately the same size; these were called *K1* and *K2*. A final culture was derived from ten small, nearly equal, non-conjugants from the same culture; it was designated *H*.

It is, of course, unfortunate that it is not possible to measure accurately the original living individuals from which the different lines are derived, but this will not alter in any way the results on the problem in which we are at present interested. The essential question is whether the lines derived from the different pairs or individuals are identical or diverse in size.

These various cultures were kept, so far as possible, in the same nutritive fluid and under the same conditions. Marked differences in size were apparent on examining the different sets with low power of the microscope. On February 21 fifty individuals of each of these eleven different sets were brought, with all the precautions mentioned on page 489, into the same culture fluid, while at the same time fifty specimens each of *D* and *g* of our earlier pure lines (see page 491) were brought into the same fluid. These were all allowed to multiply till February 26, when a random sample of 100 or more

of each was killed and measured. Later, on March 7, twenty individuals were taken anew from each of these thirteen lots, brought again with elaborate precautions into the same culture fluid, kept under the same conditions and allowed to multiply, part till March 13, part till March 19, when other samples were killed and measured. From our previous extensive experience with *i* and *g* (Table XXIII., page 488) and with five lines of Tables XXIV. (page 491), we can be assured that two sets of measurements taken at such intervals will give us reliable data as to the existence of any considerable lasting differences among the different lines. The results of the measurements of the thirteen different sets are given in classified form in Table XXV.

TABLE XXV.

Mean Dimensions in Microns, of the Thirteen Sets Described in the Text, after Cultivation under the Same Conditions, February 21 to February 26, and March 7 to March 13 (or March 19). (The conditions before; and in intervening periods were essentially the same, but elaborate precautions were taken for the periods specified). All are from the conjugating culture *M*, of January 29, save the last two sets.

Line.	Number Measured.	February 26.	Number Measured.	March 13.	Number Measured.	March 19.
		(1) Descendants of Pairs.				
L 2	100	206.360 × 60.840	100	220.560 × 59.960		
G 1	100	201.400 × 52.400	100	210.960 × 52.200		
A 1	100	193.560 × 51.840	100	203.640 × 52.560		
A 2	100	184.640 × 50.760	100	187.878 × 44.490		
I	100	132.880 × 41.960			100	138.880 × 43.120
C 2	100	128.880 × 40.400			100	119.200 × 37.280
		(2) Descendants of Single Ex-conjugants.				
F 1	100	193.000 × 50.840	56	209.643 × 56.643		
F 2	100	182.200 × 51.040	100	199.960 × 50.120		
		(3) Descended each from 8 Equal Pairs.				
K 1	100	133.680 × 39.400				
K 2	100	125.920 × 37.040			100	125.000 × 42.520
		(4) Descended from 10 Small Non-conjugants.				
H	100	131.400 × 42.000			100	128.840 × 41.360
		(5) Older Lines, not from Culture *M*.				
D	111	176.901 × 50.018	120	187.033 × 49.100		
g	100	124.440 × 35.920				140.800 × 39.640

Examination of this table shows that lines derived from different conjugating pairs or different ex-conjugants do differ from each other at the same periods in the life cycle, even though living under

identical conditions. The differences are fully as marked as those found among diverse lines derived from individuals not conjugating and taken without reference to the period in the life cycle in which they happen to be.

Besides this general result on our main problem, the following important facts are brought out by the table:

1. The six lines derived from the six different pairs (first six of the table) are clearly distinct. They show parallel differences in both sets of tests; the order of dimensions from largest to smallest is the same in both the first and the second measurements, though these are separated by at least fifteen generations.

2. The two lines, *F1* and *F2*, derived from single ex-conjugants, are likewise distinct from each other. So far as the measurements go, *F1* may possibly be the same as *A1*, *F2* as *A2*.

3. Certain different sets are likewise found in the other lots of the table.

4. The different sets fall into two very distinct groups, whose dimensions are separated by a wide interval. To the large group belong *L2*, *G1*, *A1*, *A2*, *F1*, *F2* and *D*. To the small group belong the others. The greatest mean length of any set of the smaller group (140.800 microns) differs widely from the least mean length of any set of the larger group (176.901 microns). These two groups correspond in general to what we have heretofore called the "*aurelia* form" and the "*caudatum* form."

As there was no danger of confusing any lot of the larger group with any lot of the smaller one, the second measurements of the two groups were not made for the same day; the lots of the larger group were killed March 13, while those of the smaller group were not killed till March 19, as the table shows. This was done on acount of the great labor involved in selecting, with capillary pipette, killing properly, and preserving, so many different sets on the same day. This difference of treatment of course does not alter the comparability of the different sets within a given group, which is all that we require.

5. How shall we decide which of the thirteen different sets form distinct lines? For this it will be best to take into consideration mainly the length, since we know from our earlier studies that little significance is to be attached to difference in breadth, owing to the extreme changes in that dimension with slight differences in food.

If any two sets differ in length in the same way at both measurements (taken many generations apart) and if the differences between them are each time decidedly greater than the sum of the probable errors of the measurements of the two, then we can be assured that we are dealing with really differentiated sets. Now, examination of the extensive series of measurements in Tables X. and XVIII. shows that the probable error of the mean length never reaches two microns, even when the number of specimens is much smaller than in our present measurements, and when conditions are of the most varied character. It is practically certain that the probable error of the mean length would not amount to one micron in any of the comparatively homogeneous sets with which we are here dealing. If, then, we require a difference of four microns between the mean lengths of the two sets, this difference to have the same sign (+ or —) at both measurements, we shall be within safe limits. Applying this test, we find four lines clearly distinct in the larger or "*caudatum*" group, while in the smaller or "*aurelia*" group we can be certain of but two distinct lines (represented best perhaps by *I* and *C2*). We have previously found three distinct lines in the *aurelia* group (*c*, *g* and *i*, Table XXIV.), so that all together we now have at least seven different lines of *Paramecium*, showing constant relative differences in length. It is probable that very exact tests would show the distinctness of some other lines of Table XXV.

The striking difference between adults of different races, under varied conditions, is shown in Fig. 7. Here we have two adults, one belonging to our smallest race (*i*); the other to one of the large races.

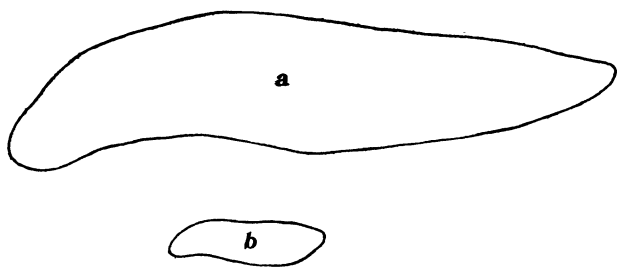

FIG. 7. Extreme adult sizes from different pure lines of *Paramecium*. *a*, large individual from a large line. *b*, small individual from the small line *i* of Table XXIII., page 488. Both magnified 235 diameters.

It is clear, then, that the question placed at the head of the present section is to be answered in the negative. The diverse lines of different size are *not* merely different stages in the life cycle.

(*c*) *Other Evidences of Permanent Differentiation in Size, Independent of the Life Cycle.*

The proof just given, that lines beginning with conjugants are differentiated in size even in the same portion of the life cycle and under the same conditions, is conclusive. But it may be worth while to give briefly certain other evidences of the same thing.

1. First we have the fact that in a given culture the conjugants themselves differ in size; this has already been shown by Pearl (1907). In a certain Culture IV., I found conjugants varying in dimensions from 148×44 to 260×60 microns. I have found (not in the same culture) conjugants with length as low as 100 microns. It is clear, therefore, that not all individuals are of the same size at conjugation. There is no reason to expect them to be so, therefore, at other definite periods in the life cycle; as we have seen, they are not. Selection of small pairs gives small progeny; of large pairs, large progeny.

2. In certain of my pure lines whose history was followed for a long time and whose dimensions were taken at intervals, conjugation occurred at times, but the dimensions at such times were not very different from the dimensions at other periods in the life history. Thus, in the earlier sections of this paper we have dealt with two pure lines, *D* and *c;* the former showed usually a mean length of about 180 microns, the latter a mean length of about 130 microns (see Table XVIII.). At a certain time an epidemic of conjugation arose in *c*. The mean dimensions were indeed higher than usual at that time, the mean length of the conjugants rising to 158.496 microns. But this does not by any means bring it up to the ordinary mean of *D,* and immediately after conjugation (in five days) the mean length of *c* fell back to 129.640 microns. Again, in the small race *g,* of Table XXIII., conjugation occurred in a number of cases; a typical pair measured but 110 microns in length. In other lines I have found for the conjugants means as high as 199.024 and as low

as 116.856, and these were correlated with corresponding measurements throughout the series.

These facts, of course, do not show that the size may not change at the time of conjugation or before or after. What they do show is that any differences thus produced do not account for the permanent differentiations we have found among different lines. We may distinguish (1) differences in size due to growth; (2) those due to nutrition and other environmental conditions; (3) those due to different stages in the life cycle (as a rule not marked in comparison with the others); (4) inherent, hereditary differences in size, persisting when all other conditions are made the same.

(*d*) *Lines Intermediate Between the Two Main Groups. The Question of Species in Paramecium.*

As we have already noted, the seven differentiated lines which we have thus far distinguished fall into two main groups, separated by a wide interval. In Table XXV. we find one group with mean lengths varying from 119.200 to 140.800 microns, while in the other group the mean lengths vary from 176.901 to 220.560 microns. Between the two there is thus a gap of 36.101 microns in which none are found. Is this gap constant and characteristic, so that our two large groups are permanently differentiated? If so, we should have some real basis for the common distinction into two species, *Paramecium caudatum* (larger) and *Paramecium aurelia* (smaller). The fact that we find in nature such cultures as that shown in Table I. (page 398), in which the individuals are distinctly separated into the two groups, seems to raise a presumption that the groups are natural ones, not due to accidents of selection.

For a long time I found no pure lines that were intermediate between these groups. It is possible that this was partly due to a tendency to choose for breeding the largest and smallest specimens, rather than intermediate ones, since my purpose at first was to determine whether there were any permanent differentiations at all; for this, marked differences were desirable.

In the course of work on certain problems connected with conjugation, I came in possession of a pure line, *Nf2,* descended from a single ex-conjugant. This, when cultivated in the usual hay infu-

sion, gave, under various different conditions, the following mean lengths in microns (each mean is based on measurements of 100 individuals): 148.197, 151.920, 158.760, 153.320, 160.852, 156.482.

It is evident that these means fall in the gap separating the "*caudatum*" group from the "*aurelia*" group. I therefore decided to cultivate these under identical conditions with a typical representative of each of the two main groups. For this purpose I chose *D* and *c*, the two lines longest cultivated, which I had used for the study of growth, environmental action, etc. (Tables X., XVIII., etc.). Twenty-five specimens, each of the three lines, *D*, *c* and *Nf2*, were brought on May 1, with the precautions described on page 489, into the same quantity of the same hay infusion and allowed to multiply till May 5. On that date a random sample of each was killed. Though the samples were large, extrinsic conditions prevented my measuring more than the numbers mentioned below; larger numbers would not have altered the results by more than one or two microns in any case. The mean dimensions of these three lines, cultivated under identical conditions, were

D (31 specimens), 202.710 × 51.871 microns.
Nf2 (33 specimens), 168.970 × 48.970 microns.
c (43 specimens), 126.605 × 44.930 microns.

Thus, the dimensions of *Nf2* lie almost precisely half way between those of *D* and *c* (the dimensions exactly half way between would be 164.658 × 48.401). We have, therefore, in *Nf2* an eighth pure line, intermediate between the "*caudatum*" and "*aurelia*" groups formed by the other seven. These two groups are then not separated by an unbridged gap.

The other character which had been held to separate *Paramecium caudatum* from *Paramecium aurelia* was the presence of but a single micronucleus in the former, while the latter had two. Calkins (1906) showed that in the same pure line we sometimes have two micronuclei, sometimes but one, so that this is not sufficient ground for distinguishing two species. Though the present study has shown that differences in size among different lines are more permanent than the data available to Calkins had seemed to indicate, this does not give any better basis for distinguishing two species, since we

have been able to isolate, not merely two permanently differentiated lines, but eight. Of course, it would require merely more extensive and intensive work to isolate others; doubtless the number to be isolated would depend only on the accuracy of the methods used.

To my great regret, I was unable to take the steps necessary to determine the number of micronuclei in the various pure lines with which I worked. The animals multiply so rapidly that with several lines in progress it is quite impossible even to keep up with the data for size alone; probably half my experiments were lost on this account, after much work had been spent on them. It was then out of the question to carry on at the same time the staining processes necessary to determine with certainty the number of micronuclei. For work of the kind presented in this paper, a syndicate of investigators is needed for keeping track of the various important aspects of the matter. In the case of two of my lines the number of micronuclei was determined; *D* (larger) had one; *c* (smaller) had two.

I may be permitted to add to the precise data thus far given a personal impression or surmise. Though, as I have shown, intermediate lines occur, I believe it will be found that most *Paramecia* can be placed in one of the two groups that we have called "*caudatum*" and "*aurelia.*" In other words, if my impression is correct, most lines will have a mean length either below 145 microns or above 170 microns; rarely will lines be found whose mean falls between these values. Such at least has been my experience in a large amount of work. Furthermore, I am inclined to believe that those belonging to the smaller group (mean length below 145 microns) will be found to have as a rule two micronuclei; those belonging to the large group but one micronucleus. This matter is worthy of special examination.

(*e*) *Do the Diverse Lines Differ in Other Respects Besides Dimensions?*

In the investigations above set forth the dimensions, and especially length, were made the basis of study, simply because they were the characters most readily examined. Most other characteristics are not easily handled in so minute and relatively undifferentiated an animal as *Paramecium.* But there is, of course, no reason to

suppose that the relations we have brought out are limited to length alone. Probably other differentiated pure lines could be distinguished on the basis of other characteristics.

The only other characteristic on which our data might give results is that of *form,* as distinguished from size. Are some races broader, some narrower, in proportion to the length?

We may first examine this question with reference to the two main groups into which most of our lines fall. Is there any general difference in the proportion of breadth to length when we compare the larger races ("*caudatum* group") with the smaller ones ("*aurelia* group")? The experiments whose results are summarized in Table XXV., page 494, give us data for a number of different lines of both groups, cultivated under the same conditions. We may, therefore, determine the proportion of breadth to length in these. The more accurate way of doing this would be by means of the formula given on page 399. This, however, would involve much computation not made for other purposes; and we may reach very nearly the same results by simply dividing the mean breadth by the mean length. If the differences between the different races are not sufficient to show clearly under this treatment, they are doubtful and inconsequential. The following table gives the ratio of mean breadth to mean length in the different lines represented in Table XXV.; the lines are arranged according to relative size, so as to exhibit any differences between the large and small groups.

The table shows that the ratio of breadth to length is almost uniformly greater in the small or *aurelia* group than in the larger. The lowest ratios of the *aurelia* group are, indeed, a little below the highest of the *caudatum* group, but the difference between the groups as a whole is unmistakable. The first column of the table is the most satisfactory in this respect, since both sets were killed at the same time. In the second column the difference between the ratios for the two groups is still more decided, but environmental differences may play some part in this case. The average ratio for the *caudatum* group is, from the first column 27.473 per cent.; from the second 25.679 per cent. For the *aurelia* group the averages are: first column 30.441 per cent.; second column 31.319 per cent. The

TABLE XXVI.

Ratio of Mean Breadth to Mean Length in the Lines and Races of Table XXV., page 494, Cultivated under Identical Conditions.

1. Caudatum Group.	February 26. Per Cent.	March 13. Per Cent.
L2	29.482	27.185
G1	26.018	24.744
A1	26.782	25.810
F1	26.342	27.019
A2	27.491	23.680
F2	27.921	25.065
D	28.275	26.252
2. Aurelia Group.	**February 26. Per Cent.**	**March 19. Per Cent.**
K1	29.473	
I	31.577	31.048
H	31.967	32.102
C2	31.347	31.275
K2	29.416	34.016
g	28.865	28.153

general average for the *caudatum* group is 26.576 per cent.; for the *aurelia* group 30.840 per cent.

In Table XXIV., page 491, we have data for certain other members of the two groups when cultivated under similar conditions. If we determine the ratio of mean breadth to mean length for this table, the results are not so clear as in the cases we have just considered. They are given in Table XXVII.

TABLE XXVII.

Ratio of Mean Breadth to Mean Length for the Races of Table XXIV., page 491.

1. Caudatum Group.	February 5. Per Cent.	February 15. Per Cent.	February 27. Per Cent.
E	31.245	26.158	26.458
D	27.615 25.651	26.010 28.723	26.893
Average	28.170	26.964	26.675
2. Aurelia Group.			
c	26.482	26.396	
g	29.568 30.853	28.298	
i	29.303 29.386	31.852	
Average	29.118	28.849	

In this table the averages for the *aurelia* group are again higher throughout than for the *caudatum* group. But the highest ratio is given by one of the *caudatum* group, and the line *c* of the *aurelia*

group gives in both cases a low ratio. But taking the averages, in connection with those of Table XXVI., it is clear that the smaller races are as a rule slightly broader in proportion to the length than are the larger races.

Turning now to the question whether there are differences in the proportion of breadth to length in different races of the same group, we have full data only for the lines *g* and *i*, as given in Table XXIII., page 488. Beginning with the data for November 23 (since before that date the number of individuals is small), we can make determinations for seven different dates of the ratio of mean breadth to mean length, the two sets being on each date as nearly as possible under identical conditions.

TABLE XXVIII.

Ratio of Mean Breadth to Mean Length for g and i (Table XXIII.).

	November 23. Per Cent.	November 26. Per Cent.	December 7. Per Cent.	December 16. Per Cent.	December 30. Per Cent.	January 2. Per Cent.	February 5. Per Cent.
g	27.011	28.238	34.091	30.370	27.797	27.686	30.853
i	34.291	28.325	34.655	30.159	25.430	24.747	29.386

Thus, in the first three determinations the ratio was greatest in the line *i*; in the last four it was greatest in the line *g*. Evidently there is no constant difference in proportions between these two lines.

For other lines our data are not sufficient to test this matter. Our only positive result on this point then is that the smaller races are as a rule proportionately broader than the larger ones.

2. RESULTS OF SELECTION WITHIN PURE LINES.

We have seen that an ordinary "wild" culture of *Paramecium* contains many lines or races, which are differentiated in size. By selection it is possible to isolate these diverse lines; so that in this way we can obtain cultures in which the mean size is large or small, or intermediate, as we prefer. In this case selection, of course, acts by isolating lines that already exist, and allowing them to propagate unmixed.

How do these diverse lines arise? Can we obtain them by selection within the limits of a single line? If from among the progeny of a single individual we select the larger and the smaller specimens,

will we obtain two diverse lines, one showing a greater mean size than the other?

As we have already seen, our first attempts to do this failed. But these first experiments were made before our study of growth and environmental effects, so that the basis of selection was wrong. The smaller specimens selected were as a rule the younger ones; they grew to full size, then, of course, produced progeny of the same size as other adults.

After the thorough study of growth, it appeared possible that a more adequate method of selection might be found. The proportions of the young differ from those of the adult (as our account has shown), so that after long practice one comes to recognize the young specimens with some accuracy. It appeared worth while, therefore, to attempt to select larger and smaller adults for further propagation.

(*a*) *Differences Due to Environmental Action Not Inherited.*

It is, of course, easy to obtain within a pure line adults of different size, by subjecting them to different environments. An analysis of our section on the effects of the environment shows that as a rule these are not inherited. Thus, if we examine Table XVIII. (page 460), we find that the same set that gave on July 17 a mean length of 184.100 microns (row 7) gave one week later, under different conditions, a mean of 146.108 microns; one day later 163.932 microns; one week later 174.400 microns; two days later 191.360 microns. The breadth changed even more, and the extremes of size in a given culture showed corresponding changes. There was no difficulty in changing the dimensions back and forth in the most varied ways. The entire Table XVIII. is an illustration of the general lack of continued inheritance of environmental effects.

Many experiments directed precisely on this point gave the same results. When, for example, the small specimens of row 8 (Table XVIII.) were cultivated under the same conditions as large specimens from row 9, the resulting cultures were soon indistinguishable.

Thus, it is clear that such environmental action as is summarized in Table XVIII. is not as a rule inherited. But I wish to point out and emphasize certain facts regarding the experiments on the action of the environment. (1) In all the experiments thus far tried, the

differential action of the diverse environments lasted but a short time. (2) The experiments were directed toward determining whether the differences produced were *permanently* inherited. Critical investigations have not yet been made to determine whether the environmental effects may not persist for one or a few generations after transference to the new fluid; nor whether long continued action of a certain environment may not produce more lasting results than brief action.

To these points I hope to devote special and extended investigations. The purpose in the present paper is to show on this matter the main general result; this unquestionably is that environmental action is not as a rule inherited in any lasting way.

(*b*) *Selection from Among Differing Individuals in the Same Environment.*

Besides the differences among individuals under different environments, we likewise find differences among individuals of the same pure line in the same culture, as a glance at the tables of the appendix will show. What will be the effect of selecting for breeding larger and smaller specimens from such a culture, avoiding, so far as possible, different stages of growth?

In order to make the selections properly, certain things must be considered. (1) It is well to bring the culture into as stable a condition as possible—a condition where there is little or no multiplication—in order that we may not be confused by different stages in growth. (2) It must be remembered that, so long as conjugation does not occur, the same results that selection would produce are brought about in the ordinary course of events, save that the large and small specimens remain mixed. That is, if there is congenital variation, producing large and small individuals, this must occur in the same way whether the different sizes are isolated or not. The progeny of every individual forms a "pure line," quite unmixed with any other, so long as no conjugation occurs. If, then, by variation a large individual *a* and a small one *b* are produced, and these differences are inherited, then later we shall find a mixture of two strains instead of a single strain. We should then expect the progeny of a

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single individual to show more and more variation as the strain became older; it would break into several or many strains, which would, however, remain intermingled.

Therefore, the best method of procedure will be to take an old strain, which, derived from a single individual, has for a long time been multiplying freely without conjugation. From this the largest and the smallest individuals should be separated and allowed to propagate under identical conditions. If hereditary variations in size have occurred, we should in this way reach the same result as by actual selection and isolation through many generations. Physiological isolation has been as complete as would be experimental isolation.

A race fulfilling these conditions we have in the pure line derived from the individual *D,* on which most of the work described in the first parts of this paper was done. On January 19, 1908, large cultures of *D* had been multiplying without conjugation since April 12, 1907, a period of about nine months. During this time about 250 generations must have been produced; these had remained physiologically isolated. The superfluous individuals had been removed by periodic "catastrophic" destruction; the greater part of the culture was thrown out, and a remnant saved, without selection, for a new culture.

On January 19, 1908, I took from the large stock culture of *D* (1) the ten largest individuals that I could find; (2) the ten smallest individuals I could find. They were separated in two watch-glasses and kept under identical conditions. The difference between the two sets was very marked; the smaller lot were certainly not more than two-thirds the length of the larger, and they were very slender, while the large ones were both long and broad. It was clear that both sets were adults.

It was found that the smaller lot multiplied much less rapidly than the large lot, and some of the small ones died. By January 30 there were but twenty of the small lot, while a very large number had arisen from the large lot. On this date the culture fluid was changed and but fifty of the larger lot retained. The small lot continued to multiply very slowly. It is clear that the small specimens

are weak, sickly ones, and the physiological difference persists at least for some generations (a matter for further study).

On February 5 about half of each lot was killed and measured. This gave 57 specimens from the larger lot, 19 from the smaller. The mean dimensions were, for the larger lot, 169.754 × 46.877 microns; for the smaller lot, 169.895 × 43.579 microns.

Thus the two were practically identical; one could not expect a closer approximation in two identical lots kept separate for seventeen days. The slight difference in breadth is only what we might expect when we consider the extreme sensitiveness of that dimension to faint environmental differences. The most striking differences that we can find as a result of physiological isolation for 250 generations have equalized themselves in a short time, when we got both sets to multiplying freely under the same conditions.

It seems hardly worth while to continue this series, since the two sets have now become equalized. However, they were continued for some time, and samples of 100 each were measured on February 15 and February 27. In these two measurements we find certain differences between the two sets, but these are in opposite directions in the two cases. The means are as follows:

	February 15.	February 27.
Large D	180.240 × 46.880	175.360 × 47.100
Small D	173.240 × 49.760	193.680 × 52.320

Evidently slight environmental differences between the two cultures had crept in. It is clear that the two sets show no constant differences, such, for example, as we find between the two lines, *g* and *i*, in Table XXIII., page 488.

Another set of experiments dealt with the two differentiated lines, *g* and *i*. The line *g* consists of individuals that are constantly larger than those of the line *i*, when the two are under the same conditions (see Table XXIII., p. 488). The experiments consisted in an attempt to separate these races still farther by propagating continually from the largest specimens of *g* and from the smallest specimens of *i*. Thus, if selection is effective, *g* must become larger, *i* smaller. The *length* was the dimension mainly attended to in these selections.

On November 23, 1907, the mean size for *g* was 129.333 × 34.933

microns; for *i* it was 88.268 × 30.268. On this date I placed in separate watch-glasses the ten largest specimens of *g* and the ten smallest specimens of *i*, keeping them under the same conditions.

On November 29 I again selected from the progeny of these the ten largest *g* and the ten smallest *i*, destroying the others.

On December 7 the same selection was repeated; the remainder of each lot was killed and measured. The mean measurements were

g, 120.590 × 41.115 microns.
i, 98.709 × 34.208 microns.

Thus, in spite of the fact that for at least fourteen generations we have selected for propagation the largest of *g* and the smallest of *i*, *g has become smaller and i has become larger!* The results of selection, if there are any, quite disappear in comparison with the effects of slight environmental differences.

In spite of this discouraging result, the experiment was continued. On December 16 I selected the five largest *g* and the five smallest *i* and again measured the rest of each. The results were

g, 127.059 × 38.588 microns.
i, 98.608 × 29.739 microns.

Thus, *i* retains the same length, while *g* has increased, but has not regained the length it had at the beginning of the experiment.

On December 25 the five largest *g* and the five smallest *i* were again selected for propagation.

On December 30, thirty-seven days after the beginning of the experiment, I again measured all but the five largest of *g* and the five smallest of *i*. The results are

g, 112.600 × 30.300 microns.
i, 86.756 × 22.062 microns.

Thus, *i* has decreased as compared with its original length, while *g*, which was selected for increase of size, has decreased a great deal more! The decrease in length of *i* is less than two microns; the decrease in *g* is more than sixteen microns! And this is the result of five selections, taking for *g* the largest, for *i* the smallest, specimens produced in the course of at least thirty generations![8]

[8] The number of specimens on which the measurements are based will be found in Table XXIII., page 488, which includes, for another purpose, the measurements from these experiments.

Evidently, selection is having no effect that can be detected. The fluctuations in the two sets are precisely what would be expected from unavoidable changes in conditions of nutrition; they show no relation to selection.

Later another experiment in selection was tried with these same races, *g* and *i*. On January 19 I selected from a large culture that had been multiplying freely for a month (1) the ten largest specimens of *g* that I could find; (2) the ten smallest specimens of *g*; (3) the ten largest specimens of *i*; (4) the ten smallest specimens of *i*.

These were allowed to multiply under identical conditions till February 5. Then a sample of fifty of each was measured. The results are as follows:

Large *g*, 114.720 × 33.920 microns.[9]
Small *g*, 116.912 × 36.070 microns.
Large *i*, 92.000 × 26.960 microns.
Small *i*, 93.583 × 27.500 microns.

The difference between the two sets of each is slight and without significance, but such as is found is in favor of the progeny of the *smaller* specimens in each case.

Evidently, we are not making a start with any effect of selection, and it is useless to continue the experiment.

Many other attempts were made to break a pure line by selection into several strains; on this point an immense amount of work was directed. But in most cases the difference between the two sets became equalized almost at once, so that the experiments were not carried farther. As soon as two unequal sets become quite equalized, there is little opportunity for further selection. In the experiments described above, though their futility seemed evident from the first results, the work was continued for many generations, in order that failure might not be due to lack of perseverance.

One other set of experiments deserves to be described, because in these the basis for selection was changed. Among the progeny of a certain individual *Nf2* conjugation occurred. The conjugants varied in size. This offered an opportunity to make a selection

[9] These measurements are found, for another purpose, in Table XXIV., page 491.

based on specimens that were evidently adults; possible confusion due to growth differences could be avoided.

On March 31 I killed and measured all but the largest and smallest pairs of conjugants; the length was found to vary from 124 to 148 microns. The smallest and largest pairs were reserved for propagating; the former, of course, measured not more than 124 microns, the latter not less than 148 microns. These were allowed to multiply separately, but under the same conditions, till April 10.

On April 10 I measured a random sample of 100 specimens of the progeny of each of these pairs. The results are as follows:

Larger pair, 151.920×43.840 microns.
Smaller pair, 158.760×38.120 microns.

Thus, the difference in size, whatever its cause, does not correspond to the difference between the ancestors; selection for size has had no evident effect.

Another experiment on the progeny of *Nf2* consisted in comparing the descendants of a single small conjugant with those of several large non-conjugants. Details of this and similar experiments will be reserved for our paper on the relation of conjugation to variation and heredity. But since it has a certain bearing on our present problem, the results may be given here.

At the same time with the cultures last described (on March 31), I isolated ten of the largest non-conjugant progeny of the same individual *Nf2*. A sample of thirty-four of these had given a mean length of 147.412 microns, so that this may be taken as the mean length of these ten specimens. With the progeny of these was compared the progeny of the smaller pair mentioned in the preceding experiment. As we have seen, this pair measured not more than 124 microns in length. The greatest pains were taken to cultivate the two sets under identical conditions. On April 20 I killed a sample of 108 of each. The mean measurements were as follows:
Progeny of small pair (124 microns) — 160.852×42.036 microns.
Progeny of ten large (147 microns) — 156.482×43.815 microns.

Thus, again, there is no correspondence between the differences in size of the parents and those of the progeny. The determining factor in the size is the fact that both sets belong to the same pure

line; the variation of the parents from the type of the pure line has no effect. The difference in the figures above is either purely statistical in character or means a faint variation in the culture fluid.

(*c*) *Summary on Selection within Pure Lines.*

Thus, we come uniformly to the result in all our experiments, that selection has no effect within a pure line; the size is determined by the line to which the animals belong, and individual variations among the parents have no effect on the progeny.

But for our results with different lines, it might be maintained that the reason why we get no constant differences between the progeny of different individuals of the same line is because the effects of environment are so much greater than the effects of selection that the latter are covered up and obscured. But as soon as we are dealing with lines that are really different (though by but a small amount) we have no such difficulty; the different lines retain their relative sizes in spite of environmental action. This is clearly shown in Tables XXIII. and XXV., pages 488 and 494.

The significance of these results will be dealt with in the next section.

VI. SUMMARY AND DISCUSSION.

1. Résumé of the Investigations.

The present paper is an experimental study of the factors involved in variation and inheritance of size in the infusorian *Paramecium*, in the period when reproduction is taking place by fission, without conjugation.

1. The first question proposed is whether the differences in size among different individuals of a culture are inherited. The preliminary study showed that in a typical culture there were two permanently differentiated groups of large and small individuals, respectively, corresponding to what had been described as the two species, *Paramecium caudatum* and *Paramecium aurelia*. But when a culture was produced from a single individual of either of these groups, forming thus a "pure line," it was found that though the different individuals of the single pure line differed much in size,

these differences were not inherited. Large and small specimens of a single pure line produced progeny of the same mean size.

2. The next question then was: What are the causes and the nature of the variations in size among the different individuals of a culture of *Paramecium?* Even in a pure line the individuals differ greatly. The "polygon of variation" of a given culture was looked upon as a mass of problems for analysis. What determines the position which any given individual holds in such a polygon, or in a correlation table? And why do different lots of *Paramecia* differ in mean dimensions; in the amount of variability; in proportions, and in the correlation between length and breadth?

The analysis of the factors in variation led to a detailed study of (1) growth, (2) the effect of the environment; (3) inherited differences in size. To these three matters the three main divisions of the paper are devoted. To one or the other of these three categories most of the variations in size were found to belong. A fourth category, consisting of variations connected with conjugation, is reserved for consideration in a later paper.

3. A large share of the differences in size to be observed in a given culture are differences in growth. In study of variation in protozoa it is as necessary to take growth into consideration as it is in the study of higher animals; the part played by it is fully as great in the protozoa as elsewhere. The paper gives a detailed study of growth, based on the measurements of 1,500 specimens of various known ages, in comparison with large numbers of "random samples." In this way a curve of growth was plotted (Diagram 5, page 449); this curve resembles essentially the curves of growth of higher animals, as the rat, or man. In different parts of this curve of growth individuals show different lengths, different breadths, and, of course, different proportions of breadth to length. A flourishing culture contains individuals in all stages of growth; so that this affects largely the mean dimensions, the observed variations, and the correlations between length and breadth. The precise effects of growth on each of these matters are dealt with in detail in the paper; they will be summarized in later paragraphs. A summarized account of growth and its effects is found in the body of the paper, pages 447 to 458; the constants for dimensions and variation in dif-

ferent stages of growth are brought together in Table X., page 428.

4. Environmental conditions were found to play a very large part in determining dimensions, variations and correlation in *Paramecium*. Conditions of nutrition were found to be particularly effective. By changes in nutrition the mean length of a given culture could be changed in a week from 146 microns to 191 microns; the breadth from 31 to 54 microns; in twenty-four hours the coefficient of variability for length was thus changed from 7.003 to 12.767, for breadth from 12.473 to 28.879; the coefficient of correlation from .3906 to .8463. Changes of the most varied sort could be produced and reversed with the greatest ease in short periods; many examples of this are summarized in Table XVIII., page 460. Within a given culture at a given time many of the differences between individuals are due to slight environmental differences in different regions. The breadth is more sensitive to environmental changes than the length; to such an extent is this true that it is difficult to use the breadth dimensions for accurate study of any other factors. A summary on the effects of the environment on dimensions, proportions, variation and correlation is found on pages 476 to 484.

5. After the study of growth and environmental action, an investigation was made of the internal factors in dimensions and variation; of the inheritance of size. Are all the observed differences between the individuals of a culture mere matters of growth and environment? Or may we find different races or lines that retain their relative sizes even in the same stage of growth and in the same environment?

A thorough experimental study showed that a given "wild" culture usually contains many different lines or races, which maintain their relative sizes throughout all sorts of changing conditions. Eight of these differing pure lines were isolated and propagated; these varied in mean length from a little less than 100 to a little more than 200 microns (see Tables XXIII. and XXV.). Other lines could unquestionably be distinguished by sufficiently accurate experimentation.

These different lines fall usually into two main groups, one group having a mean length greater than 170 microns, the other having a mean length below 140 microns. These two groups correspond to

the distinction that has been made between two species, the larger ones representing the supposed species *caudatum,* the smaller ones *aurelia.* But a line or race was found with mean length lying midway between these groups, at about 150 to 160 microns.

The smaller or *aurelia* lines were found to be, under the same conditions, as a rule a little broader in proportion to the length than the larger or *caudatum* lines. But the difference is slight and the two sets overlap extensively in this matter; slight differences in environment quite obscure the difference in proportions.

The differences among the different lines were found not to be due to different periods of the life cycle. By beginning with conjugating pairs of different sizes, distinct pure lines were as readily isolated as by beginning anywhere else in the cycle.

6. After becoming thoroughly familiar with differences due to growth, to environment, and to divergent ancestry, a further attempt was made to change by selection the characteristics of pure lines, or to break such lines into strains of differing size. In spite of much work directed on this point, it was found that selection within a pure line was quite without effect. Large individuals of the line produce progeny of the same mean size as do the small individuals. To this matter we return in later paragraphs.

2. Determining Factors for Dimensions, Variations and Correlations.

Based on the analysis of the factors in variation above set forth, a summary can be given of the various determining causes of the different dimensions, the proportions, the amount of variation and the correlations observed in samples of different cultures of *Paramecium.* We may take as an example such a sample as is shown in Table LXI. (appendix) from a "wild" culture.

1. The various different *lengths* depend upon the following factors:

(*a*) The collection embraces a number of different races or lines, having different lengths even when all conditions are the same. We have seen that different lengths varying from less than 100 to more than 200 microns may be included as a result of this fact. The *mean* length may not represent any of these races (this is the case in Table I.).

(*b*) The collection includes various growth stages of each of the lines represented. The youngest stages of each line are little more than half the lengths of the adults; all intermediate stages may be present, and the adults themselves shorten again as they approach fission. A very wide range of variation in length may be brought about by these growth stages, all within the limits of a single pure line or race. Of course when many different lines are present, an immense number of combinations are thus produced.

(*c*) The collection includes individuals of the various races that have lived under slight or considerable differences in environment, particularly in the matter of nutrition. Those that have been able to get more food will be much larger and will multiply more frequently (thus giving more young) than those that get less. Even slight environmental differences make decided differences in dimensions. While the environment shows its effects most strongly on comparison of different cultures, even within the same culture, and when all the individuals are of one race and of approximately the same age, there are marked differences due to this cause. This is shown, for example, in Table XLI. (appendix); here variations in length from 140 to 200 microns must be considered environmental effects. A few drops of water form a varied microcosm to the infusoria. When diverse pure lines, diverse growth stages, and diverse environmental conditions are found in a culture (as is usually the case), of course, the number of different sizes and forms due to the varied combinations of all these factors are very great. The same sizes may, of course, be produced in different ways; two diverse lines in different stages of growth or in different environments, or in some combination of the two, may produce forms outwardly identical. The actual variety, as defined by the physiological conditions, is therefore much greater than the measurements show, for the latter throw together heterogeneous combinations.

Combinations of all the three factors inducing diversity might give us in a single collection individuals varying in length from 50 microns to 332 microns. While these are the extremes given by our data, presumably the actual extremes would be still more divergent.

(*d*) In different collections the observed *mean* lengths depend upon the three different sets of factors just mentioned. The inclu-

sion of different *lines* or *races,* even if conditions of growth and environment are essentially the same, may give us, as we have seen, mean lengths of somewhat less than 100, or somewhat more than 200 microns, or any intermediate length. Different *stages in growth* may give us, in the same line and in the same environment, means differing to such an extent that one is nearly twice the other, or any intermediate condition. The absolute extreme values will, of course, depend upon the race employed; in the line *i* the variation of mean length caused by growth might be from about 50 to about 100 microns; in *D* it was from about 100 to about 200 microns; in *L* it would be from about 117 to 234 microns. Different *environmental conditions* give us, within the same lines, mean lengths differing to such an extent that the greater is 25 to 30 per cent. more than the less (lines *c* and *D*). In different "wild" cultures we shall have different combinations of all these factors, resulting in extreme diversities in different cases. Fig. 7 shows two extreme sizes drawn to the same scale (page 496).

2. The various different breadths depend upon the same factors as the different lengths. There are certain differences, however. As compared with length, the breadth is affected much less by growth; about the same (though a trifle less) by diversity of race; and much more by environmental differences. Environmental differences produced within the races *D* and *c* such differences in mean breadth that the greater was about twice the less.

3. The observed variation, as measured by the coefficient of variation, of course, depends upon the three sets of factors enumerated above as affecting the length and breadth. If a collection consisted of several different lines or races, all in the same condition as regards growth and environmental conditions, this would, of course, give us a considerable coefficient of variation. For example, if a collection consisted of ten individuals each of all the different lines represented in Table XXVI., page 502, and if all of each set of ten had the mean dimensions for its line (thus excluding differences due to growth and environment within the lines), the coefficient of variation when computed in the same way as for the actual collections given in the text is found to be for length 19.689; for breadth 15.679.

If a collection consists of individuals all belonging to the same

line or race, and in the same environment, then the coefficient of variation depends largely upon the stages of growth it contains. By taking specimens nearly in the same stage of growth we were able to reduce the coefficient of variation in length in some cases to 4.521, in breadth to 6.976, while by taking collections including various ages, under similar conditions, coefficients were found as high as 13.729 for length and 13.292 for breadth (Table X.). The most carefully selected lots contain specimens differing a certain amount in age, otherwise the coefficient of variation could be still further reduced in this way. Specimens beginning fission or undergoing conjugation include few growth stages, hence they show a low coefficient of variation. The coefficient for those beginning fission is less than for conjugants (see page 453).

The coefficient of variation for a given line is tremendously affected by environmental conditions. Thus, we see this coefficient changed in twenty-four hours, by a change in environment, from 7.003 to 12.767 for length; from 12.473 to 28.879 for breadth. Different environments give us all sorts of values between such extremes.

It is evident that no particular coefficient of variation can be considered characteristic of *Paramecium,* or of any line of *Paramecium;* certainly not unless the conditions as to growth, environment, etc., are very precisely defined. We have seen that the variations found among different individuals of the same pure line *do not show themselves to be heritable.* This, along with all the rest of the evidence, indicates that if all conditions of growth and environment were made identical throughout a sample of *Paramecia* belonging to a pure line, the coefficient of variation would be very near to zero. In other words, all the variations that we have been able to detect with certainty in a pure line are due to growth and environment. Presumably other variations (congenital and hereditary) must occur at times, but they appear to be so rare that it is difficult to detect them and they would have little effect on the coefficient of variation. By properly varying the conditions, we may get in a pure line all coefficients of variation in length, from a limit near zero up to 20 or more.

4. The ratio of breadth to length (serving to partly define the

form of the body), of course, varies in dependence upon all the three sets of factors with which we have dealt—difference of race, growth and environmental conditions. The smaller races are found to show, under the same conditions, a slightly greater ratio of breadth to length (see Table XXVI.). Within the same race different stages of growth show different ratios; in general, the proportion of breadth to length is greatest in the young, and gradually decreases with age; it increases again very rapidly in preparation for fission. Environmental agents affect in most marked and varied ways the proportion of breadth to length; this is connected with the fact that such agents act more upon the breadth than upon the length. A detailed summary of the different effects of the environment on the proportion of breadth to length is found on pages 478 and 479. The most important general relation is, that increase of nutriment increases the proportional breadth; decrease of nutriment produces the opposite effect. Any agent which suddenly increases the breadth likewise, as a rule, increases the ratio of breadth to length.

5. The coefficient of correlation between length and breadth is the measure of the accuracy with which breadth and length vary proportionately. If the proportion of breadth to length is the same in all individuals of a collection, then the coefficient of correlation of that collection is 1.000.[10] Since, as we have just seen, the proportion of breadth to length is altered by many factors, it follows that all these factors modify the correlation, tending to reduce it below 1.000. The correlation is affected by all the three categories of factors that affect the dimensions in essentially the following ways:

(*a*) The inclusion of different races in a collection, particularly if some of the smaller and some of the larger races occur, makes the correlation less than 1.000, because the proportion of breadth to length is greater in the smaller races. The reduction in correlation produced by this alone is very slight. If we make a collection by

[10] It is perhaps not necessary to point out that the "coefficient of correlation" is *descriptive;* it shows the observed condition in a given set of measurements. The *cause* of this condition is a matter to be determined. *Correlation* is often conceived physiologically as an underlying something that binds two things together, so that they must change correspondingly. The descriptive correlation of the statistician may be the resultant of many factors.

throwing together ten each of the different lines of Table XXV. (page 494), giving the individuals of each line the mean dimensions of its line (thus nearly excluding variations due to growth and environment), then calculate the coefficient of correlation in the same way as for our other collections, we find it to have the high value of .9735.

(*b*) The inclusion of different stages of growth in a collection reduces the correlation below 1.000, since different growth stages have different ratios of breadth to length. A detailed summary of the effects of growth on correlation is found on pages 455 to 457; here we can notice only the main points. In the earliest stages of growth the length is increasing while the breadth is decreasing; hence if we take a collection including various stages within this period, the correlation between length and breadth becomes negative; it may fall to a value of —.3138 (see Table X.). The inclusion of various early stages in a collection of adults decreases the positive correlation shown by the adults. In later growth, length and breadth increase together; the inclusion of various stages at this period has little effect on the correlation; it does, however, tend to reduce it slightly, since length and breadth do not increase at the same ratio. In old specimens, beginning fission, the length decreases while the breadth increases; a collection including different stages in this process tends again to give negative correlation, or to reduce the positive correlation due to other causes. In a collection from the same pure line, in which all specimens are in the same stage of growth, the correlation between length and breadth is high; this would be true no matter what stage of growth is the one represented. Random samples from any culture usually contain many stages of growth; this lowers the correlation between length and breadth.

(*c*) Environmental differences, like growth, affect length and breadth differently or in different proportions; if individuals thus diversely affected are included in a sample, this tends to decrease the correlation between length and breadth. A detailed analysis of the many and important effects of environmental action on the correlation will be found on pages 481 to 484; here, again, we can but summarize the important points.

1. Certain environmental agents increase the breadth while decreas-

ing the length. Inclusion of different stages of this process in a sample reduces the correlation; it may make it zero or negative.

2. Most environmental agents change the breadth more than the length, even when both are changed in the same direction. The inclusion of different stages then reduces correlation.

3. Samples in which some of the specimens are well-fed and plump, others ill-fed and thin, of course, show low correlation, since the ratio of breadth to length is not uniform. This is usually the case in cultures where food is scarce.

4. Addition of abundant nutriment causes the thin specimens to increase in breadth, by taking food, while the plump ones change little. As a result the proportion of breadth to length becomes nearly uniform throughout the lot; the correlation is therefore increased. As a rule, any agent which increases the mean breadth likewise (for the reason just set forth) increases the correlation between breadth and length.

Decrease of nutriment, for the converse reason, decreases the correlation.

5. Any agent that causes rapid multiplication decreases the correlation between length and breadth for the period of multiplication. This is owing to the inclusion in the collection of many stages of growth, showing different proportions of length to breadth.

6. Slight differences in one dimension may be produced without corresponding differences in the other, so that in a collection varying little in length the correlation may be low. But considerable changes in one dimension are usually accompanied by corresponding changes in the other. Hence, when two groups of differing lengths are thrown together, the correlation may become higher than in either one taken separately (for example, see page 437).

In any ordinary sample of *Paramecium* all these varied factors are at work in determining the observed correlation. It is clear that no particular coefficient of correlation can be considered characteristic for *Paramecium* or for any particular race of *Paramecium,* for by various combinations of these factors we may get any coefficient of correlation ranging from a pronounced negative value upward through zero to a high positive value. In Tables X. and XVIII. we

see varied collections showing extremes of value for the coefficient of correlation, from —.3138 to +.8500.[11]

3. Results on Variation, Inheritance and the Effects of Selection.

Our general results with regard to variation, inheritance and the effects of selection are then as follows:

In a given "pure line" (progeny of a single individual) all detectible variations are due to growth and environmental action, and are not inherited. Large and small representatives of the pure line produce progeny of the same mean size. The *mean size* is therefore strictly hereditary throughout the pure line, and it depends, not on the accidental individual dimensions of the particular progenitor, but on the fundamental characteristics of the pure line in question.

In nature we find many pure lines differing in their characteristic mean dimensions.

Our results with the infusorian *Paramecium* are, then, similar to those reached recently by certain other investigators working with pure lines of other organisms. Johannsen (1903) showed that in beans and in barley many pure lines, slightly differentiated from each other, exist in nature, but that selection within a pure line has no effect upon its characteristics. These plants are self-fertilized, so that there is no intermingling of different lines. Hanel (1907) has recently found the same state of affairs in *Hydra* when multiplying by budding. Certain lines tend to have a higher mean number of tentacles, others a lower mean number. But within a given line selection of parents with more or fewer tentacles has no effect on the progeny; selection has no effect within the pure line.

It is doubtless too early to draw any very positive conclusions from these facts. While the results with *Paramecium* seem clear, I intend to test them further in every way possible. It is possible that selection may be made on some other basis, with a better

[11] This fact of course does not render the study of the coefficient of correlation valueless. Its examination under varied experimental conditions is of the utmost importance for determining the real effects of various agents, and in many other ways it furnishes a valuable datum.

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chance of avoiding differences due to environment and growth. It is conceivable that congenital hereditary variations exist, but that they are few in number compared with those due to environment and to slight differences in ways of living, so that in our selection we always get the mere environmental variations. There are decided differences between the specimens of the same line beginning fission, as Table XIII. (page 442) well shows; here the length varied from 156 to 204 microns. It is possible that selection among specimens beginning fission might have a better chance for success. I have attempted this, but it is extremely difficult; I hope to return to it.

We must consider, however, that if the non-inheritable differences are so much more numerous and marked than the inheritable ones as to render conscious selection by human beings ineffective, they would apparently have the same effect on selection by the agencies of nature. The same ground for selection offered by heritable variations is offered so much more fully by those not heritable that there would be as little effect in selection by nature as in selection by man.

Certainly, therefore, until someone can show that selection is effective within pure lines, it is only a statement of fact to say that all the experimental evidence we have is against this. The results set forth in the present paper tend to strengthen that explanation of the observed facts regarding selection, regression, etc., in mixed populations, which is set forth by Johannsen (1903). We need not discuss these in detail here; they are essentially as follows:

1. Selection in a mixed population consists in isolating the various different lines already existing.

2. If selection is made, not of single individuals, but of considerable numbers having a certain characteristic, then by repeated selection it will be possible to approach nearer and nearer to a certain end.

Thus, if we select from such a heterogeneous collection as is represented in Table LXI. all the larger individuals, we shall have taken representatives of many different lines. Our selection will include the larger individuals of lines of median size, as well as the average individuals of lines of large size. The progeny of this selected lot will then consist of various lines, some larger, some smaller, but with the average higher than in the original collection. Another selection

will raise the average still further by getting rid of some of the smaller lines, etc.

3. It has been noticed that in many cases continued selection will not carry a character beyond a certain point. This is due (on the view we are setting forth) to the fact that we have finally isolated that line (or lines) of the original collection which had this character most strongly marked, and since selection of the fluctuations has no effect within the pure line, we can make no farther progress.

4. The phenomenon of so-called *regression* finds its explanation in the same way. It is found that when extremes are selected, the progeny of these extremes stand nearer the mean than did the parents, though they diverge in the same direction as the parents. The reason for this may again be seen by considering such a heterogeneous collection as that of Table LXI., with the effects of selecting the extremes of size. If we select the largest and the smallest individuals, we shall have taken (1) the largest individuals of the largest lines, and (2) the smallest individuals of the smallest lines. But these, when they propagate, produce, as we have seen, merely the *means* of the lines to which they belong. The largest individuals will produce then progeny that average smaller than themselves; the smallest individuals progeny that are larger than themselves; both sets will then approach the mean of the original collection as a whole.

In working with populations reproducing by cross fertilization among the different lines, the conditions on which these results depend become quite obscured, owing to the introduction of new factors, the union of different factors, the appearance of mendelian results, etc. Work with pure lines perhaps shows the real cause for the observed phenomena above set forth.

It must be admitted, then, that the work with pure lines, indicating that selection of fluctuations within the lines is powerless, leads to a simple and consistent explanation of many of the observed facts. But, of course, it gives no explanation of the origin of the different pure lines. Clear proof of the effectiveness of selection even within a pure line would therefore be of the greatest interest, and the present writer would find great pleasure in being the first to present such proof. But until such proof is forthcoming, it must be

admitted that the experimental results go strongly against the effectiveness of selection among slight fluctuating variations in producing new inherited characteristics.

How, then, do the different pure lines rise? This is after all the main problem. Toward its solution further investigations of this series will be directed. It is proposed to study in detail (1) the effects of conjugation on variation, heredity and the production of new races; (2) the effects of long-continued differences in environmental action on different divisions of the same line; (3) the question whether the different lines arise from something like mutations. Further, (4) additional different way of exercising selection within a single line will be tested. The question may be raised whether the production "by mutation" of such slight differences in size as we are here dealing with would not be essentially the same as their production by the inheritance of slight variations—since the extent of the "mutations" would not be greater than what we should call slight variations in size. The difference between the two conceptions almost or quite vanishes when we come to deal with such minute changes in characteristics as those we find in the different lines of *Paramecium*. The "mutation" would be merely a *rare*, heritable, variation, and it is now clear that heritable variations in size are much rarer than had been supposed; their number is so small that in *Paramecium* they are not statistically detectible among the many non-heritable fluctuations due to the environment.

RAQUETTE LAKE, NEW YORK,
August 22, 1908.

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APPENDIX.

Tables of Measurements.

The first twenty-eight tables are distributed through the text. Tables XXIX. to LXIII. follow.

Table XXIX.

Correlation Table for Length and Breadth of 59 Specimens, Age 0 to 5 Minutes. (See Lot 2, Table 10.) Descendants of *D.*

Length in Microns.

Breadth in Microns.	76	80	84	88	92	96	100	104	108	112	116	120	124	128	132	
36										1						1
40						1	1	1				2	1			6
44					2		4	1	2		5	1	3	2	1	21
48			1	2	3	1	3	1	2	2		1	2	1	1	20
52	1	1			3				1		1	1	1		1	10
56	1															1
	2	1	1	2	8	2	8	3	5	3	6	5	7	3	3	59

Length—Mean, 107.660 ± 1.296μ Breadth—Mean, 46.372 ± .332μ
St. Dev., 14.780 ± .916μ St. Dev., 3.804 ± .236μ
Coef. Var., 13.729 ± .868 Coef. Var., 8.200 ± .524

Mean Index, 44.037 per cent.; Coef. Cor., —.3138 ± .0792.

Table XXX.

Correlation Table of Length and Breadth for a Random Sample of Lot 2, Table X.—Same Lot from which came Specimens in Tables VII. and XXIX. Descendants of D. (24 hours in fresh hay infusion: July 17.)

Length in Microns.

Breadth in Microns.	140	144	148	152	156	160	164	168	172	176	180	184	188	192	196	200	204	208	212	216	
36	1				1	2	1		1	1			1								8
40				1	2	5	4	3	3	5	6	3	6	3	1	1					43
44			2	1	1	3	1	2	4	5	3	7	7	4	7	6	3		1		57
48					1	2	2	3	3	2	3	1	2	9	2	4	5	3	1		43
52					1	1	1		2			3	2	6	4	4	1	2	3	2	32
56					1	1			1		2	1			3	2	2	1		1	15
60																	1	1			2
	1	0	2	2	7	14	9	8	14	13	14	15	18	22	17	17	12	7	5	3	200

Length—Mean, 184.100 ± .776μ Breadth—Mean, 46.020 ± .251μ
St. Dev., 16.264 ± .548μ St. Dev., 5.256 ± .177μ
Coef. Var., 8.834 ± .300 Coef. Var., 11.421 ± .390

Mean Index, 25.084 per cent.; Coef. Cor., .4282 ± .0389.

TABLE XXXI.

Correlation Table for the Length and Breadth of the Young of Lot 6, between the Ages of 0 and 19 Minutes. (See Table X., row 7.)

Length in Microns.

Breadth in Microns.	108	112	116	120	124	128	132	136	140	144	148	152	
52		1							2				3
56	1	1	1	1	1		1						6
60	2							1	3	1		1	8
64				1				1	1		1		4
68			1										1
72						1							1
76		1											1
	3	3	2	2	1	1	1	2	6	1	1	1	24

Length—Mean, 128.000 ± 1.908μ Breadth—Mean, 60.168 ± .788μ
St. Dev., 13.856 ± 1.348μ St. Dev., 5.712 ± .556μ
Coef. Var., 10.825 ± 1.066 Coef. Var., 9.495 ± .933

Mean Index, 47.573 per cent.; Coef. Cor., — .0337 ± .1375.

TABLE XXXII.

Correlation Table for Length and Breadth of Young of Lot 7, between the Ages of 0 and 19 Minutes, Descendants of Individual D. (See Table X., row 13.)

Length in Microns.

Breadth in Microns.	108	112	116	120	124	128	132	136	140	144	148	152	156	160	
36												1			1
40						1		1				1			3
44		3		1	2	1				1	1	1	1	1	12
48			1	1	2	1			1	1	4	2		1	14
52	1	1		1	3	1	1					1			9
	1	4	1	3	7	4	1	1	1	2	5	6	1	2	39

Length—Mean, 134.256 ± 1.663μ Breadth—Mean, 46.768 ± .408μ
St. Dev., 15.394 ± 1.176μ St. Dev., 3.792 ± .288μ
Coef. Var., 11.468 ± .857 Coef. Var., 8.109 ± .623

Mean Index, 35.643 per cent.; Coef. Cor., — .2546 ± .1010.

Table XXXIII.

Correlation Table for Length and Breadth of Young of Lot 6, between the Ages of 18 and 28 Minutes. (See Table X., row 8.)

Length in Microns.

Breadth in Microns.	132	136	140	144	148	152	156	160	
48				5					5
52		4	6	7	2	1	2		22
56	2	4	3	2	1	1			13
60		1	2		1	1	1	1	7
64					1		1		2
	2	9	11	14	5	3	4	1	49

Length—Mean, 143.348 ± .624μ Breadth—Mean, 54.284 ± .364μ
St. Dev., 6.480 ± .440μ St. Dev., 3.788 ± .260μ
Coef. Var., 4.521 ± .309 Coef. Var., 6.976 ± .478

Mean Index, 37.921 per cent.; Coef. Cor., 1937 ± .0927.

Table XXXIV.

Correlation Table for Length and Breadth of 106 Specimens, Age 18–28 Minutes. (See row 15, Table X.) (Descendants of *D*, but taken part one day, part another.)

Length in Microns.

Breadth in Microns.	112	116	120	124	128	132	136	140	144	148	152	156	160	164	168	
36	1															1
40								1								1
44				1	2	1		1	3	2	1	2	1			14
48						1	1	6	16	3	3	2			1	33
52						1	5	6	8	3	4	3	1			31
56						4	4	3	2	3	1					17
60							1	2		1	1	1	1			7
64										1		1				2
	1	0	0	1	2	7	11	19	29	13	10	9	3	0	1	106

Length—Mean, 143.812 ± .544μ Breadth—Mean, 50.832 ± .320μ
St. Dev., 8.296 ± .384μ St. Dev., 4.900 ± .238μ
Coef. Var., 5.769 ± .268 Coef. Var., 9.640 ± .451

Mean Index, 35.438 per cent.; Coef. Cor., 1319 ± .0644.

TABLE XXXV.

Correlation Table for Length and Breadth of Young of Lot 6, between the Ages of 35 and 45 Minutes. (See Table X., row 9.)

Length in Microns.

Breadth in Microns.	132	136	140	144	148	152	156	160	
48				1	1	1			3
52	1		1		1	1	1	1	6
56			2	1	2	1	1	2	9
60			1			1		1	3
64					1	1	1	1	4
	1	0	4	2	5	5	3	5	25

Length—Mean, 149.920 ± 1.012μ Breadth—Mean, 55.840 ± .636μ
St. Dev., 7.512 ± .716μ St. Dev., 4.724 ± .452μ
Coef. Var., 5.010 ± .479 Coef. Var., 8.461 ± .813

Mean Index, 37.296 per cent.; Coef. Cor., .2799 ± .1243.

TABLE XXXVI.

Correlation Table for Length and Breadth of Young of Lot 6, between the Ages of 75 and 90 Minutes. (See Table X., row 10.)

Length in Microns.

Breadth in Microns.	140	144	148	152	156	160	164	168	172	176	180	
40	1											1
44												0
48	1		2	1	1		1	1	1			8
52			1	2	6	1	4		1		1	16
56				1			4			1		6
60						1	2	1	1	2	1	8
64												0
68						1	1		1			3
	2	0	3	4	7	3	12	2	4	3	2	42

Length—Mean, 161.524 ± 1.004μ Breadth—Mean, 54.192 ± .600μ
St. Dev., 9.648 ± .712μ St. Dev., 5.752 ± .424μ
Coef. Var., 5.974 ± .441 Coef. Var., 10.617 ± .790

Mean Index, 33.558 per cent.; Coef. Cor., .5232 ± .0756.

TABLE XXXVII.

Correlation Table for Length and Breadth of Young of Lot 9, between the Ages of 3 and 4 Hours. (See Table X., row 16.)

Length in Microns.

Breadth in Microns.	132	136	140	144	148	152	156	160	164	168	172	176	
40					1								1
44		1	1	3	1	2			1				9
48	2	1	9	1	3	3	4	3		1			27
52		5	6	4	3	2	3	4	1	1	1		30
56			3	2	1	3	3	3	2				17
60				2		1	1			1		1	6
64							1		2				3
	2	7	19	12	9	11	12	10	6	3	1	1	93

Length—Mean, 149.636 ± .688μ
St. Dev., 9.856 ± .488μ
Coef. Var., 6.587 ± .327

Breadth—Mean, 51.568 ± .322μ
St. Dev., 4.752 ± .236μ
Coef. Var., 9.212 ± .459

Mean Index, 34.546 per cent.; Coef. Cor., .3201 ± .0628.

TABLE XXXVIII.

Correlation Table for the Length and Breadth of Young of Lot 9, between the Ages of 4.20 and 5 hours. (See Table X., row 17.)

Length in Microns.

Breadth in Microns.	164	168	172	176	180	184	188	192	196	200	204	208	212	216	
52		1	1	4	2	2	1								11
56	1	2	1	2	5	5	5	3	1		1				26
60		1		2	4	3	4	2	3	3	1				23
64				1	3	3	7	6	1	2	1				24
68					1		1	1	2	1					6
72									1	2				1	4
76										1					1
	1	4	2	9	15	13	18	12	8	9	3	0	0	1	95

Length—Mean, 186.736 ± .652μ
St. Dev., 9.416 ± .460μ
Coef. Var., 5.043 ± .247.

Breadth—Mean, 60.168 ± .360μ
St. Dev., 5.224 ± .256μ
Coef. Var., 8.679 ± .428

Mean Index, 32.225 per cent.; Coef. Cor., .5557 ± .0478.

TABLE XXXIX.

Correlation Table for Length and Breadth of Paramecia at the Age of 12 Hours. (Descendants of *D;* See Table X., rows 20 and 21.)

Length in Microns.

Breadth in Microns.	136	140	144	148	152	156	160	164	168	172	176	180	184	188	192	196	200	204	208	212	216	
48	I							I														2
52		I								I												2
56									I	I	I	3	2		I	I	2					12
60											2	I	2	2	2	I	4	I				15
64											I		2	3	5	4	2	3			I	21
68														4	3	3	2	I				13
72													I	5		I						7
76																						0
80															I							I
	I	I	0	0	0	0	0	I	I	2	4	4	7	14	12	10	10	5	0	0	I	73

Length—Mean, 188.988 ± .996μ Breadth—Mean, 62.796 ± .464μ
St. Dev., 12.612 ± .704μ St. Dev., 5.872 ± .328μ
Coef. Var., 6.672 ± .374 Coef. Var., 9.350 ± .526

Mean Index, 33.275 per cent.; Coef. Cor., .4868 ± .0602.

TABLE XL.

Correlation Table for Length and Breadth of Paramecia at the Age of 18 Hours. (Descendants of *D;* See Table X., row 22.)

Length in Microns.

Breadth in Microns.	168	172	176	180	184	188	192	196	200	204	208	212	216	220	224	228	
48			I	2	I						I						5
52	I	I		I	I	5	2	6	5	4	3	I	I				31
56		I		I	4	2	4	6	5		2	5					30
60				2	I			I	5	4	4	2	5	I		I	26
64						I		I	I	4	I	I		2			11
68											2						2
	I	2	I	6	7	8	6	14	16	12	13	9	6	3	0	I	105

Length—Mean, 199.048 ± .780μ Breadth—Mean, 56.496 ± .292μ
St. Dev., 11.844 ± .552μ St. Dev., 4.428 ± .208μ
Coef. Var., 5.949 ± .278 Coef. Var., 7.837 ± .367

Mean Index, 28.427 per cent.; Coef. Cor., 4304 ± .0536.

TABLE XLI.

Correlation Table for Length and Breadth of 300 Paramecia at the Age of 24 Hours. (Descendants of *D;* See Table X., row 23.)

Length in Microns.

Breadth in Microns.	140	144	148	152	156	160	164	168	172	176	180	184	188	192	196	200	
28	1		2	1	1												5
32	1	4	2	5	8	8	7	6	2								43
36				5	8	8	9	15	10	7	2	1			1		66
40			1	2	5	3	10	16	12	7	9	3	1				69
44		1		1	2	6	9	10	12	7	6	6	4		1		65
48					2	2	4	3	1	8	6	3	6			1	36
52								2		3	3			2			10
56							1		2			1	1	1			6
	2	5	5	14	26	27	40	52	39	32	26	14	12	3	2	1	300

Length—Mean, 168.532 ± .419μ
St. Dev., 10.768 ± .296μ
Coef. Var., 6.389 ± .175

Breadth—Mean, 40.320 ± .230μ
St. Dev., 5.892 ± .162μ
Coef. Var., 14.615 ± .411

Mean Index, 23.899 per cent.; Coef. Cor., .5496 ± .0272.

TABLE XLII.

Correlation Table for Length and Breadth of 62 Dividing Specimens of Lot 2. (Descendants of *D;* See Table X., row 31.)

Length in Microns.

Breadth in Microns.	144	148	152	156	160	164	168	172	176	180	184	188	192	196	200	204	208	212	
40														1					1
44	1				2	2										1			6
48			1	1	1	2	1	3	3	2	2	1	1	1				1	20
52		1	1	2	2	7	3	3	5	1							1		26
56				1	2	1				1	2	1							8
60									1										1
	1	1	2	4	7	12	4	6	9	4	4	2	1	2	0	1	1	1	62

Length—Mean, 171.548 ± 1.188μ
St. Dev., 13.848 ± .840μ
Coef. Var., 8.072 ± .492

Breadth—Mean, 50.388 ± .308μ
St. Dev., 3.584 ± .216μ
Coef. Var., 7.111 ± .433

Mean Index, 29.583 per cent.; Coef. Cor., —.1136 ± .0840.

TABLE XLIII.

Correlation Table for Length and Breadth of Specimens in Early Stages of Fission: Constriction less than one-fourth Breadth. Lot 2. (See Table 10, row 30.)

Length in Microns.

Breadth in Microns.	144	148	152	156	160	164	168	172	176	180	
44	1				1	2					4
48			1	1	1	1	1	3	3	1	12
52		1	1	2	2	6	1	3	1	1	18
56				1	2	1				1	5
60									1		1
	1	1	2	4	6	10	2	6	5	3	40

Length—Mean, 165.200 ± .936μ Breadth—Mean, 50.700 ± .364μ
St. Dev., 8.788 ± .664μ St. Dev., 3.432 ± .260μ
Coef. Var., 5.320 ± .402 Coef. Var., 6.769 ± .513

Mean Index, 30.765 per cent.; Coef. Cor., .1048 ± .1055.

TABLE XLIV.

Correlation Table for Length and Breadth of Early Stages of Fisson, in Lot 3. (Depth of Constriction less than one-fourth Breadth.) (See Table X., row 24.)

Length in Microns.

Breadth in Microns.	152	156	160	164	168	172	176	180	184	188	192	
48			1									1
52												0
56	2				3				1			6
60		1	2			1						4
64	1		3	2		2		1		1		10
68	1		1	2		3	2		1		1	11
72				1	1	2						4
76			1	3			1					5
80								1				1
	4	1	8	8	4	8	3	2	2	1	1	42

Length—Mean, 167.620 ± .996μ Breadth—Mean, 65.716 ± .706μ
St. Dev., 9.564 ± .704μ St. Dev., 6.784 ± .499μ
Coef. Var., 5.706 ± .421 Coef. Var., 10.322 ± .768

Mean Index, 39.286 per cent.; Coef. Cor., .2215 ± .0999.

TABLE XLV.

Correlation Table for Length of Body and Depth of Constriction in 119 Dividing Specimens of the Aurelia form, Descended from c. (See Lot 4. Tables VIII. and X.)

Length in Microns.

Depth of Constriction, in Microns.	83.3	86.7	90.0	93.3	96.7	100.0	103.3	106.7	110.0	113.3	116.7	120.0	123.3	126.7	130.0	133.3	136.7	140.0	143.3	146.7	150.0	153.3	156.7	
3.3	1	1	1	5	3	10	11	6	9	3	1		2	1										54
6.7				1		4	3	3	1															12
10.0						1			1	2	2	1		1										8
13.3							1	3	2		2	2												10
16.7											1	2	1		3									7
20.0							1	1		2	1	4	2	1	1									13
23.3										1		1			3			1						6
26.7												1	1			2	1							5
30.0															1			1						2
33.3																					1		1	2
	1	1	6	3	15	16	13	13	8	7	11	6	3	8	2	1	2	0	0	1	0	1		119

Length—Mean, 111.541 ± .797μ Depth of Constriction, Mean, 10.504μ
St. Dev., 12.898 ± .564μ St. Dev. 8.431μ
Coef. Var., 11.563 ± .512

Coef. Cor., 7862 ± .0236. Increase in length with 10μ increase in depth of Constriction, 12.027μ.

TABLE XLVI.

Correlation Table for Length of Body and Depth of Constriction in 63 Dividing Specimens of the Aurelia form, Descended from c. (See Lot 5, Tables VIII. and X.)

Length in Microns.

Depth of Constriction in Microns.	93.3	96.7	100.	103.3	106.7	110.	113.3	116.7	120.	123.3	126.7	130.	133.3	136.7	140.	143.3	146.7	150.	153.3	
3.3	1		3		7		4	5	4		1									25
6.7					1		2	2	1	2	2									10
10.						1	2													3
13.3									2			1	1							4
16.7									1											1
20.											2									2
23.3										1	1									2
26.7												1				1		1		3
30.												2	1	1		1		1		6
33.3												1			2	1	1		1	6
36.7																			1	1
	1	0	3	0	8	1	8	7	8	3	6	5	2	1	2	3	1	2	2	63

Table XLVII.

Correlation Table for Length and Breadth of Dividing Specimens of Lot 4, in which the Depth of Constriction was Less than one-fourth the Breadth. (Aurelia form, Descendants of c.) (See Table X., row 33.)

Length in Microns.

Breadth in Microns.	83.3	86.7	90.	93.3	96.7	100.	103.3	106.7	110.	113.3	116.7	120.	123.3	126.7	
26.7	1														1
30.		1	1	2	1	3	2	1							11
33.3				2		11	6	2	2	1					24
36.7				2	2		5	4	4	2				1	20
40.							1	2	2		1		1		7
43.3									2				1		3
	1	1	1	6	3	14	14	9	10	3	1	0	2	1	66

Length—Mean, 103.737 ± .650μ Breadth—Mean, 34.850 ± .287μ
St. Dev., 7.823 ± .379μ St. Dev., 3.453 ± .203μ
Coef. Var., 7.541 ± .445 Coef. Var., 9.911 ± .587

Mean Index, 33.623 per cent.; Coef. Cor., .6502 ± .0479μ.

Table XLVIII.

Correlation Table for Length and Breadth of Dividing Specimens of Lot 5, in which the Depth of Constriction was Less than one-fourth the Breadth. (Aurelia form, Descendants of c.) (See Table X., row 36.)

Length in Microns.

Breadth in Microns.	93.3	96.7	100.	103.3	106.7	110.	113.3	116.7	120.	123.3	126.7	
33.3	1				2							3
36.7			1									1
40.			1		3	1		1				6
43.3			1				1	1	1			4
46.7					2		5	3	1	1	1	13
50.					1		2	2	1	1	2	9
53.3												0
56.7									2			2
	1	0	3	0	8	1	8	7	5	2	3	38

Length—Mean, 113.333 ± .850μ Breadth—Mean, 45.263 ± .597μ
St. Dev., 7.778 ± .603μ St. Dev., 5.463 ± .423μ
Coef. Var., 6.862 ± .533 Coef. Var., 12.071 ± .947

Mean Index, 39.903 per cent.; Coef. Cor., .6744 ± .0507.

TABLE XLIX.

Correlation Table for Length and Breadth of a Random Sample of Lot 4. (*See Table 10. Aurelia form, Descendants of c. Many dividing.*)

Length in Microns.

Breadth in Microns.	73.3	76.7	80.	83.3	86.7	90.	93.3	96.7	100.	103.3	106.7	110.	113.3	116.7	120.	123.3	126.7	130.	133.3	136.7	140.	143.3	146.7	150.	153.3	156.7	160.	
20.					1																							1
23.3				1	1		1				1																	4
26.7	1			2	2	1		2	6	2	3	1	2					1										23
30.		1		2	2	1	5	6	5	6	7	6	6	1	1	2		1										52
33.3	1	1		1				3	4	3	2	5	7	7	6	2	4	2	3	2								53
36.7							1	1	2	2	3	3	2	7	5	5		2	4	1		3				1		42
40.				1					1		2			4	3	1	6	4	1	2	1	2		1		1		30
43.3												1			1		1	2			1	1	3					10
46.7									1								1				2			1	2	1	1	9
50.																						1						1
	2	2	0	7	6	2	7	12	19	13	18	16	17	19	16	10	12	12	8	5	4	7	3	2	2	3	1	225

Length—Mean, 114.163 ± .784μ Breadth—Mean, 34.207 ± .241μ
St. Dev., 17.443 ± .555μ St. Dev., 5.363 ± .171μ
Coef. Var., 15.279 ± .497 Coef. Var., 15.683 ± .511

Mean Index, 30.177 per cent.; Coef. Cor., .6757 ± .0244.

TABLE L.

Correlation Table for Length and Breadth of a Random Sample of Lot 5 (Table X.). *Aurelia form; Descendants of c. 24 Hours in a Fresh Hay Infusion.*

Length in Microns.

Breadth in Microns.	86.7	90.	93.3	96.7	100.	103.3	106.7	110.	113.3	116.7	120.	123.3	126.7	130.	133.3	136.7	140.	143.3	146.7	
36.7				2	3	1	1													7
40.	1	1		3	1	3	3	1	1											14
43.3			1	2	3	1	6	2	2	3	2									22
46.7				1			1		5	5	1									13
50.							1		4	5	5	1	1		1			1		19
53.3							1			1	2	4	2	2	1					13
56.7												1	2	1		1	1			6
60.								1				2				1				4
63.3																		1		1
66.7																			1	1
	1	1	1	8	7	5	13	4	12	14	10	8	5	3	2	2	1	2	1	100

Length—Mean, 114.033 ± .820μ Breadth—Mean, 47.300 ± .437μ
St. Dev., 12.140 ± .580μ St. Dev., 6.490 ± .310μ
Coef. Var., 10.646 ± .513 Coef. Var., 13.720 ± .667

Mean Index, 41.455 per cent.; Coef. Cor., .8152 ± .0226.

TABLE LI.

Correlation Table for Length and Breadth of a Random Sample of the Culture from which came the Young of Lot 6, Table X., *after 24 hours in fresh hay infusion.* (See row 2, Table XVIII.)

Length in Microns.

Breadth in Microns.	156	160	164	168	172	176	180	184	188	192	196	200	204	208	212	216	220	224	
44		1																	1
48		2		1															3
52				3	2	1		2											8
56	1		2		3	2		1						1					10
60		1			1	1	3	1	4	1	1	1							14
64				1	3	2	1	5	1	4		1							18
68						1	4	4	2	2	4	1		1					19
72						2		3	2	1	2		3						13
76								2	2		1	1	2	1					9
80												1							1
84									2									1	3
88													1						1
	1	4	2	5	9	9	8	18	13	8	8	5	6	3	0	0	0	1	100

Length—Mean, 184.680 ± .848μ Breadth—Mean, 64.880 ± .580μ
St. Dev., 12.596 ± .600μ St. Dev., 8.624 ± .412μ
Coef. Var., 6.821 ± .327 Coef. Var., 13.292 ± .645

Mean Index or Ratio of Breadth to Length, 35.131 per cent.; Coef. Cor., .6469 ± .0392.

TABLE LII.

Correlation Table for Length and Breadth of Descendants of D, in Culture Fluid where Injurious Bacteria have Multiplied. June 25. (See row 5, Table XVIII.)

Length in Microns.

Breadth in Microns.	140	144	148	152	156	160	164	168	172	176	180	184	188	192	196	200	204	208	212	216	220	224	228	232	236	240	244	248	252	256	
36														1																	1
40					1					1																					2
44	1				1				1	2	1	1	1	1		2		1													12
48						1	2	1	3	1	1	6			1	3	2	1	1	1											24
52							1		2	3	8	5	4	2	5	1	3		1	1											36
56			1		1						4	1		1	2	6	1	5	1	5	4	1	1	3	1	1					39
60							1			1		2	1		1	3	2		3	2	2	4	3		1							26
64															1			2		2	3	2		2	1		1		1		15
68															1		1	2		1	1	1	1	2	3	2					15
72																			1	1						1	1				4
76																	1								1				1		3
80																												1			1
	1	0	1	0	3	1	4	1	6	8	14	15	6	5	11	15	10	11	7	13	10	8	5	7	7	4	2	1	1	1	178

Length—Mean, 201.888 ± 1.147μ Breadth—Mean, 56.112 ± .395μ
St. Dev., 22.680 ± .811μ St. Dev., 7.808 ± .279μ
Coef. Var., 11.233 ± .407 Coef. Var., 13.913 ± .507

Mean Index or Ratio of Breadth to Length, 27.850 per cent.; Coef. Cor., .6771 ± .0274.

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TABLE LIII.

Correlation Table for Length and Breadth of a Starving Culture of Descendants of D. Eleven days in small watch glass of hay infusion, not renewed. (See row 6, Table XVIII.)

Length in Microns.

Breadth in Microns.	128	132	136	140	144	148	152	156	160	164	168	172	176	180	184	188	
28		3				1	2										6
32	1		1	6	2	5	1	2									18
36	1		2	6	7	2	4	3			1			1			27
40	1	1	1	1	2	4	6	1	2	3	1	1					24
44				3	1	3	6		2	1	2					1	19
48							1	1	2								4
52									1			1					2
	3	4	4	16	12	15	20	7	7	4	4	2	0	1	0	1	100

Length—Mean, 149.360 ± .736μ Breadth—Mean, 38.080 ± .356μ
St. Dev., 10.896 ± .520μ St. Dev., 5.288 ± .252μ
Coef. Var., 7.296 ± .350 Coef. Var., 13.881 ± .675

Mean Index or Ratio of Breadth to Length, 25.515 per cent.; Coef. Cor., .4481 ± .0539.

TABLE LIV.

Correlation Table for Length and Breadth of Descendants of D, in a rather Ill-fed Culture. September 15. (See row 13, Table XVIII.)

Length in Microns.

Breadth in Microns.	160	164	168	172	176	180	184	188	192	196	200	204	208	212	216	220	224	228	232	
40							2	2	2	1										7
44				1		1	1	3		1	2		2			1			1	13
48	1					1	1	2	2	7	6	1	5	1	2	1				30
52					1	1	2	3	3	3		2	4	3	3	3	4	3		35
50							1	2	1		1	2		1		1	1	2	1	13
60																	1		1	2
	1	0	0	1	1	3	7	12	8	12	9	5	11	5	5	6	6	5	3	100

Length—Mean, 202.280 ± 1.031μ Breadth—Mean, 49.600 ± .298μ
St. Dev., 15.284 ± .729μ St. Dev., 4.412 ± .210μ
Coef. Var., 7.556 ± .362 Coef. Var., 8.896 ± .428

Mean Ratio of Breadth to Length, 24.593 per cent.; Coef. Cor.. .4085 ± .0562.

TABLE LV.

Correlation Table for Length and Breadth of the Same Lot Shown in Table LIV., but after 48 hours in fresh hay infusion. September 15.
(See row 14, Table XVIII.)

Length in Microns.

Breadth in Microns.	124	128	132	136	140	144	148	152	156	160	164	168	172	176	180	184	188	192	196	200	204	208	212	216	
44								1																	1
48												1													1
52					1	1		1	1		1	2	1		2										10
56									2	2	3		1		1		1								10
60								1	1	3	3	2	3	4	2	3	2		1						25
64			1						3		1	1	1		1	1	4	1							14
68	1											3		3	4	4	1	1	2					1	20
72											1	1		1		1	3	4	2	1	1				15
76																	1	1		1					3
80																		1							1
	1	0	1	0	1	1	0	3	7	5	9	10	6	8	10	9	12	8	5	2	1	0	0	1	100

Length—Mean, 175.320 ± 1.060μ Breadth—Mean, 63.160 ± .472μ
St. Dev., 15.708 ± .749μ St. Dev., 7.000 ± .334μ
Coef. Var., 8.959 ± .431 Coef. Var., 11.083 ± .535

Mean Ratio of Breadth to Length, 36.123 per cent.; Coef. Cor., .5376 ± .0480.

TABLE LVI.

Correlation Table for Length and Breadth of Descendants of c. August 9.
(See row 17, Table XVIII.)

Length in Microns.

Breadth in Microns.	100	103.3	106.7	110.	113.3	116.7	120.	123.3	126.7	130.	133.3	136.7	140.	143.3	146.7	150.	153.3	156.7	160.	
23.3	2	1	2		1	1		1	1											9
26.7	1		1	5	1	3		1				1		1						14
30.			2	1	3	1	4			1	1									13
33.3				2	2	3	2	2	1	4	3	2								21
36.7					2	1	4	4	2	1	3		1	3	1					22
40.						1	2	1		5	1				2					12
43.3									1	2		1	1		1				1	7
46.7												1								1
50.													1							1
	3	1	5	8	9	10	12	9	5	13	8	5	3	4	4	0	0	0	1	100

Length—Mean, 123.666 ± .813μ Breadth—Mean, 33.600 ± .400μ
St. Dev., 12.040 ± .573μ St. Dev., 5.917 ± .283μ
Coef. Var., 9.736 ± .469 Coef. Var., 17.608 ± .865

Mean Ratio of Breadth to Length, 27.136 per cent.; Coef. Cor., .6528 ± .0410.

TABLE LVII.

Correlation Table for Length and Breadth of a Sample of the Non-Conjugants of a Conjugating Culture of Descendants of the Individual c. Flourishing culture in a large vessel. September 25, 1907. (See row 21, Table XVIII.)

Length in Microns.

Breadth in Microns.	124	128	132	136	140	144	148	152	156	160	164	168	172	176	180	184	188	192	196	200	
16				1																	1
20			1																		1
24		2	1	1	2																6
28	2		4	4	1	5	1	1	1												19
32		1	3	2	1	3	3	3	3		2	1		1			1				24
36			1	5	5	8	5	2	7	2	5	1		1							42
40					2	3	2	9	5	7	3	1	5	4	2	3	1			1	48
44							2	2	1	2	4		2	5	1	3		2		1	25
48							1	1	2		1	4		2	2	3	3	2	1		22
52												1		1	1	3		1	2		9
56																				1	1
60																		2			2
	2	3	10	13	11	19	14	18	19	11	15	8	7	14	6	12	5	7	3	3	200

Length—Mean, 158.800 ± .877μ Breadth—Mean, 38.560 ± .353μ
St. Dev., 18.384 ± .620μ St. Dev., 7.396 ± .249μ
Coef. Var., 11.578 ± .396 Coef. Var., 19.176 ± .670

Mean Ratio of Breadth to Length, 24.244 per cent.; Coef. Cor., .7135 ± .0234.

TABLE LVIII.

Correlation Table for Length and Breadth of Descendants of c, Five Days after Cessation of Conjugation. Food getting scarce. September 30, 1907. (See row 22, Table XVIII.)

Length in Microns.

Breadth in Microns.	100	104	108	112	116	120	124	128	132	136	140	144	148	152	
20		1			1										2
24	1			1				2							4
28			4	5	1		3			1					14
32			2	3	4	3	1	2	2	2	1				20
36				1	1	1	2	2	5	4		1	2		19
40						1	1	1	3	5	10	6	2	2	31
44											4	2	1		7
48										1	1		1		3
	1	1	6	10	7	5	7	7	10	13	16	9	6	2	100

Length—Mean, 129.640 ± .867μ Breadth—Mean, 35.440 ± .400μ
St. Dev., 12.848 ± .613μ St. Dev., 5.928 ± .283μ
Coef. Var., 9.911 ± .477 Coef. Var., 16.730 ± .820

Mean Ratio of Breadth to Length, 27.262 per cent.; Coef. Cor., .7576 ± .0287.

TABLE LIX.

Correlation Table for Length and Breadth in a Large, Old Culture of Descendants of c, January 23, 1908. (See row 23, Table XVIII.)

Length in Microns.

Breadth in Microns.	100	104	108	112	116	120	124	128	132	136	140	144	148	152	156	160	164	168	172	176	
32						2															2
36				1	3	1															5
40					1	1		1	1	2		1									7
44							2	2	3	1	1										9
48	1					1		1	2	4	2	7		1							19
52									1	1	2	1	3	1		1					10
56									1	1	2	1	1	5		2	1				14
60												2	2	1	1	3	1				10
64											1	1	1	1		1	2	1			8
68															1		2		3		6
72																	1	2	1		4
76															1	1			2		4
80																					0
84																		1		1	2
	1	0	0	1	4	5	2	4	8	9	8	13	7	9	3	8	7	4	6	1	100

Length—Mean, 144.880 ± 1.097μ Breadth—Mean, 54.160 ± .765μ
St. Dev., 16.264 ± .776μ St. Dev., 11.346 ± .541μ
Coef. Var., 11.224 ± .542 Coef. Var., 20.948 ± 1.042

Mean Ratio of Breadth to Length, 37.106 per cent.; Coef. Cor., .8500 ± .0187.

TABLE LX.

Correlation Table for Length and Breadth of Descendants of c. Same Culture shown in Table LIX, but cultivated in small watch glass, January 30 to February 15, 1908. (See row 27, Table XVIII.)

Length in Microns.

Breadth in Microns.	76	80	84	88	92	96	100	104	108	112	116	120	
16			1										1
20	1			6	2	3	1						13
24			2		6	11	6	3	2				30
28					3	1	11	11	11	1	1		39
32							3	5		4	1		13
36									1	2		1	4
	1	0	3	6	11	15	21	19	14	7	2	1	100

Length—Mean, 100.320 ± .528μ Breadth—Mean, 26.480 ± .266μ
St. Dev., 7.828 ± .373μ St. Dev., 3.944 ± .188μ
Coef. Var., 7.804 ± .374 Coef. Var., 14.895 ± .753

Mean Ratio of Breadth to Length, 26.321; Coef. Cor., .7671 ± .0278.

TABLE LXI.

Correlation Table for Length and Breadth of a Random Sample of the "Wild" Conjugating Culture M, January 29, 1908. 200 Non-conjugants, 38 Conjugants.

Length in Microns.

Breadth in Microns.	132	136	140	144	148	152	156	160	164	168	172	176	180	184	188	192	196	200	204	208	212	216	220	224	228	232	236	24	
28				1					1				2																1
32		1				1	3		3		1	1	1																3
36					2		2	2	7	4	4	5	1	2		1	1												11
40						1	2	1	5	6	7	5	4	1	2	6	2	1	2	1			1						31
44	1				1	1		3	1	4	5	5	6	2	6	7	1	5	2	1		1							47
48		1						1	1	5	2	4	3	1	2	5	6	1	4	3					1				52
52												2		1	3	2	4	1	1	1	3	2						1	40
56																	2						1						18
60												2				2	2	1	1	2			1					1	4
64										1		2			3		2	1			1				1				10
68																1													11
72															2			1		1			1						2
76																	1												4
80																		1			1								2
84																													0
88																		1					1						2
	1	2	0	1	3	3	7	7	18	20	19	24	18	9	17	26	18	12	10	8	5	3	4	0	2	0	0	1	238

TABLE LXII.

Correlation Table for Length and Breadth of Dividing Specimens of Lot 1 (Table X.), in which Lengthening had begun. (Constriction more than 4 microns deep.)

Length in Microns.

Breadth in Microns.	160	164	168	172	176	180	184	188	192	196	200	204	208	212	216	220	224	
40					2						1		1				1	5
44	2	1		3	2		2	5	2	4	2	2	3	1			1	30
48		2	10	9	5	7	8	8	4		3	2		3	2			71
52		2	2	10	10	4	12	2	2	4	1	1		1				51
56					3	4	2		1	1	3	3	1					18
60					1	1		3		1								6
64																		0
68															1			1
	2	5	12	22	23	16	22	15	16	12	9	9	6	3	6	2	2	182

Length—Mean, 186.066 ± .710μ Breadth—Mean, 49.540 ± .215μ
St. Dev., 14.208 ± .502μ St. Dev., 4.296 ± .152μ
Coef. Var., 7.636 ± .271 Coef. Var., 8.671 ± .309

Mean Ratio of Breadth to Length, 26.796 per cent.; Coef. Cor., —.0938 ± .0496.

TABLE LXIII.

Correlation Table for Length and Breadth of Dividing Specimens of the Aurelia Form (Descendants of c), in which Lengthening had begun.

(See Lot 4, Tables VIII. and X.)

Length in Microns.

Breadth in Microns.	100.	103.3	106.7	110.	113.3	116.7	120.	123.3	126.7	130.	133.3	136.7	140.	143.3	146.7	150.	153.3	156.7	
26.7			1		1					1									3
30.			1	1	1	1		2		2	1		1						10
33.3	1	1	2	2	2	2	4	1		1	1	1							18
36.7		1				3	5			2									11
40.					1		2	1		2	1					1		1	9
43.3									1										1
46.7													1						1
	1	2	4	3	5	6	11	4	1	8	3	1	2	0	0	1	0	1	53

Length—Mean, 121.383 ± 1.053μ Breadth—Mean, 34.590 ± .383μ

St. Dev., 11.367 ± .743μ St. Dev., 4.147 ± .273μ

Coef. Var., 9.365 ± .613, Coef. Var., 11.989 ± .797,

Mean Ratio of Breadth to Length, 28.648 per cent.; Coef. Cor., .3100 ± .0837.

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