ON THE EFFECT OF SELECTION IN PARAMECIUM¹

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In 1908, JENNINGS (p. 511) reported on the effect of selection in *Paramecium* as follows:

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

While these results seemed conclusive, the fact that they were based upon separate lots of from one hundred to as few as a dozen individuals, together with the desirability of testing the principle independently, led to the present series of experiments in which somewhat larger numbers of *Paramecia* have been measured.

These experiments were begun in 1911 at the suggestion of Professor CHARLES ZELNY to whom I take pleasure in expressing my indebtedness for help and kindly criticism.

MATERIAL AND METHODS

Two species of *Paramecia* were used: *Paramecium caudatum*, the common large form having a single micro-nucleus and a pointed posterior end, and *P. aurelia*, the small species with two micro-nuclei and a blunt caudal end.

As the number of factors which may affect the metabolic processes, and incidentally the size of the animals, is large, the methods are given in some detail. In a typical experiment a *Paramecium* was placed in a few drops of hay infusion on a depression slide kept in a moist chamber. After several generations, the longest and the shortest *Paramecium* were selected from its descendants. This was accomplished by removing several of the largest and several of the smallest animals, making the final selections from these individuals. The *Paramecia* selected were isolated in equal quantities of infusion and kept under environmental

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conditions as nearly identical as it was possible to make them. As the descendants of each group increased in number, they were transferred to watch glasses. Later, all animals in each group were killed and measured. Separate capillary pipettes were used for transference, feeding, and killing. To lessen the harmful effects of excretion products, the *Paramecia* were transferred to fresh culture media every second day. In all cases, the transference pipette was rinsed in boiling water immediately after caring for each group to avoid the possibility of mixing cultures. The food consisted of separate infusions of timothy hay, alfalfa, wheat straw, oat straw, beef, and liver, left open to the air for twenty-four hours after boiling. Scrupulous care was taken to give the same quantity of food to each group of *Paramecia* under comparison.

For killing and preserving the animals, two kinds of fluids were used: (1) Worcester's fluid (a saturated solution of mercuric chloride in 10 percent formol), and (2) 10 percent formol. Both fluids killed instantly and preserved the animals satisfactorily. However, specimens kept in Worcester's fluid some weeks became partially imbedded in mercuric chloride crystals. No harmful effects have been noted from the use of 10 percent formol. In killing, approximately fifty individuals were placed in a small receptacle and overwhelmed with the hot killing fluid. All *Paramecia* in the groups under comparison were, as nearly as possible, killed at the same time.

Measuring was accomplished by two methods. By the first, the images of the animals were thrown upon a screen with a projection microscope, giving a magnification of three hundred and twenty diameters. The length and breadth of the image of each individual were measured with a millimeter ruler. Here each unit of measurement was equal to three and one-eighth microns. By the second, measurements of the animals were made with a compound microscope and ocular micrometer with a combination of lenses such as to make one unit of measurement equal to seven and one-half microns. In each method, the use of a checkered glass slide and a mechanical stage made it possible to locate all of the *Paramecia* without duplicating measurements. The well known formulæ brought together by DAVENPORT (1899) have been employed in computing the constants. All computations were checked by the aid of a Monroe mechanical calculator.

In making the polygons for length of groups A, B, and C (figs. 1, 3, 5), four units of measurement were grouped together to make a single unit on the abscissa of the polygon. This was done in order to destroy any irregularities due to an unconscious prejudice on the part of the

measurer for certain numbers. At the bottoms of these polygons, the numbers are the measurements in millimeters of the images of the Paramecia. In the polygons for breadth of groups A, B, and C (figures 2, 4, 6), two units of measurement were grouped together to make one unit on the abscissa of the polygon.

In the polygons for length and also those for breadth of groups D_1 and D_2 (figures 7, 8), two units of measurement were grouped together to make a single unit on the abscissa. There seemed to be little evidence of error due to unconscious preference for certain numbers in measuring the breadths of these groups. This was probably due to the greater ease of taking this measurement, since it was possible to hold both of its limits on the scale, in the eye at once. The numbers at the bottoms of the polygons of D_1 and D_2 (figures 7, 8) are in microns. In all of the polygons, the numbers to the left indicate numbers of Paramecia.

EXPERIMENTS

The main question in these experiments is: Can diverse groups of Paramecia be obtained by selection within a single group, i.e., if, from the progeny of a single animal, the largest and the smallest individual be selected, will there develop two diverse groups, one of greater mean size than the other? To answer this question, four extensive tests have been made. The groups, in which the selections were made, were designated as A, B, C, and D, respectively.

Experiment 1. The original Paramecium in group A (table 1) was a descendant of a *P. aurelia* taken from a stream seven months previously. The former was isolated on a depression slide in three drops of hay infusion. When its descendants numbered 110, two individuals were selected from them,—one, the shortest Paramecium, whose approximate length and breadth were 90 and 36 microns, respectively, the other, the longest Paramecium, approximately 162 microns in length and about 45 microns in breadth. The progeny of the shortest individual became known as group A_1 , that of the longest as group A_2 . When the descendants in each of these two groups numbered several hundred, they were killed and measured. The 705 animals of group A_1 had a mean length of 124.996 microns with a probable error of ± 0.281 microns and the 724 individuals of group A_2 had a mean length of 124.346 microns with a probable error of ± 0.300 microns. The average breadth of group A_1 was 55.050 ± 0.138 microns, that of group A_2 was 54.290 ± 0.146 microns.

FIGURE 1.—Frequency polygons of the lengths of groups A_1 and A_2 . Continuous line, A_1 , number of *Paramecia* 705. Broken line, A_2 , number of *Paramecia* 724.

From these data, it is seen that the longer ancestor gave individuals which were, on the average, slightly shorter than those derived from the shorter ancestor, but the difference is much less than three times the sum of the probable errors of the mean lengths and thus fails to come near the limit of what is ordinarily considered as significant. Similarly, the progeny of the larger ancestor have the smaller mean breadth. The difference appears to be without significance, since it is less than three times the sum of the probable errors of the means.

Experiment 2. The original *P. caudatum* in group B (table 2) was taken from a stream and isolated on a depression slide in a medium consisting of three drops of stream water, one drop of hay infusion,

TABLE 1.—Diagram of experiment 1.

TABLE 2.—Diagram of experiment 2.

FIGURE 2.—Frequency polygons of the breadths of groups A_1 and A_2 . Continuous line, A_1 , number of Paramecia 705. Broken line, A_2 , number of Paramecia 724.

and one drop of beef infusion. From approximately 425 of its descendants, one of the shortest Paramecia, whose approximate length and breadth were 162 and 45 microns, respectively, and one of the longest Paramecia, having a length of approximately 235 microns and a breadth of about 54 microns, were selected. The progeny of the shortest one were designated as group B_1 , those of the longest as group B_2 . When the descendants in each of these groups numbered several hundred, they were killed and measured. The 703 individuals of group B_1 had a mean length of 179.540 microns with a probable error of ± 0.465 microns and the 923 of group B_2 had a mean length of 174.762 microns with a probable error of ± 0.403 microns. The average breadth of group B_1 was 56.359 ± 0.200 microns, and that of B_2 was 57.640 ± 0.168 microns.

FIGURE 3.—Frequency polygons of the lengths of groups B_1 and B_2 . Continuous line, B_1 , number of Paramecia 703. Broken line, B_2 , number of Paramecia 923.

An examination of these data shows that the longer ancestor gave descendants which were, on the average, slightly shorter than those derived from the shorter ancestor. But the fact that the progeny of the larger ancestor increased (approximately $3\frac{1}{2}$ microns) in mean breadth may account, in part, for the slight difference in the lengths of these groups. At any rate, the descendants of the longest Paramecium selected have a smaller mean length than do the progeny of the shortest Paramecium selected. The measurements thus indicate a negative effect of selection, but obviously a more probable explanation is the existence of some uncontrolled environmental difference between the two cultures.

Experiment 3. The original *P. caudatum* in group C (table 3) was taken from a stream and isolated in a medium consisting of three drops of stream water, one drop of hay infusion and one drop of beef infusion. When its descendants numbered 380, selections were made as follows: (1) the shortest Paramecium with an approximate length and breadth of 162 and 36 microns, respectively, and (2) the longest individual with a length of approximately 198 microns and a breadth of about 45 microns. The progeny of the shortest animal was known as group C_1 , those of the longest as group C_2 . When the descendants in each of these two groups became numerous, they were killed and measured. The 815

TABLE 3.—*Diagram of experiment 3.* TABLE 4.—*Diagram of experiment 4.*

individuals of group C_1 had a mean length of 167.105 microns with a probable error of ± 0.465 microns and the 710 of group C_2 had a mean length of 164.034 microns with a probable error of ± 0.496 microns. The average breadth of group C_1 was 66.093 ± 0.184 microns, and that of C_2 was 67.800 ± 0.231 microns.

FIGURE 4.—Frequency polygons of the breadths of groups B_1 and B_2 . Continuous line, B_1 , number of Paramecia 703. Broken line, B_2 , number of Paramecia 923.

These data show that the difference between the mean lengths of these two groups, is slightly more (0.088 microns) than three times the sum of the probable errors of their means, and thus exceeds the limit of what is ordinarily considered a significant difference. But, here, also, the longer ancestor gave descendants whose mean length was less than that of the progeny of the shorter ancestor, so that the difference can hardly be considered as significant. It seems probable that the difference may be due to an uncontrolled factor in the environment. A comparison of the

breadths shows a slight increase in favor of the descendants of the longer ancestor, but it seems doubtful if such a difference can be accepted as significant without question.

FIGURE 5.—Frequency polygons of the lengths of groups C_1 and C_2 . Continuous line, C_1 , number of *Paramecia* 815. Broken line, C_2 , number of *Paramecia* 710.

FIGURE 6.—Frequency polygons of the breadths of groups C_1 and C_2 . Continuous line, C_1 , number of *Paramecia* 815. Broken line, C_2 , number of *Paramecia* 710.

Experiment 4. In making selections of shortest animals in previous experiments, it seemed possible that some of them might be young individuals, so a test was made with *Paramecia* that were known to be mature. The original *P. aurelia* (table 4, p. 394) of group D was taken from a laboratory culture and isolated in three drops of straw infusion. From approximately 900 of its descendants, five small *Paramecia* and five large ones were selected. Each of these ten individuals was placed in a separate receptacle and watched until the first sign of a constriction appeared, whereupon the approximate measurement of the animal was made. From these ten, two individuals were selected,—one, the shortest *Paramecium* whose approximate length and breadth were 135 and 60 microns, respectively, the other, the longest one, with a length of approximately 184 microns and a breadth of about 64 microns. The progeny of the shortest *Paramecium* was designated as group D_1 , those of the longest as group D_2 . When the descendants in each of these two groups numbered several hundred, they were killed and measured. The 694 individuals of group D_1 had a mean length of 125.307 microns with

FIGURE 7.—Frequency polygons of the lengths of groups D_1 and D_2 . Continuous line, D_1 , number of *Paramecia* 694. Broken line, D_2 , number of *Paramecia* 591.

FIGURE 8.—Frequency polygons of the breadths of groups D₁ and D₂. Continuous line, D₁, number of *Paramecia* 694. Broken line, D₂, number of *Paramecia* 591.

a probable error of ± 0.354 microns and the 591 of group D_2 had a mean length of 117.996 microns with a probable error of ± 0.355 microns. The average breadth of D_1 was 44.923 ± 0.159 microns, and that of D_2 was 48.211 ± 0.348 microns.

An examination of these data shows that the longer ancestor gave descendants which were, on the average, slightly shorter than those derived from the shorter ancestor. But the fact that the mean breadth of group D_2 is greater than that of D_1 , may account in part for the difference in the mean lengths of these groups. At any rate, the descendants of the longest *Paramecium* selected have a smaller mean length than do the progeny of the shortest *Paramecium* selected. The measurements thus indicate a negative effect of selection, but here, also, a more probable explanation is the existence of some uncontrolled environmental difference between the two cultures.

DISCUSSION AND CONCLUSIONS

From the results of these tests, it appears that diverse groups of *Paramecia* cannot be obtained by selection within the progeny of a single individual. Even when the difference in length between the shortest and the longest *Paramecium* selected is as much as 57 microns (average difference in the four tests), diverse groups failed to develop, each of the two groups under comparison either having the same mean lengths or the progeny of the longest *Paramecium* having the smaller mean length. When repeated differences in size such as these exist (i.e., when one of two animals under comparison is approximately twice as large as the other) with no corresponding difference appearing in the mean size of their respective progeny, and when similar results are obtained from large numbers of animals, it would seem obvious that variations in size in *Paramecia* descended from the same ancestor are not inherited, but that the mean size of the descendants is determined by the group to which the individuals belong.

In dealing with the question of how such variations arise, the effects of growth and of environment may be considered. No tests were made by the writer on variation of size due to growth, but JENNINGS (1908, table 15) has shown that the mean length of the progeny of a single *Paramecium* varies greatly at different ages. The difference in mean length of young individuals, two and one-half minutes after separation, and of adults of the same line eighteen hours old was more than 90 microns. These differences of size (due to growth) are almost sufficient

to explain the appearance of the variations without taking into account the effect of the environment. That the small *Paramecia* selected in the present experiments (groups A, B, and C) were young ones is obvious from the fact that the large animals selected divided first (table 5). At the end of the first three days, the descendants of the larger individuals were from one and one-half to two times as numerous as those of the smaller ones.

The environment presents a condition too complex to permit a full discussion here. It is known that the effect of the environment on the size of *Paramecia* may be considerable, especially when the animals are subjected to opposite extremes. JENNINGS (1908, table 18) shows that, by varying the amount of nutrition of animals in the same line, differences of 38 microns in mean lengths and 11 microns in mean breadths may be obtained. WOODRUFF (1911, p. 569) is of the opinion that "the variations in the daily rate in the different volumes of water is due to the excretion products of the *Paramecia* themselves." It is well known that the mean size of a group of individuals is affected by a change in division rate, for the more rapid the latter, the larger will be the number of young *Paramecia* present in the culture at that time, and vice versa. Even in cultures in which an attempt was made to keep environmental conditions constantly favorable, the effect on the mean size of the progeny of a single individual was noteworthy, as will be seen in the comparison which follows. A "new wild" *P. aurelia* (from which was descended group A of this series) was taken from a stream and isolated in straw infusion. Its descendants were kept under conditions similar to those in the tests. In two weeks, 799 (lot 21) of its progeny, taken at random, were killed and measured (table 6). The remainder of the culture was continued as before (except that alfalfa infusion was used instead of straw infusion). Two months later, 337 (lot 22) of them, also taken at random, were killed and measured (table 6). In five months, groups A_1 and A_2 (table 1), descendants of this line, were killed and measured. Even though these four lots were kept under favorable conditions, there was a difference in mean lengths of 25 microns (between lot 22 and group A_2 table 7,). The same two groups differed 19 microns in breadth.

The variations in size of *Paramecia* due to the combined effects of growth and environment are so great that the cause of the appearance of such variations cannot be in doubt. In the fourth experiment (table 4), it will be recalled that the selection was made from mature animals,

TABLE 5.—Comparison of number of progeny of *Paramecia* during first three days after selection.

Date	Group A		Group B		Group C		Group D	
	A ₁ Smaller	A ₂ Larger	B ₁ Smaller	B ₂ Larger	C ₁ Smaller	C ₂ Larger	D ₁ Smaller	D ₂ Larger
1st day isolated	1	1	1	1	1	1	1	1
2nd day	2	8	4	8	4	4	2	1
3rd day	14	28	12	18	10	20	6	4

TABLE 6.—Comparison of different lots of the descendents of a single *Paramecium* (*aurelia*); all lots kept under favorable environmental conditions. TABLE 7.—Comparative lengths and breadths (in microns) of four groups of descendents of a "new wild" *P. aurelia*, all groups having been kept under favorable environmental conditions.

	Lot 21	Lot 22	Group A ₁	Group A ₂
Mean length	100.666 ± 0.296	98.760 ± 0.517	124.996 ± 0.281	124.346 ± 0.300
Mean breadth	39.705 ± 0.146	34.837 ± 0.224	55.050 ± 0.138	54.290 ± 0.146

and that the result was negative. Here the factor of growth, of course, did not enter, but the differences in the respective lengths and breadths of the animals selected in group D were such as could readily be due to the environment. Hence, it seems evident that variations in size among descendants of a single *Paramecium* arise from the effects of growth and environment and that such variations are not inherited. It is possible that variations due to heredity may occur, but if they do, they are few in number compared with those arising from growth and environment, so that in the selections, variations due only to these two factors, are obtained.

A comparison of groups A and D (*P. aurelia*), and of B and C (*P. caudatum*) indicates that lines which differ in their characteristic mean sizes occur in nature (table 8).

In examining the results of the four experiments (table 8), it is seen that, in the first, the difference between the mean length of the progeny of the shortest *Paramecium* selected and of that of the longest one selected is less than three times the sum of the probable errors of their means and thus fails to reach the limit of what is ordinarily considered a significant difference. In each of the other three experiments (second, third, and fourth), the mean length of the progeny of the largest *Paramecium* was less than that of the descendants of the smallest one (table 8). Here, what was lost in length was compensated for, in part, by breadth. While the latter was not sufficient in any case to account for

TABLE 8.—Mean sizes of the different groups of *Paramecia* under comparison.

	Group	Length in microns			Breadth in microns		
		Parents	Offspring	Difference	Parents	Offspring	Difference
Group A, Experiment 1	A ₁	90	124.996 ± 0.281	0.650	36	55.050 ± 0.138	0.760
	A ₂	162	124.346 ± 0.300		45	54.290 ± 0.146	
Group B, Experiment 2	B ₁	162	179.540 ± 0.465	4.778	45	56.359 ± 0.200	1.281
	B ₂	235	174.762 ± 0.403		54	57.640 ± 0.168	
Group C, Experiment 3	C ₁	162	167.105 ± 0.465	3.071	36	66.093 ± 0.184	1.707
	C ₂	198	164.034 ± 0.562		45	67.800 ± 0.231	
Group D, Experiment 4	D ₁	135	125.307 ± 0.354	7.311	60	44.923 ± 0.159	3.288
	D ₂	184	117.996 ± 0.355		64	48.211 ± 0.348	

the difference in mean lengths, it is without doubt, significant. From these results and from the fact that the groups were large (minimum of 591, maximum of 923, total nearly 6000 individuals), it seems evident that selection within the progeny of a single *Paramecium* is without effect.

These results are in accord with those of certain other investigators working with organisms descended from single individuals. After making his well-known tests on self-fertilizing beans and barley, JOHANNSEN (1903) showed that, in nature, there are many pure lines, differing slightly from each other, but that selection within the progeny of a single seed had no effect upon the characteristics of that line. As stated elsewhere in this paper, JENNINGS (1908), in his extensive work on *Paramecium*, came to a similar conclusion in regard to the occurrence in nature of many "pure lines" differing slightly in their characteristic mean dimensions, and to the ineffectiveness of selection within a pure line. HANEL (1907), who experimented with *Hydra grisea* reproducing by budding, found strains differing with respect to numbers of tentacles. On selecting within a strain, however, such variations were not inherited. LASHLEY (1915) working with *Hydra viridis* found a slight difference, in the direction of selection, in the averages of the earlier buds of two groups, but this difference did not persist in the buds produced later by the same parents; regression was complete in a single generation. Even by continued selection in the same lines, LASHLEY (1916) was unable to obtain any positive results, selection within the progeny of a single *Hydra* being without effect. KELLY (1913) found in the parthenogenetic insect, *Aphis rumicis*, variations in the relative lengths of the third and fourth segments of the antennae. While his selections were too limited to be conclusive, they indicate that "the somatic differences in the parthenogenetic line are not inherited." EWING (1914) obtained similar results in parthenogenetic strains of *Aphis avenae*. In a given strain, he found large variations from the mean, but they were not transmitted to the offspring, selection within the descendants of a single parthenogenetic *Aphis* being without effect.

It is obvious that the findings of these investigators, together with the results of the present tests, support the conception of the non-variability of the character in inheritance. Clearly, these results do not support the recent statement of CASTLE (1916, p. 253) who says, "No race of animals has yet been shown to be devoid of genetic variations in size." So far as the writer has been able to ascertain, races of *Paramecium*

aurelia descended from single ancestors are not known to possess genetic variations in size, while JENNINGS (1908, table 23, i and its progeny), and the writer in this paper (experiments 1 and 4, table 8) have shown that variations in size among the progeny of a single *P. aurelia* are not inherited. However, evidence of genetic variations among the descendants of single organisms, reproducing asexually, has recently been set forth. CALKINS and GREGORY (1913) found heritable variations in size and in conjugating power within the progenies of single ex-conjugants of *Paramecium caudatum*. STOCKING (1915) has shown that, in the progeny of a single ex-conjugant of *Paramecium caudatum*, abnormalities were inherited through a large number of generations. In certain lines, however, such abnormalities were not hereditary. MIDDLETON (1915) by long continued selection within the progeny of a single *Stylonychia pustulata* (reproducing by fission), obtained two sets ('clones'?) differing characteristically from each other in rate of fission, under identical conditions. JENNINGS (1916), working on *Diffugia*, reproducing asexually, has found selection within the progeny of a single individual effective. From these findings, it is evident that certain morphological and physiological variations occurring within the progeny of a single individual (reproducing asexually) may be inherited. However, before drawing definite conclusions, tests should be made on still other kinds of uniparental organisms, using as bases of selection a large variety of characters.

SUMMARY

1. Variations in size of *Paramecia* descended from a single animal appear to be due to the environment and to growth.
2. In these experiments, in which nearly six thousand animals were measured, the results indicate that selection within the progeny of a single *Paramecium* is without effect. Even when one of the animals selected was twice as long as the other, diverse groups failed to develop, each of the groups under comparison either having the same mean lengths, or the progeny of the longest *Paramecium* having the smaller mean length.

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