HERITABLE EFFECTS OF TEMPERATURE DIFFERENCES ON THE FISSION RATE OF *STYLONYCHIA PUSTULATA*¹

AUSTIN RALPH MIDDLETON

University of Louisville, Louisville, Kentucky

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TABLE OF CONTENTS

	PAGE
INTRODUCTION	535
Methods	536
FIRST SERIES OF EXPERIMENTS.....	537
(1) General outline	537
(2) The direct effect of the diverse temperatures on the fission rate (A, figure 1)	539
(3) First test of the permanence of modification (B, figure 1).....	541
(4) Second test of the permanence of modification (D, figure 1).....	543
(5) Third test of the permanence of modification (F, figure 1).....	543
(6) General results of the first series of experiments.....	545
SECOND SERIES OF EXPERIMENTS.....	545
(7) General outline (figure 3).....	545
(8) Results of keeping the basic stocks for eight months under diverse temperatures (A, figure 3)	547
(9) Test of modifications produced after 20 days in diverse temperatures (B, figure 3)	555
(10) Test of modifications produced after 40 days in diverse temperatures (C, figure 3)	555
(11) Test of modifications produced after 60 days in diverse temperatures (D and E, figure 3)	558
(a) Both sets in the same intermediate temperature (D, figure 3)	559
(b) Both sets in the same high temperature (E, figure 3).....	559
(12) Tests of modifications produced after 120 days in diverse temperatures (F, G and H, figure 3)	560
(13) Test of the modifications after 170 days in diverse temperatures (K, figure 3)	563
(14) General results of the second series of experiments.....	564
THIRD SERIES OF EXPERIMENTS.....	564
(15) General outline (figure 5)	564
(16) The basic cultures, kept for 60 days at diverse temperatures (A, figure 5)	566
(17) Tests after 30 days at diverse temperatures.....	566
(a) Test at high temperature (B, figure 5).....	566
(b) Test at intermediate temperature (C, figure 5).....	567
(c) Test at low temperature (D, figure 5).....	567
(18) General results of the third series of experiments	567
GENERAL RESULTS AND CONCLUSIONS.....	571
LITERATURE CITED	572

¹ From the Zoological Laboratory of the JOHNS HOPKINS UNIVERSITY.

INTRODUCTION

The present paper is a contribution to the study of the question of how living systems may become modified so that the modifications remain in later generations, even after the modifying factors are removed. The living system shows a great power of compensating for disturbances induced in it; if the compensation does not come in the same generation that is affected, it usually comes in the next one, so that "acquired characters are not inherited." Can we change the living system so that the change will persist into later generations?

To attack this question, the simplest possible conditions are here selected. The organism is an infusorian multiplying by simple fission. The character selected is one most easily modified, namely, the rate of fission. This can be changed by all sorts of conditions; but a given fission rate is, under given conditions, characteristic for a given stock; it is thus heritable in this kind of reproduction. As a modifying agent temperature was chosen. The experiments are therefore most simple in plan. Half the progeny of a given individual are subjected to high temperature, half to low temperature. The former divide rapidly, the latter slowly. They are kept for many generations under these diverse conditions. Finally, we bring both sets again to the same temperature and allow them to multiply. Will any effect have been produced, such as to show in the later history of the race? Or will both sets compensate at once their different fission rates, and return to the rate that they would have shown had they not lived under different conditions? If a lasting modification has been produced, will it be in the same direction as the immediate action of the external agent, so that those that have been in the high temperature continue to divide more rapidly at the intermediate temperatures? Or will there possibly be a swing in the opposite direction, so that those that have been dividing slowly, in the low temperatures, now divide more rapidly in the intermediate temperature?

So far as I have been able to discover, no studies have been made to test these matters. Many investigations have been made of the immediate effect of diverse temperatures on the rate of fission, such as those of MAUPAS (1888, 1889), CALKINS (1902), WOODRUFF and BAITSELL (1911) and others. But none of these studies touches the specific problem here investigated.

The organism employed, *Stylonychia pustulata*, is the same one used in my former study, on the effects of selection on the fission rate (MIDDLETON 1915).

In the experiment here described, tests of the lasting effect of divers-

ity of temperature were made under many conditions; when the diversity of conditions had lasted but a short time, and when it had acted for moderate and for long periods. Further, sometimes, the tests were made with both sets under a common intermediate temperature; sometimes with both under the same high temperature, sometimes with both under a low temperature.

Three long series of experiments were carried on. In one of these the two halves of a single stock were kept for eight months under diverse temperatures, with tests of their progeny at various intervals and under various conditions. The other two series were shorter; one was a preliminary series; the other followed the main series. Diagrams of the course of the three series are given in figure 1 (page 538), figure 3 (page 546), and figure 5 (page 564).

The work was undertaken in the Zoölogical Laboratory of the JOHNS HOPKINS UNIVERSITY, and I am gratefully indebted to Professor H. S. JENNINGS for valuable criticisms.

METHODS

In order that results of the study of the differential effects of a particular environmental agent on a character so delicately responsive to all sorts of agents as the fission rate of an infusorian may be of value, other conditions must be kept uniform. To secure this uniformity and to guard against contamination of the lines by exchange of individuals, or by introduction of an outside individual, a modification of the method described by JENNINGS (1913) was used.

As culture medium, 1/16 percent Horlick's malted milk was employed, prepared as described in my previous paper (MIDDLETON 1915, p. 455). The animals were cultivated on ground-glass slides, each bearing two circular depressions capable of holding four or five drops of liquid. These were kept in moist chambers. Three drops of culture medium were used in each depression, and no cover glasses were employed. Half the slides were subjected to higher temperatures (giving the "high lines"), half to lower temperatures (giving the "low lines"). The word "line" is here used as a convenient designation for the series of individuals derived in a given experiment from a single parent. All the progeny of a single parent through fission are designated a *clone*.

Uniformity of bacterial content in the culture medium was secured by washing the animals in fresh culture medium before every transfer to a new slide. The animals were allowed to swim about for a time in the fresh medium, in order to wash themselves largely free of bacteria;

they were then transferred to the definitive slide in new fluid. The pipette used in transferring the individuals was invariably sterilized in boiling water after each transfer, thus preventing the unintentional introduction of any individual which might cling to the pipette; there was thus no possibility of admixture of lines. The slides were labelled in lead pencil; the number of fissions and selections at each examination were likewise recorded on them, to be later transferred to permanent records. The individual lines were designated in accordance with the plan set forth by JENNINGS (1913).

Each concavity contained characteristically one individual. The slides were examined every other day or oftener, and all but one of the individuals that had been produced were removed. Thus the number of lines and of individuals was kept constant. The remaining individual was transferred to a new slide, with fresh fluid. In deciding which individual should be thus retained to carry on the line, "balanced selection" was practiced throughout, in accordance with the plan set forth in my former paper (MIDDLETON 1915, p. 459). This is based on the following considerations. Often one of the two individuals formed by the division of a given parent divides before the other. In my earlier paper I showed that by continued selection, on the one hand of the individuals that divide early, on the other hand of those that divide late, two sets are finally produced with characteristically diverse fission rates. For our present purposes it is necessary to avoid this effect of selection. This is done by choosing on alternate days first the faster, then the slower individual, if any difference exists. The effect of any selection toward rapid fission is thus compensated at the next transfer by a reverse selection toward slow fission. (For details see my previous paper.)

In drawing off progeny from each of the two sets of lines ("high" and "low") for tests under intermediate temperatures, the original stocks were still kept under the diverse temperatures. That is, when test lines are to be started, half the individuals of a given line are left under the old temperature, half placed under the new intermediate temperature. Each line is thus doubled, its two halves serving as controls one for the other, as to the effects of the new conditions.

FIRST SERIES OF EXPERIMENTS

(1) *General outline*

The first series of experiments was a preliminary one, to feel out the problem and to determine the best methods of procedure. The plan of

the experiments was not so well worked out, nor the conditions so accurately controlled as in later experiments.

The course of the series, with the conditions under which the organisms were kept, the periods of time, and the various tests made, are indicated in figure 1. The plan was in brief as follows:

One hundred and twenty animals of the tenth generation from a single individual of *Stylonychia pustulata*, from a 'wild' laboratory culture, were isolated on the slides, one animal in each concavity (on June 17, 1915). Sixty of the 120 lines so formed constituted the "high" set, being kept at a temperature of 30° to 32° centigrade; the other sixty formed the "low" set, being kept at 18° to 20° centigrade. They were thus propagated for twenty days, records being kept of the numbers of fissions (A, figure 1). At the end of twenty days, the first test was made; progeny from both sets were placed at the room temperatures (B, figure 1). Meanwhile, the two original sets were allowed to propa-

FIGURE 1.—Diagram of the first series of experiments. The horizontal lines represent the cultures, the vertical lines show the number of days through which the cultures are carried. The numbers above the horizontal lines show the temperatures (centigrade) at which the cultures were kept. The upper lines show the basic cultures with their direct continuations. The double lines below are the parallel test cultures, derived from the basic cultures and kept in progress at the same time, but with both sets under the same temperature. The beginning, duration and end of each culture or test are shown. A + sign above the test shows that the high-temperature set divides more rapidly; below, it shows that the low-temperature set divides more rapidly.

A. Two sets of 60 lines each derived from the same parents, kept for 20 days at diverse temperatures; the high-temperature set divide more rapidly. B. The first test, both sets at room temperature for 16 days; the ex-high set continues to divide the more rapidly. C. The basic cultures continued for 33 days as mass cultures at slightly differing temperatures. D. The second test at room temperatures; the ex-high set continues to divide the more rapidly. E. Continuation of the two cultures of the first test as mass cultures at the same room temperature. F. Third test, both sets on slides at room temperature for 10 days; no marked difference in fission rate.

gate in mass cultures for 33 days, at only slightly differing temperatures (C, figure 1)—the high set at 30° to 32°, the low set at 25° to 29°. Now the second test was made, both sets were placed on slides at room temperature for 10 days (D, figure 1), and records were kept of their rate of fission. A third test was taken from the descendants of the two sets of test 1. These at the end of test 1 were kept for eleven days in mass cultures at room temperatures (E, figure 1), then placed for ten days on slides at room temperatures, and records kept of their fission rates (F, figure 1).

In this series therefore we have to deal with (1) the direct effect of the diverse temperatures on fission rate during the first twenty days (A, figure 1); (2) with the fission rates in the three tests (B, D, F, figure 1) in which the members of the two sets were kept at the same temperature, after various periods in diverse temperatures.

(2) *The direct effect of the diverse temperatures on the fission rate (A, figure 1)*

Table 1 gives the actual numbers of fissions for each set for the two ten-day periods during which they were subjected to the diverse temperatures. Each set contained sixty lines; in order to make it possible to judge of the uniformity of the effects, each set is divided into six groups of ten lines each; a given group of the high set is compared with the similarly numbered group of the low set. This grouping is based entirely on the numbering of the lines at the beginning, so that it is purely random.

TABLE 1

SERIES I.—*Number of fissions (generations) per line for the two sets of sixty lines each, of the first clone, during the period A of figure 1, the high set kept at 30° to 32° C., the low set at 18° to 20° C., with the excess in favor of the high set. (Differences given the minus sign show an excess of the low set.)*

	Lines number						Total 60 lines	Average per line per 10-day period	Percent that the difference is of the total in all
	1 to 10	11 to 20	21 to 30	31 to 40	41 to 50	51 to 60			
First 10-day period									
30° to 32° C. high	299	191	237	168	163	215	1273	21.21	
18° to 20° C. low	198	176	218	202	227	225	1246	20.76	
Excess of high	101	15	19	—34	—64	—10	27	.45	1.07
Second 10-day period									
30° to 32° C. high	378	369	308	291	161	304	1811	30.18	
18° to 20° C. low	139	141	161	155	165	142	903	15.05	
Excess of high	239	228	147	136	—4	162	908	15.13	33.47

As table 1 shows, during the first ten days the animals kept at the higher temperature divided little more rapidly than those kept at the lower temperature; indeed, in three of the six groups of each set those kept at the lower temperature divided more rapidly than the corresponding group kept at the higher temperature, and the average for the two sets was almost the same. But in the second ten days the set kept at the higher temperature divided on the average twice as rapidly as that kept at the lower temperature. The "high" group averaged 3.018 fissions per day for each line, the "low" group but 1.505 fissions per day. In the first half of figure 2 is plotted the difference in number of fissions between the two sets for the successive two-day periods of the experiment. In this figure the straight base line or abscissa represents the number of fissions for each two days, in the sixty lines that were kept at low temperature, while the irregular line shows the number of fissions above or below this, produced in the same period by the 60 lines kept at high temperature. Throughout the second ten days the set at high temperature are consistently above the others, the excess increasing rapidly toward the end of the twenty-day period. The difference in temperature seems to have a cumulative effect.

FIGURE 2.—First series of experiments. Curve of the excess in number of fissions in the set cultivated at high temperature over the number in the set cultivated at low temperatures. The first part of the curve to the vertical line at 20 days, shows the excess while the two sets were in the different temperatures, the high-temperature set cultivated at 30 to 32 degrees, the low set at 18 to 20 degrees (A, figure 1). The second half, to the right of the vertical line, shows the excess of the ex-high set when both sets are cultivated at the same room temperature (B, figure 1). The curve is plotted for two-day periods, the horizontal scale showing the number of days, the vertical scale the excess in fissions of the high-temperature set over the low-temperature set.

(3) *First test of the permanence of modification (B, figure 1)*

Now the two sets of sixty lines each, one of which has lived for twenty days at 30° to 32° , the other for the same period at 18° to 20° , are both brought to room temperature. Will they continue to show a difference in fission rate? And, if so, of what nature will be the difference?

The period of this test was 16 days, from July 7 to July 22, inclusive. The two sets were kept at the same room temperature, but at this period of the year the temperature is high in Baltimore, and it gradually rose throughout the period of test. The records show that for each of the first four days the maximum temperature reached was 28.5 degrees; in each of the next four it was 30 degrees; in each of the next four, 32 degrees, and on the fourteenth day it reached its highest point, at 32.5 degrees. The minimum temperatures for the various days varied from 25 degrees to 29 degrees.

Thus the temperature at which both sets were now kept was much nearer that at which those cultivated under high temperature had been kept than to that at which the "low" set had been kept. In other words, there was now relatively little change of temperature for the "high" set, while the "low" set are raised to a much higher temperature than that to which they have hitherto been accustomed. But the two sets were now at all times under the same temperature.

Table 2 gives the number of fissions for each set of lines, by groups of ten, during these 16 days of subjection to the same temperature, with the excess in favor of the set that had previously been kept under the high temperature. This set continues throughout the period to maintain its higher rate of fission, falling only slightly from the average rate of 3.018 fissions per day for the 10 days preceding this test to 2.889 per day for the 16 days of the test. The set which had been kept in low temperature increases its fission rate on being placed at the higher temperature of the test, but not so much as to reach that of the other set. During the last 10 days of low temperature, its fission rate had averaged for each line 1.505 per day; during the 16 days of the test, it rises to an average of 1.844 fissions per day. The second half of figure 2 gives the curve of the difference in number of fissions by two-day periods, while the two sets were at the same temperature. The ex-high set remains consistently above the ex-low set.

Thus in this first test it appears that a heritable difference has been produced of the same nature as the direct difference produced by the diversity of temperature. The ex-high set retains its higher rate; the

TABLE 2

SERIES I.—First test (B, figure 1). Number of fissions (generations) per group of ten lines for the two sets of sixty lines each, both sets now kept at the same temperature (after twenty days at diverse temperatures), with the average number of fissions for each line, and the excess in favor of the ex-high lines.

	Lines number						Total	Average per line for the whole period	Average per line per day	Percent that the difference is of the total in all
	1 to 10	11 to 20	21 to 30	31 to 40	41 to 50	51 to 60				
First 10-day period										
Ex-high lines.....	298	287	302	342	264	299	1792	29.86	2.986	
Ex-low lines.....	187	185	195	196	204	192	1159	19.31	1.931	
Excess of ex-high....	111	102	107	146	60	107	633	10.55	1.055	21.46
Following 6-day period										
Ex-high lines.....	156	150	164	199	131	181	981	16.35	2.725	
Ex-low lines.....	106	106	109	107	98	90	616	10.27	1.712	
Excess of ex-high....	50	44	55	92	33	91	365	6.08	1.013	22.83

ex-low in a measure also its lower rate, impressed upon them by the previous differences in temperature.

(4) *Second test of the permanence of modification (D, figure 1)*

After the first twenty days on slides in diverse temperatures, parts of the lines not employed in the first test were transferred, unwashed, to small mass cultures, the high temperature lines forming one culture, the low temperature lines another. The culture fluid was .031 percent malted milk. The high-temperature mass culture was placed in an incubator kept at the same temperature as the high-temperature slides, i.e., at 30 to 32 degrees centigrade. The low-temperature culture was kept in an incubator at 25 to 29 degrees centigrade. The two were allowed to multiply at these slightly differing temperatures in the mass cultures for 33 days (see C, figure 1). Thus the two sets had been kept at diverse temperatures for 53 successive days, since the beginning of the experiment.

Now, on August 2, thirty individuals from each of the cultures were isolated on slides and allowed to multiply for 10 days at room temperature, records being kept of their rate of fission (D, figure 1). The room temperature varied during this period from 25 to 32.5 degrees, so that again the common temperature at which the two sets were kept was high. Table 3 gives the number of fissions during the ten days for each of the thirty lines of the two sets, with comparison between the two lines designated by the same number in the two sets. (These designations are of course given at random, before the records are taken.)

It will be observed that every member of the ex-high set showed a greater number of fissions than any member of the ex-low set. On the average, each ex-high line produced during the ten days 6.37 more generations than each ex-low line. Here again therefore, it appears that the earlier differences in temperature have produced a modification of the fission rate that lasts after the diversities in temperature have disappeared, and this lasting modification is in the same direction as the immediate effect produced by the diverse temperature.

(5) *Third test of the permanence of modification (F, figure 1)*

At the close of the first test (B, figure 1), the two halves of the clone had been first kept for 20 days at diverse temperatures, then for 16 days at the same temperatures; during these 16 days we had found that the ex-high set continued to multiply more rapidly than the ex-low set. To

TABLE 3

SERIES I.—Number of fissions per line for the two sets (*ex-high* and *ex-low*), in the second test of 10 days under the same temperature (*D*, figure 1). Thirty lines in each set. The table gives the number of fissions for each line during the ten days of the test, with comparison between the like-numbered lines of each set, together with the excess in favor of the *ex-high* lines. The room temperature at which both were kept ranged from 25 to 32.5 degrees.

	Lines number																														Total	Aver- age per line	Percent that the difference is of the total in all
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30			
Ex-high	33	32	33	33	34	34	32	29	34	32	34	34	34	32	34	32	36	31	34	33	32	32	36	34	32	32	35	34	35	998	33.27		
Ex-low	29	29	27	25	25	27	28	28	25	27	23	28	24	28	22	26	26	26	29	27	23	29	29	31	27	27	28	27	29	28	807	26.90	
Excess of ex-high...	4	4	6	8	9	7	4	1	9	5	11	6	10	4	12	6	10	10	2	7	10	3	3	5	7	5	4	8	5	7	191	6.37	
																																	10.58

TABLE 4

SERIES I.—Number of fissions per line for the two sets (*ex-high* and *ex-low*), in the third test of 10 days under the same temperature (*F*, figure 1). Thirty lines in each set; both kept at room temperature (25 to 32.5 degrees). The previous history of the two sets can be seen in figure 1. The table gives the number of fissions for each line, with comparison between the like-numbered lines of each set, together with the excess in favor of the *ex-high* lines. The minus sign indicates an excess in favor of the *ex-low* lines.

	Lines number																														Total	Aver- age per line	Percent that the difference is of the total in all
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30			
Ex-high	23	22	23	21	26	26	27	29	23	27	27	26	28	25	28	26	24	26	26	24	22	26	25	26	26	25	23	29	27	760	25.33		
Ex-low	28	28	24	25	28	29	28	30	31	28	30	28	28	30	30	29	29	28	28	27	28	30	31	30	28	29	27	26	27	852	28.40		
Excess of ex-high...	-5	-6	-1	-4	-2	-3	-1	-1	-8	-1	-3	-2	0	-5	-2	-4	-5	-3	-2	-4	-3	-6	-4	-6	-4	-2	-4	-4	3	0	-92	-3.07	
																																	-5.71

see whether this difference would continue, the two sets were converted into two small mass cultures which were kept at room temperature (25 to 34.5 degrees) for 11 days (E, figure 1), then the fission rate of the two was again compared, by cultivation on slides for ten days at room temperature (25 to 32.5 degrees). Thirty individuals were isolated from each of the two sets, numbered at random, and cultivated on slides under identical conditions (F, figure 1). Table 4 gives the actual number of fissions for each of the thirty lines of each set during the ten days of the test.

Table 4 shows that the ex-high lines no longer divide more rapidly than the ex-low lines, indeed, in most cases the ex-low lines divide more rapidly. In 26 out of the 30 pairs made by comparing lines of like designation, the ex-low lines show more divisions than the ex-high lines. The average excess per line for the ten days is 3.07 fissions in favor of the ex-low lines.

Thus the higher fission rate produced by the earlier high temperature has disappeared; it is possibly even replaced by a lower fission rate, although the difference is small and perhaps does not justify drawing conclusions on this point.

(6) *General results of the first series of experiments*

The experiments of this first series tend to show that diversities of temperature produce a diversity of fission rate which lasts after the diversity of temperature has ceased. In two tests made immediately after the subjection to diversity of temperature, the lasting difference in fission rate was of the same character as the direct effect produced by the different temperatures; the lines that had been subjected to the higher temperature continue to divide more rapidly after the two sets are returned to the same temperature, and this difference lasts as much as 16 days. But in a test at common temperature made 37 days after the lines had been returned to the common temperature, the difference in favor of the ex-high lines was no longer in evidence; it was indeed replaced by a slight excess in fission rate in favor of the ex-low lines.

SECOND SERIES OF EXPERIMENTS

(7) *General outline*

The results of the preliminary series of experiments, described above, appeared to justify a careful study of the phenomena, on a larger scale, and with more precise control of the conditions. Accordingly, on Oc-

FIGURE 3.—Diagram of the extensive second series of experiments. The horizontal lines show the duration of the cultures, the vertical lines showing the number of 10-day periods through which they are carried. The numbers above horizontal lines show the temperatures at which the cultures were kept.

The two upper horizontal lines show the basic cultures, kept at diverse temperatures. The double lines below are the parallel test cultures, derived from the basic cultures and kept in progress at the same time, but with both sets of cultures under the same temperature. The beginning, duration and end of each culture or test are shown. A + sign above the test lines shows that the high temperature set (or the ex-high-temperature set) divides the more rapidly; a + sign below the test lines shows that the low-temperature set (or ex-low-temperature set) divides the more rapidly.

- A. Two sets of 30 lines each derived from a single parent and kept at diverse temperatures for eight months. The high temperature set divides more rapidly at first, but decreases after about four months; and after 150 days its rate falls below that of the low-temperature set; all its members are dead at the end of 171 days. The low-temperature set is discontinued at the end of 240 days.
- B. First test after 20 days; the ex-high set divide more rapidly.
- C. Second test, after 40 days; the ex-low set divide more rapidly.
- D to K. Tests at the diverse periods and temperatures shown; in every case the ex-low-temperature set divide more rapidly.

tober 4, 1915, an extensive series of experiments was set on foot, using the progeny of a single individual taken from a 'wild' laboratory culture of *Stylonychia pustulata*.

A diagram of this second series of experiments is shown in figure 3; the reader will find it well to keep this diagram before him in following the experiments. The general course of the series was as follows: The basic cultures consisted of two sets of thirty lines each, both derived from the same parent, kept at diverse temperatures for 240 days, or eight months (figure 3, A). One set ("high") was kept at a temperature of 28 to 30 degrees centigrade, the other set ("low") was kept at 6 to 9 degrees centigrade. The difference in temperature was thus much greater than that employed in the earlier series, and the temperatures were kept more constant. The number of fissions for each set was recorded throughout these eight months. (It may be noted that at the end of four months the last 10 lines of each set were discarded, so as to leave but twenty lines in each set, in place of thirty.)

At intervals, tests were made as to the permanency of any modifications induced, by taking progeny from each set and keeping the two at the same temperature. Tests were made at intermediate temperatures after subjection to diverse temperatures for 20 days (B, figure 3); again after 40 days (C); after 60 days (D and E); after 120 days (F, G, and H), and after 170 days (K, figure 3). Further, at the 60-day period, besides the test at intermediate temperatures there was a test with both sets of lines at high temperature, and at the 120-day period there were tests with both sets at low temperatures, both at intermediate temperatures, and both at high temperatures. There is of course no difficulty in having several parallel tests in progress at once, since the number of progeny produced within a few days is sufficient to set on foot many lines. All these tests are clearly indicated, with their relation to the basic cultures, in figure 3.

(8) *Results of keeping the basic stocks for eight months under diverse temperatures (A, figure 3)*

Of the sixty lines derived from the single parent individual, thirty were placed on October 8, 1915, in a refrigerator regulated to 6 to 9 degrees centigrade, the other thirty in an electric self-regulating incubator regulated to 28 to 30 degrees centigrade. The two were cultivated at these temperatures, but under otherwise similar treatment and conditions, for 240 days. The temperatures at times varied somewhat from the limits above mentioned, as shown in table 5.

A. R. MIDDLETON, TEMPERATURE DIFFERENCES AND THE FISSION RATE OF STYLONYCHIA

TABLE 5

SERIES II.—Basic cultures (A, figure 3). Number of fissions (generations) for the two sets of thirty lines each, of the second clone; one set kept in high temperature, the other set in low temperature; with the excess in favor of the high set. (Differences given the minus sign show an excess of the low set.)

Lines number																														Total	Average per line	Percent that the difference is of the total in all
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30			
First 10-day period																														609	23.30	49.84
28°-30° C. high																																
6°-9° C. low																																
Excess of high																																
Second 10-day period																														911	30.37	78.80
28°-30° C. high																																
8°-10° C. low																																
Excess of high																																
Third 10-day period																														740	24.67	76.60
28°-29° C. high																																
8°-10° C. low																																
Excess of high																																
Fourth 10-day period																														867	28.00	91.39
26°-30° C. high																																
6°-8° C. low																																
Excess of high																																
Fifth 10-day period																														953	31.76	91.96
24°-30° C. high																																
6°-10° C. low																																
Excess of high																																
Sixth 10-day period																														564	18.80	81.36
24°-28° C. high																																
6°-11° C. low																																
Excess of high																																

TABLE 5 (continued)

SERIES II.—Basic cultures (A, figure 3). Number of fissions (generations) for the two sets of thirty lines each, of the second clone; one set kept in high temperature, the other set in low temperature; with the excess in favor of the high set. (Differences given the minus sign show an excess of the low set.)

	Lines number																														Total	Average per line	Percent that the difference is of the total in all
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30			
Seventh 10-day period																																	
24°-26° C. high	24	20	22	20	20	23	22	20	20	26	22	18	18	18	21	21	22	20	23	26	21	20	21	21	22	20	26	24	25	24	650	21.66	
6°-10° C. low	1	1	1	0	4	3	5	0	2	1	4	3	1	2	5	3	2	2	1	2	4	2	2	2	2	2	2	4	3	63	2.10		
Excess of high	23	19	21	19	20	23	18	17	15	26	22	16	17	14	18	20	15	20	24	19	18	20	19	18	18	24	22	21	21	587	19.56	82.32	
Eighth 10-day period																																	
26°-28° C. high	16	14	16	11	19	21	18	15	24	24	18	19	14	14	13	12	15	12	10	11	12	12	14	12	13	13	24	20	17	19	472	15.73	
6°-15° C. low	1	1	2	1	2	2	2	3	1	2	2	3	1	2	3	5	4	2	1	2	4	4	2	2	3	4	2	1	3	69	2.30		
Excess of high	15	13	14	10	17	19	16	13	21	23	16	17	11	13	11	9	10	8	10	10	8	10	10	11	10	20	18	16	16	403	13.43	74.48	
Ninth 10-day period																																	
24°-26° C. high	21	25	20	23	29	30	30	25	23	23	24	28	30	29	27	21	23	25	24	25	21	22	24	17	25	19	14	21	19	25	712	23.73	
6°-10° C. low	1	2	1	3	0	0	1	3	0	0	1	0	2	1	0	0	2	1	0	0	1	0	1	0	1	0	0	0	1	23	.76		
Excess of high	20	23	19	20	29	30	29	22	23	23	23	28	28	26	21	23	23	25	21	21	24	16	24	19	14	21	19	24	689	22.97	93.74		
Tenth 10-day period																																	
23°-28° C. high	8	13	13	12	13	18	14	20	19	18	21	24	13	23	23	15	12	8	9	10	22	22	23	22	12	12	23	24	16	498	16.60		
5°-11° C. low	2	1	3	2	0	0	3	1	2	2	1	3	0	1	2	1	3	1	2	3	2	2	0	2	0	2	3	0	1	45	1.50		
Excess of high	6	12	10	10	13	18	11	19	17	16	20	21	13	22	21	14	9	7	7	20	20	23	20	12	12	21	21	16	15	453	15.10	83.42	
Eleventh 10-day period																																	
24°-28° C. high	16	16	12	18	17	19	23	25	26	19	27	26	27	18	25	25	22	26	18	19	26	24	23	24	24	24	32	29	24	23	677	22.56	
4.5°-14° C. low	2	4	3	2	2	3	1	0	2	4	0	2	3	2	2	2	1	3	2	2	1	2	2	2	2	2	2	4	3	2	65	2.16	
Excess of high	14	12	9	16	15	16	22	25	24	15	27	24	16	23	23	21	23	16	17	25	22	21	22	22	22	28	26	22	20	612	20.40	82.52	
Twelfth 10-day period																																	
23°-29.5° C. high	24	24	27	30	27	31	29	31	28	29	27	26	32	31	34	34	27	31	38	33	24	29	20	25	30	28	35	34	27	32	877	29.23	
6°-13° C. low	4	4	1	2	3	2	3	2	3	3	3	2	3	2	3	5	3	3	3	3	4	4	3	3	1	2	1	4	5	87	2.90		
Excess of high	20	20	26	28	24	29	26	29	25	20	24	23	30	28	32	31	22	28	35	30	21	25	16	22	27	27	33	33	23	27	790	26.33	81.95

A. R. MIDDLETON, TEMPERATURE DIFFERENCES AND THE FISSION RATE OF STYLONYCHIA

TABLE 5 (continued)

SERIES II.—Basic cultures (A, figure 3). Number of fissions (generation s) for the two sets of thirty lines each, of the second clone; one set kept in high temperature, the other set in low temperature; with the excess in favor of the high set. (Differences given the minus sign show an excess of the low set.)

	Lines number																				Total	Average per line	Percent that the difference is of the total in all
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20			
Thirteenth 10-day period																							
27°-29.5° C. high	11	10	8	7	21	18	22	23	16	13	10	10	15	14	9	15	15	18	12	16			
5°-11.5° C. low	5	5	1	1	2	2	2	3	4	4	4	3	4	3	4	4	5	5	3	3			
Excess of high	6	5	7	6	19	16	20	20	12	9	6	7	11	11	5	11	10	13	9	13			
Fourteenth 10-day period																							
27°-28° C. high	4	7	6	10	9	6	9	11	10	10	8	8	7	8	8	20	13	22	11	7			
7°-15° C. low	3	3	1	2	2	3	5	4	5	2	4	5	3	5	2	5	3	4	3	5			
Excess of high	1	4	5	8	7	3	4	7	5	8	4	3	4	3	6	15	10	18	8	2			
Fifteenth 10-day period																							
27°-28° C. high	7	8	9	5	8	6	7	7	4	3	3	5	5	4	8	3	5	5	5	5			
7°-11° C. low	3	3	1	2	2	3	3	3	2	3	4	3	4	3	2	3	3	4	4	4			
Excess of high	4	5	8	3	6	3	4	4	2	0	-1	2	1	1	6	0	2	1	1	1			
Sixteenth 10-day period																							
27°-28° C. high	5	5	6	8	5	7	5	7	10	5	1	3	3	6	2	2	4	4	4	5			
8°-14° C. low	3	6	6	6	5	5	5	6	7	8	6	7	6	6	7	6	6	6	6	6			
Excess of high	2	-1	0	2	0	2	0	1	3	-3	-5	-4	-3	0	-5	-4	-2	-2	-2	-1			
Seventeenth 10-day period																							
27°-28° C. high	3	3	5	6	3	8	8	5	6	8	8	7	3	3	4	3	2	3	3	3			
8°-14° C. low	4	6	5	5	7	8	8	7	8	7	7	7	8	7	5	7	6	7	7	7			
Excess of high	-1	-3	0	1	-4	0	0	-2	-2	1	1	0	-5	-4	-1	-4	-4	-4	-4	-4			

TABLE 5 (continued)

SERIES II.—Basic cultures (*A*, figure 3). Number of fissions (generations) for the two sets of thirty lines each, of the second clone; one set kept in high temperature, the other set in low temperature; with the excess in favor of the high set. (Differences given the minus sign show an excess of the low set.)

	Lines number																				Total	Average per line	Percent that the difference is of the total in all
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20			
Eighteenth 10-day period																							
27°-28° C. high	1	3	3	3	1	1	2	2	2	0	1	0	2	1	0	0	0	0	0	0	22	1.10	
8°-14° C. low	5	5	8	7	7	6	7	6	5	5	6	7	7	5	6	5	7	7	6	7	124	6.20	
Excess of high	-4	-2	-5	-4	-6	-5	-5	-4	-3	-5	-5	-7	-5	-4	-6	-3	-7	-7	-6	-7	-102	-5.10	-69.86
Total, 180 days																							
Ex-high	290	307	297	313	347	378	362	359	348	342	313	333	331	317	336	321	309	326	313	314	6556	327.8	
Ex-low	53	62	56	54	53	55	63	70	62	58	62	58	62	63	65	55	67	72	63	58	1212	60.6	
Excess of ex-high	237	245	241	259	294	323	297	296	278	280	255	271	268	252	280	267	242	254	250	256	5344	26.72	68.80
Nineteenth 10-day period																							
6°-10.5° C. low	1	2	2	2	2	2	2	2	3	3	2	1	2	3	2	1	2	3	2	2	41	2.05	
Twentieth 10-day period																							
9°-11.5° C. low	3	3	3	2	3	2	2	3	4	2	2	3	6	6	1	0	5	6	2	2	60	3.00	
Twenty-first 10-day period																							
8.5°-17° C. low	5	5	3	2	5	2	3	3	3	1	5	8	7	9	1	2	9	9	8	2	92	4.60	
Twenty-second 10-day period																							
10°-15° C. low	4	6	4	2	5	4	4	4	1	0	5	5	4	5	4	5	6	6	5	4	83	4.15	
Twenty-third 10-day period																							
8°-14° C. low	8	9	8	8	7	8	8	9	8	8	9	9	9	10	8	10	10	10	9		173	8.65	
Twenty-fourth 10-day period																							
90°-13° C. low	7	7	4	6	6	6	6	6	9	6	4	5	9	7	6	8	6	9	5	9	131	6.55	
Total, 240 days low	81	94	80	76	81	79	90	90	98	82	85	93	100	105	78	79	105	115	95	86		89.60	

The number of fissions for each line have been grouped by the successive ten-day periods, and are given in table 5. As in previous tables, the two lines of each set that had been designated by the same number are compared in each case. The table gives for each case also the excess in favor of the lines at high temperature; the totals for each set in each ten-day period, and the average number of fissions for each ten days for the lines of each set.

Examination of the table shows that at first the set kept at the higher temperature divided much more rapidly than that kept at the lower temperature, but that in the later periods the fission rate of the set kept at high temperatures fell off, until in the later periods the set kept at low temperature divided the more rapidly; and that finally the set kept at high temperature all died out, while the low-temperature set continued to multiply strongly. This course of the experiment is well shown in figure 4, in which the average number of fissions for each 10-day period is plotted for both sets, the continuous line showing the high-temperature set, the broken line the low temperature set.

Certain details shown in table 5 and figure 4 are of interest. In the first five ten-day periods the high-temperature set gradually increased its fission rate, rising in the fifth ten-day period to a maximum average of 31.76 fissions in ten days for each line, or more than three fissions in 24 hours. During the same five ten-day periods the low-temperature set gradually decreased in fission rate, from an average of 7.8 fissions in ten days, in the first period, to 1.3 fissions in ten days in the fourth and fifth periods. Thus on the fifth ten-day period the high-temperature set averaged 24 fissions to each single fission in the low-temperature set. In the sixth period the fission rate of the high-temperature set dropped precipitately to 18.8 fissions in 10 days. Now it remained with considerable fluctuations till the thirteenth ten-day period, when it again fell off strongly to 14.15 fissions per line. From thence onward it continued to fall rapidly, to 9.7, to 5.6, to 4.7, and finally on the eighteenth period to 1.10 fissions in ten days. During these last periods the high-temperature set was evidently weakened, and many deaths occurred. Five lines died out on the 8th day of the fifteenth ten-day period, and the number of deaths gradually increased until the fifth day of the eighteenth ten-day period, when the last two lines of the high-temperature set died out. Thus in the high-temperature set there were roughly three phases distinguishable. First there was a fifty-day period of increase of fission rate, then seventy days of very gradual decrease; then a final period of sixty days of rapid decrease in fission rate, culminating in the

death of all the lines of the high-temperature set in the 175th day of the culture.

In the set kept at low temperature the fission rate is consistently low, as compared with the other set before it had degenerated. With relation to the changes from week to week in the average fission rate of the low-temperature set, a disturbing factor exists in the fact that it was not possible to keep the low temperature so constant as could be done for the high temperature. Thus the maximum temperature varied somewhat from week to week, as is shown in table 5 (at the left). Examination of these maximum and minimum records of table 5 will show that on

FIGURE 4.—Polygons showing the effects on the fission rate of keeping two sets belonging to the same clone for eight months under opposed extreme temperatures (second series of experiments, basic lines, figure 3, A). The polygons show the average number of generations per line for each 10-day period, the continuous line representing the high-temperature set (kept at 28 to 32 degrees), the broken line the low-temperature set (kept at 6 to 9 degrees). The base line shows the 24 ten-day periods of the experiment, the vertical scale the number of fissions per line. The high-temperature set at first divided much the more rapidly, but fell off till in the 16th period it was below the low-temperature set; at the end of 180 days the high-temperature lines were all dead.

the whole as the maximum for any period is higher, the fission rate for that period is correspondingly higher. There is apparently no other significance to the fact shown in figure 4, that in the low-temperature set the fission rate at first decreased, remained low for about 100 days, then gradually increased, and was highest toward the end. If the temperature could have been kept constant, the fission rate would apparently have remained constant. What is significant is that there was no decrease in the fission rate in this set, that no lines died out, and that all remained vigorous and multiplied rapidly until the cultures were discontinued after 240 days.

Thus the experiment indicates that keeping *Stylonychia pustulata* at the high temperature of 28 to 32 degrees centigrade has a cumulative injurious effect,—an effect hardly noticeable till four months have passed, but resulting in two months more in the degeneration and death of all the lines. On the other hand, keeping them at the low temperature (which actually varied from 6 to 15 degrees, and probably averaged about 10 degrees) had no injurious effect; at the end of eight months they were as vigorous as at the beginning.

The question may be raised whether the ultimate death of the high-temperature set was not due to the fact that the heat increased the rate of all vital processes, so that the "life cycle" was passed through more quickly in this set than in the other. The cause of death would then be merely the fact that the vegetative cycle was completed. The total number of fissions in the different lines of the high-temperature set varied from 290 to 378 before extinction occurred. In the same period of time (180 days) the low-temperature lines had each gone through only 53 to 72 fissions; and when the cultures were discontinued with the low-temperature set still vigorous, the total fissions that they had gone through were but 76 (in the lowest line) to 115 (highest line). It might be maintained by anyone that holds to the theory of a definite life cycle limited to a given number of vegetative reproductions, that if the low-temperature set should be kept long enough to attain as many generations as the high-temperature set (which would require about two years), this set too would die out. BAITSELL (1912), however, kept *Stylonychia pustulata* under cultivation for 572 generations, so that it would not seem probable that extinction after 378 generations is due to the completion of the vegetative cycle, even assuming that there is such a cycle. My own experiments give no direct evidence on the point.

(9) *Test of modifications produced after 20 days in diverse temperatures (B, figure 3)*

Progeny from each line of the two sets of the basic experiment were placed, after 20 days in the diverse temperatures, in an intermediate temperature of 16 to 20 degrees, and allowed to multiply there for 20 days. The number of fissions for the entire 20 days, and for the first and second 10-day periods is given for each line of each set in table 6. The like-numbered lines of each set are compared, as in previous tables, and the difference between them given.

As table 6 shows, the set that has been multiplying in the higher temperature continues to multiply the more rapidly after the two sets have been brought to the same temperature. In 23 out of the 30 lines of each set compared, the ex-high line has produced more generations than the ex-low line. The average number of fissions per line for the twenty days is for the ex-high set 29.7, for the ex-low set, 27.6. The average difference is thus but 2.1 fissions in 20 days, but this difference is consistent, showing (as we have seen) in 23 lines out of 30, and being equally evident in the first and second ten days of the culture period.

In this test therefore, as in the two earlier tests of our first series of experiments (pages 541 and 543), the higher fission rate induced by the higher temperature clearly persists in some measure after restoration of the two sets to the same temperature.

(10) *Test of modifications produced after 40 days in diverse temperatures (C, figure 3)*

After one set had been 40 days in the temperature of 28 to 30 degrees, the other in that of 6 to 9 degrees, progeny were again taken from each line of each set and allowed to multiply for 20 days at an intermediate temperature of 14 to 18 degrees. The comparative results are shown in table 7, for the entire period of 20 days, and for each of the two component 10-day periods.

In this test we find a reversal of the conditions found in the previous tests. The set that has been kept at low temperature now divides more rapidly than the set that has been kept at high temperature. In every one of the thirty lines compared from each set, the ex-low set has produced more generations than the ex-high set; and this is true for each of the two ten-day periods taken separately. All but three of the 30 lines of the ex-low set exceed the number of fissions (16) found in the

TABLE 6

SERIES II.—*Test after 20 days (B, figure 3). Number of fissions (generations) for the two sets of thirty lines each kept for 20 days in 16°-20° C., after having been kept for 20 days, one set in 30°-32° C., the other in 6°-10° C., with the excess in favor of the high set. (Differences given the minus sign show an excess of the ex-low set.)*

	Lines number																														Total	Aver- age per line	Percent that the differ- ence is of the total in all
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30			
First 10-day period																																	
18°-20° C. ex-high...	15	20	17	18	18	21	20	22	16	15	12	14	15	14	15	15	16	17	14	15	17	16	14	19	16	14	14	16	15	15	485	16.16	3.63
18°-20° C. ex-low...	19	16	14	15	14	16	19	15	14	12	14	13	18	12	14	14	12	15	15	18	16	13	13	18	15	16	16	12	17	16	451	15.03	
Excess	-4	4	3	3	4	5	1	7	2	3	-2	1	-3	2	1	4	2	-1	-3	1	3	1	1	1	-2	-2	4	-2	-1	34	1.13		
Second 10-day period																																	
16°-18° C. ex-high...	12	16	14	16	14	13	14	12	14	13	12	14	12	14	16	16	15	16	12	15	11	13	11	15	15	14	13	10	11	13	406	13.53	3.71
16°-18° C. ex-low...	14	12	13	12	14	17	19	17	10	12	14	10	9	13	11	12	11	11	15	10	8	10	17	12	12	11	12	13	15	377	12.56		
Excess	-2	4	1	4	0	-4	-5	4	1	-2	4	3	1	5	4	4	5	1	0	1	5	1	-2	3	2	2	-2	-2	-2	29	.97		
Entire 20 days																																	
16°-20° ex-high.....	27	36	31	34	32	34	34	30	28	24	28	27	28	31	31	31	33	26	30	28	29	25	34	31	28	29	26	26	28	891	29.70	3.67	
16°-20° ex-low	33	28	27	27	28	33	38	32	24	24	28	23	27	25	26	23	26	26	33	26	21	23	35	27	28	27	24	30	31	828	27.60		
Excess of ex-high.....	-6	8	4	7	4	1	-4	2	6	4	-4	5	0	3	6	5	8	7	0	-3	2	8	2	-1	4	0	2	-4	-3	63	2.10		

TABLE 7

SERIES II.—*Test after 40 days (C, figure 3). Number of fissions (g enerations) for the two sets of thirty lines each kept for twenty days in 14° to 18° C. subsequent to forty days in extreme temperatures (28°-32° and 6°-10° C.) with the excess in favor of the high set. (Differences given the minus sign show an excess of the ex-low set.)*

	Lines number																														Total	Aver- age per line	Percent that the differ- ence is of the total in all
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30			
First 10-day period																																	
14°-18° C. ex-high..	6	7	6	6	5	6	5	7	6	6	6	7	6	6	5	6	6	5	6	5	6	6	5	6	6	6	6	5	6	176	5.87		
14°-18° C. ex-low...	7	8	8	7	8	8	7	10	8	9	9	8	9	10	10	7	7	9	8	8	7	9	7	10	8	8	7	7	8	244	8.13		
Excess	-1	-1	-2	-2	-2	-3	0	-4	-2	-3	-2	-3	-2	-3	-4	-5	-1	-1	-4	-2	-3	-1	-3	-2	-4	-2	-2	-1	-2	-68	-2.26		
Second 10-day period																																	
14°-16° C. ex-high...	10	9	7	10	8	8	8	9	8	9	9	7	9	9	7	8	9	9	9	8	9	9	9	9	9	9	8	8	7	10	257	8.57	
14°-16° C. ex-low...	12	10	11	13	13	14	11	11	12	10	11	11	8	15	12	14	11	9	10	13	9	10	12	13	10	10	9	9	12	14	339	11.30	
Excess	-2	-1	-4	-3	-5	-6	-3	-2	-4	-1	-2	-4	1	-6	-5	-6	-2	0	-1	-4	-1	-1	-3	-4	-1	-1	-1	-1	-5	-4	-82	-2.73	
Total 20 days																																	
14°-18° ex-high....	16	16	13	16	13	14	13	16	14	15	15	14	15	15	13	13	15	15	14	15	13	15	15	14	15	15	14	14	12	16	433	13.43	
14°-18° ex-low....	19	18	19	21	20	22	19	18	22	18	20	20	16	24	22	24	18	16	19	21	17	17	21	20	20	18	17	16	19	22	583	19.43	
Excess	-3	-2	-6	-5	-7	-8	-6	-2	-8	-3	-5	-6	-1	-9	-9	-11	-3	-1	-5	-6	-4	-2	-6	-6	-5	-3	-2	-7	-6	-150	-6.00		
-14.76																																	

highest line of the ex-high set, and no line of the ex-high set exceeds any line of the ex-low set. The average difference per line is six fissions in twenty days in favor of the ex-low set.

The twenty days of this test are coincident with the fifth and sixth ten-day period of the stock cultures still remaining in the diverse temperatures. It was within this 20-day period that the great falling off took place in the fission rate of the set kept at high temperature (see figure 4). In the fifth ten-day period the average number of fissions per line had been 31.76; in the next period it fell to 18.80. That is, apparently the injury due to the long-continued stay in the high temperature began to be manifest at about this time. It would appear probable that this is connected with the fact shown in our present test (table 7), that when the two sets are subjected to the same intermediate temperature, the ex-high set no longer continues to divide more rapidly than the ex-low set, as had been the case in the previous test. The high-temperature set, on being placed in the lower temperature of 14 to 18 degrees, drop from an average of 50.56 fissions per line in 20 days (table 5, periods 5 and 6) to an average of 13.43 fissions per line in the same period, that is, to about one-fourth their former rate. The low-temperature set, raised to the same intermediate temperature (14 to 18 degrees) increase their average fission rate per line from 3.26 in twenty days to 19.43 in twenty days, that is, to about six times their former rate.

(11) *Test of modifications produced after 60 days in diverse temperatures (D and E, figure 3)*

The reversal of the relative fission rates of the two sets when kept at the same temperature, described in the last section, seemed of such interest as to demand further and thorough study, with variation of the conditions. So at the end of that test, when the basic cultures had been for sixty days in the diverse temperatures, two sets of test progeny were taken from each of the basic lines, and tests were made (a) with both sets of lines at intermediate temperature, and (b) both sets at high temperature. The fission rate was determined in each of these test sets for a period of 60 days. This 60-day period corresponds, as figure 3 shows, with periods 6 to 12, inclusive, of the basic cultures.

Thus at this time (periods 6 to 12, inclusive) there were in progress six sets of cultures, each of 30 lines each; three belonging to the originally high set; three to the originally low set. They formed three pairs of cultures, as follows: (1) A high set still kept at the original temperature of 28 to 30 degrees, with a low set still kept at 6 to 9 degrees; (2)

an ex-high set kept at the intermediate temperature of 14 to 18 degrees, with an ex-low set kept at the same intermediate temperature; (3) a high set kept at 28 degrees, with an ex-low set kept also at this high temperature (28 degrees).

(a) Both sets at the same intermediate temperature (D, figure 3)

During these periods 6 to 12, as figure 4 shows, the high sets of the basic culture still kept up their rapid fission, with little or no indication of injury, while the low-temperature set divided slowly. When the two sets were kept in the intermediate temperature of 14 to 18 degrees, the ex-high set decreased and the ex-low set increased in fission rate to such an extent as to reverse their relative positions, so that the ex-low set now divided more rapidly than the ex-high set. This is the same phenomenon observed in our previous test (section 10). The actual number of fissions for each of the thirty lines of each set is given by 10-day periods in table 8, with comparison of the like-numbered lines. In all of the 180 comparisons thus made, except 2, the ex-low lines divided more rapidly than the corresponding ex-high lines. For the entire period of 60 days, every one of the ex-low lines produced more generations than *any* of the ex-high lines. In every one of the six ten-day periods the total number of generations produced by the thirty ex-low lines was much above the total number produced by the thirty ex-high lines, and the average number of fissions per line per ten-day period was in every case correspondingly greater for the ex-low lines. All these relations are shown in detail in table 8.

Thus this test shows again that cultivation in diverse temperatures has produced in the two halves of the clone a diversity that is inherited in vegetative reproduction. But when the two sets are restored to the same intermediate temperature, the ex-high set, which was dividing the more rapidly, now divides the less rapidly. The inherited effect of the diversity of temperature is the reverse of the direct effect.

(b) Both sets in the same high temperature (E, figure 3)

In this experiment both sets were kept at a temperature about the same as that of the high set in the basic cultures. A different incubator was used, so that the temperature was not absolutely identical with that of the basic high set; apparently it was, for a part of the time at least, a little higher, for the ex-high set divided somewhat more rapidly than did the high set of the basic cultures.

When the two sets were kept at the same high temperature, the ex-low lines increased greatly their fission rate, but during the first ten days it did not reach that of the ex-high set. For these first ten days in 29 out of 30 of the pairs the ex-high line divided more rapidly. The detailed figures are given in table 9. But after these first ten days the ex-low lines increased still further in their fission rate, while the ex-high lines fell off, so that now their positions became reversed (just as happened when the two were in intermediate temperatures). That is, in the last five 10-day periods of this test with both sets at high temperature, the ex-low lines multiplied more rapidly than the ex-high lines. For the entire period of 60 days, every ex-low line produced more generations than any ex-high line. The total number produced in the 30 ex-high lines varied from 78 to 133; the totals in the ex-low lines varied from 148 to 189 generations. Thus the test with both sets at high temperatures gives essentially the same results as that with both sets at intermediate temperatures, save that equilibrium is not reached in the high temperature, until after the first 10-day period.

(12) *Tests of modifications produced after 120 days in diverse temperatures (F, G and H, figure 3)*

At the end of the tests described in section 11, and after the basic lines had been for four months in the diverse temperatures (the high set at 28 to 30 degrees, the low set at 6 to 9 degrees), tests were again made of the permanence of the modifications produced. Three sets of tests were made at this time; (a) with both sets at intermediate temperature (12 to 21 degrees); (b) with both sets at high temperature (28 degrees); (c) with both sets at low temperature (5 to 15 degrees). The tests lasted for 20 days. In this case there were used for each test the progeny of only 20 lines (numbers 11 to 30) of each of the two basic cultures. These three tests coincide in time with periods 13 and 14 of the basic cultures. As figure 4 shows, it was during these two periods that there occurred the extreme decrease in the fission rate of the set kept in the basic cultures at high temperature. The high temperature had apparently produced a marked injurious effect.

In the test at intermediate temperature (F, figure 3, and table 10), and in that at high temperature (G, figure 3, and table 11), the results were the same as in the tests at 40 and at 60 days. The ex-low set divided more rapidly than the ex-high set. In the high-temperature test both sets divided more rapidly than in the intermediate-temperature test, and the result is that in the high temperature the excess in favor of the

A. R. MIDDLETON, TEMPERATURE DIFFERENCES AND THE FISSION RATE OF STYLONYCHIA

TABLE 8

SERIES II.—Test at intermediate temperature after 60 days (D, figure 3). Number of fissions (generations) for the two sets of thirty lines each kept for sixty days in intermediate (room) temperature subsequent to sixty days in extreme temperatures, with the excess in favor of the ex-high set. (Differences given the minus sign show an excess of the ex-low set.)

	Lines number																														Total	Average per line	Percent that the difference is of the total in all
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30			
First 10-day period																																	
Ex-high	6	5	6	3	6	6	6	5	6	8	7	5	5	8	6	5	7	7	7	8	4	5	3	3	4	4	6	4	6	5	166	5.53	
Ex-low	8	9	8	7	6	9	9	11	7	9	8	8	8	8	8	10	8	9	7	8	8	9	8	8	9	9	8	9	9	8	250	8.33	
Excess	-2	-4	-2	-4	0	-3	-3	-6	-1	-1	-1	-3	-3	0	-2	-5	-1	-2	0	0	-4	-4	-5	-5	-5	-5	-2	-3	-3	-3	-84	-2.80	-20.20
Second 10-day period																																	
Ex-high	4	5	3	3	6	7	6	4	6	7	7	8	5	5	5	4	6	3	6	4	2	4	6	4	6	4	3	5	2	4	144	4.80	
Ex-low	9	7	7	6	9	8	9	8	9	8	9	8	10	8	9	8	11	8	10	10	9	7	8	7	9	8	9	8	8	8	252	8.40	
Excess	-5	-2	-4	-3	-3	-1	-3	-4	-3	-1	-2	0	-5	-3	-4	-4	-5	-5	-4	-6	-7	-3	-2	-3	-3	-4	-6	-3	-6	-4	-108	-3.60	-27.27
Third 10-day period																																	
Ex-high	6	3	4	5	8	5	7	6	6	7	6	6	6	6	6	5	6	6	5	4	7	6	7	6	8	4	4	4	5	4	168	5.60	
Ex-low	6	6	7	9	7	8	9	8	7	8	7	8	10	8	8	8	9	10	8	8	8	6	8	8	10	9	9	9	7	7	240	8.00	
Excess	0	-3	-3	-4	1	-3	-2	-2	-1	-1	-1	-2	-4	-2	-2	-3	-3	-4	-3	-4	-1	0	-1	-2	-2	-5	-5	-5	-2	-3	-72	-2.40	-17.65
Fourth 10-day period																																	
Ex-high	3	2	5	1	6	4	5	5	2	6	6	5	5	5	5	6	4	6	5	4	3	4	5	5	6	5	5	4	1	1	129	4.30	
Ex-low	6	6	6	5	6	6	6	8	6	6	8	6	6	6	6	7	6	8	6	7	7	6	6	7	6	7	7	7	7	10	197	6.56	
Excess	-3	-4	-1	-4	0	-2	-1	-3	-4	0	-2	-1	-1	-1	-1	-1	-2	-2	-1	-3	-4	-2	-1	-2	0	-2	-2	-3	-6	-9	-68	-2.26	-20.81
Fifth 10-day period																																	
Ex-high	11	9	9	9	12	15	15	13	12	10	13	13	14	14	14	14	15	14	9	13	9	12	10	12	13	11	12	11	12	12	362	12.06	
Ex-low	12	14	15	20	16	16	18	16	16	15	16	16	16	16	17	16	14	19	16	18	14	16	13	16	16	16	14	15	15	16	473	15.76	
Excess	-4	-5	-6	-11	-4	-1	-3	-3	-4	-5	-3	-3	-2	-2	-3	-2	1	-5	-7	-5	-5	-4	-3	-4	-3	-5	-2	-4	-3	-4	-111	-3.70	-13.29
Sixth 10-day period																																	
Ex-high	6	9	3	7	10	12	10	12	9	9	9	10	11	10	10	9	12	11	8	6	7	11	3	11	11	9	9	8	10	9	271	9.03	
Ex-low	11	12	12	12	11	13	13	13	9	13	12	11	14	14	13	12	12	13	12	11	11	14	12	13	11	13	13	15	14		372	12.40	
Excess	-5	-3	-9	-5	-1	-1	-3	-1	0	-4	-3	-1	-3	-4	-3	-4	0	-1	-5	-6	-4	0	-11	-1	-2	-2	-4	-5	-5	-5	-101	-3.37	-15.72
Total, 60 days																																	
Ex-high	36	33	30	28	48	49	49	45	41	47	48	47	46	48	46	43	50	47	40	39	32	42	34	41	48	37	39	36	36	35	1240	41.33	
Ex-low	52	54	55	59	55	60	64	64	54	59	60	57	64	60	61	62	60	66	60	63	57	55	57	58	63	60	60	61	61	63	1784	59.47	
Excess of ex-high...	16	-21	-25	-31	-7	-11	-15	-19	-13	-12	-12	-10	-20	-12	-15	-19	-10	-19	-20	-24	-25	-13	-23	-17	-15	-23	-21	-25	-25	-28	-544	-18.14	-19.14

A. R. MIDDLETON, TEMPERATURE DIFFERENCES AND THE FISSION RATE OF *STYLONYCHIA*

TABLE 9

SERIES II.—Test in high temperature (*E*, figure 3). Number of fissions (generations) for the two sets of lines kept for sixty days in 28° C. subsequent to sixty days with the low lines in 8° C. and the high lines in 28° to 30° C., with the excess in favor of the ex-high sign. (Differences given the minus sign show an excess of the ex-low set.)

	Lines number																														Total	Average per line	Percent that the difference is of the total in all
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30			
First 10-day period																																	
Ex-high	27	26	27	24	26	25	27	27	27	30	28	28	28	24	28	24	27	27	23	25	25	26	27	27	27	28	27	23	25	25	788	26.26	
Ex-low	20	23	21	20	21	21	22	24	24	18	21	21	22	21	22	25	22	23	23	22	22	22	21	21	24	23	24	20	23	23	659	21.96	
Excess	7	3	6	4	5	4	5	3	3	12	7	7	6	3	6	-1	5	4	0	3	3	4	6	6	3	5	3	3	2	2	129	4.30	8.91
Second 10-day period																																	
Ex-high	13	18	19	17	22	20	20	31	20	16	19	21	12	12	17	14	10	11	9	12	13	15	18	17	10	18	14	17	12	14	481	16.03	
Ex-low	28	28	29	27	25	26	25	26	28	27	22	25	26	25	26	24	23	24	27	25	27	24	25	27	26	26	24	27	27	27	776	25.86	
Excess	-15	-10	-10	-10	-3	-6	-5	5	-8	-11	-3	-4	-14	-13	-9	-10	-13	-13	-18	-13	-14	-9	-7	-10	-16	-8	-10	-10	-15	-13	-295	-9.83	-23.46
Third 10-day period																																	
Ex-high	12	17	18	21	18	13	14	25	13	14	17	17	8	12	10	26	13	10	10	4	10	12	6	13	9	9	10	12	4	8	390	13.00	
Ex-low	28	23	30	26	28	28	30	33	25	25	27	25	31	31	23	30	29	31	26	26	30	27	28	27	27	28	29	29	26	28	834	27.80	
Excess	-16	-6	-12	-5	-10	-15	-16	-8	-7	-11	-10	-8	-23	-19	-13	-4	-16	-21	-16	-22	-20	-15	-22	-14	-18	-19	-19	-17	-22	-20	-444	-14.80	-36.27
Fourth 10-day period																																	
Ex-high	8	9	9	10	11	12	13	19	17	12	22	20	16	16	8	16	18	17	15	14	9	6	9	10	8	9	6	9	16	16	380	12.66	
Ex-low	25	24	26	30	22	24	26	27	25	27	26	26	22	25	26	28	25	27	24	23	23	24	24	24	26	24	23	24	23	20	743	24.76	
Excess	-17	-15	-17	-20	-11	-12	-13	-8	-8	-15	-4	-6	-6	-9	-18	-12	-7	-10	-9	-9	-14	-18	-15	-14	-18	-15	-17	-15	-7	-4	-363	-12.10	-32.33
Fifth 10-day period																																	
Ex-high	19	19	22	21	17	18	21	12	20	20	21	17	20	20	19	14	21	16	17	19	11	12	13	11	9	16	5	10	12	15	487	16.23	
Ex-low	41	39	38	49	41	40	41	39	35	41	36	41	38	40	41	38	37	34	41	32	42	42	42	48	46	40	44	40	43	45	1214	40.46	
Excess	-22	-20	-16	-28	-24	-22	-20	-27	-15	-21	-15	-24	-18	-20	-22	-24	-16	-18	-24	-13	-31	-30	-29	-37	-37	-24	-39	-30	-31	-30	-727	-24.23	-42.74
Sixth 10-day period																																	
Ex-high	14	17	14	12	14	14	14	7	12	10	17	19	12	12	6	6	12	14	14	10	15	7	7	13	15	15	17	15	13	12	393	13.10	
Ex-low	27	27	30	29	29	26	33	31	30	30	33	33	36	35	35	30	26	27	34	29	36	37	30	35	31	31	30	32	32	32	936	31.20	
Excess	-13	-10	-16	-17	-15	-12	-19	-24	-18	-20	-16	-14	-24	-23	-29	-24	-14	-13	-20	-19	-21	-30	-23	-22	-16	-16	-13	-17	-19	-20	-543	-18.10	-40.85
Total, 60 days																																	
Ex-high	93	106	109	105	108	102	109	121	114	102	124	122	96	96	88	100	101	95	88	84	83	78	80	91	78	95	79	86	82	90	2910	97.30	
Ex-low	169	164	174	181	166	165	177	180	167	168	165	171	175	177	173	175	162	166	175	157	180	176	170	182	180	172	174	172	174	175	5162	172.07	
Excess	-76	-58	-65	-76	-58	-63	-68	-59	-53	-66	-41	-49	-79	-81	-85	-75	-61	-71	-87	-73	-97	-98	-90	-91	-102	-77	-96	-86	-92	-85	-2243	-74.77	-27.76

A. R. MIDDLETON, TEMPERATURE DIFFERENCES AND THE FISSION RATE OF STYLONYCHIA

TABLE 10

SERIES II.—*Test in intermediate temperature (F, figure 3). Number of fissions (generations) for the last twenty lines of the two sets of lines kept for twenty days in intermediate temperature subsequent to one hundred and twenty days in extreme temperatures; with the excess in favor of the ex-high set. (Differences given the minus sign show an excess of the ex-low set.)*

	Lines number																														Total	Aver- age per line	Percent that the differ- ence is of the total in all	
	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30														
First 10-day period																																		
Ex-high	4	4	2	4	5	6	4	6	3	7	2	7	5	3	5	5	4	7	3	5											91	4.55		
Ex-low	7	8	14	14	13	14	14	13	12	14	9	12	10	10	10	10	13	14	11	10											232	11.60		
Excess	-3	-4	-12	-10	-8	-8	-10	-7	-9	-7	-7	-5	-5	-5	-7	-5	-5	-9	-7	-8	-5											-141	-7.05	-43.65
Second 10-day period																																		
Ex-high	6	1	3	2	4	4	8	7	4	5	6	7	4	3	8	5	4	4	5	5											95	4.75		
Ex-low	7	8	13	14	14	13	12	13	12	13	11	13	10	10	9	12	13	14	14	15											240	12.00		
Excess	-1	-7	-10	-12	-10	-9	-4	-6	-8	-8	-5	-6	-6	-7	-1	-7	-9	-10	-9	-10											-145	-7.25	-43.24	
Total, 20 days																																		
Ex-high	10	5	5	6	9	10	12	13	7	12	8	14	9	9	13	10	8	11	8	10											186	9.30		
Ex-low	14	16	27	28	27	27	26	26	24	27	20	25	20	20	19	22	26	28	25	25											472	23.60		
Excess	-4	-11	-22	-22	-18	-17	-14	-13	-17	-15	-12	-11	-11	-11	-6	-12	-18	-17	-17	-15											-286	-14.30	-43.47	

TABLE II
 SERIES II.—*Test at high temperature (G, figure 3). Number of fissions (generations) for each of the two sets kept for twenty days in high temperature (28° C.) subsequent to 120 days in extreme temperatures; with the excess in favor of the high set. (Differences given the minus sign show an excess of the low set.)*

		Lines number																				Total	Average per line	Percent that the difference is of the total in all
		11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30			
First 10-day period																								
Ex-high		11	10	8	7	21	18	22	23	16	13	10	10	15	14	9	15	15	18	12	16	283	14.15	
Ex-low		25	26	34	37	29	34	30	36	37	35	26	30	29	32	26	33	31	30	28	29	617	30.85	
Excess of ex-high		-14	-16	-26	-30	-8	-16	-8	-13	-21	-22	-16	-20	-14	-18	-17	-18	-16	-12	-16	-13	-334	-16.70	-37.11
Second 10-day period																								
Ex-high		4	7	6	10	9	6	9	11	10	10	8	8	7	8	20	13	22	11	7	194	9.70		
Ex-low		33	35	32	42	39	38	32	35	33	35	30	34	30	32	33	32	31	35	32	31	674	33.70	
Excess of ex-high		-29	-28	-26	-32	-30	-32	-23	-24	-23	-25	-22	-26	-23	-24	-25	-12	-18	-13	-21	-24	-480	-24.00	-55.32
Total 20 days																								
Ex-high		15	17	14	17	30	24	31	34	26	23	18	22	22	17	35	28	40	23	23	477	23.85		
Ex-low		58	61	66	79	68	72	62	71	70	70	56	64	59	64	59	65	62	65	60	1291	64.55		
Excess of ex-high		-43	-44	-52	-62	-38	-48	-31	-37	-44	-47	-38	-46	-37	-42	-42	-30	-34	-25	-37	-37	-814	-40.70	-46.04

ex-low lines is absolutely greater. But relative to the actual rate of fission in the two cases the results are much the same, as shown by the percentage that the excess is of the total fissions (tables 10 and 11). In both of these tests every ex-low line produced during the 20 days more generations than any ex-high line.

In the test with both sets at low temperature (5 to 15 degrees), (H, figure 3) the results were peculiar. The ex-high set on being placed in the low temperature divided several times on the first day, the 20 lines giving 69 fissions during the first day of low temperature. After this the ex-high set almost completely ceased to divide. During the other nine days of the first ten-day period there were but 12 fissions among the twenty lines of the ex-high set, and during the second ten-day period a total of but 7 fissions occurred in this set. During the second ten-day period many of the ex-high lines died, and at the end of the 20 days in low temperature only seven of the original twenty remained. Meanwhile, the ex-low set were dividing normally; during the first 10 days they showed 67 fissions; during the second 10 days, 65 fissions.

Thus it appears clear that long-continued cultivation at high temperature had so affected the animals that they could not survive continued exposure to low temperatures. Aside from the burst of reproduction in the ex-high set on the first day of exposure to low temperature, in this test as in the others the ex-low set multiplies much more vigorously than the ex-high set.

Throughout these three tests, therefore, it is clear that when the sets living under high and low temperatures respectively are placed at the same temperature (whether this be high, low or intermediate), the ex-low set are more vigorous and multiply more rapidly than the ex-high set. The high temperature has injured the vitality and reproductive power of the set exposed to it for a long period, while the low temperature has had no such effect.

(13) *Test of the modifications after 170 days in diverse temperatures (K, figure 3)*

After 5 months and 20 days in the diverse temperatures, the set exposed to the high temperature had become much weakened, and was now multiplying less rapidly than the set at low temperature (see table 5, and figure 4). Progeny of the two sets were now placed in an intermediate temperature of 18 to 20 degrees. This failed to revive the high temperature set. During 10 days the 20 ex-high lines gave a total of but five fissions, and all were dead before the 10 days were up. The 20

lines of the ex-low set meanwhile divided vigorously, producing 325 fissions in the 10 days.

(14) *General results of the second series of experiments*

This very extensive second series of experiments shows that when one set of lines of *Stylonychia* are kept for a long time at a temperature as high as 28 to 30 degrees, the other at 6 to 9 degrees, the set at the high temperature at first divides much more rapidly than the other. But after some months at the high temperature the vigor of the high-temperature stock is gradually undermined and destroyed; their rate of multiplication becomes less, finally falling below that of the low-temperature set; many of the lines die out, and at the end of six months all are dead. The low-temperature set on the other hand continue their slow rate of division, and show no indications of decreased vitality at the end of eight months.

After but 20 days in these diverse temperatures, if the two are brought to the same intermediate temperature, the set that had been in the high temperature continue to divide more rapidly than the set that had been in low temperature. This confirms the result of the first series of experiments. But after the two sets have been in the diverse temperatures for longer periods (forty days or more), then when the two are brought to the same temperature, the set that have been kept at the lower temperature (and were dividing more slowly), now divides more rapidly than the other. This is true whether the common temperature at which the two are cultivated is high, intermediate or low. It was true in tests made after 40 days; after 60 days; after 120 days, and after 170 days. The different fission rate thus induced lasted for at least six weeks after the two sets were restored to the common temperature.

Thus exposure to diverse temperature induces in a single stock diversities that are heritable in vegetative reproduction. After a limited period the inherited effect is manifest in the same direction as the immediate effect of the different temperature. After a longer period the inherited effect on the fission rate is the reverse of the immediate effect.

THIRD SERIES OF EXPERIMENTS

(15) *General outline (figure 5)*

The results of the first and second series of experiments were further tested by a relatively brief third series, lasting all together 60 days. A diagram of the tests made in this series is shown in figure 5.

On April 10, 1916, forty animals of the sixth filial generation from a single individual propagating asexually, taken from a 'wild' laboratory culture of *Stylonychia pustulata*, were isolated in the usual way. Of the forty lines of propagation obtained from these, twenty were kept at a high temperature of 24 to 27 degrees; the other 20 at a low temperature of 8 to 18 degrees. They were cultivated at these diverse temperatures for sixty days. These form the basic cultures for this series (A, figure 5).

FIGURE 5.—Diagram of the third series of experiments. The horizontal lines represent the duration of the cultures; the vertical lines show successive 10-day periods.

A. The two basic cultures, derived from a single parent, and kept for 60 days at the diverse temperatures shown by the numbers above the lines. B, C and D, tests with progeny of both the basic sets under the same temperature, after 30 days in opposite extreme temperatures. B, test at high temperature; C, at intermediate temperature; D, at low temperature.

A + sign above the test lines shows that the high-temperature set (or ex-high set) divides more rapidly; below the test lines it shows that the low-temperature set (or ex-low) divides more rapidly.

After thirty days in these diverse temperatures, three tests were made. In one, lines from both the basic sets were kept at a high temperature (B, figure 5); in the second both sets were kept at an intermediate temperature (C, figure 5); in the third both sets were kept at a low temperature (D, figure 5). In all these six sets (twenty lines in each set) the fission rate was determined for thirty days. The tests were coincident in time with the last thirty days of the basic cultures.

(16) *The basic cultures, kept for 60 days at diverse temperatures (A, figure 5)*

The number of fissions for each of the twenty lines is shown for each of the six 10-day periods of the experiment in table 14. On the whole the lines kept at the high temperature increased their fission rate in the later periods, while those in the lower temperature decreased their rate. There was some fluctuation in the temperatures from period to period, as table 12 shows, and it is not improbable that the variations in fission rate were directly correlated with these. The important points in the records are that there was a very great difference between the two sets in respect to their fission rates, and that there is no indication of any injurious effect of either the high or of the low temperature.

(17) *Tests after 30 days at diverse temperatures*

(a) Test at high temperature (B, figure 5)

In the two sets kept at 22 to 28 degrees, those that had before been kept at higher temperature continued to divide more rapidly than the set that had been kept at low temperature. The fissions for each line of each set for the three ten-day periods of the test are shown in table 13. Out of the sixty comparisons made, in all but four the ex-high set divided more rapidly than the ex-low set. In the entire period of 60 days, the lowest line of the ex-high set produced more generations than the highest line of the ex-low set. Thus the two sets derived from the same parent, and kept for these thirty days under the same temperature, are distinctly diverse in their fission rate, as a result of the earlier diversity of temperature under which they lived.

It is notable that in the last ten-day period of the thirty days at the same temperature, the difference between the two sets did not become less. It was in fact somewhat greater in the last 10 days.

A. R. MIDDLETON, TEMPERATURE DIFFERENCES AND THE FISSION RATE OF STYLONYCHIA

TABLE 12

SERIES III.—Basic cultures (A, figure 5). Number of fissions (generations) for the two sets of twenty lines each; one set kept for 60 days in high temperature, the other set in low temperature, with the excess in favor of the high set. (Differences given the minus sign below show an excess of the low set.)

	Lines number																				Total	Average per line	Percent that the difference is of the total in all
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20			
First 10-day period																					304	15.20	
24° to 26° C. High	16	14	13	15	16	16	15	14	14	15	17	16	16	16	15	15	16	15	14	16			
8° to 11° C. Low	2	1	2	3	3	5	3	1	3	2	3	1	2	1	3	1	3	1	3	1	44	2.20	
Excess	14	13	11	12	13	11	12	13	11	13	14	15	14	15	12	14	13	14	11	15	260	13.00	75.86
Second 10-day period																					441	22.05	
24.5° to 26° C. High	22	20	19	20	24	22	21	20	25	23	26	21	23	21	21	22	22	22	24	23			
8.5° to 12° C. Low	5	5	2	4	5	5	4	3	4	5	3	3	4	4	4	1	4	4	4	4	77	3.85	
Excess	17	15	17	16	19	17	17	17	21	18	23	18	19	17	17	21	18	18	20	19	364	18.20	70.27
Third 10-day period																					401	20.05	
24.5° to 27° C. High	19	18	20	17	22	19	22	19	25	21	22	21	21	19	18	20	18	21	20	19			
9.5° to 17° C. Low	8	7	7	8	7	7	7	6	6	8	4	7	6	7	5	3	7	7	6	6	129	6.45	
Excess	11	11	13	9	15	12	15	13	19	13	18	14	15	12	13	17	11	14	14	13	272	13.60	51.32
Fourth 10-day period																					320	16.00	
24° to 26.5° C. High	18	15	17	14	16	15	19	18	17	16	16	16	18	14	15	16	15	16	14	15			
10° to 16° C. Low	8	10	5	7	9	7	7	6	8	9	8	8	8	7	7	8	8	8	10	10	158	7.90	
Excess	10	5	12	7	7	8	12	12	9	7	8	8	10	7	8	8	7	8	4	5	162	8.10	33.67
Fifth 10-day period																					406	20.30	
25° to 27° C. High	17	14	14	17	20	19	22	17	20	30	19	29	18	31	20	23	22	23	17	14			
8° to 18° C. Low	2	4	4	4	2	4	2	2	3	3	1	2	4	3	3	3	3	3	2	3	57	2.85	
Excess	15	10	10	13	18	15	20	15	17	27	18	27	14	28	17	20	19	20	15	11	349	17.45	75.37
Sixth 10-day period																					593	29.65	
21.5° to 27° C. High	26	25	24	22	25	25	21	17	22	27	21	27	25	27	26	24	29	28	26	26			
8° to 13° C. Low	3	2	3	2	1	2	5	3	5	3	5	3	3	3	0	1	5	3	5	2	60	3.00	
Excess	23	23	21	20	24	23	16	14	17	24	16	24	22	24	26	23	24	25	21	24	533	26.65	81.62
Total, 60 days																					2465	123.25	
High	118	106	107	105	123	116	120	105	123	132	121	130	121	128	115	120	122	125	115	113			
Low	28	29	23	28	27	30	28	21	29	30	24	24	27	25	22	17	30	26	30	26	525	26.25	
Excess	90	77	84	77	96	86	92	84	94	102	97	106	94	103	93	103	92	99	85	87	1940	97.00	64.88

(b) Test at intermediate temperature (C, figure 5)

When the two sets that had lived for thirty days under diverse temperatures were placed in the same intermediate temperature of 14 to 20 degrees, the set that had been kept at the lower temperature divided somewhat more rapidly than the set that had been kept at high temperature. The exact figures are given in table 14. The difference is not very great, but is constant. In only three cases out of a possible forty in the first and second ten-day periods did the ex-high line show an excess over the corresponding ex-low line, and in only five cases out of a possible twenty in the third period. In the entire 60 comparisons made in table 14, the ex-low line showed the higher fission rate in 43. For the entire period of 60 days the ex-high lines varied from 26 to 40 fissions, with the average at 30.45, while the ex-low lines varied from 31 to 42, with the average at 36.30.

Thus when the two are tested at intermediate temperatures they give a result the reverse of that reached when tested at high temperatures. In the high temperature the set that have before been at high temperatures multiply faster; in the intermediate temperature those that have before been at low temperature multiply faster.

(c) Test at low temperature (D, figure 5)

The two sets that had been living at diverse temperatures for thirty days were both placed under a common low temperature of 10 to 15 degrees, (D, figure 5). During the first 10-day period of the test the set that had been living under low temperature multiplied distinctly more rapidly than the ex-high set (table 15). Of the 20 pairs compared, in all but four the fission rate was greater in the member from the ex-low set. In the second and third ten-day periods however, the ex-high lines multiplied more rapidly than the ex-low lines. The difference is not so great as the opposite difference in the first ten-day period, but appears uniform enough to be significant (see table 15). This result is difficult to interpret.

(18) *General results of the third series of experiments*

The results of this relatively brief third series of experiments are not entirely clear. But in the earlier series of experiments we had found that when the two sets had been cultivated for so short a period as twenty days, on a test under common conditions the ex-high set retain their higher rate of fission; while after forty days they fall, when tested, behind the other set. In the present series the exposure had been for

TABLE 13

SERIES III.—*Test at high temperature (B, figure 5). Number of fissions (generations) for the two sets of twenty lines each, both kept for thirty days in high temperature, 22°-27° C., subsequent to 30 days in extreme temperatures, 21°-27° C. and 8°-17° C.; with the excess in favor of the ex-high set. (Differences given the minus sign show an excess of the ex-low set.)*

	Lines number																				Total	Average per line	Percent that the difference is of the total in all
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20			
First 10-day period																					324	16.20	
Ex-high	18	15	17	14	16	15	19	18	17	16	16	17	19	15	16	16	15	16	14	15			
Ex-low	12	12	12	11	11	10	12	13	11	11	12	11	12	12	10	9	12	9	14	13	229	11.45	
Excess	6	3	5	3	5	5	7	5	6	5	4	6	7	3	6	7	3	7	0	2	95	4.75	17.17
Second 10-day period																					407	20.35	
Ex-high	17	14	14	17	20	19	22	17	20	30	19	29	18	31	20	23	22	23	17	14			
Ex-low	14	12	8	9	12	15	15	27	18	32	12	14	18	15	13	12	15	14	17	17	309	15.45	
Excess	3	2	6	8	8	4	7-10	2	-2	7	15	0	16	7	11	7	9	0	-3		98	4.90	13.68
Third 10-day period																					493	24.65	
Ex-high	26	25	24	22	25	25	21	17	22	27	21	27	25	27	26	24	29	28	26	26			
Ex-low	19	21	18	18	20	23	21	26	22	27	15	17	18	19	18	17	18	18	17	16	388	19.40	
Excess	7	4	6	4	5	2	0	-9	0	0	6	10	7	8	8	7	11	10	9	10	105	5.25	11.91
Total, 30 days																					1224	61.20	
Ex-high	61	54	55	53	61	59	62	52	59	73	56	73	62	73	62	63	66	67	57	55			
Ex-low	45	45	38	38	43	48	48	66	51	70	39	42	48	46	41	38	45	41	48	46	926	46.30	
Excess	16	9	17	15	18	11	14-14	8	3	17	31	14	27	21	25	21	26	9	9		298	14.90	13.86

TABLE 14

SERIES III.—*Test at intermediate temperature (C, figure 5). Number of fissions (generations) for the two sets of twenty lines each, both kept for thirty days in intermediate temperature, 14° to 20° C., subsequent to thirty days in extreme temperatures (21° to 27° C. and 8° to 17° C.); with the excess in favor of the ex-high set. (Differences given the minus sign show an excess of the ex-low set.)*

	Lines number																				Total	Average per line	Percent that the difference is of the total in all
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20			
First 10-day period																					203	10.15	
Ex-high	12	10	11	11	11	11	14	9	7	12	10	8	11	9	9	11	10	9	9	9			
Ex-low	12	12	11	12	11	11	13	12	10	14	13	13	13	16	13	16	15	12	10	10			
Excess	0	-2	0	-1	0	0	1	-3	-3	-2	-4	-5	-2	-4	-7	-2	-6	-6	-3	-1	-46	-2.30	-10.17
Second 10-day period																					205	10.25	
Ex-high	10	10	9	9	12	8	16	7	11	14	12	10	10	11	9	11	8	9	10	9			
Ex-low	15	16	9	11	15	14	15	14	12	12	8	13	13	15	12	14	11	11	13	13			
Excess	-5	-6	0	-2	-3	-6	1	-7	-1	2	4	-3	-3	-4	-3	-1	-6	-2	-1	-4	-50	-2.50	-10.86
Third 10-day period																					201	10.05	
Ex-high	9	10	9	11	9	11	13	11	14	10	9	11	8	12	9	8	8	8	13	13			
Ex-low	12	10	14	11	11	12	10	10	9	10	12	10	10	14	11	14	10	12	10	10			
Excess	-3	0	-5	0	-2	-1	3	1	5	0	-3	1	-2	-2	-2	-6	-2	-4	-2	3	-21	-1.05	-4.96
Total, 30 days																					609	30.45	
Ex-high	31	30	29	31	32	30	43	27	32	36	31	29	32	27	30	26	26	27	31	31			
Ex-low	39	38	34	34	37	37	38	36	31	32	34	36	36	42	39	39	40	38	33	33			
Excess	-8	-8	-5	-3	-5	-7	5	-9	1	4	-3	-7	-7	-10	-12	-9	-14	-12	-6	-2	-117	-5.85	-8.69

TABLE 15

SERIES III.—Test at low temperature (*D*, figure 5). Number of fissions (generations) for the two sets of twenty lines each, both kept for thirty days in low temperature, 8° to 15° C., subsequent to thirty days in extreme temperatures (21° to 27° C. and 8° to 17° C.) with the excess in favor of the ex-high set. (Differences given the minus sign show an excess of the ex-low set.)

	Lines number																				Total	Average per line	Percent that the difference is of the total in all
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20			
First 10-day period																					117	5.85	
Ex-high	6	6	6	5	6	7	8	6	7	7	6	5	5	5	5	6	4	6	5	6			
Ex-low	8	5	10	7	9	7	7	6	8	9	8	8	7	7	8	8	8	10	10	10	158	7.90	
Excess	-2	1	-4	-2	-3	0	1	0	-1	-2	-2	-3	-3	-2	-2	-2	-4	-2	-5	-4	-41	-2.05	-14.90
Second 10-day period																					67	3.35	
Ex-high	4	4	5	4	3	3	4	3	3	3	3	3	3	3	3	3	3	3	3	4			
Ex-low	2	4	4	4	2	4	2	2	3	3	1	2	4	3	3	3	3	3	2	3	57	2.85	
Excess	2	0	1	0	1	-1	2	1	0	2	1	-1	0	0	0	0	0	0	1	1	10	.50	+8.06
Third 10-day period																					78	3.90	
Ex-high	5	4	5	5	4	3	2	4	4	4	3	3	4	5	3	4	4	4	3	5			
Ex-low	3	2	2	3	2	2	5	3	5	3	5	3	3	3	0	1	5	3	5	2	60	3.00	
Excess	2	2	3	2	2	1	-3	1	-1	1	-2	0	1	2	3	3	-1	1	-2	3	18	.90	+13.04
Total, 30 days																					262	13.10	
Ex-high	15	14	16	14	13	13	14	13	14	14	12	11	12	13	11	13	11	13	11	15			
Ex-low	13	11	16	14	13	13	14	11	16	15	14	13	15	13	10	12	16	14	17	15	275	13.75	
Excess	2	3	0	0	0	0	0	2	-2	-1	-2	-3	0	1	1	-5	-1	-6	0	-13	-65	-2.40	

the intermediate period of thirty days, when apparently one of these conditions was transforming into the other. It is therefore perhaps not surprising that the results are not uniform.

GENERAL RESULTS AND CONCLUSIONS

All the experiments show that when infusoria of the same stock are cultivated for considerable periods under diverse temperatures, there result differences between the two sets that are hereditary under vegetative fission. When the two sets are restored to the same temperature, they continue to show diverse fission rates, and this for long periods.

The precise nature of the differences depends upon the length of the period during which the two sets have been kept at different temperatures. After a relatively short period (twenty days or somewhat more), when the two are transferred to a common intermediate temperature, the set that has been at the higher temperature continues to divide more rapidly. But after long periods of exposure to high and low temperatures, when the two are placed at a common temperature the ex-low set divides more rapidly than the ex-high set. The difference thus produced is very great, and is uniform. Apparently the vigor of the organisms is impaired by long continuance of high temperatures, while cultivation at low temperatures has no such effect. Thus on restoration to common intermediate temperatures the ex-low set take and hold the lead in reproduction.

If the cultivation in high temperatures (30 to 32 degrees) is prolonged to six months, the organisms all die, while the low-temperature cultures (6 to 9 degrees) continue to flourish. The latter showed no signs of injury when the cultures were discontinued at the end of eight months.

The most interesting result is perhaps that which follows when the exposure to diverse temperatures has not been so prolonged as to injure the high-temperature set. In this case the diversity in fission rate impressed upon the organisms by the direct action of the different conditions is continued after the conditions have become the same for both sets. But after injury has been induced by high temperature, the inherited effect is manifested in a reversal of the direct action of the different conditions.

It would be of great interest to produce diversities of fission rate by other means, particularly by diverse chemical action, and to determine the inherited results of these. Our results suggest that the numerous

lines or races differing slightly in inherited characteristics, which seem to occur in all lower organisms, have been produced in part by past diverse environmental conditions.

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