

THE EFFECT OF CONJUGATION IN PARAMECIUM¹

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TWO FIGURES

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I. INTRODUCTION

What is the effect of conjugation on the individual or stock that undergoes it? This question reduces experimentally to the following: In what respect does a stock that has conjugated differ from one that is in other respects similar, but has not conjugated? What difference is produced if half a given stock is allowed to conjugate while the other half is not?

When one examines the evidence for the conclusions commonly drawn as to the effects of conjugation on the stock in the infusoria, it is curious that almost no direct experimental evidence is found. The conclusions as to the rejuvenating or other physiological effects of conjugation are based almost exclusively on reasoning of the following character: "Since without conjugation such and such processes of degeneration (or other phenomena) occur, it must be that conjugation has the effect of preventing or curing this degeneration (or these other phenomena)." The experimentation is almost all devoted to testing the premise, while direct experimental demonstration that the conclusion is correct, that conjugation actually does rejuvenate (or the like), is almost unattempted. Such exceptions to this generalization as exist we shall later take up in detail.

There is then need of an investigation in which conjugation itself—rather than what happens without conjugation—shall lie at the center of experimentation. Such a study this paper pre-

sents. The fundamental experiment is to divide a given stock into two parts, kept under identical conditions, permitting one part to conjugate and preventing the other; to keep these further under identical conditions, and to determine in what respects they differ. In the few attempts that have heretofore been made to observe directly the results of conjugation, the control series (of the same stock *without* conjugation) has almost invariably been omitted, so that it is uncertain how far the phenomena observed would have occurred equally if there had been no conjugation.²

In the investigation here set forth this fundamental experiment has been many times repeated, with careful study of the various characteristics of the set that have conjugated, as compared with those of the set that have not conjugated. Besides an account of the results of this fundamental experiment, the paper deals with certain other problems connected with the physiology of conjugation. The effect of conjugation on the size of the individuals of the stock has been set forth in a former paper (Jennings '11).

Thus the matters dealt with in the present paper are mainly the following: the effects of conjugation on the rate of multiplication; on survival and mortality; on the general vigor; its relation to 'rejuvenescence;' the effects of conjugation among close relatives; the effects of continued inbreeding; the results of allowing a stock to conjugate many times in a given period, as compared with causing it to multiply without conjugation for the same period; the relation of conjugation to inheritance, and the effect of conjugation on variation.

Each experiment gives evidence on most of the matters just mentioned, so that it is not possible to separate fully the different subjects. The experimental results will first be presented systematically, with more particular reference to the effects of conjugation on vigor, multiplication, survival and variation, then each of the topics will be taken up, and an analysis given of the experimental evidence bearing upon it.

² The only exception to this that I have found is in the experiment of R. Hertwig, briefly set forth in his paper of 1889; this will be taken up later.

II. METHODS

Most of the experiments followed a general plan, the chief features of which may be here set forth. The object was, to compare, under similar conditions, a set of the animals that had conjugated with another set that was ready to conjugate, but was prevented from doing so. Both *Paramecium caudatum* and *Paramecium aurelia* were employed in the work. Abundant conjugation was obtained in the way described by Maupas ('89), Calkins and Cull ('07), and others. In the evening large numbers of the animals were taken from the large cultures and placed in watch glasses; early the following morning they were usually beginning conjugation. *Paramecium caudatum* is especially favorable for obtaining with certainty the first stages of the process, since as Maupas ('89, pp. 171, 182) has noted, this animal conjugates in the early morning, commencing at about five o'clock. If therefore there were no conjugations when the watch glasses were set, one can be certain that any pairs found early the following morning have just united.

Split pairs. At the beginning the pairing animals fit loosely together; they at first, as a rule, adhere together only by their anterior ends. At such a time it is easy to separate them, by drawing them repeatedly into a fine pipette. The separated individuals are then isolated and cultivated separately.

Pairs. Other pairs are allowed to complete conjugation. They separate spontaneously after about twelve hours; the two members are then isolated and cultivated separately, under the same conditions as the members of the 'split pairs.'

In this way two sets are obtained, taken from the same culture, both ready to conjugate and beginning the process at the same time; the only difference between them lies in the fact that one is allowed to complete the process, while the other is not. By cultivating the two sets under identical conditions it becomes possible to determine what difference is made by conjugation.

Designation. The terms 'pairs' and 'split pairs' will be used in referring to the members of the two sets, and to their progeny. The two members of any pair, or of any split pair, will be desig-

nated *a* and *b*. It is important to understand that these designations do not imply any characteristic differences, the two letters being assigned arbitrarily and at random to the two members, in order to make it possible to speak of them separately. But the individual and its progeny to which a given letter is assigned of course retain this designation throughout. The pairs (and split pairs) of any experiment are designated by serial numbers, so that any individual is indicated by a number and a letter; thus *8b* signifies the individual *b* of pair 8. The lines of progeny from a given individual receive the same designation as the parent individual, so that in later stages of the experiment *8b* signifies the line of progeny derived from the individual *b* of the pair 8.

Culture. The isolated individuals were transferred to the concavities of hollow ground glass slides, each concavity containing two or three drops of culture fluid. Thick slides with two concavities were found most convenient. At the beginning the animals were usually left for one or two days in water from the dish in which they were found, in order not to disturb the processes of conjugation by the shock of removal to a different fluid. For the later cultivation an infusion of pure Timothy hay was usually employed. This was made by boiling one gram of Timothy hay for ten minutes in 100 cc. of tap water, then adding to this infusion, after it was cool, 100 cc. of filtered but unboiled water. Sometimes this filtered water was taken from the parent culture; a procedure that, in some cases, though not in all, works well. The infusion was tried in varying strengths at different times, but all the animals of a given experiment were treated throughout in exactly the same way. The infusion was invariably made up fresh just before it was used.

In the last experiments tried, it was found that $\frac{1}{16}$ per cent Horlick's malted milk, as recommended by Peebles ('12), was preferable in some respects to the hay infusion, particularly in summer. It was found necessary, for the best results, to make up this culture fluid fresh each day.

The animals were transferred to two drops of fresh infusion, on a clean slide; in some experiments every day, in others every

other day. In some experiments two individuals of each line were transferred to the new slide, in others only one; the remainder being destroyed.

The slides were kept in moist chambers, on strips of glass which were supported above water covering the bottom of the vessel.

Records. At each transfer the number of fissions undergone since the last transfer was recorded; so that these records were made either every day or every other day. Since either one or two individuals had been left on the slide at the previous transfer, there was no difficulty in determining how many fissions had occurred. In some cases of course the same number of fissions had not occurred in the two individuals left the day before, but this made absolutely no difficulty in practice. If, for example, ten individuals were found on the slide, invariably four of these were distinctly smaller than the other six. This showed that each of the two original individuals had divided twice, producing 8, and that two of these 8 individuals had divided again, giving the four small specimens out of the ten. If therefore two of these small individuals were transferred to the new slide (as would usually be done in such a case), the number of fissions was recorded as three.

For keeping records of large numbers of cultures (the numbers ran up to 480 in some cases), the following procedure is convenient. Procure hollow ground slides of which the upper surface has been ground, so that one can write on them with a lead pencil. Then at transfer write upon the new slide (besides the designation of the line) the number of specimens found in the old slide, the number left in the new one, and an indication of the generation to which they belong. Thus in the case just cited, where 10 individuals were present and two of the smaller ones were transferred, the legend on the new slide would be simply '10 (16)-2'; which indicates that 10 were present; that 2 were retained, and that if the fission from which these two resulted were complete, there would have been 16 on the slide. After all the slides have been thus transferred, labeled, and placed in

the moist chambers, the latter are examined and the records on the slides copied to permanent records on large sheets of paper. From these records the exact number of fissions can be obtained at any time; thus, since in the above case the generation containing 16 had been obtained from 2, it is clear that three fissions had occurred.

Tabulation and tables. For analysis, the records of fissions have in most cases been tabulated for definite periods, as of one week or of ten days. In analyzing the records it has been necessary to make from the original tables of records a very large number of secondary tables, particularly correlation tables. In place of publishing these secondary tables, the original tables of record will be published in the Appendix of the present paper. These contain all that would be found in the correlation and other secondary tables, and anyone who desires can reconstruct the latter from them, so that they furnish every possibility for testing the results here given. Furthermore, the original record tables show much that is lost when they are transformed into correlation tables; particularly do they show much that is of interest from the point of view of 'pure line' studies.

Besides these tables giving the original records, the present paper will contain as a rule only tables giving the data—the constants, et cetera—resulting from the analysis, by biometrical methods, of these records; these are found in the body of the paper.

III. EFFECT OF CONJUGATION ON MULTIPLICATION, SURVIVAL AND VARIATION

As previously noted, the experiments described below furnish evidence on other matters besides those set forth in the heading above, but we shall examine them first from this point of view. We shall divide the experiments into two sets; the first including those dealing with 'wild' cultures; the second those dealing with pure strains.

EXPERIMENTS WITH WILD CULTURES: *PARAMECIUM CAUDATUM**Experiment 1: May 4 to June 7, 1909*

As giving typical results, an experiment which was in progress from May 4 to June 7, 1909, will first be presented.

The animals were taken from a 'wild' culture of *Paramecium caudatum*, which was brought from a pool on May 3, 1909. It was found to swarm with the infusoria, and on the evening of May 3 numbers of them were placed in watch glasses; at that time none were conjugating. Early the next morning conjugation was beginning. Thirty-five pairs in the first stages of union were separated in the way already described, but in 11 of these one of the members was killed or lost, so that there remained 59 individuals that had gone through the first stages of pairing; among these 59, both members were present in 24 of the 'split pairs.'

Thirty-one pairs were allowed to complete conjugation, then the two members isolated. One member of one pair was lost, so that from the pairs there were derived 61 lines of propagation, as against 59 from the split pairs.

In this experiment the 120 lines of propagation were changed, and the records taken, every other day. The numbers of fissions were grouped in weekly periods for each line.

Thus we have before us 120 individuals undergoing propagation; one set of 61 have just conjugated, while another set of 59 were ready to conjugate, but were prevented from doing so. They differ in no other way but in regard to conjugation. What later difference does this make in the two sets?

1. The first thing that we discover is that the individuals which were ready to conjugate but were prevented, are by no means in a depressed, degenerate condition, unable to propagate farther. On the contrary, they continue to propagate in an active, healthy manner. They continued to do this till the experiment was discontinued five weeks later.

2. Secondly, we notice that those which have conjugated multiply less rapidly than those which have not. This difference is

very great, and will be well brought out by examining side by side certain weekly records for the first fifteen individuals of each set (table 1).

TABLE 1

Experiment 1. Paramecium caudatum. Number of fissions per week for the first 15 lines of each set (d = dead).

First week.																
Pairs.....	0	1	5	5	4	2	1	5	0	5	6	3	2	4	0	
Split pairs.....	6	6	8	7	7	7	7	7	7	8	7	8	7	7	7	
Second week:																
Pairs.....	1	2	6	5	6	6	0	5	2	4	5	5	1	2	6	
Split pairs.....	4	6	6	7	6	5	4	6	6	4	5	6	5	6	6	
Third week:																
Pairs.....	d	d	8	6	8	1	0	5	0	6	6	3	3	d	8	
Split pairs.....	7	9	6	9	7	6	6	8	6	5	9	10	9	8	7	

3. All those which have not conjugated multiply, while among those that have conjugated are a considerable number that either never divide again, although they may live for a long time; or divide but few times. This will be evident from examination of the general record table (table 29, Appendix).

4. A considerable number of the lines derived from those that have conjugated die out, while none of the others die out (table 29).

5. It is evident on a cursory examination of the records that among the lines derived from the conjugants there is much greater variation in the rate of fission than among those derived from the individuals that have not conjugated.

Each of these points will now be taken up in detail and the facts precisely brought out.

The weekly records for the entire experiment are given in table 29 (Appendix), which serves as a basis for the following discussion.

Fifth week not typical. One point should be brought out at the beginning of the analysis. During the fifth week the lines of propagation were in an unhealthy condition owing to extraneous reasons. On May 31, at the end of the fourth week, the experiment was tried of mixing a little starch from boiled bread

with the culture fluid, in the hope that this would improve the latter. It had the reverse effect, making the animals unhealthy, and almost or quite stopping multiplication. As a result, the figures for the fifth week are very low and irregular for both sets. It would beyond doubt give a more correct idea of the real relations if we should exclude the fifth week entirely, and consider the experiment as ending with the fourth week. But I have not felt justified in suppressing any part of the record, and the main results are clear in spite of the irregularity due to the exceptional conditions of the fifth week. But it will be well to keep in mind the fact that the results obtained from the fifth week have little or no significance on our main problems.

Rate of fission. Table 2 gives the mean number of fissions for each of the two sets, for each week and for various combinations of weeks; also the ratio of the means for those that have not conjugated (split pairs) to the means for those that have conjugated.

TABLE 2

Experiment 1. Mean numbers of fissions per week and for certain other periods, in those that have conjugated (pairs) as compared with the same for those that have not conjugated (split pairs); also ratio of the means for the two sets.

	MEMBERS OF PAIRS		MEMBERS OF SPLIT PAIRS		RATIO OF MEAN FOR SPLIT PAIRS TO MEAN FOR PAIRS
	No.	Mean	No.	Mean	
First week.....	61	3.279 \pm 0.148	59	6.729 \pm 0.099	2.052
Second week.....	56	4.661 \pm 0.205	59	5.932 \pm 0.093	1.273
Third week.....	45	5.000 \pm 0.264	59	6.678 \pm 0.199	1.336
Fourth week.....	42	3.976 \pm 0.210	59	5.102 \pm 0.130	1.283
Fifth week.....	38	2.737 \pm 0.156	59	2.593 \pm 0.113	0.947
First two weeks.....	56	7.589 \pm 0.363	59	12.661 \pm 0.144	1.668
Second two weeks.....	42	9.306 \pm 0.415	59	11.780 \pm 0.287	1.266
Four weeks:					
(a) Those that lived through.....	42	18.857 \pm 0.526	59	24.441 \pm 0.352	1.296
(b) All, including those that did not live to end.....	61	13.918 \pm 0.775	59	24.441 \pm 0.352	1.756
Five weeks:					
(a) Those that lived through.....	38	21.842 \pm 0.602	59	27.034 \pm 0.384	1.238
(b) All.....	61	15.902 \pm 0.833	59	27.034 \pm 0.384	1.700

Table 2 shows that in every week (save the fifth), and in every combination of weeks, the average number of fissions was greater for those that had not conjugated than for those that had conjugated. The fifth week, as we have already seen, gives, for extrinsic reasons, atypical results. In that particular case the difference in the means is not significant, as is shown by the probable errors in the two cases. For the entire four (or five) weeks, the average number of fissions was about 25 per cent greater in those that have not conjugated. If we take the total number of fissions for each line that was alive at the beginning of the experiment, we find that the average number of fissions was 70 to 75 per cent greater for those that had not conjugated. This is of course partly due to the fact that none of the latter died before the end of the experiment, while a considerable number of the conjugant lines died out early.

It is of interest to compare the number of progeny produced by the two sets. This is of course obtainable from the number of fissions. The potential progeny produced by the two sets in each of the five weeks is given in table 3.

As the table shows, each line of those that have not conjugated produced weekly on the average almost exactly two-and-a-half times as many progeny as a line of the conjugants.

The 61 lines derived from the conjugants had a potential production all together during four weeks of the experiment of

TABLE 3

Experiment 1. Potential number of progeny from those that have conjugated, as compared with those that have not, based on the number of fissions in table 2.

WEEK	PAIRS			SPLIT PAIRS		
	Number of lines	Number of progeny	Average per line	Number of lines	Number of progeny	Average per line
1	61	991	16.246	59	8008	135.729
2	56	3038	54.250	59	4296	72.813
3	45	4712	104.711	59	17066	289.254
4	42	1489	35.452	59	2962	50.203
5	38	479	12.605	59	537	9.102
Average per week			44.653			111.420

1 billion, 256 million progeny, while the 59 lines derived from those that had not conjugated had a production of 48 billion, 467 million, so that the non-conjugants produced somewhat more than 38 times as many progeny as the conjugants. The very great difference between the two in this respect arises from the fact that many of the conjugant lines died out before the end of the experiment and the further fact that the number of progeny increases in geometrical ratio as the number of fissions increases in arithmetical ratio. To this latter fact is due also the seemingly excessive differences in the number of progeny produced in the different weeks, as shown in table 3. Unfavorable temperature or culture medium, decreasing the number of fissions by a small number, decreases the progeny enormously.

To sum up on this point, the experiment shows clearly that those that have not conjugated multiply more rapidly than those that have conjugated, and the difference persists for at least four weeks after conjugation.

Variation. A careful examination of the data given in table 29 will show that there is more variation (among the different lines) for the number of fissions in any given period, for those that have conjugated than for those that have not. To determine accurately the differences in this respect, it is necessary to determine the standard deviations and coefficients of variation for each period. These are given in table 4 together with a comparison showing what the ratio of the variation among the non-conjugants is to that among the conjugants.

Table 4 shows that in every week, and in every combination of weeks, without exception, the variation is greater in those that have conjugated than in those that have not. It is greater in the conjugants, whether measured absolutely, by the standard deviation; or relatively to the mean, by the coefficient of variation. In many of the periods the coefficient of variation is for the non-conjugants but one-third to one-fourth of that for the conjugants.

It is then a simple statement of fact to say that in this case conjugation increased greatly the variability in the fission rate. Examination of table 29 shows that this great increase of varia-

tion in the progeny of the conjugants is due mainly to the fact that many of the lines descended from them multiply but slowly, while others multiply at nearly the same rate as do the progeny of non-conjugants. Among the 59 lines of non-conjugants, there are but two that gave fewer than 20 fissions in the five weeks, while among the 38 lines of conjugants that lived through the entire five weeks there are 12 that fall below 20. On the other

TABLE 4

Experiment 1. Relative variability in number of fissions for given periods, in those that have conjugated (pairs), and those that have not (split pairs).

	PAIRS			SPLIT PAIRS			RATIO OF SPLIT PAIR TO PAIR	
	No.	Standard deviation	Coefficient of variation	No.	Standard deviation	Coefficient of variation	Standard deviation	Coefficient of variation
First week.....	61	1.709 \pm 0.104	52.131 \pm 3.955	59	1.132 \pm 0.070	16.829 \pm 1.074	0.662	0.323
Second week....	56	2.270 \pm 0.145	48.704 \pm 3.769	59	1.055 \pm 0.066	17.792 \pm 1.139	0.465	0.365
Third week....	45	2.625 \pm 0.189	52.494 \pm 4.648	59	2.266 \pm 0.141	33.929 \pm 2.337	0.863	0.646
Fourth week....	42	2.018 \pm 0.149	50.743 \pm 4.596	59	1.481 \pm 0.092	29.027 \pm 1.948	0.734	0.572
Fifth week.....	38	1.427 \pm 0.110	52.135 \pm 5.011	59	1.290 \pm 0.080	49.759 \pm 3.778	0.904	0.954
First two weeks	56	4.030 \pm 0.257	53.103 \pm 4.232	59	1.643 \pm 0.102	12.975 \pm 0.819	0.408	0.244
Second two weeks.....	42	3.991 \pm 0.294	42.870 \pm 3.689	59	3.268 \pm 0.203	27.743 \pm 1.850	0.819	0.647
Four weeks:								
(a) Those that lived through	42	5.055 \pm 0.372	26.806 \pm 2.110	59	4.010 \pm 0.249	16.405 \pm 1.046	0.793	0.612
(b) All.....	61	8.901 \pm 0.544	63.951 \pm 5.430	59	4.010 \pm 0.249	16.405 \pm 1.046	0.451	0.257
Five weeks:								
(a) Lived through	38	5.499 \pm 0.425	25.174 \pm 2.068	59	4.376 \pm 0.272	16.188 \pm 1.031	0.796	0.643
(b) All.....	61	9.571 \pm 0.585	60.186 \pm 4.829	59	4.376 \pm 0.272	16.188 \pm 1.031	0.457	0.269

TABLE 5

Experiment 1. Paramecium caudatum. Number of lines that died out during different periods, among those descended from the pairs (conjugation consummated).

	WEEK					FOUR WEEKS	FIVE WEEKS
	1	2	3	4	5		
Number died.....	0	5	11	3	4	19	23
Per cent of those alive at beginning of week.....	0	8.2	19.6	6.7	9.5		
Per cent of all.....	0	8.2	18.0	4.9	6.6	31.15	37.7

hand, the upper extreme for the non-conjugants (38) is higher than that for the conjugants (32), but most of the non-conjugants are so grouped near the high figure that the variation is relatively small.

Mortality. None of the 59 lines of non-conjugants died out during the five weeks of the experiment. Of the 61 lines of conjugants, on the other hand, 23, or 37.7 per cent, died out during the experiment. The number of lines descended from conjugants that died out during each week is given in table 5.

Thus in this case conjugation greatly increased the mortality. Although the 'split pairs' were ready to conjugate, and had actually taken the first steps in the process, they are not in the least injured by being prevented from consummating the process; while those that finished mating showed a high mortality.

Abnormalities. Besides the actual deaths, the descendants of those that had conjugated showed many abnormalities, while among the descendants of the non-conjugants there were none. For example, on May 14, I noted that there were among the descendants of the conjugants 24 abnormal individuals, belonging to 12 different lines, while in the other set there were none.

The abnormalities take the most diverse forms: bodies of irregular shape, crooked, truncate, or with projections; double or multiple monsters: some are abnormally large, others extremely thin. The structural abnormalities are in many cases connected with abnormalities in fission. In some cases the ex-conjugants do not divide for many days after separation. During this time they grow larger till they reach an immense size, many times greater than that ever reached at other times. Some of these immensely large individuals never divide again, and after living a week or two die. Others after a time divide irregularly, producing progeny of diverse sizes and forms. Thus the individual 10a, of the pairs, in this experiment did not divide until eight days after the separation from its mate. It then divided during the night into seven, of four diverse sizes. The individual 17b divided immediately into two specimens, which became immensely large; these did not divide again for six days, then each produced two large abnormal individuals which soon died.

Abnormal individuals appear again and again in certain of the lines derived from conjugants, while in others they do not appear at all. The conditions which induce them are thus evidently inherited from generation to generation in the fissions. As a rule, a given abnormality is not inherited in its special form, but only the tendency to produce abnormalities of various sorts.

Lines which show abnormalities in structure commonly have a slow rate of fission, are thin, succumb easily to unfavorable conditions, and in general, appear to lack vitality. Often they die out after a number of generations.

There are likewise found lines which show the thinness, slow fission rate, and general lack of vitality, without structural abnormalities.

It appears probable that these abnormalities have a cytological basis, and are due to irregularities in the nuclear processes accompanying conjugation. A precise study is greatly needed, as to the minute characteristics of these abnormalities, their heritability, their experimental cause, and their cytological basis. Such a study I hope will soon be made.

The data obtained from this experiment, and presented in table 29, will be analyzed in later papers with reference to the problems of sexuality, and of uniparental and biparental inheritance.

We may summarize the results of this experiment, so far as they bear on the problems now under consideration, as follows:

Conjugation decreases the rate of fission, causes a great increase in variation in the fission rate, brings about many abnormalities, and greatly increases the death rate.

Experiment 2: April 7 to June 7, 1909

This extensive and long continued experiment was the first one undertaken for comparing the fission-rate and vitality of animals that had conjugated and animals that had not. Owing to lack of experience the method of culture was not good, so that the mortality was very high; this makes the results less sharp and clear than in the experiment just described. The chief mistakes in culture were: (1) the culture fluid was not made up

by measure, so that it varied in strength from day to day. All the specimens were treated alike at each change, so that no difference between the sets resulted from this; but the changes in concentration of the infusion caused many deaths. (2) The hay of which the infusion was made was not sorted over, to exclude all but Timothy; thus at times injurious plants were included, increasing the mortality. (3) As a rule, only one individual of each line was retained at each change, so that if this individual died, the line became extinct. As a consequence of all these things, the number of lines decreased rapidly in all the different sets.

However, such an experiment, lasting eight weeks, is not likely to be often repeated, and the results are of much value in certain relations.

Three sets of the animals were employed in this experiment. One set ('pairs') consisted of individuals that had just conjugated; a second ('split pairs') included individuals that had begun to unite for conjugation, but were separated, in the manner previously described; the third set ('free') consisted of ordinary individuals that had not begun union, taken from the same culture as the others. This third set is known not to have conjugated recently, since they were taken (like the others) in the early morning, from watch glasses which contained no conjugants when set the evening before. Comparison of the 'split pairs' and the 'free' will show whether entrance upon the condition preparatory to conjugation alters the animals in any way, such as to affect their multiplication and vigor.

The 'free' specimens were given paired designations, each two being called *a* and *b*, as in the 'pairs' and 'split pairs'. In this case of course *a* and *b* were related in no way; the paired designations were given at random, in order to test the question whether one individual of a pair of conjugants dies or is weak more often than occurs as a result of mere chance causes, in specimens paired at random and merely by designation. This is a matter that will be dealt with fully in a later paper.

The three sets were treated in exactly the same way, the slides of pairs, split pairs and free alternating in the same moist cham-

bers. The culture fluid was changed as a rule every other day.

Since usually members of pairs of conjugants do not divide till the second day after conjugation, the comparison of the rate of fission for the three sets was not begun till this second day. Thus, the animals were isolated on the morning of April 8, but the tabulation of the fissions begins, for all sets, on April 10.

For purposes of comparison, the fissions were tabulated by weeks for each of the three sets. The experiment may best be divided into two periods, the first comprising the first two weeks; the second the last six weeks. At the end of the second week a considerable number of each set were lost by accident, so that the number to be dealt with is much smaller in the second period.

The experiment included at the beginning 57 lines ($28\frac{1}{2}$ pairs) of those that had finished conjugation; 39 lines ($19\frac{1}{2}$ pairs) of split pairs, and 58 lines of 'free' individuals.

The actual number of fissions per week is given for the first two weeks in table 30; for the last six weeks in table 31 (Appendix).

It should be noted that the data given are, so far as numbers of fissions go, of little value after the sixth week, and particularly is this the case for the seventh week. Pressure of other duties forced me to neglect these experiments at that time, so that during the seventh week the slides were changed but once; as a result they hardly multiplied at all. The figures for the seventh and eighth weeks are given only in order not to suppress any part of the record.

It is evident, as in the previous experiment, that the animals which were ready for conjugation were by no means in a depressed or degenerate condition. The split pairs continue to multiply, somewhat more rapidly than those that have conjugated. We shall examine in detail the rate of fission, the variation, and the mortality, in the three sets.

Rate of fission. Table 6 gives the mean numbers of fissions in each set, for each week, and for certain other periods.

As this table shows, in practically all of the 15 means given, the rate of fission is less for those that have conjugated than for those that have not. The only exception is in the seventh

week, where the rate is nearly the same, with a slight excess in favor of the conjugants. But as we have already noted, the animals were changed and (records made) but once that week, and in consequence there was almost no multiplication; the figures for that week are of no significance. In all the other cases (14 out of 15) the non-conjugants show a greater rate of fission, the excess varying from 6 to 85 per cent, with an average of 23 to 31 per cent.

The split pairs and the free individuals show no significant difference so far as rate of fission is concerned; so that the specimens that have taken the first steps in conjugation do not differ in this respect from those that have not.

TABLE 6

Experiment 2. Mean number of fissions for each week, and for certain other periods, in the three sets, together with a comparison of all that have conjugated with all that have not.

WEEK	PAIRS		SPLIT PAIRS		FREE		ALL NON-CONJUGANTS SPLIT + FREE		COMPARISON OF CONJUGANTS AND NON-CONJUGANTS	
	Number of lines	1	Number of lines	2	Number of lines	3	Number of lines	4	5	6
		Mean number of fissions		Mean number of fissions		Mean number of fissions		Mean number of fissions	Excess of non-conjugant rate over that of conjugants	Per cent of excess in terms of conjugant mean
1	50	4.080 ± 0.150	37	7.378 ± 0.162	54	7.685 ± 0.131	91	7.560 ± 0.102	3.480	85.3
2	34	2.000 ± 0.126	21	2.810 ± 0.174	30	2.233 ± 0.126	51	2.471 ± 0.106	0.471	23.6
3	22	3.591 ± 0.236	10	4.800 ± 0.230	19	4.947 ± 0.197	29	4.897 ± 0.160	1.306	36.4
4	19	3.211 ± 0.335	9	4.666 ± 0.237	17	4.471 ± 0.178	26	4.538 ± 0.241	1.327	41.3
5	14	7.143 ± 0.609	8	9.125 ± 0.186	15	9.333 ± 0.164	23	9.261 ± 0.126	2.118	29.7
6	11	5.182 ± 0.272	8	5.125 ± 0.512	14	5.714 ± 0.159	22	5.500 ± 0.215	0.318	6.1
7	11	1.455 ± 0.133	8	1.125 ± 0.186	14	1.429 ± 0.089	22	1.318 ± 0.091	-0.137	-9.4
8	9	4.111 ± 0.430	6	5.333 ± 0.130	11	4.636 ± 0.264	17	4.882 ± 0.185	0.771	18.8
1 and 2	34	6.176 ± 0.229	21	10.381 ± 0.296	30	10.100 ± 0.281	51	10.216 ± 0.206	4.040	65.4
3 and 4	19	7.158 ± 0.520					26	11.692 ± 0.253	4.534	63.3
5 and 6	11	13.727 ± 0.451					22	14.773 ± 0.267	1.046	7.6
6 weeks	11	29.364 ± 0.645	8	35.000 ± 0.860	14	35.143 ± 0.486	22	35.091 ± 0.440	5.727	19.5
8 weeks	9	34.111 ± 0.962	6	42.500 ± 0.910	11	41.545 ± 0.645	17	41.882 ± 0.532	7.771	22.8
Mean of Mean per week for those that survived 8 weeks	9	4.264	6	5.313	11	5.193	17	5.235	0.971	22.8
		3.846		5.045		5.056		5.053	1.207	31.4

TABLE 7

Experiment 2. Relative variability in fission rate for those that have conjugated and those that have not.

WEEK	CONJUGANTS ('PAIRS')			NON-CONJUGANTS ('SPLIT PAIRS') AND ('FREE')		
	No.	Standard deviation	Coefficient of variation	No.	Standard deviation	Coefficient of variation
1	50	1.573 \pm 0.106	38.549 \pm 2.962	91	1.447 \pm 0.072	19.134 \pm 0.991
2	34	1.085 \pm 0.088	54.235 \pm 5.590	51	1.126 \pm 0.075	45.592 \pm 3.623
1 + 2	34	1.977 \pm 0.162	32.011 \pm 2.874	51	2.181 \pm 0.146	21.350 \pm 1.489
3 + 4	19	3.360 \pm 0.368	46.944 \pm 6.166	26	2.671 \pm 0.250	22.847 \pm 2.246
5 + 6	11	2.219 \pm 0.319	16.168 \pm 2.385	22	1.857 \pm 0.189	12.570 \pm 1.298
6 weeks....	11	3.170 \pm 0.456	10.796 \pm 1.571	22	3.059 \pm 0.311	8.716 \pm 0.893
8 weeks....	9	4.280 \pm 0.680	12.548 \pm 2.026	17	3.252 \pm 0.376	7.764 \pm 0.904

The split pairs and the free may therefore properly be considered together, as non-conjugants, as in the fourth column of table 6. With these total results for all the individuals that have not conjugated may then be compared the results for those that have conjugated, as in columns 5 and 6 table 6. As there shown, for the first week the excess of the non-conjugants was 85 per cent, so that their rate was nearly double that of the conjugants. After this the excess for the non-conjugants decreased, although even in the eighth week it is 18.8 per cent. Thus the conjugants had not regained a rate equal to that of the non-conjugants even after so long a period.

Variation. The variation in the rate of fission is shown comparatively for conjugants and non-conjugants in table 7. In this table the two classes of non-conjugants—the 'split pairs' and the 'free'—have been put together, since we have already seen from table 6 that there is no characteristic difference between them. This fact is shown equally if we compute the variation separately for the two classes. Thus, for the first week the split pairs give a standard deviation of 1.458 and a coefficient of 19.767, while the corresponding figures for the free are 1.425 and 18.542. For the second week the figures are: split pairs, 1.180 and 41.998; free, 1.023 and 45.785; first two weeks, split pairs, 2.011 and 19.375; free, 2.285 and 22.628. Throwing the two together, as in table 7, gives the great advantage of larger numbers.

As the table shows, although the means for the conjugants are throughout less (table 6), their standard deviations are as a rule greater than the standard deviations for those that have not conjugated. As a result, the coefficient of variation (standard deviation divided by the mean) is in every case much greater for those that have conjugated. For the first week the variation, as measured by this coefficient, is twice as great in the conjugants. For the entire eight weeks it is nearly twice as great.

Thus in this experiment, as in the former one, conjugation has the effect of greatly increasing the variability of the fission rate.

Mortality. Owing to the high general mortality, due to imperfect culture methods, the distribution of deaths in this experiment is of much less significance than in Experiment 1. It is summarized in table 8.

As the table shows, the death rate was greater in those that had conjugated than in those that had not, in every week save the second. In the second week I tried the experiment of adding to the cultures water from a pool that was extremely foul, but contained many *Paramecia*. It proved disastrous; many of my lines were killed, and among these were a larger proportion of the split pairs and free than of the pairs. I doubt if the distribution in such a catastrophe is of any significance; though possibly it indicates that those that have conjugated are more resistant to such decidedly injurious conditions.

Throughout the remainder of the experiment (as throughout the entire time in Experiment 1), the mortality was highest among those that had not conjugated. For the entire eight weeks together the mortality is nearly the same for all three classes, but is a little greater for those that have conjugated.

There appears to be no significant difference, as to mortality, between the split pairs and the free. There is thus no indication that prevention of a conjugation that had been initiated is in any way injurious.

Abnormalities. In this experiment, as in Experiment 1, I noted frequent abnormalities among the progeny of the conjugants; none among the other sets. No detailed study was made of these.

TABLE 8¹*Experiment 2. Death rate in pairs, split pairs and free individuals.*

	NUMBER AT BEGINNING	DIED	PER CENT DIED		NUMBER AT BEGINNING	DIED	PER CENT DIED
First week:				First two weeks			
Pairs.....	57	7	12.3	Pairs.....	57	21	36.8
Split pairs.....	39	2	5.1	Split pairs.....	39	18	46.2
Free.....	58	4	6.9	Free.....	58	28	48.3
Second week				Last six weeks.....			
Pairs.....	50	14	28.0	Pairs.....	28	19	67.9
Split pairs.....	37	16	43.2	Split pairs.....	13	7	53.8
Free.....	54	24	44.4	Free.....	23	12	52.2
Third week:				Last five weeks			
Pairs.....	28	6	21.4	Pairs.....	22	13	59.1
Split pairs.....	13	3	16.7	Split pairs.....	10	4	40.0
Free.....	23	3	13.0	Free.....	19	8	42.1
Fourth week				Total eight weeks			
Pairs.....	22	3	13.6	Pairs.....	49	40	81.6
Split pairs.....	10	1	10.00	Split pairs..	31	25	80.6
Free.....	19	2	10.5	Free.....	49	38	77.6
Fifth week:							
Pairs.....	19	5	26.3				
Split pairs.....	9	1	11.1				
Free.....	17	2	11.1				
Sixth week:							
Pairs.....	14	3	21.4				
Split pairs.....	8	0	0				
Free.....	15	1	6.7				
Seventh week:							
Pairs.....	11	0					
Split pairs.....	8	0					
Free.....	14	0					
Eighth week:							
Pairs.....	11	3	27.3				
Split pairs.....	8	2	25.0				
Free.....	14	3	21.4				

¹ Eight of the pairs, 8 of the split pairs, and 9 of the free, were accidentally lost during the experiment, so that their disappearance is not accounted for in this table.

Summary of results. Experiment 2 gives the following general results:

In three sets of individuals taken from the same culture and treated in the same way, one set that was allowed to complete conjugation, another separated before union was complete, and a third that had not yet begun conjugation:

1. Those that had completed multiplied throughout less rapidly than those that did not complete conjugation, and less rapidly than those that had not begun conjugation. This difference persisted throughout the experiment; that is, for eight weeks after conjugation had occurred.

2. The descendants of those that had completed conjugation were much more variable in their rate of fission than those that did not conjugate.

3. The mortality was slightly greater among those that had completed conjugation than among the others.

4. There was no marked difference in these respects between the set that were separated after beginning conjugation, and the set that had not yet begun.

Experiment 3: June 20 to June 24, 1909: Effect of high temperatures

This experiment lasted but four days, and was designed primarily to test the relative variability in the dimensions of the progeny of conjugants and of non-conjugants. The results on this point have been given in my paper of 1911, on Assortative mating, et cetera (table 32, p. 99). Here will be given the results of the experiment so far as they bear upon the comparative vitality and the rate of reproduction in conjugants and non-conjugants.

In the experiments which we have thus far described, the conjugants reproduced more slowly than the non-conjugants, while at the same time the mortality of the conjugants was higher. It appears possible that under some conditions the greater rapidity of fission of the non-conjugants might be disadvantageous, causing greater mortality in them than in the conjugants. This possibility is realized in the present experi-

TABLE 9

Experiment 3. Paramecium caudatum. Relative number of fissions for the conjugants and non-conjugants: during the four days, June 20 to June 24, 1909. (Including only those that lived throughout the four days.)

	NUMBER OF FISSIONS													TOTAL	MEAN
	2	3	4	5	6	7	8	9	10	11	12	13			
Number of conjugant lines	1	1	4	7	8	6	4	4	1				36	6.222	
Number of non-conjugant lines.....							1	2	3	5	3	2	16	10.813	

ment. The temperature during the four days that the experiment lasted was excessively high, the thermometer standing much of the time above 90° F. (above 32°C.). The non-conjugants multiplied with furious rapidity, at the rate of two to four fissions a day (one fission in 6 to 12 hours), so that the average for all that lived through was a little over two and a half per day (one fission in 9½ hours). The conjugants, on the other hand, multiplied much less rapidly, the rate being but one-and-a-half per day, or one fission in eighteen hours.

Correlative with this excessively rapid rate of reproduction, the non-conjugants showed a very high mortality. At the end of four days, 35 of the original 51 lines were dead, so that the mortality was 68.6 per cent. In the conjugants, on the other hand, of the original 47 lines, only 11 died, or but 23.4 per cent.

The data for the rate of fission of the conjugants and non-conjugants in this experiment are given in table 9.

The results of this experiment agree with those of all the others in showing that conjugation decreases the rate of fission. They differ from those of all others in the fact that the mortality is much greater in the non-conjugants. The result, due, as it evidently is, to the excessive rate of fission induced in the non-conjugants by the very high temperature, shows that under certain conditions conjugation may have a directly protective effect, owing to its decreasing the rate of multiplication. Interesting results would be obtained by comparing conjugants and non-conjugants of the same stock under diverse conditions; high and low temperatures, different chemical conditions, et

cetera. Possibly it would be found that under all conditions tending to cause excessive rapidity of fission, conjugation is protective by decreasing this rate.

The usual relations are found as to the relative variability of the conjugants and non-conjugants. In my paper on Assortative mating ('11, p. 99), I have shown that the progeny of the conjugants are in this experiment much more variable in size than the progeny of the non-conjugants, for at least seven generations. Here we need to consider only the variability in fission rate.

Of the non-conjugants, as we have seen, but sixteen lived through the four days. Their mean rate of fission is 10.813 ± 0.233 , the standard deviation is 1.379 ± 0.164 , and the coefficient of variation is 12.756 ± 1.546 . Of the conjugant lines, thirty-six lived through; their mean number of fissions was 6.222 ± 0.205 , the standard deviation 1.827 ± 0.145 , and the coefficient of variation 29.369 ± 2.528 . Thus the variation is both absolutely and relatively much greater in the conjugants; if we measure it by the coefficient of variation, the variability in fission rate was more than twice as great in the progeny of the conjugants as in that of the non-conjugants.

EXPERIMENTS ON PURE STRAINS: CONJUGANTS ALL DESCENDED FROM A SINGLE INDIVIDUAL

A large number of experiments, some of them extensive and long continued, were undertaken with cultures descended from a single individual. The conditions in such pure strains (or 'pure lines', as I have called them in previous papers), are of special interest in some respects, while the results bear likewise upon the same general problems as does the work with wild cultures.

Race k. These experiments were mostly carried on with the race *k*, some account of which has been given in my previous papers of 1910 and 1911. This race, belonging to the species *Paramecium aurelia*, is distinguished by a tendency to conjugate frequently, making it most favorable material for a study of

matters connected with conjugation. It is easy to induce epidemics of pairing at intervals of about a month, and they sometimes occur at much shorter intervals. In order to make clear the conditions with which we are dealing, it is necessary to give a brief account of the history of this race.

As a pure strain, the race *k* is derived from a single ex-conjugant isolated November 9, 1908. This individual itself came from a culture derived from eight pairs of conjugants taken February 4, 1908, these eight pairs being themselves derived from 10 single individuals of similar size, taken from a wild culture January 29, 1908. Thus even before the destruction of all but this single individual of November 9, the race *k* was derived from few individuals, which very possibly all came from one. Our first experiment given below (Experiment 4) made use of this race *k* before its absolutely certain derivation from a single individual; all the rest employed *k* as known to be a pure strain.

Epidemics of conjugation were observed in this race eight times between January 29 and its absolute purification on November 9. Since November 9 a great number of conjugations have been observed; records have been kept of at least twenty.

That portion of the race still in existence (July, 1912), and the part on which some of the chief experiments were performed, has descended from many successive conjugations, in which all the surviving progeny were derived from a single member of a pair. Eight such conjugations have been observed, so that all the animals now existing (and used in Experiments 13 and 14, below) are derived from eight generations of the strictest inbreeding—all the members of each of these generations being derived from the fission of a single individual. This inbred race seems healthy and vigorous, so long as cultivated in mass culture. But it appears to have lost the ability, which it had at the beginning, to propagate for any considerable period on slides. On this account it has of late become unavailable for comparative work on such questions as the rate of reproduction and the way this is affected by conjugation or by other conditions; a fact which has caused much trouble and loss of time. Many extensive experiments

have been undertaken, but after two or three weeks of intense labor, all representatives of this race *k* cultivated on the slides have become unhealthy and died. Earlier in its history it was kept on slides for months in succession, multiplying vigorously throughout. Whether its present peculiarity in this respect has any connection with the long continued inbreeding, or whether it may be due only to weakening from previous long cultivation on slides, it is difficult to say; there is some indication, as we shall see, that the latter is the case.

Experiment 4: October 19 to November 8, 1908: Paramecium aurelia

The first experiment on conjugation in race *k* was designed primarily to permit a comparison of the dimensions of the progeny of conjugants and non-conjugants in the same race. The results so far as dimensions are concerned are given in my paper of 1911 on Assortative mating (pp. 96-97). Incidentally, the records kept give data as to the relative rate of fission, and as to mortality. As we have noted above, in this experiment (alone of all those with *k*), the race is not yet known to be absolutely pure, in the sense of derived from a single individual, without admixture from others; it is, however, extremely homogeneous, and probably quite pure, even at this time.

The experiment was begun with 46 paired individuals (23 pairs), and 46 that were non-conjugants, derived from the same culture. During the course of the experiments 4 of the conjugant and 8 of the non-conjugant lines were accidentally lost, leaving 42 of the former and 38 of the latter.

This was one of the earliest experiments of the sort that I tried, and the mortality was very high, doubtless owing to inexperience in handling. Of the 42 lines derived from the conjugants, but 17 lived throughout the twenty days of the experiment, while of the non-conjugants 18 lines lived through. The total number of fissions for each of these 35 surviving lines is given in table 10.

For the 17 conjugant lines the mean number of fissions is 13.294 ± 0.670 , with a standard deviation of 4.098 ± 0.474 and

a coefficient of variation of 30.828 ± 3.890 . For the 18 non-conjugant lines the mean is 13.500 ± 0.425 ; the standard deviation 2.672 ± 0.300 , the coefficient of variation 19.792 ± 2.310 .

Thus here, as in all other cases, the rate of fission is a little greater in the non-conjugants, while the variability is much greater in the progeny of the conjugants than in that of the non-conjugants.

TABLE 10

Experiment 4. Paramecium aurelia. Number of fissions for the conjugants and non-conjugants during the twenty days of experiment 4 (October 19 to November 8, 1908).

	NUMBER OF FISSIONS																	TOTAL	MEAN
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		
Number of conjugant lines . .	1						1	1					2	4	3	2	3	17	13.294
Number of non-conjugant lines.....						1		1				1	3	8		2	2	18	13.500

Where the mortality is so high as in this case, it is doubtless due mainly to extrinsic causes, so that its distribution is of little significance. The facts are these: of 42 lines descended from conjugants, 25 died out during the twenty days of the experiment, a mortality of 59.52 per cent. Of the 38 lines descended from non-conjugants, 20 died out, a mortality of 52.63 per cent. Thus the mortality is, as usual, greatest among the descendents of the conjugants.

Experiments 5 to 14: Comparative effects of repeated conjugation, and of long abstention from conjugation

During the year 1910 a very extensive series of experiments was carried on with the pure race *k*, for testing the relative effects of conjugation and of abstention from conjugation. The whole series was so bound together that it might well be considered one prolonged experiment; it will be convenient, however, in giving an account of it, to designate as separate experiments the various phases of it.

Diagram of history of these experiments. We have already (page 302) given some account of the race *k*. To make clear the conditions in the present series of experiments, I give a diagram (fig. 1) showing the history of the various divisions of this race with which we are dealing; reference to this diagram should frequently be made in reading the text. The race *k* as dealt with in these experiments was derived from a single ex-conjugant of November 9, 1908; before these experiments were undertaken, it had passed through three self-fertilizations, or conjugations with inbreeding; that is, all the surviving members of the race were descendants by fission of a single individual of the preceding conjugation; so that the two individuals that make up a pair were thus descended from one. (In a fourth conjugation in the series, on March 9, 1909, 25 pairs were saved, so that all came from these; see diagram, fig. 1.)

After the fourth conjugation, of May 24, 1909, the culture was allowed to rest till January 29, 1910; during the interval there may have been many conjugations, in which of course the individuals would mate at random. On January 29, 1910, a pair was isolated, from a single member of which came the line of cultures which we shall call *B*; it forms the branch designated *B* in our diagram (fig. 1).

In the remainder of this culture a new conjugation occurred March 4, 1910. At this time there were isolated certain ex-conjugants, one of which gives the series forming the branch *C*, one of the three main branches in our diagram; there were also

Fig. 1. Diagram showing the nature and history of Experiments 5 to 14, and the history of the pure strain *k*, employed in this work. The rectangles (bounded by broken lines) indicate each an experiment, and show what sorts of individuals were compared, with the history of each. A united pair indicates the progeny of conjugants; a single individual, the progeny of non-conjugants. Thus, in Experiment 9, there were compared the progeny of non-conjugants (of branch *A*), and of conjugants (branch *B*) that had gone through four conjugations since the others had conjugated. The dates beside each rectangle show the length of time that this experiment lasted. The numbers in or (near) the rectangles give the number under which the experiment is described in the text. The pairs at the left show (with dates) the known self-fertilizations that the race had undergone before these experiments began, the survivors being in each case derived from a single ex-conjugant, save after the conjugation of March 9, 1909.

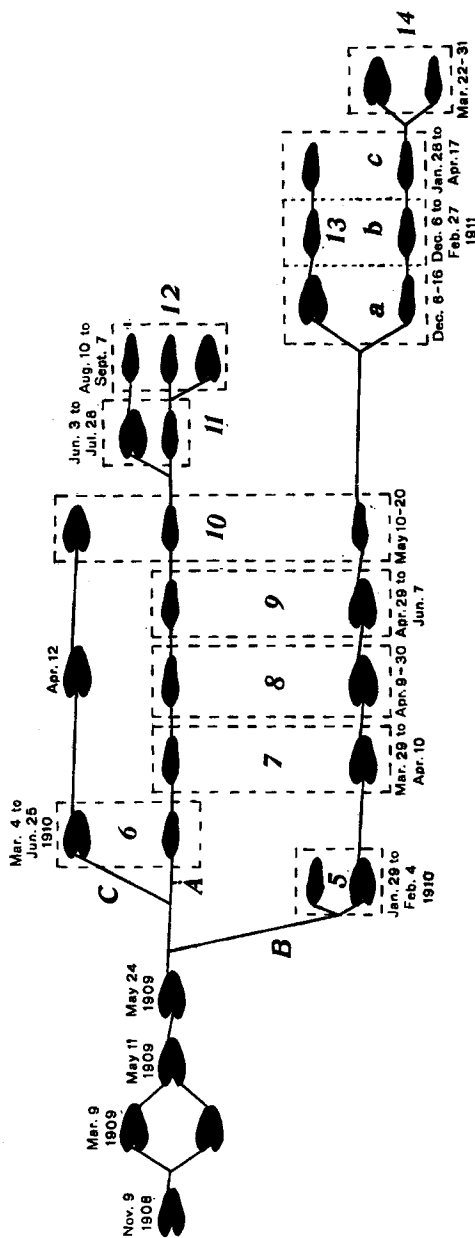


Figure 1

isolated certain split pairs, in which conjugation had not been consummated; one of these gives the long line forming the branch *A* of our diagram.

This branch *A* was then propagated without conjugation, from some period before March 4, 1910, while the other two lines *B* and *C* conjugated repeatedly; the experiments consisted mainly in comparison of the progeny from these conjugations with the progeny of the non-conjugating branch *A*. Finally, on June 4, a part of *A* was allowed to conjugate, the rest not, and these two parts compared. Again, on August 10, a part was allowed to conjugate, and compared with the part that had propagated from the beginning of the experiment without conjugation. In the following an account is given of the results of these various comparisons.

Experiment 5: January 29 to February 4, 1910: Paramecium aurelia

Experiment 5 was a brief one, dealing with 10 pairs (20 individuals) and 10 split pairs (20 individuals), belonging to the branch *B* of race *k* (fig. 1). These 40 lines were cultivated side by side under the same conditions for six days. Of the descendants of the pairs, three were accidentally lost during the course of the experiment, so that we have finally but 17 lines descended from conjugants; 20 descended from non-conjugants.

Of the 17 lines of conjugants, 8 died out during the experiments, a mortality of 47.06 per cent. Of the 20 lines of non-conjugants, 9 died out, a mortality of 45 per cent.

The number of fissions for the six days of the experiment is given for the surviving conjugant and non-conjugant lines in table 11, while the results are summarized in table 12.

Thus, as the tables show, this experiment, so far as it goes, illustrates the usual conditions:

1. Those that have conjugated multiply less rapidly than those that have not.
2. The rate of fission is much more variable among those that have conjugated—both the standard deviation and the coeffi-

TABLE 11

Experiment 5. Paramecium aurelia. Number of fissions for the descendants of pairs and split pairs, during the six days from January 29 to February 4, 1910.

	NUMBER OF FISSIONS							TOTAL	MEAN
	0	1	2	3	4	5	6		
Conjugant lines (pairs).....	1			2	2	3	1	9	3.889
Non-conjugants (split pairs)					2	3	6	11	5.364

TABLE 12

Experiment 5. Summary of results as to mortality, rate of fission and variability, in conjugant and non-conjugant lines, for the six days of the experiment.

	NUMBER	DIED	PER CENT DIED	MEAN FISSIONS	STANDARD DEVI- ATION	COEFFICIENT OF VARIATION
Pairs.....	17	8	47.06	3.889 ± 0.272	1.663 ± 0.192	42.762 ± 5.781
Split pairs.....	20	9	45.00	5.364 ± 0.116	0.771 ± 0.082	14.382 ± 1.565

cient of variation being more than twice as great as for the non-conjugants.

3. The mortality is higher among those that have conjugated.

Experiment 6: March 5 to June 25, 1910: Paramecium aurelia

From March 5 to June 25, 1910, a period of sixteen weeks, a further experiment was carried on with this pure line *k*, giving results as to the difference between those that have conjugated and those that have not. The experiment was primarily a study of the fission rate and its inheritance in different races and under different conditions, so that it included a large number of lines of propagation, of diverse character. Among these were twelve lines from the same culture, six beginning with members of pairs that had just conjugated, and six others derived from individuals that were beginning conjugation, but were separated before the process had been accomplished (split pairs). It is only with the results from these twelve sets, bearing on the effects of conjugation, that we shall deal here, reserving the remainder of the experiment for a paper dealing with the inheritance of the rate of fission.

A watch glass culture of the race *k* showed on March 4 the beginnings of conjugation, many of the individuals being observed in the act of uniting; (for the history of the race before and after this time, see the diagram, fig. 1). Three pairs were separated as they were beginning conjugation; the component individuals of these three split pairs were called 1, *a* and *b*; 2, *a* and *b*; 3, *a* and *b*. Three other pairs, designated 6, 7 and 8, were allowed to finish conjugation, then their members (*a* and *b*) were isolated and cultivated on slides side by side with the others. Thus we have six lines that have just conjugated; six others that were attempting to conjugate, but were prevented.

The experiment may be divided into three stages. The first stage is from March 6 until April 11 (37 days). (Those that had conjugated did not divide until March 6, so that the fissions of those that had not conjugated are counted, for comparison only, from that day also.)

All the six lines of those not allowed to conjugate lived and multiplied vigorously throughout this period of five weeks and two days. Of the six lines derived from those that had conjugated, on the other hand, four died out completely within two weeks, while the others multiplied more slowly than did those that had not conjugated. The detailed records for the 12 lines are given in table 13. Here the classification by weeks begins March 8, so as to omit in the weekly record the irregularities due to the first two or three days after conjugation; this gives us just five weeks.

As table 13 shows, even the two lines of conjugants that lived multiplied less rapidly than did those that had not conjugated, the weekly average for all the former being 8.8, while for the latter it is 10.2. The next stage of the experiment was of a character to determine whether this difference in rate of fission continues beyond five weeks, as well as to decide whether it might be due to accidental causes, or was a result of inherent differences. Of the non-conjugants, the pairs 1 and 3 were continued till April 12 (31 days additional), while the conjugants were represented by 7 *a* only. Several lines of each set were kept in progress. The results are given in table 14 for two periods of

two weeks each, also for the total, thirty-one days; and for the entire sixty-eight days from the beginning.

As table 14 shows, the progeny of the conjugant line 7 *a* still reproduced somewhat more slowly than did the non-conjugant lines, during these last four weeks of the nine weeks during which the experiment had lasted. The average in the conjugants is less in each of the partial periods, as well as in the period as a whole. The average rate of fission is somewhat less in all lines than during the first five weeks; this is a common result of continued cultivation on slides.

In the third portion of the experiment two of the lines of non-conjugants (1 *a* and 1 *b*) and one of the lines of conjugants (7 *a*)

TABLE 13

Experiment 6. Paramecium aurelia. Comparative number of fissions in six lines derived from conjugants and in six derived from non-conjugants (split pairs), from a watch glass culture of the race k; for five weeks and two days. The fissions are given by days for the first nine days; for the rest only by the week. The numbers give the number of divisions that occurred in the time specified. The first week is counted from March 8 to March 14. (d = died out.)

		DAILY RECORD; DAYS OF MARCH									RECORD BY WEEKS					TOTAL 37 DAYS
		6	7	8	9	10	11	12	13	14	1st	2d	3d	4th	5th	
A. Those that have conjugated:																
6 a.....			1	2	1	1	0	0	0	d	d					
6 b.....			1	1	2	0	1	0	1	d	d					
7 a.....			1	1	2	2	2	1	1	0	9	7	7	9	9	42
7 b.....	d										d					
8 a.....			1	2	0	0	d				d					
8 b.....			1	1	2	2	1	2	1	1	10	7	10	10	10	48
Mean per week, 8.80; mean for 37 days, 45.00																
B. Those that did not conjugate																
1 a.....	1	2	2	1	2	1	2	2	0	10	11	10	13	11	58	
1 b.....	1	1	2	1	2	2	1	2	1	11	6	11	12	10	52	
2 a.....	1	2	1	2	2	1	3	1	1	11	10	11	13	11	59	
2 b.....		1	1	1	2	2	1	1	1	9	9	9	13	9	50	
3 a.....	1	2	1	2	1	2	2	1	1	10	11	10	11	9	54	
3 b.....	1	1	1	2	1	2	2	1	0	9	9	9	8	10	47	

Mean per week, 10.20; mean for 37 days, 53.33.

were cultivated six weeks longer, making a total of sixteen weeks or 112 days. The comparative rates of reproduction for this period, as well as for the entire time, are shown in table 15. One point in this table requires explanation. During this period of forty-four days there were left of each set for various purposes separate lines of propagation which lasted less than the total period; for example, one line was continued seven days, another twelve, et cetera. These diverse periods have been summed for

TABLE 14

Experiment 6. Comparative rates of fission for certain lines that had conjugated March 4, and for others that were prevented from conjugating at that time. The number of fissions is given by periods of two weeks, for weeks 6 to 9; also for the last 31 days of a period of 68 days; and for the entire period.

	WEEKS 6 AND 7	WEEKS 8 AND 9	LAST 31 DAYS	TOTAL 68 DAYS
A. Conjugant				
7 a (line 1).....	13	14	30	72
(line 2).....	13	15	30	
(line 3).....	15	11		
Mean for 7a.....	13.7	13.3	30	72
B. Non-conjugant				
1 a (1).....	15	16	35	88
(2).....	15	17	36	94
(3).....	13	14	31	
(4).....	15			
Mean.....	14.5	15.7	34	91
1 b (1).....	18	15	37	88
(2).....	15	15	35	
(3).....	20			
(4).....	18			
Mean.....	17.75	15	36	88
3 a.....	17	13	36	90
3 b (1).....	12	15	31	78
(2).....	13	16	32	
(3).....		14		
Mean.....	12.5	15	31.5	78
Mean of means for all non-conjugants.....	15.94	14.68	34.38	86.75

each set, and are presented, with the total number of fissions during the periods, in the entry numbered 2.

Examination of table 15 shows that the line 7 *a*, derived from a conjugant, no longer differs in any very marked or constant way in its rate of fission from the two derived from the non-conjugants; it is certainly not slower in its rate than the others. So far as the experiment goes, it indicates that after about two months the rate of fission of the conjugants, which had been made slower by conjugation, has regained about the usual rate. Owing to the small numbers of diverse lines involved, such a conclusion is of course not very secure.

TABLE 15

Experiment 6. Paramecium aurelia. Relative numbers of fissions in certain periods, and rates of fission, in certain conjugant and non-conjugant lines, for the last 44 days of an experimental slide culture that lasted 112 days from the time of conjugation; also totals for the entire 112 days of the experiment.

	CONJUGANT LINE 7a			NON-CONJUGANT LINE 1a			NON-CONJUGANT LINE 1b		
	Days	Fissions	Daily rate	Days	Fissions	Daily rate	Days	Fissions	Daily rate
A. Changed every 48 hours during last 44 days									
1. Single consecutive line.....	44	48	1.091	44	38	.864	44	45	1.023
2. Sum of diverse periods for parts of line..	157	155	.987	141	123	.872	135	139	1.030
3. Single consecutive line from beginning.....	112	120	1.071	112	133	1.188	112	133	1.188
B. Changed every 24 hours during last 44 days:									
1. Single consecutive line.....	44	62	1.409	32	40	1.250	44	60	1.364
2. Sum of diverse periods, for parts of line.	140	176	1.257	88	115	1.307	133	176	1.323
3. Consecutive line from beginning	112	134	1.196	100	128	1.280	112	148	1.321

The experiment as a whole shows the fact that after conjugation the organisms are in a condition such that many may die, while those that have not conjugated live; and the further fact that the rate of reproduction is made slower by conjugation, remaining in this condition for about two months. This is true even when all the lines concerned belong to the same race (derived originally from the same single individual).

Experiment 7: March 29 to April 10, 1910: Paramecium aurelia

On March 29 there was a conjugation in the progeny of a single ex-conjugant of January 29; the relation of these to the remainder of the experiments will be seen from the diagram, figure 1. These animals belong to the branch *B* of the diagram. They have conjugated once, and probably twice, since those of the branch *A*, which are known not to have conjugated since some period before March 4, and to have been ready for conjugation March 4. A comparison as to rate of fission was made between these non-conjugants of branch *A* and the conjugants of March 29, branch *B*, lasting for nine days (April 1 to April 10).

Of the conjugants of branch *B*, 19 lines were in progress; their average rate of fission per line for the nine days was 1.409 per day. Of the non-conjugants (branch *A*), 21 lines were in progress; their mean rate for the nine days was 1.455 per day.

Thus the non-conjugants of branch *A* give no indication as yet of injury through having omitted conjugation. The difference in rate between conjugants and non-conjugants was slight, but in favor of the non-conjugants.

Experiment 8: April 9 to April 30, 1910: Paramecium aurelia

On April 9 there was conjugation among the progeny of one of the ex-conjugants of Experiment 7 (conjugation March 31) shown in branch *B* of figure 1. There have now been seven generations of inbreeding in this branch; and it has conjugated twice (probably three times) since those of branch *A* have conjugated at all. What difference will this make between the rate of fission in the members of the two branches?

Eight lines descended from these conjugants of April 9 (branch *B*) were kept under observation till April 30. As the first fission did not occur till April 12, this gives nineteen days during which the rate of fission was determined for these. Of the non-conjugants of March 4 (branch *A*, fig. 1), fifteen lines were in progress at this time.

The average number of fissions for the nineteen days was, in the conjugants of branch *B*, 21.375, while the average rate of fission was 1.125 per day. In the non-conjugants of branch *A*, the average number of fissions for nineteen days was 21.533, the daily rate 1.133.

There was thus no appreciable difference in rate of fission. The branch *A* shows no sign of injury as a result of having omitted several conjugations which the branch *B* has undergone.

Experiment 9: April 29 to June 7, 1910: Paramecium aurelia

On April 29 there was another conjugation in branch *B* (9, fig. 1) in the same direct line as the conjugations of Experiments 7 and 8. That is, the conjugants of our present experiment are all descended from a single ex-conjugant of Experiment 8, these from a single ex-conjugant of Experiment 7, and so on. Thus there have now been in this branch *B* three conjugations (probably four), since there has been a conjugation in branch *A*. The members of branch *A* have been cultivated on slides since March 4, while those of branch *B* have been cultivated part of the time on slides, part of the time in watch glasses.

Precise comparison of branch *A* (fig. 1) with the progeny of one of the ex-conjugants of April 29 in branch *B* was not made till about two weeks after the conjugation of the latter. During this time observation with the eye seemed to indicate that the members of *B* were a little larger than those of *A*. In view of this apparent differentiation between the two, experiments were set on foot for comparing the fission rate and the dimensions.

Fission rate. Four separate series or lines of propagation were carried on from May 22 to June 7, both for the conjugants (*B*) and the non-conjugants (*A*).

We may divide this time into two periods of eight days each. The results for the two sets are given in table 16, the four parallel lines of each set being numbered (1) to (4).

The table shows that for the total period, each of the four lines of *A* multiplied more rapidly than any of the four lines of *B*. For any of the eight-day periods, the lowest record for *A* is at least equal to the highest for *B*, save in one single case. The lines of *A* average for the entire period 20.5 per cent more fissions than those of *B*.

When we recall that *B* has conjugated recently, and three times since *A* has conjugated at all, we see that the dropping out of the conjugations has not unfavorably affected the rate of reproduction in *A*.

Dimensions. To the eye it appeared that *B* was a little larger, under the same conditions, than *A*. As these belong to the same pure strain, this is a matter of interest, as it would show that hereditary differences in size may arise within the pure strain possibly as a result of conjugation. A careful comparison of the dimensions was therefore made. Keeping all under the same cultural conditions, I first measured a number of individuals of each set at the same age, choosing the age of thirty minutes after fission. Three other measurements were taken; the results of all are given in table 17.

The fact that, as table 17 shows, *B* was larger at each of the four measurements, seems to indicate that there has indeed arisen a slight hereditary differentiation in size within the pure

TABLE 16

Experiment 9. Paramecium aurelia. Comparative number of fissions May 22 to June 7, for two sets, one of which (B) has conjugated three times in series since the other (A).

	FIRST 8 DAYS	SECOND 8 DAYS	TOTAL		FIRST 8 DAYS	SECOND 8 DAYS	TOTAL
B. Line (1)	13	8	21	A. Line (1)	15	11	26
(2)	14	5	19	(2)	15	9	24
(3)	14	7	21	(3)	14	13	27
(4)	14	8	22	(4)	11	12	23
Mean	13.75	7.0	20.75	Mean	13.75	11.25	25.00

TABLE 17

Experiment 9. Paramecium aurelia. Comparative lengths in microns of A and B. These belong to the same pure strain, but A has been cultivated for a long time without conjugation, while B has conjugated at least three times in succession since A.

	A		B	
	No.	Mean length	No.	Mean length
May 19: age, 30 minutes.....	10	116.600 \pm 0.551	11	125.454 \pm 1.063
May 22: adults, ill fed.....	46	99.000 \pm 1.195	50	123.680 \pm 0.913
May 28: adults, well fed.....	64	135.469 \pm 1.042	70	141.429 \pm 0.785
June 1: adults.....	65	131.877 \pm 0.616	41	132.237 \pm 0.747

line *k*. However, the fact that the difference was so extremely small at the last measurement taken admonishes us not to lay too much stress upon this case; the matter must be tested further.

Determination of conjugation. With *A* and *B* (fig. 1) a study was made as to the relative influence of external and internal conditions in inducing conjugation. In *B*, as we have seen, there had been at least four successive conjugations since there has been one in *A*. Will *A* be therefore readier to conjugate than *B*? If conjugation depends mainly upon an internal condition of need, then certainly we should expect this.

To test this, watch glasses of *A* and *B* were set side by side May 13, two weeks after the last conjugation of *B*, and the conditions for inducing conjugation supplied, so far as possible. On June 3 conjugation occurred in both *A* and *B*.

Thus under the proper conditions both sets conjugate at the same time, in spite of the fact that one has conjugated at least four times since the other. The experiment indicates that the recent external conditions are of more importance in determining conjugation than a progressive internal need arising through the fact that conjugation has not lately occurred.

Experiment 10: May 10 to May 20, 1910: Paramecium aurelia

On May 10 there was a conjugation in branch *C* (fig. 1, page 307), making the third in series in this division since any conjugation occurred in *A*. Four pairs were isolated from this new conjugation in branch *C*, and experiments were set on foot for

comparing these as to vitality, reproductive power, et cetera, with *A* (which had not conjugated for some months), also with those of branch *B* (which had conjugated two weeks before).

The four ex-conjugants of two pairs of *C* were placed on slides and treated like the non-conjugants of branch *A*. Two of these ex-conjugants divided once, two did not divide at all, and all died after three to ten days. Meanwhile, the members of branch *A* multiplied actively, at about the rate of once per day.

Two other pairs from branch *C* were allowed to multiply in a watch glass. This they did very slowly, so that on May 13 but 10 individuals had been produced from the four. These were then carefully brought into identical conditions with an equal number of specimens of *A*, and of *B*, the three being placed side by side in watch glasses.

On May 16, all the specimens of *C* were dead, while the members of *A* and *B* were flourishing.

Thus in this case, the recent conjugants (*C*) multiplied very slowly or not at all, and soon died; while others that had not conjugated so recently nor so often (*A* and *B*) flourished.

Experiment 11: June 3 to July 28, 1910: Paramecium aurelia

Comparison of conjugants and non-conjugants of the branch *A* (fig. 1, page 307).

On June 3 there was conjugation in a watch glass (taken from the slides May 15) of members of the branch *A* (fig. 1), derived from a split pair of March 4. Other divisions of this same stock (branches *B* and *C*, fig. 1) had conjugated four times in succession since any conjugation in *A*. Thus we have in *A* a set that has gone long past the normal conjugation period. Part of it now (June 3) conjugates, while the remainder (part in the watch glass, part on slides) does not. Thus we have an opportunity to test the effects of conjugation on the vitality and reproductive power of a stock that has long gone without it. Experiments for this purpose were conducted on slide cultures, and also in watch glass cultures.

To make clear the conditions in these experiments (which when described in words alone are a little confusing), I give in

figure 2, a diagram, which shows the relations of the various parts of the experiment. If the reader will make frequent references to this, he will have no difficulty in following the account of the experiments, and appreciating their bearings.

We have, derived from the branch *A* of figure 1, at first two divisions, designated *D* and *E* in figure 2; *D* has been cultivated continuously on slides, since March 4, while *E* was transferred on May 15 from the slides to a watch glass.

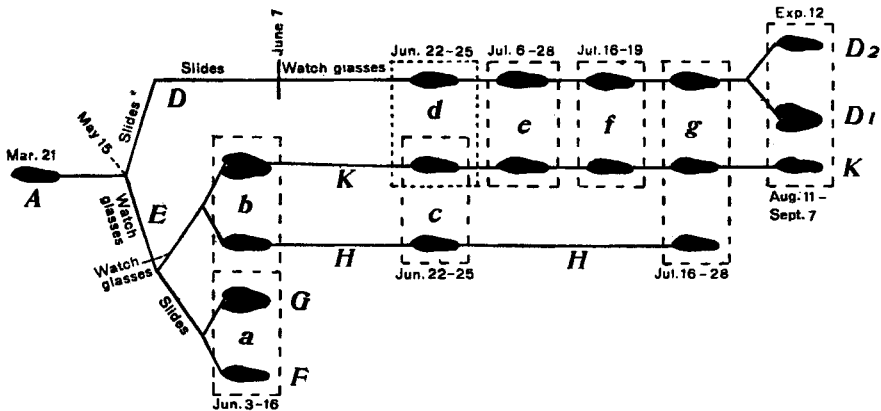


Fig. 2. Diagram showing the history and the nature of the comparisons made in the various divisions of Experiment 11, and in Experiment 12. Each rectangle represents one of the parts of Experiment 11, save the one to the right, which represents Experiment 12. The lower case letters *a* to *g*, within the rectangles, show the letters under which the various parts of Experiment 11 are described in the text; thus the one marked *e* shows Experiment 11 *e*. The capital letters *A* to *K* show the diverse branches of the race *k*, described in the text. The duration of the experiments is given by the dates below or above each rectangle. The legends at the branches *D*, *E*, etc., show the manner of cultivation; the branches *D* and *E* being separated on May 15, the former placed on slides, the latter in watch-glasses, etc.

Now, on June 3, this branch *E* is divided into four parts, which we may call *F*, *G*, *H* and *K*. The first two are kept on slides; the second two in watch glasses. The lots *F* and *H* are non-conjugants, while *G* and *K* are conjugating pairs kept under the same conditions. Comparison of *F* and *G* we may call Experiment 11 *a*; comparison of *H* and *K*, Experiment 11 *b*.

Experiment 11 a: cultivation on slides. From the watch glass (fig. 2, *E*), thirty-two ex-conjugants, from 16 pairs (*G*, fig. 2) and thirty-two non-conjugants (*F*, fig. 2) were isolated. These 64 lines were cultivated side by side, on slides, under identical conditions.

In both sets the mortality was high (as I have invariably found to be the case in attempting to cultivate *Paramecium aurelia* in hot summer weather). By June 13, 25 of the 32 lines of ex-conjugants (*G*) had died. By June 16 all the ex-conjugant lines were dead, while nine of the non-conjugant lines (*F*) were still alive.

In the ex-conjugants the average number of fissions, for those that lived to June 13, was 5.445, while in the non-conjugants, for the same period, it was 7.857. The average rate of fission for all the ex-conjugants (reckoning the rate for each one as long as it lived), was 0.5485 per day, while for the non-conjugants, reckoned the same way, the average rate was 0.7365 per day; so that the rate for the non-conjugants was 34.27 per cent greater than for the ex-conjugants.

Experiment 11 b: cultivation in watch glasses. Parallel to the slide cultures of 11 *a*, two watch glass cultures, one of conjugants (fig. 2, *K*), one of non-conjugants (*H*), were propagated. One of these contained at the beginning 30 ex-conjugants, the other 30 non-conjugants. On each of the following days the animals were removed one by one to a new watch glass, counted, and the number reduced so as to be the same for each. The ratio of the number present on each day to the number present the day before was thus obtained, this may be called the multiplication ratio. It was as follows for eight successive days, beginning June 5:

DAY	1	2	3	4	5	6	7	8	MEAN
Progeny of conjugants (<i>K</i>).....	1.67	1.84	2.17	1.10	1.11	1.26	1.47	1.51	1.52
Progeny of non-conjugants (<i>H</i>).....	2.80	2.08	1.92	1.52	1.80	1.80	1.74	1.56	1.90

Thus the rate for the progeny of the non-conjugants was greater every day except one; and the mean rate for the non-conjugants was almost exactly 20 per cent greater than for the progeny of the conjugants.

It is clear therefore that the progeny of the non-conjugants have a great advantage, both as to rate of reproduction and as to mortality.

Experiment 11 c. The two sets in the watch glasses (fig. 2, *H* and *K*) were allowed to multiply till June 22, then 10 individuals were removed from each, isolated on slides, and their rates of fission followed individually and compared. All multiplied vigorously, in two days 8 of the conjugant progeny had divided five times; 1 three times, 1 six times. Of the non-conjugant progeny, 5 had divided six times, 2 five times, 2 three times, while 1 died. Thus the two are now nearly equally vigorous, the rate of fission being still a trifle higher for the non-conjugants.

Experiment 11 d. The non-conjugants dealt with thus far (Experiments 11, *a* to *c*) had come originally from the same watch glass as did the conjugants (that is, from branch *E*, fig. 2). But there was under propagation at the same time, another set of non-conjugants (branch *D*, fig. 2), cultivated on slides since March 4, while those just described (*F* to *K*, fig. 2) had been cultivated in watch glasses (hence with more fluid) since May 15. On June 7 a watch glass culture of this slide series (*D*, fig. 2) was made, and left uniform with a watch glass culture (branch *K*, fig. 2) derived from the conjugants of June 3. On June 22, 10 individuals were taken from each of these two watch glasses (*D* and *K*, fig. 2), and cultivated on slides, in order to compare their fission rates and general vigor. The results were strikingly different from those thus far obtained; on account of their great interest I give them in detail.

Table 18 shows that, contrary to all our previous results, the progeny of the conjugants *K* are much more vigorous than those of the non-conjugants *D*.

Now, in the experiment which just preceded this (11 *c*), we saw that in a test made on the same date as the present one, and with conditions identical, the non-conjugants (*H*) with the same history

TABLE 18

Experiment 11 d. Paramecium aurelia. Number of fissions for three successive days, in ten non-conjugants from the slide series D (of fig. 2), and in ten progeny of the conjugants (K, fig. 2), that had lived in watch-glasses since May 15 (d = dead).

LINE	NON-CONJUGANTS (D)				CONJUGANTS (K)			
	June			Total	June			Total
	23	24	25		23	24	25	
1.....	1	1	d	(2)	3	2	2	7
2.....	1	0	d	(1)	d			(0)
3.....	1	d		(1)	3	2	1	6
4.....	d			(0)	3	2	1	6
5.....	0	d		(0)	3	2	1	6
6.....	2	0	0	2	3	3	1	7
7.....	2	0	0	2	3	3	1	7
8.....	d			(0)	3	2	2	7
9.....	0	d		(0)	3	2	2	7
10.....	2	1	0	3	2	3	3	8

as the conjugants (*K*) (cultivation in watch glasses since May 15) were not less vigorous than the progeny of the conjugants, but at least equally vigorous with them. The only difference between the non-conjugants of this present experiment (*D*) and those of the previous one (*H*) is that those in the present experiment were cultivated about a month longer on slides (May 15 to June 7). Apparently this is the cause of the weakness of these animals.

But it is clear that the progeny of the non-conjugants of this slide series *D* are now in a weakened, depressed condition, while both conjugants and non-conjugants from watch glass cultures are vigorous. This gives us an opportunity to determine the effects of conjugation in such a depressed culture. Before undertaking this, two additional tests were made to see if the depressed condition of the progeny from the slide series *D* was beyond doubt.

Experiment 11 e. The first of these consisted again of ten lines of the non-conjugant slide series *D*, ten from the conjugants (watch glass series, *K*). The experiment continued from July 6 to July 28.

In this experiment, as soon as any line of either set died out, it was replaced from some other line of the same set. The number

of such necessary replacements will give a comparative measure of the mortality in the two sets. In the non-conjugants *D* (slide series) there were necessary 34 such replacements; in the conjugant progeny *K* (watch glass series), there were 22. Of the conjugant line (*K*) six lived to the end (21 days), the average number of fissions for these being 21. Of the non-conjugant lines (*D*), four were alive at the end, their average number of fissions being 16.25. On the sixteenth of July ten lines were alive in each set; the average number of fissions at that time was for the conjugants (*K*) 15.6; for the non-conjugants (*D*), 13.00.

Experiment 11 f. On July 16, an additional comparison was made, taking fourteen each of the conjugants (*K*) and non-conjugants (*D*). In the nature of the results this experiment lasted but a short time. Of the non-conjugants *D* (slide series), only two divided, and all were dead by the third day. Among the conjugants (*K*), all divided; three died out during the three days; the remaining 10 averaged six fissions in three days. The non-conjugants (*D*) are clearly depressed and weak.

Experiment 11 g. A crucial question is whether the two sets of non-conjugants of unlike history—the slide series *D* and the watch glass series *H*—are still unlike in their vigor, as comparison with the conjugants (Experiments 11 *a-f*) indicates. Therefore, on July 16 comparative tests were made of these two, and also of the progeny of the conjugants of June 3 (*K*).

The three lines compared are those designated *D*, *K*, and *H*, figure 2. Of line *D* (non-conjugants cultivated on slides up to June 7) two watch glasses, containing five specimens each, were taken. Of the other non-conjugant line *H* (cultivated in watch glasses since May 15), the same number was propagated, in the same way. Of the ex-conjugants of June 3—the line *K*, with the same cultural history as the non-conjugants of line *H*—three watch glasses of five specimens each were propagated. In all three series the conditions were made exactly alike, all the animals being washed in the same water before they were introduced into the culture fluid. At intervals of some days the animals were removed one by one to a new watch glass, counted, and the number reduced, so as to be the same in all. The ratio of those

present to those that had been introduced was taken at each; this gives the ratio of multiplication for the two days. These ratios are given in table 19.

From the results given in table 19 the following are clear: (1) the non-conjugants, *D*, cultivated on slides till June 7, are still much depressed, and much less vigorous than either the non-conjugants (*H*) which came from the watch glass with the conjugants, or than the conjugants themselves (*K*); (2) the non-conjugants (*H*) which have the same cultural history as the conjugants (*K*) are still somewhat superior in vigor to the conjugants. In every one of the four periods their ratio of multiplication is greater.

The results of this experiment are then throughout consonant with those of Experiments 11 *a*, 11 *b* and 11 *c*. From all, the

TABLE 19

Experiment 11 g. Paramecium aurelia. Ratio of multiplication for a number of periods of time, in watch glass cultures of the three sets D, H and K, described in the text.

	JULY 16-18	JULY 18-20	JULY 20-24	JULY 24-28
(D) Non-conjugants, cultivated on slides till June 7.				
Culture 1.....	1.8	1.778	7.3	2.5
Culture 2.....	1.	1.	<i>d</i>	<i>d</i>
Mean.....	1.4	1.389	3.65	1.25
(H) Non-conjugants from same watch-glass as the conjugants of K, set May 15.				
Culture 1.....	2.8	2.857	6.5	6.2
Culture 2.....	4.02	2.476	5.5	10.0
Mean.....	3.41	2.667	6.00	8.1
(K) Conjugants of June 3, from same watch-glass as the non-conjugants of H.				
Culture 1.....	3.00	1.533	3.9	6.6
Culture 2.....	2.00	2.110	6.2	3.7
Culture 3.....	3.00	1.867	5.9	6.4
Mean.....	2.667	1.833	5.333	5.567

conclusion is evident that the reason why the conjugants (*K*) are more vigorous than the non-conjugants (*D*) of the slide series, is because the latter were cultivated for a month longer on slides. For the non-conjugants (*H*), cultivated throughout in the same way as the conjugants, are still more vigorous than the latter.

To this the only objections that could be raised in favor of the view that conjugation has caused rejuvenescence would be as follows: (1) It might be held that it is not possible to be certain, in taking free specimens from a watch glass containing conjugants, that one is not taking specimens that have already conjugated. I believe that there is no ground for this objection in the present case, since the cultures in question were watched with the utmost care in order to detect conjugation at its beginning. Further, Experiments 11 *a* and 11 *b* show that there was an actual difference between conjugants and non-conjugants, of the same sort as that found in all our other experiments, the non-conjugants being the more vigorous. It is clear therefore that in this case we were actually dealing with non-conjugants. (2) It might be said that it would be difficult to be certain that in the period that had elapsed since June 3 there had not been conjugation in the vessels of 'non-conjugants.' It may be admitted that this difficulty exists, though in this case the organisms were inspected daily, and I believe that no conjugations occurred. But this objection is in fact negated by the experimental results themselves. The greater vigor of the non-conjugants seen in Experiment 11 *g*, of July 16, is of just the same character as that seen immediately after conjugation (Experiments 11 *a* and 11 *b*) and as that characteristic of the non-conjugants in all our other experiments, to which the present objection is not applicable.

But is the explanation given above—the fact that the non-conjugants of one set (*D*) were cultivated a month longer than the other (*H*) on slides—a credible one? I believe that everyone who has had extensive experience with experiments of this sort will agree with me that long continued cultivation on slides does produce a depressed condition. There are some stocks that will not stand it at all, though they live perfectly in mass culture. And in fact, my experimental records show that in this partic-

ular stock of *A* (fig. 1) those cultivated on slides became very unhealthy in June, so that all died out June 18; the branch was preserved only because some of them had thus been removed to watch glasses on June 7, forming our present stock *D* (fig. 2).

Thus all the evidence, from many distinct sources, points to the explanation we have set forth above, that the non-conjugants of the slide series *D* are less vigorous than either the non-conjugants (*H*) or the conjugants (*K*) of the watch glass series, because they were cultivated longer on slides.

Experiment 12: Conjugation in a depressed stock: August 10 to September 7: Paramecium aurelia

Whatever the cause, we now have on hand, after the Experiments 11 *a* to 11 *g*, a much depressed stock, which has omitted at least four normal conjugations. Now, it might be maintained that our uniformly negative results thus far as to rejuvenescence by conjugation are due to the fact that we were not dealing with depressed stocks. It is of interest, therefore, to determine the effects of conjugation within a stock thus known to be depressed. There is, however, great difficulty in carrying out such an experiment, for such depressed stocks cannot easily be induced to conjugate. The main condition for conjugation appears to be, that there shall be a period of rapid multiplication, followed by a decline in the conditions inducing it. But in such depressed stocks it is almost impossible to induce rapid multiplication. After many efforts, I finally succeeded on August 10 in getting a scanty conjugation in a watch glass culture of this depressed set of the slide series of *D* (fig. 2) which has descended without previous conjugation from the split pair of March 4. I was able to obtain for study but three pairs, the ex-conjugants of course forming when isolated six lines of propagation. I call these sets *D* 1.

From the same watch glass culture I isolated at the same time ten of the individuals that were not conjugating, and from these, ten lines of propagation were derived, which were kept under the same conditions as those from the conjugants. These may be called *D* 2.

For further comparison, I placed beside these, ten lines derived from a culture of this same series that had conjugated June 3, and had lived in small mass cultures since. These belong to the line *K*, figure 2.

As this experiment gives the only results obtained that could be interpreted as showing a favorable influence of conjugation on survival and reproduction, it appears best to give in some detail the records for the lines of propagation. This is done in table 32, in the Appendix.

To understand table 32, the following must be considered: Each line of any of the three sets was started with a single individual August 11. The animals were changed to new fluid every day or every other day (all being alike), and all those that had been produced were retained, save on certain days, when the number was reduced, by removal of certain of the animals. To give the essential facts in the history of the cultures, it is therefore necessary only to give the number of individuals on these dates, before and after reduction. This is what is done in table 32. Thus, in line 1 of set *K*, the single individual of August 10 had on August 14 produced 6, of which 4 were removed, leaving 2. On August 16, these 4 had produced 8, of which all but 2 were removed, *et cetera*.

The last column of table 32 gives the total number of fissions undergone by the line in question, up to September 7, or to its death. As the lines of set *K* were all obviously vigorous, only five were kept under observation till September 7, when the last line of set *D* 2 died out.

To grasp the results, it will be best to examine first the facts for set *K*, which had lived in mass cultures since May 15, and had conjugated June 3. In this case, as will be observed, all the ten lines flourished well. Number 4 was lost by accident August 28, and numbers 3, 5, 7 and 9 were discontinued September 3, because the results were clear.

Now, compare with these the results given for the conjugants and non-conjugants of August 10 (the depressed race) in sets *D* 1 and *D* 2. It is clear from the data of set *D* 1 that the conjugation of August 10 has by no means restored this depressed series to the

level shown by the other set *K* of table 32. In spite of the utmost care, and the division of the lines as soon as possible, so as to have more than one from each of the ex-conjugants, the progeny of three of them had died out within eight days, and a fourth died out later. Only two of them survived till September 7, when this experiment was discontinued; and the nine lines derived from these two were then multiplying much less rapidly than those derived from set *K* (table 32). It should be stated further that two tests made respectively three and four months later (one December 1, 1910, the other January 7, 1911), showed that the members of set *K* (conjugants of June 3) were still far more vigorous than conjugants of August 10 (set *D* 1). In the test comparison of December 1, all the twelve lines of set *D* 1 (conjugants of August 10) died out after a week of cultivation on slides, while those of set *K* flourished.

When however we compare the records for the conjugants of August 12 (set *D* 1) with those for the non-conjugants of the same culture (set *D* 2) in table 32, we find that the conjugants have a decided advantage. The non-conjugants ceased multiplication almost entirely, after the first week, and gradually died out, the last one dying on September 7. At this time the descendants of two of the ex-conjugants were multiplying well, so that an indefinitely large number of progeny were later produced from them.

Thus in this case two of the six conjugants were more vigorous than any of the non-conjugants of the same stock and cultural history. Most of the conjugants died out, but in the natural course of events the entire set would have been replaced by the progeny of the few more vigorous lines.

This is the only case, out of a very large number of experiments, that gives any indication of a beneficial effect of conjugation on vigor and survival. Just what has happened here? First, attention should be called to the fact, already set forth, that conjugation in this depressed stock was very scanty; in connection with the further fact that the condition for producing conjugation is a period of rapid multiplication, followed by a check. Now, from this and from the data of tables 18 and 19, it is evident that there

were few in this depressed race that could be induced to multiply sufficiently to furnish the conditions required for conjugation. Those that did conjugate evidently represent then *those members of the stock that are most vigorous and active in multiplication*. Their later vigor and survival, as compared with the non-conjugants, may therefore have been due to this, and not to the conjugation; in other words, conjugation may have been the effect, not the cause, of their greater vigor. If the same individuals that conjugated could have been cultivated without conjugation, it is probable that they would have multiplied equally well or better.

However this may be, it is clear that conjugation did not cause rejuvenescence in any simple direct way, since the majority of the conjugants died out, and those that survived were weak. But in one respect this experiment gives the same results as all others. Conjugation resulted in an increase of variability, as regards vigor and rate of reproduction. Among the extreme variates were some whose vigor was sufficient to keep them alive, while among the more uniform non-conjugants all died. The advantage of the conjugants, so far as it did not exist before conjugation, is then in this case due to the effect of conjugation in increasing variation.

Experiment 13: Production of inherited differentiation by conjugation: December 6, 1910, to May 15, 1911:

Paramecium aurelia

A very extensive and long-continued series of experiments was carried on in the winter and spring of 1910-1911, with the same pure strain *k*, of *Paramecium aurelia*, that was used in the experiments just described (Experiments 6 to 12). The main purposes of this new set were, to determine whether as a result of conjugation differentiations may arise within a pure strain, and to bring out the rules of inheritance within the pure strain. Most unfortunately, in the later and most critical part of the experiment the conditions became such that multiplication almost ceased, and this made futile a large part of the work, particularly that designed to discover the rules of inheritance. Whether this

cessation of reproduction was due to something in the cultural conditions, or to weakening of the stock as a result of long continued culture on slides is perhaps not absolutely clear, though the evidence is strong that the latter alternative is the correct one. But in spite of this, the experiment gave definite results on some important questions. I shall give the experimental data only so far as they throw light on definite problems.

From many other experiments the general impression had been obtained that conjugation produces inherited differentiation even within the pure strain. By 'pure strain' is meant here simply a series of animals all derived from one single individual. Experiments set forth in previous papers indicate that no inherited differentiation within such a pure strain arises, as a rule, during multiplication by fission; and this agrees essentially with most other work on inheritance in vegetative reproduction. The evidence, so far as *Paramecium* goes, was based mainly on studies of the inheritance of size. If, as these indicate, heritable differentiations do not arise in fission, then the question comes up as to how the existing differentiations into diverse races do arise. The indications just mentioned, that conjugation produces such differentiation, then of course call for investigation; this was attempted in the present series of experiments.

If inherited differentiation does result from conjugation, this might be held to be due to Mendelian inheritance, or something similar. If the individual with which the pure strain began was a heterozygote, and its progeny through fission were identical heterozygotes, then of course when these interconjugated, new combinations of various sorts might be produced, exactly as differentiations may arise by self-fertilization of heterozygotes in plants.

Eight self-fertilizations. To avoid, so far as possible, the heterozygotic condition, I used the race *k*, of *Paramecium aurelia*, already described in connection with Experiments 5 to 11 (see diagram of its history, fig. 1, page 000). At the time when the present series of experiments (13 and 14) begins, self-fertilization had occurred in this race eight times in series. That is, the progenitor of the race was a single individual; its progeny con-

jugated among themselves; from these conjugants a single ex-conjugant was taken and allowed to multiply till there was conjugation among these. A single member of a pair was again allowed to propagate till there was conjugation; and thus the process was repeated eight times, all the members of each of the eight non-sexual series being the progeny of a single ex-conjugant of the previous series. The known history of this race is illustrated in the diagram of figure 1. This diagram shows also the relation of the organisms employed in the present experiment to those used in previous experiment. They belong to branch *B* of figure 1, and are derived from a single ex-conjugant of the conjugation of April 29; they are thus the same stock as the conjugants employed in Experiment 9.

Self-fertilization for eight generations in succession, of course goes far in getting rid of heterozygotism in most characters. East and Hayes ('12) have given the general formula for determining what proportion of the organisms would be homozygotic with respect to any given number of characters after a given number of self-fertilizations; this being based on the formula originally given by Mendel ('66). In a recent note, written before the paper of East and Hayes had appeared, I went into some details on the matter (Jennings '12). If we call x the proportion of the organisms that will be homozygotic, letting n be the number of successive self-fertilizations and m the number of pairs of characters, then the formula for use is

$$x = \left(\frac{2^n - 1}{2^n} \right)^m$$

From this formula we find that after eight successive self-fertilizations the proportion of the organisms that would be homozygotic for any one, two, or more characters, up to ten, is as follows:

NUMBER OF CHARACTERS	PROPORTION HOMOZYGOTIC	NUMBER OF CHARACTERS	PROPORTION HOMOZYGOTIC
1.....	0.99609	6.....	0.97679
2.....	0.99220	7.....	0.97297
3.....	0.98833	8.....	0.96917
4.....	0.98447	9.....	0.96539
5.....	0.98062	10.....	0.96162

Thus, after eight self-fertilizations, more than 96 per cent of the organisms would be homozygotic with respect to all ten characters.

Of course we do not know on how many independently heritable characters depends the rate of fission (which was the characteristic chiefly examined). If it depends on not more than 10 such characters, the chances are thus at least 26 to one that we are dealing with a pure homozygotic organism, when we select a single individual after the eighth successive self-fertilization of the line.

The above analysis is based on the view that there is no separation of the zygotic constituents in the reproduction by fission, this being indicated by the evidence thus far brought forward. If it were not true, then we would expect the organisms constituting a pure strain (descended by fission from a single individual) to become more and more diverse as fission was repeated, for as any individual became homozygotic with respect to any character it could produce forever after only progeny that were homozygotic in that respect. The result would be in the course of 20 or 30 generations to produce a set of individuals, each of which was homozygotic with respect to all the characters it bore, though the different ones would have diverse homozygotic characters. Selection among such individuals would then give rise readily to diverse races; this is opposed to the evidence hitherto obtained.

The eighth of the conjugations in succession took place April 29, 1910. A single ex-conjugant gave rise to a culture, which propagated without admixture, till this experiment was begun, December 6, 1910. On the evening of December 5 a watch glass of the animals was taken from the large culture; on the following morning those in this watch glass were conjugating, while those that remained in the large culture dish were not.

Experiment 13 a. Fifty-two pairs were taken from the watch glass, 100 non-conjugants from the culture dish; all these were isolated on slides, in the way already described. Thirteen of the pairs were later lost by an accident. This left 78 lines derived from animals that had conjugated, 100 from animals that had

not conjugated. Of the conjugants 20 died during the first week; of the non-conjugants 18, leaving 58 and 82 respectively in the two groups.

RATE OF FISSION. The ex-conjugants, as usual, began dividing the second day after conjugation. Beginning for both sets at this time, daily records were kept of the number of fissions in each line. Table 20 gives the fissions for the first week, in each of the two sets. As in all our other experiments, the rate of fission was somewhat greater in those that have not conjugated.

TABLE 20

Experiment 13 a. Paramecium aurelia. Comparative number of fissions in conjugants and non-conjugants of the same culture, for a period of one week, beginning two days after the separation of the pairs.

	NUMBER OF FISSIONS											TOTAL	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
	0	1	2	3	4	5	6	7	8	9	10				
Conjugant lines	4		1	2	8	4	6	12	14	5	2	58	6.155 \pm 0.220	2.484 \pm 0.106	40.350 \pm 2.910
Non-conjugant lines					2	5	16	33	15	8	3	82	7.098 \pm 0.093	1.246 \pm 0.066	17.550 \pm 0.953

VARIATION. As in other cases, the variation in the rate of fission is much greater among the descendants of the conjugants than among those of the non-conjugants. The standard deviation is twice as great, and the coefficient of variation two-and-a-half times as great, in the descendants of the conjugants (table 20).

MORTALITY. Of the 78 conjugant lines, 20 died out during the first week, or 25.6 per cent. Of the 100 non-conjugant lines, 18 died out, a mortality of 18 per cent.

Experiment 13 b: inherited differentiations in the pure strain. At the end of this first week, those lines of each set that showed indications of differentiation in rate of fission were selected for farther propagation. That is, from both the conjugant set and the non-conjugant set the extreme lines were taken; also certain of the intermediate ones. Thus, from the conjugant lines there were selected the four that had not divided at all (table 20), the

one that had divided but twice; two whose record had stood at 3 fissions, six at 4, two at 5; then two at 8, two at 9, and the two at 10. In the non-conjugants, the two with a record of 4 were taken, three at 5, three at 6, four at 7, two at 9, and two at 10. In all, 21 of the conjugant lines and 16 of the non-conjugant lines were thus continued. Two of the former (15 and 16) were, however, derived originally from one ex-conjugant.

The purpose of continuing these 37 lines was to determine whether the varying rates of fission are inherited; this would show that inherited differentiation had arisen within the pure line, in this respect at least.

All but one of those conjugant lines which had not divided during the first week died out during the second week. Sixteen of the conjugant lines and fourteen of the non-conjugant ones were cultivated under identical conditions from December 6 to February 27, a period of ten weeks; a few of these lines died out, however, before the end of the period. It will be well to divide the period into five homogeneous divisions of about two weeks each, giving the fission rates for each line in each of these divisions. During the first four periods the organisms were changed daily; during the last one, every other day, a regimen under which they did not thrive. On this account, the first four periods are more characteristic and significant than the last. The fissions for these five periods are given in table 33 (Appendix). I have arranged them in the order of their relative rates of fission, as determined by comparing the total numbers of fissions in the first three periods (given in the last column).

Examination of table 33 shows clearly that in some cases at least the different rates of fission are inherited. Compare for example among the conjugants, line 4 and line 15 (or 16). In every one of the five periods, line 4 shows a higher rate of fission than does line 15. The same thing appears in other lines, of which details will be taken up later.

These constant differences appear in spite of the fact that all of the lines were treated in exactly the same way throughout the ten weeks' experiment. All were kept together, in the same moist chambers, and in the same culture fluid. In order that the drop belonging to one line should not have a continuously

different bacterial content from that of another, the animals of different lines were frequently interchanged; line 1 being transferred into a drop in which line 2 has been living, and vice versa. The drops were for the same purpose frequently intermixed.

If two such lines as No. 4 and No. 16 (of the conjugants) showed in the long run about the same rate of fission, but with accidental fluctuations from period to period, then of course in some periods No. 4 would show a greater number than No. 16, while sometimes the reverse would occur. When we find however that such a line as No. 4 has uniformly a greater number of fissions than another, and this continues for so long a time as ten weeks, with no external differences to cause these results, we must conclude that the lines themselves are differentiated.

We may make then as the test of inherited differentiation the condition that one line shall show in every one of the five periods of table 33 a distinctly higher fission rate than another. This is an extremely severe test, and one that is beyond question more than sufficient to show actual inherited differentiation. In the slow process of experimentation these repeated differences are most striking and surprising. Our first period covers eighteen days; during this time one finds that conjugant No. 1 divides more rapidly than No. 8 or No. 16. To test the latter, the three are kept under identical conditions for twelve days longer (second period). Again No. 1 shows the highest rate, No. 3 a lower one, No. 16 a still lower one. To make assurance doubly sure, we keep them fourteen days more (third period); again they show the same relative rates. We keep them a fourth period of twelve days; a fifth one of fourteen days; these confirm the differences shown in the first three periods. There can be no question but that the cause of the diversities is in the lines themselves; in other words there is differentiation inherited from generation to generation.

On this basis it is clear that among the conjugants, Nos. 1, 8 and 16 represent three lines with inherited differentiation in rate of fission. It is hardly doubtful but that other differentiated lines exist, accidental fluctuations bringing these equal to one

of the above three at one of the five periods. But we may hold rigidly to our test and still demonstrate the existence among the conjugants of the three diverse lines 1, 8 and 16. As will be observed, the fission rate is on the average more than twice as great in No. 1 as in No. 16.

Among the non-conjugants also there are inherited differentiations. In every period but one, non-conjugant line 1 has twice as high a fission rate as line 14. On the basis of our severest test, it is clear that lines 1, 12 and 14 are diverse in their inherited rate of fission.

It is clear therefore that heritable differentiations do arise within the pure line, so far as the rate of fission is concerned. How are these differentiations brought about?

At this point a weak spot in the plan of the present experiment appears. All our experiments show that conjugation increases the variability in the rate of fission; this is true both in wild cultures and in pure lines, and holds for the present experiment, as table 20 shows. It would appear probable therefore that some of these variations are inherited, and that this is precisely what the results given in table 33 demonstrate. But we find inherited differentiations also, as we have seen, among what we have called the non-conjugant lines of the present experiment. The weak point mentioned relates to the applicability of the term 'non-conjugant' to these lines. As already set forth, the last previous recorded conjugation took place for this line *k* on April 29. The present experiment began December 6. Now, it is almost certain that in the intervening time the animals had conjugated one or more times, since this race *k* conjugates once in one or two months, when conditions are favorable. Therefore, if conjugation produces differentiations, my 'non-conjugants' of the present experiment have had much opportunity to become differentiated in that manner; they are not properly 'non-conjugants' for present purposes. That they have become in some way differentiated is clearly shown by comparison of No. 1 with No. 14 in the non-conjugants of table 33.

This, of course, does not vitiate our main result, that inherited differentiation does arise within a pure line, and it leaves it

probable, or perhaps certain, that such differentiation arises in consequence of conjugation. But it leaves unsettled the question whether such inherited differentiations may not arise also in other ways.

To give clear results on this point, the experiment should have been performed as follows: A single individual of *k* should have been isolated, allowed to multiply by fission; watched continuously till the first conjugation occurred, then the experiment should have been performed with these conjugants and non-conjugants. If inherited differentiation appeared among the non-conjugants in such a case it could not be held to be due to conjugation.

These conditions are fulfilled in Experiment 15 on another race, to be described. They were likewise fulfilled in the latter part of the present experiment, and repeatedly in experiments on race *k* in 1912. But unfortunately race *k* has lost its power to flourish in slide cultures; in every case with the later experiments on this race all the lines have died out after a few weeks of culture. It would be of interest to carry out the experiments with race *k*, in view of its history of eight repeated self-fertilizations, and efforts will be made to find a successful method of slide culture for it. In the meantime the results of Experiment 15, with race *E*, give clear results on the main questions at issue.

The results of the present experiment therefore leave open the possibility that heritable differentiations may arise in other ways than by conjugation. Do they furnish positive evidence that heritable differentiation actually does arise as a result of conjugation? As we have seen, all our many experiments show that conjugation increases the variation in rate of fission between the lines. This is true (as already set forth) for the first week of the present experiment. Furthermore, if we compare the variability of the conjugant and non-conjugant lines of table 33, we find again that the conjugants are much more variable. We are of course not here dealing with random samples, but since both sets were selected to give as much variation as possible, a comparison of the variations may be of significance. The means, standard deviations and coefficients of variation for various periods are given

for the conjugant and non-conjugant lines, in table 21. The constants are given, not only for each of the five periods of table 33, but also for certain of these periods taken together; likewise for the first week (column 1).

Table 21 shows that: (1) in every case the mean rate is higher in the non-conjugants; (2) in every case the standard deviation (measure of the absolute amount of variation) is greater in the conjugants; (3) in every case the coefficient of variation (measure of the variation relative to the mean) is much greater in the conjugants.

Since these measures are based on the number of fissions for long periods under identical conditions, they can hardly be held to represent meaningless accidental fluctuations, but rather actual differentiations. They show further that these differentiations are much greater in those that conjugated during the last epidemic than in those that did not. This conjugation therefore caused inherited differentiations within the pure line. Whether the fewer inherited differentiations among those that did not conjugate during the last epidemic are due to previous conjugations we cannot tell in this case, but must refer the reader to the account of Experiment 15.

The inheritance of the rate of fission in these cases may be demonstrated, for those that prefer this method, by working out the coefficients of correlation. The numbers we are dealing with are of course small, but significant, owing to the great number of generations dealt with. We may take the fissions during the first two periods of table 33 and by determining their correlations with the fissions in the same lines for the second two periods, get a numerical expression of the inheritance. For the conjugant lines we find that the coefficient of correlation thus taken is 0.5031 ± 0.1346 . For the non-conjugant lines it is 0.5627 ± 0.1331 .

(A full treatment of the inheritance of the fission rate, by biometric methods, with adequate numbers, will be given in another connection; together with an analysis of the relation of this method of measuring inheritance to other ways of dealing with the matter.)

TABLE 21

Experiment 13 b. Paramecium aurelia. Comparative variability in fission rate for descendants of conjugants and of non-conjugants, for various periods. (The constants in the first column are based on the data of table 20; the remainder on the data of table 33, in the appendix).

	1 FIRST WEEK DECEMBER 8-26	2 FIRST PERIOD DECEMBER 8-26	3 SECOND PERIOD JANUARY 2-14	4 THIRD PERIOD JANUARY 15-26	5 FOURTH PERIOD JANUARY 30- FEBRUARY 12	6 FIFTH PERIOD FEBRUARY 13-27	PERIODS 1 AND 2	PERIODS 3 AND 4	PERIODS 1 TO 3
Mean—Conj.....	6.155±0.220	12.647±0.908	9.250±0.263	11.188±0.629	9.786±0.668	6.167±0.762	22.750±0.913	21.214±1.126	33.875±1.243
Non-Conj.....	7.098±0.093	15.786±0.520	10.143±0.235	12.857±0.653	11.417±0.439	9.364±0.550	25.786±0.661	25.083±0.982	38.786±1.149
Stand. Dev.—Conj.....	2.484±0.156	5.551±0.642	1.561±0.186	3.733±0.445	3.707±0.473	3.912±0.539	5.414±0.646	6.247±0.796	7.373±0.879
Non-Conj.....	1.246±0.066	2.883±0.367	1.301±0.166	3.622±0.462	2.253±0.310	2.706±0.339	3.668±0.468	5.041±0.694	6.372±0.812
Coef. Var.—Conj.....	40.350±2.910	43.893±5.976	16.878±2.069	33.370±4.400	37.879±5.478	63.442±10.345	23.798±2.904	29.447±4.066	21.765±2.715
Non-Conj.....	17.550±0.963	18.263±2.405	12.832±1.662	28.176±3.866	19.735±2.821	28.399±4.489	14.225±1.850	20.096±2.876	16.428±2.150

Experiment 13 c. In order to test more fully the inheritance of the differences in fission rate shown in table 33, certain lines were next selected for propagation on a more extensive scale. Beginning January 28, 1911, the attempt was made to propagate 16 parallel lines each of conjugant numbers 1, 2, 3, 11, 14 and 16 (of table 33), the purpose being (1) to determine whether the results with 16 lines of a given number confirm those obtained with but one line; (2) to discover whether there arise differentiations within any of the series derived from a single individual. This second point, as we have before seen, is fundamental for a full understanding of the results thus far reached.

These objects were not fully attained, owing to the cessation of active propagation on slides in the race *k*, but certain results of importance were reached.

CONJUGANT LINES. The sets derived from the different ex-conjugants of table 33 showed great differences in vitality as well as in rate of fission. Lines 1, 2, 3 and 11 began strongly, 16 parallel sets being derived from the original single set in one to three days. With conjugant lines 14 and 16, on the other hand, there was great difficulty in getting 16 sets established; multiplication was extremely slow, and many of the sets died out almost as soon as they were isolated. It was a week from the beginning of the experiment before 16 sets were in operation in conjugant lines 14 and 16.

The relative rates of fission that had characterized the various lines from the beginning continued to show themselves in the sets of 16 from each line. The slower lines showed much greater mortality than the faster ones. As fast as any set of a given line died out it was replaced from another set of that line. The number of deaths for each line was thus recorded. It will be instructive to give for each of these lines of ex-conjugants the number of fissions and the number of deaths, up to February 15. This is done in table 22.

The mortality in the slow lines increased from February 15 on, so that on February 16 there were but three sets left (out of 16) in line 14. By February 26 all the 16 sets of lines 14 and 16 were dead, so that these two lines became extinct. In the meantime,

TABLE 22

Experiment 13 c. Paramecium aurelia. Number of fissions, and number of deaths, in each of the sets of 16 parallel cultures belonging to six of the conjugant lines of table 33, between January 28 and February 15. For each line, the minimum and maximum number of fissions in the cultures that lived through the period are given. Thus, in line 1, one of the 16 sets gave 15 fissions, another 20.

	LINE					
	1	2	3	11	14	16
Fissions, January 28 to February 15.....	15-20	9-15	15-16	10-13	5-6	3-11
Number of deaths.....	1	4	0	8	19	20

the 16 sets of line 1 were flourishing under precisely the same treatment.

Later, lines 2 and 3 began also to die out. On March 7, line 2 was extinct, while of line 3 one set still existed. Line 1 continued to flourish; 16 sets still existed March 7. For other purposes 64 sets of line 1 were kept in propagation till April 17.

'NON-CONJUGANT' LINES. Later, the rapid line 1 and the slow line 14 of the 'non-conjugants' (of table 33) were compared similarly, beginning February 26. Sixteen sets of each were put in progress. Line 1 continued to multiply rapidly, line 14 slowly; by March 20 the maximum number of fissions in the former was 17, in the latter 6. At this date an attempt was made to increase the numbers to 64 parallel sets for each line. But it was found impossible to get 64 sets of line 14 into existence, owing to the great number of deaths. On March 31 the last set died out, and the non-conjugant line 14 became extinct. At this time there were 64 sets of line 1, which were continued till April 17, when the experiment was abandoned. During the last two weeks there were few fissions even in line 1.

Thus all of the lines having a slow rate of fission died out, even though the attempt was made to keep up 16 parallel sets; and this under conditions in which the lines with rapid fission continued to flourish. Although all were treated alike, only the two most rapid lines, No. 1 of the conjugants and No. 1 of the non-conjugants, continued to live till the close of the experiment, April 17.

This appears to indicate that the lines with slower fission are defective in some way. Of course it is possible, perhaps probable, that under more natural conditions they would have continued to exist, in spite of their slow multiplication. The extremely slow line 16 (conjugant) had lived from December 6 to February 26, a period of two months and twenty days, comprising forty successive generations. But slow multiplication and high mortality are decidedly correlated.

It had been planned to employ the 64 sets that were kept for a number of different lines in biometrical studies of the inheritance of the fission rate; and in an attempt to determine whether heritable differentiations in fission rate arise in the progeny of a single individual multiplying by fission. But the death of all the slow lines, and the extremely slow multiplication of the others for the last weeks of the experiment rendered the extensive data obtained valueless.

Summary of Experiment 13. We may summarize the results of this entire experiment as follows:

In a pure strain, all the individuals derived originally from a single one; and all derived from eight successive conjugations with self-fertilization of the strain:

1. Conjugation decreased the rate of fission,
2. Conjugation increased greatly the variability in rate of fission.
3. The differences in rate of fission were found to be inherited, so that in this respect heritable differentiations arise within the pure strain.
4. These heritable differentiations are due partly, if not entirely, to conjugation, since the latter increases greatly the variability. But whether such heritable differentiation may arise within the pure strain by other means is not determined in this experiment.
5. A low fission rate is correlated with a high mortality. Conjugation produces many lines with low fission rate; these lines die out in the course of time, if the conditions become severe, although the lines with rapid fission continue to live. But the slow lines may live for many generations (forty in this experiment).

Experiment 14: Paramecium aurelia

This was a direct continuation of the foregoing, dealing with the rapidly multiplying line 1 of the non-conjugants. On March 20, 1911, a considerable number of these were placed in a watch glass; on March 22, conjugating pairs were found among these. Of these 48 pairs were isolated, making after separation 96 lines, which were cultivated on slides as usual. At the same time there were in progress 64 lines of those that had not conjugated, and to these were now added 48 more. Thus we have now propagating, under identical conditions, 96 lines of ex-conjugants and 144 lines of non-conjugants, all derived from individual No. 1 of the non-conjugants of the previous experiment.

Conditions were unfavorable for multiplication, the temperature being low and the university buildings not heated. Of the 96 lines of conjugants, all but four died without dividing; that is, 95.8 per cent. Of the 48 non-conjugants set at the same time, 27, or 56.25 per cent died without dividing.

By March 31, nine days after conjugation, all but 4 of the 96 conjugant lines were dead, while 27 of the 48 non-conjugant lines were dead. Of the entire 144 non-conjugant lines, kept under the same conditions as the conjugant ones, 37 had died during the same period. Thus the proportion of deaths was, for the conjugants, 95.83 per cent; for the non-conjugants 25.69 per cent.

This experiment shows that under such unfavorable conditions the animals that have recently conjugated are much less resistant than those of the same descent that have not recently conjugated.

*Experiment 15: inherited differentiation produced by conjugation:
Paramecium caudatum*

In the summer of 1912, after several months spent in vain attempts to repeat with the race *k* the essential features of Experiment 13, under such conditions as would show beyond question whether all the inherited differentiations were due to conjugation or not, a successful experiment for this purpose was carried through with a race of *Paramecium caudatum* which I called *E*.

The race *E* was derived from a single individual taken July 31 from a wild culture of *Paramecium caudatum*. This individual and its progeny were allowed to multiply on slides till a large number were obtained. On August 19 many of these were transferred to a mass culture, and on August 22 watch glass cultures containing many individuals were removed from this mass culture. Early the following morning conjugation was beginning in these watch glasses. In the way set forth in our general account of methods (page 282), I picked out 67 pairs and 68 split pairs (pairs which had begun to unite, but which were separated before conjugation was consummated). The two members of each pair (and of each split pair) were designated *a* and *b*. The products of the first division of each of these were retained, becoming the progenitors of two lines which I called *x* and *y*. Thus from each pair (and each split pair), four lines were propagated, two from *a* and two from *b*. This of course gave 268 lines derived from conjugants and 272 derived from the non-conjugants of the split pairs.

During the heat of summer the cultivation of many lines of *Paramecium* is very difficult, owing to excessively rapid development of bacteria in the drop cultures. This has the effect of inducing a high mortality, and also of making it very difficult to keep the environmental conditions uniform throughout a large number of lines. This latter condition is essential in the present experiment, since if it is not fulfilled, differentiations in fission rate due to environmental conditions simulate those due to heritable or intrinsic differences in the diverse lines.

Owing to these difficulties the mortality among the conjugants was high, and the measures required for making the conditions uniform were so time-consuming that I was compelled to abandon a large number of the lines of propagation of the non-conjugants, so that I succeeded in keeping to the end of the experiment but 88 lines of conjugants, derived from 44 original ex-conjugants, and 174 lines of the non-conjugants, derived from 87 original members of split pairs. These however were sufficient for the solution of the problem that gave rise to the experiment. These 262 lines were propagated from August 24 to September 16

(non-conjugants) or September 18 (conjugants), a period of 24 (or 26) days.

Culture methods necessary to secure uniformity of conditions. In order that the conditions should be uniform throughout the large number of lines, the following method of culture was found necessary:

As a culture medium, Horlick's malted milk was employed, following the example of Miss Peebles ('12), one-sixteenth of 1 per cent being found the most favorable proportion. This was made fresh each day, with boiling water. The animals were changed every other day. The chief difficulty in making the conditions uniform throughout all the lines is as follows: A number of diverse bacteria are found in the cultures, falling into them at the time of changing, or reaching them in other ways. Some of these multiply strongly in certain of the slide cultures while others get a better foothold in others. The effect of the diverse bacteria on the rate of reproduction differs greatly; as a result therefore some of our lines of *Paramecium* multiply rapidly, others slowly, even though there is no intrinsic differentiation among them. Now, in transferring with a capillary pipette a single individual to a new drop, as is done at the time of changing the animals, inevitably a certain amount of the bacterial culture is transferred with them, serving to infect the new drop. Thus one line will be accompanied always by the bacterium *x*, causing rapid multiplication; another by the bacterium *y*, causing slow multiplication. The results simulate those of inherited differentiation in the fission rate.

Experience showed that this difficulty is obviated by the following method of procedure, which was adopted for the present experiment on August 28:

The new fluid (1/16 per cent malted milk) was made sterile by boiling. It was then infected with bacteria from a mass culture of the race *E*, in which the animals were flourishing strongly. This was done by filtering (through two thicknesses of filter paper) a quantity of the fluid from this culture (in order to remove the *Paramecia*). I added four pipettes full of this filtered fluid to 100 cc. of the fresh culture fluid.

For changing the animals to this, two fresh slides are prepared, each containing three drops of this fresh fluid. A vessel of boiling water is at hand; also a supplementary vessel of the fresh culture fluid. The capillary pipette is first dipped in boiling water, then into the fresh culture fluid, then a single individual is removed with it from the old slide to the first new slide. The pipette is then again disinfected in boiling water and washed in the supplementary dish of culture fluid. Meanwhile, the removed *Paramecium* has been swimming about violently in the three drops of fresh culture fluid, thus washing itself largely free from the bacteria introduced with it. Now, with the cleaned pipette, it is retransferred from this wash water to the second slide of fresh fluid. (In much of my work I gave each animal a second washing in the same way.)

A new 'wash slide' is then prepared, the pipette is disinfected and washed as before, and we proceed to transfer in the same way an individual from the second slide to the wash water and then to its definitive slide. After every transfer the pipette must be disinfected and washed, and new wash water must be used for every individual transferred.

Experience shows that all the details of this painful process are quite necessary if the conditions are to be kept uniform in a large number of lines. Carrying this out for some 250 lines for nearly a month I found so exhausting as to make it practically impossible to continue the experiment for a longer period.

Records. The records of the conjugant and non-conjugant lines for this experiment, conducted in the manner just described, are given in tables 34 and 35 (Appendix). The results of this experiment are of so fundamental an importance for the subject with which the present series of papers deals that I feel it necessary to give the records in detail, showing the number of fissions that occurred in each period of two days. These records will be used farther in studies on inheritance, to follow the present paper.

Explanation of tables 34 and 35. These tables give, for the conjugants and non-conjugants, respectively, of the pure strain *E*, the records of fissions for each line for the entire period (twenty-four days for the

conjugants; twenty-one days for the non-conjugants). The records given are the numbers of fissions that have occurred during the two days ending on the date at the head of the column. (In only one case, for the non-conjugants, in the column headed November 6, is the elapsed period three days instead of two.)

Each pair or split pair consisted of the two mated individuals *a* and *b*. From each *a* and *b* the two sister lines *x* and *y* were kept in progress. Thus from each pair there were derived four lines, *ax*, *ay*, *bx* and *by*. But the lines from both *a* and *b* were kept throughout the experiment in but few cases (16 in the pairs, 22 in the split pairs).

The lines from *a* and *b* were kept in separate moist chambers and changed at different times, so that there is no opportunity for resemblance between them to arise through special similarity of treatment. The two lines *x* and *y*, from a single individual, were however kept in the two concavities of the same slide, in the same moist chamber, and changed in succession. (This was for convenience in replacing one from the other, but in repeating such an experiment, *x* and *y* should be kept in separate moist chambers and handled separately; otherwise the significance of any correlation between *x* and *y* is not entirely clear.)

In working out constants of variation, the period August 27 to September 6 (twelve days) was considered the 'first half' for the conjugants; August 28 to September 6 (eleven days) for the non-conjugants. The second half for the conjugants included twelve days (September 8 to 18); for the non-conjugants, ten days (September 8 to 16).

The blanks left in the column under certain dates indicate that the line in question died out on that date, and *its place was supplied by taking an individual from the sister line x or y, derived from the same parent, that is, from the same a (or b, as the case may be)*. But a blank in the final column of totals indicates only that the line in question did not live independently throughout the experiment, but was supplied from its sister line at some date, indicated as just set forth.

In determining mean, standard deviation or coefficient of correlation for any period or periods, only lines that lived independently throughout that period are included. However, for the entire period, the few totals included in parenthesis, in the last column, *are employed* also, since the lines for which they stand coincided with another for only two or three fissions at the beginning.

In working out coefficients of correlation, for successive periods, it is of course necessary to correlate any line with its real ancestral line, and to do this it is necessary to pay careful attention to the blanks left in certain columns and the replacement of certain lines which they indicate. Thus, if in table 34 we wish to correlate the fissions in the first half of the entire period with those in the second half, then when, for example, we enter the fissions for the second half of the time (September 8 to 18) in line 8 *by*, we see that this second half descended partly from 8 *bx*; there is no difficulty, however, in determining exactly how many fissions occurred in the first half. We take in this case for the first half of the period the sum of the fissions for 8 *bx* to September 2, plus those for 8 *by*

for September 4 and 6 (that is, $0 + 2 + 2 + 1 + 3 + 3 = 11$); for the second half, 8 by, September 8 to 16 ($=17$); and similarly for all analogous cases. Thus in the correlation tables a given preceding period may sometimes be counted twice, since it gives rise to two lines of progeny, and is therefore correlated with both. This of course introduces no error into the coefficient of correlation. The other constants (given in table 23) were computed with each period counted but once.

Results. Comparative examination of tables 34 and 35 shows a very great difference between the progeny of the pairs, and those of the split pairs, in respect to variation and differentiation in the rate of fission. This is well shown by observing the range of variation in the two cases. In the split pairs the slowest lines show in twenty-one days 18 fissions, the fastest, 28 fissions. In the pairs the range is (for twenty-four days), from 10 to 35. If we reduce these latter numbers by one-eighth, in order that they may compare directly with those for the split pairs, the range becomes for the pairs 8.75 to 30.6, as compared with 18 to 28 for the split pairs. From September 8 to 16 the range for those derived from the pairs is 1 to 17; for those from the split pairs, 8 to 15.

Working out the mean number of fissions, with the standard deviation and the coefficient of variation for the pairs and split pairs during a number of different periods, we obtain the results shown in table 23. Here the data are given for the first and second halves of the experiment; also for the entire period. In order to have certain periods which are absolutely identical in every respect for the two sets, I give also for the pairs the data for the ten days extending from September 6 to September 16, this period coinciding with the 'second half' for split pairs.

As the last columns of table 23 show, the variability in fission of the lines descended from conjugants was, for the entire period, four times as great as that for those descended from non-conjugants. In the first half of the time it was about twice as great; in the second half five times as great.

Examining tables 34 and 35 to discover the cause of this great difference in variation, we find that the descendants of those that have conjugated are differentiated into a number of distinct lines, with different rates of fission. This will at once be evident

if one compares, among the pairs (table 34) line 1 with line 6, and the latter again with line 4. Line 1 *ax* shows a total during the twenty-four days of 33 fissions, line 6 *ax* of 16 fissions; line 4 *ax* of 13 fissions. Corresponding differences are shown in the other divisions of lines 1, 6 and 4, the differences extending *even to lines descended from the two mates* of a pair. Thus line 1 *ax* has 33 fissions; line 1 *bx*, descended from its mate, 31 fissions; line 6 *ax* has 16 fissions; line 6 *by*, descended from its mate, 17 fissions; line 4 *ax* has 13 fissions; line 4 *bx*, descended from its mate, has 12. If we compare similarly the two lines *x* and *y*, derived from a single member of a pair, we find that their fission rates are close together, while lines derived from different pairs differ greatly.

Certain peculiarities of the fission rate are evident. During the first five or six days after the beginning of fission the different lines descended from the conjugants are more nearly uniform in their rate. Then a number of the lines, such as those belonging to pairs 4, 5, 15 and 26, show a marked decrease in the fission

TABLE 23

Experiment 15; Pure strain E. Constants of variation in fission, for the lines descended from conjugants (pairs), and for those descended from non-conjugants (split pairs), for certain periods of time. The total time is, for the pairs, 24 days, for the split pairs, 21 days. The first half includes for the pairs the twelve days, August 27 to September 6; for the split pairs eleven days, August 28 to September 6; the second half, September 8 to 18 (pairs), September 8 to 16 (split pairs).

	NUMBER OF LINES		MEAN NUMBER OF FISSIONS		DAILY RATE	
	Pairs	Split pairs	Pairs	Split pairs	Pairs	Split pairs
Total time.....	69	145	26.333 \pm 0.613	24.034 \pm 0.096	1.097	1.144
First half.....	78	158	12.154 \pm 0.081	11.424 \pm 0.072	1.013	1.039
Second half.....	83	171	14.241 \pm 0.444	12.614 \pm 0.056	1.187	1.261
September 8-16.....	83	171	12.060 \pm 0.367	12.614 \pm 0.056	1.206	1.261

	STANDARD DEVIATIONS		COEFFICIENT OF VARIATION	
	Pairs	Split pairs	Pairs	Split pairs
Total time.....	7.544 \pm 0.433	1.712 \pm 0.063	28.650 \pm 1.734	7.122 \pm 0.284
First half.....	2.370 \pm 0.128	1.347 \pm 0.051	19.501 \pm 1.065	11.789 \pm 0.453
Second half.....	6.003 \pm 0.314	1.083 \pm 0.039	42.154 \pm 2.885	8.585 \pm 0.315
September 8-16..	4.954 \pm 0.259	1.083 \pm 0.039	41.074 \pm 2.485	8.585 \pm 0.315

rate, which persists throughout the remainder of the experiment. In other lines, such as those derived from pairs 1, 2, 3, 7, 12, the rate remains high throughout the entire period.

The inherited differences between the lines will perhaps be best brought out if we divide the twenty-four days of the experiment into four periods of six days each, and give the number of fissions for each of these periods, for a number of diverse lines. We shall re-group these lines in such a way as to bring out strongly the diversities. The results are shown in table 24. It is observable, for example, that in every one of these four periods the line 3 *ax* has a greater rate of fission than 6 *ax*; similarly 3 *ay* and 3 *bx* show in every period a greater rate than 6 *ay* and 6 *bx*. Comparison of other lines shows the same relations.

TABLE 24

Experiment 15. Pure strain E. Fissions in certain of the conjugant lines, for four successive periods of six days each, so arranged as to exhibit the differences between the lines.

LINE	AUGUST 27-31	SEPTEMBER 2-6	SEPTEMBER 8-12	SEPTEMBER 14-18
3 a x.....	6	10	9	9
6 a x.....	3	3	5	5
4 a x.....	7	4	1	1
3 a y.....	6	9	9	10
6 a y.....	3	3	5	5
4 a x.....	(7)	4	1	0
3 b x.....	6	9	9	8
6 b x.....	3	5	6	(5)
4 b x.....	6	5	1	0
9 b x.....	6	7	10	9
5 b x.....	4	5	1	0
1 b x.....	4	9	10	8
15 b y.....	7	5	2	1
20 b y.....	6	8	11	9
27 b y.....	5	5	5	6
26 b x.....	6	5	3	1

In some cases a line begins with a high rate of fission, then runs down to a very low one, dividing but once or not at all during the last six-day period. Such is the case, for example, in the lines derived from pair 4. In such cases careful and extended tests were made to determine whether the slow fission rate was characteristic of all the members of the given line. Thus, of line 4 *ax*, seven sets; of 4 *ay*, nine, and of 4 *bx*, eight sets; were kept in progress during the last twelve days of the experiment; all showed the same extremely low rate of fission characteristic for the lines derived from pair 4 in tables 34 and 24.

In the same way, eleven sets of No. 5 *a*, eight of No. 5 *b*, twenty sets of No. 6 *b*, six sets of No. 26 *a*, and eight sets of No. 15 *b* were kept in progress during the last twelve days of the experiment; all of them showed slow rates of fission corresponding closely to those given for the lines in question in table 34.

Of the rapid lines, No. 9 *a* was tested by keeping eighteen parallel lines in progress during the last twelve days of the experiment. All divided rapidly, giving 18 to 20 fissions during the twelve days.

It is thus clear that *the lines descended from the ex-conjugants are differentiated in their inherited characteristics*, some having a rapid rate of fission, some a slow rate, and some an intermediate one (although all were kept under absolutely identical conditions). One result of this inherited differentiation is the production of the very high coefficients of variation shown in table 23.

Are there likewise inherited differentiations among the lines derived from the non-conjugants—the members of the split pairs? Examination of the coefficients of variation in table 23, as well as a general inspection of table 35, shows at once that if there is any such differentiation, it is very slight compared with that among the descendants of the conjugants. If we compare very carefully the records of the different lines in table 35, we find a few cases in which it is doubtful whether there may not be inherited differentiation. Line 3 *ax*, for example, shows a rate of fission somewhat below that of most others, while 6 *by*, 22 *ax* 40 *ay* and 44 *ax* show rates rather above the average. But the differences between even the extreme cases are very small com-

pared with those between the diverse lines derived from the conjugants. Furthermore, taking the most extreme case of line 3 *ax*, with but 18 fissions, we find that the sister line, 3 *ay*, derived from the same parent, does not show a low rate of fission; so that the slow rate is not characteristic of this entire line. In the conjugant lines, on the other hand, the rates of the two or more sets derived from a single individual we found to correspond closely, showing that the characteristic is an inherited one. It would then appear on the whole probable that all the differences seen among the lines derived from the non-conjugants are simply the slight fluctuations unavoidable where a large number of lines are cultivated.

The question may be tested for both sets in another way. If the differences between different lines are matters of inherited differentiation, then of course lines having a fast or a slow rate in one part of the period of the experiment should have a corresponding rate in the other parts. That is, the rates of fission for earlier and later periods should be correlated. We may therefore determine the coefficients of correlation for successive periods, in both the conjugants and non-conjugants; this will tell us whether the rates of fission are, as a rule, inherited in the different lines.

I have worked out for both sets the correlation between the numbers of fissions in each line (1) in the first half of the experiment compared with the second half; (2) in the second third (September 3 to 8 or 4 to 10) compared with the last third (September 10 to 16 or 12 to 18). This latter comparison was made owing to the fact that the direct physiological effect of conjugation appears to obscure the characteristic differentiations, for some days after conjugation.

Furthermore, I have worked out, for the entire period of the experiment, the correlation between the sister lines, *x* and *y*, derived originally from a single member (of a pair or split pair). If the differences in rate of fission are inherited, these two sister lines should of course be similar, giving a positive coefficient of correlation. The correlation, for both conjugants and non-conjugants, is given in table 25.

As table 25 shows, the lines derived from conjugants give an extremely high correlation. In other words the fact that we are here dealing with lines differentiated in inherited characters is demonstrated by this method as well as by the other evidence. After passing the disturbance due to the direct physiological effect of conjugation, the correlation between successive periods rises to 0.8957 (practically to 0.9), an extraordinarily high coefficient. The correlation between the sister lines *x* and *y* is likewise 0.9, showing an almost perfect correspondence.

In the lines descended from the non-conjugants, on the other hand, there is no correlation between the numbers of fissions in successive periods, the coefficients being practically 0. That is, so far as this method can show, *the diversities in fission rate are not inherited*, among the members of a pure race which have not conjugated.

On the other hand, the numbers of fissions for the sister lines *x* and *y*, do give a small coefficient of correlation (0.2119).

TABLE 25¹

Experiment 15. Pure strain E. Coefficients of correlation in number of fissions, for successive periods, and for the sister lines x and y, in the descendants of conjugants (pairs) and of non-conjugants (split pairs).

	PAIRS			SPLIT PAIRS	
	Num- ber of lines	Coefficient	Num- ber of lines	Coefficient	
First half (10-12 days) with second (10-12 days)	82	0.5743 \pm .050	174	.0120 \pm .051	
Second third (6-8 days) with last third	81	0.8957 \pm .015	172	-.0020 \pm .051	
Total time, <i>x</i> with <i>y</i>	50	0.9017 \pm .018	58	.2119 \pm .085	

¹ For the pairs the 'first half' comprises the 12 days August 27-September 6 of table 34; the 'second half' the remaining 12 days. For the split pairs the 'first half' is 11 days, August 28-September 6; the 'second half' is the remaining 10 days. The 'second third' comprises September 4-10 (pairs) or September 3-8 (split pairs); the 'last third,' September 12-18 (pairs), or 10-16 (split pairs). The total time is, for the pairs, 24 days; for the split pairs, 21 days. The correlation tables for successive periods are formed by taking the number of fissions of a given line in an early period and entering this on the table in connection with the number of fissions for the same line in the later period.

Whether any significance is to be attached to this is doubtful, since the value of the coefficient is but two-and-a-half times its probable error; and a coefficient of this amount would occur once in ten times as a result of chance distribution. Further, the two sister lines x and y were kept in the two concavities of the same slide, and one was changed immediately after the other. The result of this may have been to keep the two under slightly more uniform conditions than prevails for two individuals in different moist chambers, giving rise to the slight correlation. The matter will be investigated farther, but in any case it is clear that any differentiation that may exist between the non-conjugant lines is extremely slight; so that correlating the fissions of successive periods gives no trace of it.

The present experiment therefore clears up the difficulty left by the results of Experiment 13. In that experiment, as shown in table 33, the 'non-conjugants' exhibited inherited differentiations, as did the conjugants. It seemed practically certain however that these 'non-conjugants' had gone through previous conjugations, so that the observed heritable differentiations were probably due to these previous conjugations. On page 337, I pointed out the necessity for an experiment in which this matter should be controlled. Our present experiment supplies this need; we know that our non-conjugants here have not conjugated since they came from a single parent individual. And our results show that the inherited differentiations in Experiment 13 were indeed due to conjugation; they do not appear when we deal with actual non-conjugants (lines which have not conjugated since they were all derived from a single individual).

Even if it should turn out that the slight correlation shown by x and y in the non-conjugants of the present experiment is due to real differentiations between the lines, this result would not modify our present conclusion in any essential way, since the differentiation so indicated would be so slight as to be of quite a different order from that produced by conjugation, the latter giving rise, as we have seen, to coefficients as high as 0.9. Even a slight differentiation arising during vegetative reproduction would be of the highest interest, but it would not alter the positive fact of the

immediate production of strongly marked heritable differences by conjugation.

The data of our present experiment, given in tables 34 and 35, bring to light many other important relations, which will be dealt with in subsequent papers. For our present purposes it is sufficient that the experiment demonstrates that conjugation produces within a pure race heritable differentiations; so that as a result races diverse in their heritable characters arise from a single race with uniform heritable characters.

Our previous experiments had shown that conjugation increases variation; and that the variations observed to follow conjugation are heritable. The present experiment puts the finishing touch on this demonstration by showing that these heritable variations do not arise without conjugation.³ Thus we find that one method of producing new strains is by conjugation.

We have now in hand the essential facts for drawing conclusions as to the actual effects of conjugation on the stock.

IV. RÉSUMÉ OF RESULTS: DISCUSSION, AND CONCLUSIONS

In the foregoing sections are detailed the results of a large number of experiments in which conjugants were compared with non-conjugants of the same stock and the same cultural history. What effects do we find conjugation to produce?

The prevailing view as to the effects of conjugation is that it produces rejuvenescence in the stock. This view is excellently stated in Calkins' recent Protozoology ('09), particularly in chapter III. The essentials are somewhat as follows:

If we could take such an entire succession of cells thus formed from the repeated divisions of a fertilized protozoön, and if at any given period could combine them in one mass of cells, we should have the analogue of a metazoön and would find that the protoplasm represented by the aggregate of cells would manifest the same successive periods of vitality as those of youth, adolescence, and old age in Metazoa. We would find that the young cells divided more rapidly than they do later in the cycle; we should find that after a certain time they become sexually mature and are able to conjugate and so to perpetuate the

³ There remains the possibility that heritable variations of a totally different (lesser) order of magnitude may arise during vegetative reproduction.

race; and we would find that, ultimately, evidences of weakened vitality and degeneration appear in the aggregate of cells, and that they finally die of old age (p. 103).

It is conjugation that reinvigorates the stock; for succinct, explicit statements of this we may quote from other papers of Calkins and his associates:

Conjugation between two cells results in the complete reinvigoration of all activities, both physiological and germinal (Calkins and Cull '07, page 376). As with the fertilized egg of a metazoön, the copula or fertilized egg of a protozoön is endowed with a great power of cell reproduction and with a high potential of vitality, and this is the main characteristic of the first period of the life cycle (Calkins '06, page 233). As with the metazoön so with the aggregate of protozoa cells, we note a period of youth characterized by active cell proliferation; this in both groups of organisms is followed by the gradual loss of the division energy accompanied by morphological changes in type of the cells preliminary to conjugation and fertilization and to the renewal of vitality by this means (Calkins '06, p. 232).

The experiments described in the present paper constitute an examination as to how far conjugation actually exhibits these effects in *Paramecium*; as well as how far it shows other results. We shall here summarize and discuss the evidence as to the effects of conjugation on the rate of reproduction; on the vigor or vitality, as evidenced by the comparative mortality; on abnormalities; on its production of variation; on inheritance; and the relation of the results as a whole to the theory of rejuvenescence.

EFFECT OF CONJUGATION ON RATE OF REPRODUCTION

Practically all the experiments show that the average rate of reproduction is less after conjugation than before. That is, if we take two sets of animals of the same stock and history, both ready to conjugate; permit one set to conjugate, and prevent the other, we find that those which have conjugated divide thereafter on the average less rapidly than the others.

In most cases the rate of fission was very considerably greater in those that had not conjugated, the excess usually varying from 25 per cent up to 80 per cent or more. In some cases, however,

the difference is very slight. In no case did the conjugants have a higher rate of fission, although in Experiments 4, 7, 8, 12 and 14 the difference between conjugants and non-conjugants was so small as to be without significance. But in the majority of the experiments, and particularly those which included many cases and were little disturbed by extrinsic factors, those that had not conjugated showed a fission rate higher in a marked degree. And this higher fission rate of the non-conjugants persisted for weeks and months (see the results of Experiments 1, 2 and 6).

So much has been said of the greater reproductive power, the "active cell proliferation," et cetera, of the period following conjugation, that this result appears surprising. Yet those investigators who have examined the matter with the greatest care, came long ago to the same result. Maupas insists again and again, at great length, in opposition to the prevailing views, that conjugation does not increase the rate of reproduction. Since the matter is an important one, and one on which incorrect ideas are prevalent, and since Maupas had evidently done much careful work on the question, it may be worth while to give a résumé of the points he makes. The following passage might well be designed as a statement of the present condition of affairs:

On a affirmé que la faculté fissipare des Ciliés était modifiée par la conjugaison, et que cet acte sexuel avait, pour principal effet, de la renforcer et de l'accélérer. Les Ciliés, au sortir de la conjugaison, se multiplieraient beaucoup plus rapidement qu'ils ne le font plus tard. Cette opinion est devenue courante, et on la trouve reproduite dans les Mémoires et les Traités Généraux, comme une vérité définitivement acquise. Elle a été émise pour la première fois, par Bütschli en 1876, et reprise ensuite par Balbiani, en 1882, qui s'en est emparé, et a même cru en avoir fourni la démonstration expérimentale ('88, pages 254-255).

Maupas then examines the supposed evidence of Bütschli and Balbiani, showing that it amounts to nothing. He sets forth that in his own records of fissions, beginning in a number of cases with ex-conjugants, there is no indication of a greater rate of fission in the early part of the cycle. He says of the fissions:

Elles se succèdent avec une marche uniforme, modifiée uniquement par les variations de température. Je ne me suis pas contenté de cette

unique expérience. J'ai isolé d'autres ex-conjugués de la *Stylonychia pustulata*, puis de l'*Onychodromus grandis*, de l'*Euplotes patella*, du *Paramecium aurelia* et de la *Leucophrys patula*. J'ai suivi, jour par jour, les générations successives de leur descendants, pendant des durées de temps qui ont varié depuis quinze jours jusqu'à un ou deux mois. Chez aucune de ces espèces je n'ai constaté la moindre différence dans la succession de bipartitions. Anciennement ou nouvellement conjugués, tous les individus se sont comportés de la même façon. ('88, pages 255-256).⁴

In the paper of 1889 Maupas details experiments with *Paramecium aurelia* (p. 227), *Colpidium colpoda* (p. 247), *Leucophrys patula* (p. 261), *Onychodromus grandis* (p. 321), *Stylonychia pustulata* (p. 329), and *Euplotes patella* (p. 353), all showing that after conjugation these animals do not reproduce more rapidly than later in the history of the strain. In *Onychodromus* and *Stylonychia*, indeed, Maupas found that those which had recently conjugated multiplied more slowly, but he believed this to be due merely to individual variations, and to have no connection with conjugation. He sums up, in opposition to Bütschli, as follows:

J'ai affirmé, en outre, que cette puissance de multiplication se maintient régulière et égale pendant le cycle entier, sans qu'il se produise un affaiblissement graduel depuis la première génération post-syzygienne, jusqu'au retour d'une nouvelle période de maturité karyogamique. Autrement dit, je nie que les Infusoires, au sortir de la conjugaison, jouissent d'un faculté de reproduction plus énergique que plus tard ('89, p. 504).

Richard Hertwig ('89) came, through an experimental study, to similar conclusions, save that he discovered the fact that animals which have conjugated actually reproduce more slowly than those which have not. He was apparently the first to perform the experiment employed on a large scale in the present paper, of separating pairs before conjugation was completed, and comparing these members of split pairs with specimens that had finished conjugating. He gives only a general account of his

⁴ Maupas notes that of course in the last stages of morphological degeneration just before death, there is a cessation of fission; but when this condition is reached *rejuvenescence is no longer possible*. "Je suis convaincu que si, dans les générations d'un cycle, il se produit un ralentissement, celui-ci se fait sentir seulement dans la période effectuée de dégénérescence sénile; c'est à-dire, lorsque les Infusoires sont devenus incapable de rajeunissement karyogamique" ('89, p 504).

experiment, not even mentioning the number of cases examined; but some cultures obtained from the split pairs were kept as long as three months:

Als erstes Resultat ergab sich mir eine auffallende Fruchtbarkeit der an der Conjugation verhinderten Thiere; obwohl ich meine Versuche noch nicht abgeschlossen habe, so möchte ich jetzt schon hervorheben, dass die künstlich getrennten Thiere lange Zeit über sich energischer theilten als Paramaecien, welche die Conjugation durchgemacht hatten ('89, p. 223).

These observations led Hertwig to endeavor to save the theory of rejuvenescence through conjugation, by holding that lack of conjugation results in a rate of fission so great as to be harmful; conjugation would then rejuvenate by slowing and regulating this immoderate rate of reproduction ('89, p. 226).

But the facts appear to be clear, so far as the infusoria go. In view of the large number of experiments made by Maupas on this point, the absolute agreement of his results with those of Richard Hertwig; the fact that these men are perhaps the most thorough investigators that have ever worked along these lines; the further fact that there exist no careful experimental results opposed to these; and finally, the very large body of evidence presented in the present paper, all giving the same results—is it not time that the statements or implications that in the infusoria conjugation results in increased reproduction should disappear from the literature of science?

EFFECT OF CONJUGATION ON MORTALITY

The experiments show that as a rule mortality is much higher, under the same conditions, among those that have conjugated than among those that have been prevented from conjugation. This is true both for conjugation among unrelated individuals, and for that among individuals belonging to the same pure strain.

Accidental influences increasing the death rate quite without relation to conjugation are so numerous, especially in experiments carried on under unfavorable conditions, that here the principle is particularly important that one extensive experiment carried through under ideal conditions, without extrinsic disturbing

factors, gives a truer insight than many imperfect experiments. Such a model experiment is, for present purposes, Experiment 1. In this experiment none of the lines descended from non-conjugants (split pairs) died out during the five weeks of the experiment. Of the lines descended from conjugants, though kept under exactly the same conditions, 38 per cent died out during the same period. In the other experiments some of both sets died, though as a rule with more deaths among the lines derived from conjugants.

In two out of the ten or twelve experiments in which this matter was tested the usual relation was reversed; in both these experiments we are dealing with exceptional conditions. In Experiment 3 the temperature was abnormally high, standing much of the time above 32°C. I have found by long experience in Baltimore that it is not possible to carry on slide cultures of *Paramecium* at such a temperature; from whatever source, the animals rapidly die out. Thus, in this experiment the conditions were so bad that a large proportion of both conjugants and non-conjugants died within the four days of the experiment. But under these conditions the lines descended from non-conjugants died out still more rapidly than those descended from conjugants. Of the former 68.6 per cent died in the four days; of the latter, but 23.4 per cent. The difference seemed clearly due to the furious rapidity at which the non-conjugants multiplied, while the conjugants (as is the rule after conjugation) divided but slowly. There is little doubt but that under usual temperature conditions the advantage would have been, in this case also, with the non-conjugants. The fact, however, that conjugation may be physiologically advantageous under very exceptional conditions is an important one.

The other case in which the advantage was with the conjugants is Experiment 12. Here we are dealing with a much depressed stock, in which reproduction is slow and mortality high before conjugation. Such a stock can hardly be induced to conjugate; so that but three pairs could be obtained from it. With the six lines derived from these were compared ten lines derived from non-conjugants of the same culture. It is important to note that the latter were not split pairs; in other words, they were not

ready to conjugate (as were the non-conjugants in most of our experiments). Such split pairs could not be had in the present case. Of the six conjugant lines, four (or 66 per cent) died out; but of the non-conjugant lines *all* died.

As set forth in the account of this experiment, the ground for this difference seems to lie in the fact that a certain vigor and power of multiplication are a prerequisite for conjugation; so that in the only three pairs that conjugated are included the only vigorous members of the stock; the others died for the same reason that they did not conjugate.

What are the grounds for the greater mortality of the conjugants, found in the great majority of cases? Two possible grounds occur to one:

1. Conjugation involves extremely complex and delicate cytological processes. It seems possible that these processes are easily diverted into abnormal courses, resulting in abnormalities and death.

2. Conjugation, like fertilization, is a process of uniting diverse germ plasms; of producing new combinations of germ plasm (evidence bearing directly on this will be given in a paper to follow the present one). Possibly some of these combinations are incompatible; or produce results not fitted for continued existence under the conditions.

EFFECT OF CONJUGATION ON ABNORMALITIES

Throughout the experiments it was observed that frequent abnormalities of all sorts occur among the descendants of the conjugants, while among the descendants of non-conjugants such are relatively rare. The grounds just set forth as possibly accounting for the greater death rate of the conjugants, perhaps play a part also in the production of abnormalities.

EFFECT OF CONJUGATION ON VARIATION

The most striking effect of conjugation that appears in comparing the conjugants and non-conjugants, is the great *increase in variability* in the rate of reproduction. In all of the experiments the conjugants are much more variable in this respect than

are the non-conjugants. It will be well to summarize here the coefficients of variation for conjugants and non-conjugants in certain periods of each experiment. This is done in table 26.

As the coefficient of variation was computed for several different periods in most of the experiments, it hardly appears practicable to bring together in table 26 all the coefficients given in the tables of the body of the paper. I have therefore selected the longer periods, with some typical partial periods.

As table 26 shows, the difference in variability between conjugants and non-conjugants is not a slight one, but is very great. The coefficient of variation averages at least twice as great for the conjugants, and in some of the cases given in table 26 it is three or four times as great. There can be no question but that *conjugation increases greatly the variation in rate of reproduction*, both in wild cultures and in pure races.

If in place of studying the variation relative to the mean rate, as shown by the coefficient of variation, we examine the absolute amount of the variation, as shown by the standard deviation, we

TABLE 26

Comparative variability, as measured by the coefficient of variation, for the lines descended from conjugants and for those descended from non-conjugants, in numbers of fissions to a given period; for various experiments of the present paper.

EXPERIMENT	TIME	CONJUGANTS (PAIRS)		NON-CONJUGANTS (SPLIT PAIRS)	
		Number of lines	Coefficient	Number of lines	Coefficient
(Wild Cultures)					
1	First 2 weeks.....	56	53.103 \pm 4.232	59	12.975 \pm 0.819
1	Second 2 weeks.....	42	42.870 \pm 3.689	59	27.743 \pm 1.850
2	First 2 weeks.....	34	32.011 \pm 2.874	51	21.350 \pm 1.489
2	Second 2 weeks.....	19	46.944 \pm 6.166	26	22.847 \pm 2.246
3	Four days.....	36	29.369 \pm 2.528	16	12.756 \pm 1.546
(Pure strains)					
4	Twenty days.....	17	30.828 \pm 3.890	18	19.792 \pm 2.310
5	Six days.....	17	42.762 \pm 5.781	20	14.382 \pm 1.565
13a	Seven days.....	58	40.350 \pm 2.910	82	17.550 \pm 0.953
13b	18 Days, December 8-26.....	14	43.893 \pm 5.976	16	18.263 \pm 2.405
13b	January 2-14.....	14	16.878 \pm 2.069	16	12.832 \pm 1.662
13b	January 30-February 12.....	12	37.899 \pm 5.478	14	19.735 \pm 2.821
13b	December 8-January 29.....	16	21.675 \pm 2.715	14	16.428 \pm 2.150
15	First 12 (11) days.....	78	19.501 \pm 1.065	158	11.789 \pm 0.453
15	Later September 8-16.....	83	41.074 \pm 2.485	171	8.585 \pm 0.315
15	24 (21) days.....	69	28.650 \pm 1.734	145	7.122 \pm 0.284

shall come to the same result, finding that in every case the variations are not only relatively, but absolutely, greater among the conjugants. The standard deviations corresponding to the coefficients of variation given in table 26 will be found in the tables included in the body of the paper, under the different experiments.

In just what way is the variation increased in the conjugants? That is, do we find that after conjugation there are more specimens with a lower rate of fission, or with a higher rate of fission, or with both? What is the nature of the distribution of the fission rates in each case?

The fact that the mean rate is lower for the conjugants would cause us to suspect that the increase in variation is at least partly due to a decrease in the rate of fission of some of the lines, while others remain high. Examinations of the data shows that this is largely true. To bring out this point, it will be well to note the comparative range of variation in number of fissions, for the conjugants and non-conjugants, in the various experiments. This is exhibited in table 27. In this table are included the number of fissions for only the lines that lived through the period specified.

As table 27 shows, at the lower extremity the conjugant lines range much farther than the non-conjugant lines; in every case the lower extreme for the conjugants is below that for the non-conjugants, and in many cases the difference is very considerable. At the other extremity of the range no such great difference is found. The maximum is, as a rule, higher for the non-conjugants, but this is not invariable; in some cases the maximum for the conjugants is equal to that for the non-conjugants; or even a little greater.

It appears therefore that conjugation increases the variation mainly toward the lower extremity of the range; it produces many lines whose rate of fission is lower than that for the non-conjugants, while others remain high. But even in the middle regions of the range, the conjugant lines are less heaped up about the mean than the non-conjugants. These peculiarities may be illustrated by examination of the distribution of the variations in the experiments with larger numbers, as given in table 28, for Experiments

1 and 15. With this table may also be examined tables 9, 10, 11 and 20, which give the same data for various other experiments.

The spreading out toward the lower end of the range in the lines descended from pairs is very striking in table 28. In some cases it appears that the lines descended from conjugants tend to differentiate into two groups, one with a low fission rate, the other with a higher one. This is particularly notable in the data for Experiment 15, in table 28, but is observable also in Experiment 1.

TABLE 27

Comparative range of variation in lines descended from conjugants, and in those descended from non-conjugants, for fissions in a given period.

EXPERIMENT	TIME	CONJUGANT		NON-CONJUGANTS	
		Minimum number of fissions	Maximum number of fissions	Minimum	Maximum
(Wild cultures)					
1	First 2 weeks.....	0	12	7	15
1	Second 2 weeks.....	0	16	6	20
1	Total 4 weeks.....	9	28	13	35
2	First 2 weeks.....	0	11	7	15
2	Second 2 weeks.....	0	13	6	11
2	Eight weeks.....	25	38	37	47
3	Four days.....	2	10	8	13
(Pure strains)					
4	Twenty days.....	1	17	6	17
5	Six days.....	0	6	4	6
7	Nine days.....	10	14	11	16
9	Sixteen days	19	22	23	27
13a	Seven days.....	0	10	4	10
13b	December 8-16.....	4	21	12	21
13b	January 2-14.....	7	12	8	12
13b	January 30-February 12	3	15	9	14
13b	December 8-January 29	21	46	24	50
15	12 (11) days.....	6	16	8	15
15	September 8-16.....	1	17	8	15
15	24 (21) days ¹	{ 10 35 (8.075 30.06)		18	28

¹ In the last entry, for experiment 15, the time for the conjugant lines is 24 days, that for the non-conjugants but 21. If we reduce by one-eighth the fissions for the conjugants, they will then be comparable with those for the non-conjugants; this gives the figures shown for this case in parenthesis.

It will be observed from table 28 that even in that part of the range where the non-conjugant lines are found, the conjugant figures are much less heaped up near the mean than are those for the non-conjugants. This shows clearly that the greater variability of the conjugants is not due alone to an extension of the range of variation toward the lower end; but also to a scattering of those lying near the mean. If, for example, we omit in Experiment 15 all the conjugant lines lying lower (in table 28) than any of the non-conjugant lines, we still find the variation for the conjugants to be much greater than that for the non-conjugants. In Experiment 15, making the omission mentioned, the coefficient of variation for the conjugants would be 16.776, as compared with but 8.585 for the non-conjugants.

Conjugation, then, increases variability in reproductive power. The next question is: Are these differences inherited, so that in this way differentiated races are produced? To this question were mainly dedicated Experiments 13 and 15, and, as the account given in the text shows, *the differences thus produced are inherited*. In wild cultures, such as that of Experiment 1, this question cannot be answered so clearly, since the differences in fission rate existing before conjugation are likewise inherited and the effect of conjugation is only to increase the number and extent of these

TABLE 28

Distribution of the number of fissions for the lines of descendants of conjugants, as compared with those from non-conjugants, for certain periods in experiments 1 and 15. (The table shows, for example, that in the first two weeks of experiment 1, three of the conjugant lines did not divide: four divided once, etc.)

	NUMBER OF FISSIONS																	TOTAL LINES	
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		17
Experiment 1—																			
First two weeks:																			
Pairs.....	3	4	1	2		1	3	3	6	4	13	11	2	1					56
Split pairs.....								2		1	2	3	15	18	13	5			59
Second two weeks																			
Pairs.....	2		1	2		2	2	2	4	5	7	2	4	2	3	2	2		42
Split pairs.....							2	3	3	3	6	5	11	4	5	7	3	3	59
Experiment 15—																			
September 8-16																			
Pairs.....		6	5	2	1				2	2		2	5	9	13	20	12	4	83
Split pairs.....									1		6	13	52	68	27	4			171

inherited differences. But working with a pure race, as in Experiments 13 and 15, it is found (1) that differences in rate of fission among those that have not conjugated since they were derived from a single parent are not inherited (unless possibly certain differences of a minimal character are to be excepted; differences of an order of magnitude far below those with which we are dealing); (2) that conjugation among the members of such a pure race does result in differentiations that are inherited, so that from a race homogeneous with respect to fission rate, we get many races, differing in their rates. The hereditary differences thus produced are not small and inconstant but so decided as to give coefficients of correlation up to 0.9 between earlier and later generations, in spite of fluctuations due to environmental differences.

To what is due the production of inherited differentiations by conjugation? Here for the present we can only speculate. It would seem probable that we have before us something of the process that we see in Mendelian inheritance. If the members of a culture differ in their germinal make-up, conjugation among them would produce many new combinations of germinal characteristics. The fact that we find such heritable differentiations produced by conjugation among the members of the same pure race would be accounted for if the members of the race are heterozygotic, although all alike in germinal composition. Interconjugation among such similar heterozygotes would, on Mendelian principles, produce many new combinations of germinal constituents, just as happens in the self-fertilization of higher organisms.

In connection with such a view of the matter, it needs to be recalled, however, that in our Experiment 13 we were dealing with a stock that had gone through eight successive self-fertilizations, the stock being derived, after each of these, from a single ex-conjugant. Such a series of eight self-fertilizations would, as set forth in the account of Experiment 13, go far in getting rid of heterozygotism, unless the character we are studying depends on a very large number of independent factors. In that stock we nevertheless found that inherited differentiations as to fission rate were produced by conjugation. This may indicate that Men-

delian recombination is not the whole secret of the matter; it does not, however, demonstrate this.

In a previous paper ('11), I have shown that conjugation likewise increases variability in size. In the account of Experiment 9, of the present paper, some data are given indicating that the size differentiations so produced are likewise inherited. But the results there given are by no means conclusive, the matter requires further study.

It seems best to reserve for a later paper on inheritance a comparative review of what is known as to the production of variation by conjugation in other organisms, with the various theories that have been held.

CONJUGATION AND BIPARENTAL INHERITANCE

In a paper to be published at once, by the present author and K. S. Lashley, it will be shown that conjugation results in inheritance from both the parents that enter into the pair. All details are reserved for the paper referred to; the matter is mentioned here merely to complete the outline as to effects of conjugation.

CONJUGATION AND THE THEORY OF REJUVENESCENCE

The chief positive results from the present investigation are: (1) that conjugation increases variation, giving rise to heritable differentiations; (2) that it results in biparental inheritance (to be taken up in a separate paper); (3) that the fission rate is lower after conjugation; (4) that the mortality is as a rule higher, and abnormalities are more common, among the descendants of conjugants than among those of non-conjugants.

What is the relation of these results to the theory that conjugation produces rejuvenescence?

A number of diverse things have been included under rejuvenescence, the theory meaning for some authors one combination of these, for others another combination. The main points included appear to be the following:

1. The structural changes—the replacement of the old macronucleus by a new structure derived from the micronuclei—has

sometimes been held to constitute a visible rejuvenescence, a rejuvenescence of the macronucleus. This in some forms is accompanied by a renewal of other structures, as for example, of the bodily appendages in the *Hypotricha*. Engelmann ('76) emphasized these changes as constituting in themselves "ein wahren Verjüngung" (p. 629).

This actual replacement of old structures by new no one will of course deny, and it seems not inappropriate to call it a rejuvenescence, if we mean by this word nothing more than these observed facts.

2. But the theory has as a rule gone far beyond these observed facts. Thus, Maupas says, after a statement of these structural changes:

Ce nouveau appareil nucléaire agit sur tout l'organisme, auquel il appartient, comme une sorte de ferment régénérateur, lui restituant, sous leur forme parfaite et intégrale, toutes les énergies vitales caractéristiques de l'espèce. Cet être se trouve donc rajeuni dans le sens littéral et absolu du mot. Il peut dès lors redevenir le progéniteur d'un nouveau cycle de multiplications agames, dont toutes les générations successives seront douées des mêmes facultés rajeunies, jusqu'à ce que celles-ci s'usent et s'affaiblissent peu à peu, par leur exercice même, et en arrivant ainsi à ressentir le besoin réparateur d'une nouvelle période d'activité fécondatrice ('89, p. 434).

Now, in the passage, we have quoted, Maupas evidently affirms certain things that by no means follow from the structural changes observed, but can only be demonstrated by the results of experimentation. We shall have to inquire how far these have been thus demonstrated. But before doing this, we must proceed to one farther development of the theory.

3. As we have seen (page 357), Maupas did not hold that the vigor and rate of reproduction are increased by conjugation, although such a general statement as the one above quoted would seem to imply that this is true. This idea has, however, been held by many as a fundamental part of the theory of rejuvenescence. The rate of reproduction has been held to become less and less as the number of vegetative generations increases, until by a new conjugation it is brought back again to its original level (see the quotations on page 356).

This part of the theory of rejuvenescence which holds that the vigor of reproduction is increased by conjugation appears to be definitely a mistake, for the infusoria, as we have already shown (page 359). We shall therefore consider it no farther.

The experimental results of the present paper of course do not alter the facts as to the 'structural rejuvenescence' if one desires so to call it. Certain points are worthy of notice in this connection.

1. So far as the 'rejuvenescence' or renewal of structures other than the macronucleus is concerned (locomotor organs, et cetera), this takes place equally in vegetative reproduction. It furnishes therefore no foundation for a theory that conjugation is in any special way a rejuvenating process.

2. The replacement of the macronucleus by parts of the micronuclei of the two individuals of the pair is of course thoroughly in consonance with the results of the present study, furnishing not the slightest difficulty for interpretation. The micronuclei are to be conceived as corresponding to the nuclear apparatus of the germ cells of higher organisms, each one consisting of a certain combination of 'determinants' or 'genes.' When the macronucleus is replaced by parts of two micronuclei, a new combination of 'determinants' is thus produced; the progeny may therefore differ from the parents. In other words, 'variation' is induced in conjugation—through the production of many new combinations, in different cases. Again, since the new macronucleus is produced by the union of parts from two diverse individuals, the progeny may inherit from these two; in other words, conjugation results in biparental inheritance, as we have actually found to be the case.

Now, it is a priori not impossible that the effects of the renewal of the macronucleus are completed in those two results; it is not a priori certain that the new macronucleus must otherwise function any better than the old one.

We must therefore inquire as to the experimental ground for the assertion made in the quotation given above from Maupas, to the effect that this new apparatus acts on the entire organism as a

sort of regenerating ferment, restoring all its vital energies, et cetera.

The grounds for this view have consisted, almost exclusively, not in actual observation of any such rejuvenizing action by conjugation, but in the observation that during vegetative reproduction under experimental conditions the organisms become depressed, degenerate, and finally die. From this it was concluded that conjugation must be what remedies this.

This line of argument has, however, quite lost its force, in view of the modern work of Calkins, Enriques, Woodruff, and others. These authors' results demonstrate that the very limited periods within which Maupas observed degeneration has no significance for the question as to whether degeneration is an inevitable consequence of continued reproduction without conjugation, for they kept vegetative reproduction in progress for periods many times as long as those which Maupas found to result in degeneration. The work of Woodruff, in particular, seems to show that *Paramecium* may be kept multiplying vegetatively for an indefinite period. Furthermore, the work of Enriques and of Woodruff has shown to what the degeneration observed by Maupas was due. Under proper nutritive and chemical conditions no such degeneration appears.

It is not necessary to review in detail this vast subject, but there will hardly be any dissent from the statement that the modern work has largely, if not entirely, deprived of its force this argument for the necessity of conjugation.

All the more therefore we are driven to examine the direct evidence as to the rejuvenating effect of conjugation. And in doing so, we must reflect that if the argument above mentioned were valid, there should be no difficulty in observing experimentally the rejuvenating effect; so that a fortiori we must demand what this direct evidence is.

In reading Maupas' great works ('88, '89) in search of this direct evidence for a rejuvenating effect of conjugation, one is astonished at the way it eludes one at every step. Most of the actual observations that bear on the matter at all, seem indeed

opposed to the rejuvenating action of conjugation. Maupas demonstrated, as we have seen, by extended experimentation, that conjugation is not followed by an increase in the vigor of multiplication. He found, in repeated observations, that conjugation within his degenerating stocks did not help them, but attributed this to their being closely related. But he observed further that *when the depressed stocks that interconjugated were not related, they still died after conjugation*, so that conjugation did not remedy degeneration in the one case or the other ('89, p. 409). He found that conjugation is often sterile (followed by death) in *wild* cultures of *Stylonychia* ('89, p. 331). He found that ex-conjugants of *Spirostomum*, *Climacostomum* and *Didinium* did not reproduce farther ('89, pp. 277, 295, 297). In *Leucophrys* a large proportion of the conjugants die ('89, p. 254-255). He found that in some cases a second conjugation follows a first one after but a few generations (*Leucophrys*, '89, p. 409). He found that animals which are ready to conjugate may be prevented, and they will then continue to multiply with uninterrupted vigor ('89, p. 306). All these observations speak against rather than for the idea of a regular cycle of vegetative reproduction, resulting in degeneration, and requiring conjugation at a certain stage, this remedying the degeneration.

Has Maupas absolutely no evidence that conjugation rejuvenates? He seems possibly to have held that the following fact is evidence of this effect. In his long continued cultures, he found that when the animals derived from a single parent interconjugated, they later died. It is notable that this result has not been confirmed by later investigation, and Maupas himself noted certain exceptions. But Maupas found that when he mixed individuals from different cultures, the pairs were fertile (provided both did not belong to degenerated cultures). It would appear that Maupas supposed that rejuvenescence had taken place in these cases. But of course there is absolutely no evidence that such has occurred, unless it is shown experimentally that the ex-conjugants are more vigorous and propagate longer than similar parents who did not conjugate. In view of the

results given in the present paper, where the reverse is shown to be the rule, it is clear that these observations of Maupas do not touch the matter at all.

One single case only Maupas has which makes even an approach to the form of this necessary demonstration, and this, as we shall see, really gives no evidence at all. This is the case of one of his cultures of *Stylonychia pustulata* ('88, pp. 196-201). A line of propagation was begun with a single individual, November 1, 1885. This line died out on March 26, 1886, after 215 generations. On February 22 a single specimen of the 156th generation was taken from this line and allowed to conjugate with an individual from outside. Maupas tells us on page 323 of his paper of '89 that these individuals from outside, which he mixed with those from the long-continued cultures "were taken at hazard in my small aquaria." Thus such an individual had not been living under the peculiar conditions of these experiments. Derived from this pair a new line of propagation was continued for 316 generations (till July 10, 1886), while the old line from which one of these ex-conjugants came, died out after but 59 generations more.

Now, the work of Enriques, Woodruff, Baitsell ('12), et cetera, has shown that the conditions with which Maupas worked result after a time in depression of the vital functions, but that animals kept under more favorable conditions do not show such depression, even though they have lived as long without conjugation as the depressed race. The depression is due to the conditions, not to lack of conjugation. What Maupas did was to take from outside a fresh, vigorous specimen, and mate it with one of these depressed ones. He then found that the progeny were vigorous. *He does not note whether the line of progeny he used came from the depressed member of the pair, or from the vigorous one;* although this is an absolutely essential point for determining whether the depressed stock was rejuvenated even by conjugation with a vigorous one. The probability is strong that the new line of propagation came from the new, vigorous individual. But such an individual would have given an equally long series of vegetative propagations if it had not been mated at the beginning. Its

vigor was due to the fact that it had been living under favorable conditions, not to conjugation.

There is absolutely nothing in this experiment to demonstrate that a partially exhausted race is rejuvenated by conjugation. A real test would be the following: Two unrelated lines should be allowed to multiply till both become depressed. Then they should be allowed to conjugate, to determine whether the conjugation remedies the depression. It will manifestly not do, in testing the question whether conjugation remedies depression, to take a vigorous, undepressed specimen as one member of the pair. According to the cyclical theories, *all* lines of propagation become depressed after a series of vegetative reproductions, so that if conjugation is to maintain the race, it must be effective when it occurs between two lines, both of which are depressed.

Now, as we have briefly mentioned above, *Maupas performed this crucial experiment*. He kept lines of propagation of *Stylonychia* of diverse origin till they became depressed, then allowed them to conjugate one with another. This fact is briefly set forth on page 409 of his paper of 1889. Such cross-conjugation of two diverse lines *did not result in rejuvenescence*; the animals died just as happened when the two members of a pair came from the same parents. Speaking of sterile conjugations, Maupas says, "Elles s'effectuent, en effet, aussi bien entre individus appartenant à un même cycle ou proches parents, qu'entre individus étrangers l'un à l'autre et provenant de cycles différents" ('89, p. 409).

Anyone who goes critically through the 480 pages of Maupas' two great papers for the purpose of finding out what evidence there is that conjugation rejuvenates, will, I believe, be forced, as I have been, to realize that they contain no evidence for this whatever, although they do contain evidence against it. Maupas' conclusion was evidently due to the supposed theoretical necessity for something to remedy the degeneration induced by long vegetative reproduction under the conditions of his experiments. *All that his experiments show is that long continued propagation under the given conditions results in injury to the stock—and this equally whether there is or is not conjugation within the stocks, or between*

two such stocks of diverse origin. There is thus not even any indirect evidence that conjugation rejuvenates, since the stocks that conjugated underwent the same fate as those that did not.

So far as I have been able to discover, there is no experimental evidence from any other source that conjugation rejuvenates. In Miss Cull's paper entitled "Rejuvenescence as the result of conjugation" ('07), the evidence consists merely in showing that a considerable fraction of those that had conjugated continued thereafter to multiply. But control experiments show, as set forth in the body of the present paper, that they would have continued equally if they had not conjugated; in fact a larger proportion would have continued to multiply if they had not been allowed to conjugate. There is thus in these results no evidence of rejuvenescence through conjugation; and this must be said of all observations which merely show that some of the ex-conjugants continue to multiply. Control experiments with animals prevented from conjugating are necessary for a correct understanding of the results.

In the long series of studies set forth in the present paper as Experiments 5 to 14, the effects of conjugation were studied when one division of a race is allowed to conjugate frequently, while another is kept from conjugating; also the effects of conjugation in a race that is actually depressed. As to the first point, the animals that did not conjugate were found throughout to be more vigorous than those that conjugated frequently.

With regard to the effects of conjugation in a depressed race, it is to be recalled that Maupas had repeatedly tried this experiment, finding always that conjugation has no beneficial effect under such conditions. The question might then be regarded as settled, since there is no expectation of beneficial effect even accepting the views of the great upholder of the theory of rejuvenescence; positive results would be directly opposed to the experimental results of Maupas.

Yet it was in one of these experiments alone that any result was reached that could possibly lend themselves to an attempt to maintain that conjugation has a beneficial effect on vigor and vitality. In Experiment 12 the stock was so depressed that it

multiplied scarcely at all, and the mortality was high. It was almost impossible to get conjugation among its members, since a prerequisite to conjugation is a period of rapid multiplication. The necessary conditions were fulfilled only for three pairs. From these, six ex-conjugants were obtained. The six lines of propagation derived from these were compared with ten lines from individuals that did not conjugate (and did not attempt to do so).

There was no general rejuvenescence due to conjugation. Three of the six conjugant lines died out within a week, and a fourth a little later; so that two-thirds of the conjugant lines were dead. But two continued to multiply. But in the mean time *all of the ten non-conjugant lines died out.*

What has happened here? We can hardly speak of rejuvenescence where two-thirds of the ex-conjugants die out. The survival of some of the conjugants may have been due to the greater vigor that was a prerequisite to their conjugation, the lack of which caused the others not to conjugate. Aside from this we can only say that the results of conjugation were here the same as usual; *it induced variation in the reproductive power.* As always, some lines derived from the conjugants had a low reproductive power and died at once. Two out of the six had greater reproductive power; they therefore continued to multiply. In the meantime, the uniform non-conjugants, retaining the original depressed condition, all died out after a short time.

This experiment therefore gives, in fact, the same result as all the others, an increase of variation as a result of conjugation. It differs from the others merely in the fact that in two of the six cases the extremes of variation reached a higher level than that which characterized the animals before conjugation. The same result is reached in Experiment 15, where the conjugants at the upper extreme of the range exceeded in their rate of reproduction the uniform non-conjugants. But in this latter case there is no temptation to speak of rejuvenescence, since the non-conjugants still continue to multiply vigorously.

Thus, under exceptional conditions the production of variation by conjugation results in preserving some representatives of a stock which would otherwise die out completely.

The results of the present investigation on the effect of conjugation need to be considered in connection with the results of the investigations of Calkins, Enriques, Woodruff, and others, on the results of long continued vegetative reproduction. The two lines of work complement each other and lead to harmonious and definite conclusions. In a recent brief paper ('12 a) I have reviewed the two in their relation to each other. Here I shall not attempt to review the work on vegetative reproduction but merely to summarize the common result of both lines of work.

GENERAL CONCLUSION

Comparing conjugation with the fertilization of higher animals, we find the following to be the state of the case:

In higher animals fertilization has two diverse effects, which recent investigation, particularly that of Loeb and his associates, has clearly disentangled. (1) On the one hand, it initiates development; it prevents the egg from dying, as it would do if not fertilized. This function of fertilization is the one that is replaced by the processes which induce artificial parthenogenesis. (2) But, secondly, fertilization brings about in some way inheritance from two parents. When there is inheritance from but one parent, the inheritance is as it were complete; the child as a rule resembles its parent in all hereditary characteristics; this is the result of the so-called 'pure line' work. But when we have biparental inheritance, a great number of different combinations of the characteristics of the two parents are produced, so that the process of fertilization is one that in this respect completely alters the face of organic nature, producing infinite variety in place of relative uniformity.

These two functions of fertilization, the initiation of development, on the one hand, and the production of inheritance from two parents, on the other, are logically independent; they might conceivably be performed at different times and by different mechanisms. The fact that in many organisms the same mechanism that brings about biparental inheritance is likewise the one that initiates development might from certain points of view be

called an adaptation. Its result is to insure that in *all* organisms that develop there shall be inheritance from two parents, not from one. In the work on artificial parthenogenesis these two functions have been separated experimentally; the initiation of development takes place alone.

Now, in endeavoring to understand conjugation, attention has been given hitherto almost exclusively to the first of these two functions. It was held that the function of conjugation must be to make possible life and development where it was otherwise impossible, just as fertilization arouses the egg to further life and development. But it turns out that in the infusoria conjugation, instead of having this one of the two functions of fertilization, has the other. The two functions are in the infusorian separated, just as they are in artificial parthenogenesis, but it is the second, not the first, that we have before us. Conjugation is not necessary in order that life and reproduction shall continue; they continue without it. There is no evidence that conjugation in the infusoria increases the reproductive power, or rejuvenates the organism physiologically in any way.

But the life which thus continues is uniform and unchanging. To give biparental inheritance, with varying mixtures of the characteristics of the two parents; to produce these new combinations in great variety, conjugation is necessary. And when this happens under such conditions that the original combinations were not adapted to survival, then some of the new combinations produced often are adapted to the conditions; conjugation then results in a survival of an organism that would have been completely destroyed without it. It is most interesting in this connection to observe that conjugation is usually induced by an unfavorable change of conditions, a change of such a nature that the organisms begin to decline. Thereupon conjugation occurs, so that new combinations are produced, adapted to varied conditions, some of which may survive.

Thus the whole series of investigations on vegetative reproduction and on conjugation leads to a unified result, and one that is in consonance with what we observe in higher animals.

Our main results may then be summed up as follows: So far as physiological effects are concerned, conjugation does not produce rejuvenescence, for after conjugation most of the animals are less vigorous than before. *What conjugation does is to bring about new combinations of germ plasm, just as is done in the sexual reproduction of higher animals. One result of this is to produce biparental inheritance; another is to give origin to many variations, in the sense of inherited differentiations between different strains. Some of the new combinations are better adapted to the existing conditions than others; these survive while the others die out.*

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APPENDIX: FUNDAMENTAL TABLES

TABLE 29

Experiment 1. Paramecium caudatum. Number of fissions per week in the 61 lines derived from conjugants, and in the 59 lines derived from those that have not conjugated. May 4 to June 7, 1909. Numbers in parenthesis indicate that the line in question had died out before the end of the experiment. (d = died out, during the week indicated.)

Pairs (conjugation consummated)

PAIR	INDIVIDUAL	WEEK					TOTAL	PAIR	INDIVIDUAL	WEEK					TOTAL	PAIR	INDIVIDUAL	WEEK					TOTAL
		1	2	3	4	5				1	2	3	4	5				1	2	3	4	5	
1	a	0	1	d			(1)	12	a	6	5	3	4	2	20	23	a	4	5	5	2	d	(16)
	b	1	2	d			(3)		b	4	7	7	5	2	25		b	5	6	4	1	2	18
2	a	5	6	8	5	1	25	13	a	5	7	7	5	4	28	24	a	4	6	6	2	2	20
	b	5	5	6	4	1d	(21)		b	1	6	6	3	1	17		b	1	0	d			(1)
3	a	4	6	8	6	2	26	14	a	4	7	6	6	3	26	25	a	4	4	1d			(9)
	b	2	6	1	1	d	(10)		b	4	6	7	3	3	23		b	4	6	4	5	0	19
4	a	1	0	0	d		(1)	15	a	1	0	1	d		(2)	26	a	3	6	5	6	2	22
	b	5	5	5	4	2	21		b	2	5	d			(7)		b	3	7	7	8	5	30
5	a	0	2	0	d		(2)	16	a	3	2d				(5)	27	a	0	d				(0)
	b	5	4	6	4	2	21		b	3	2	3	3	3	14		b	4	4d				(8)
6	a	6	5	6	4	7	28	17	a	5	7	7	2	3	24	28	a	4	4	d			(8)
	b	3	5	3	0	6	17		b	1	d				(1)		b	4	6	3d			(13)
7	a	2	1	3	3	4	13	18	a	5	6	9	7	2	29	29	a	4	7	4	6	5	26
	b	4	2	d			(6)		b	3	5	5	4	1	18		b	4	8	9	5	4	30
8	a	0	6	8	4	3	21	19	a	5	6	0	0	2	13	30	a	3	5	7	6	3	24
	b	4	6	5	5	3	23		b	5	5	0	0	2	12		b	3	6	4	4	3	20
9	a	0	0	d			(0)	20	a	4	2	2	1	1	10	31		0	0	d			(0)
	b	0	0	d			(0)		b	3	4	3	2	2	14								
10	a	4	d				(4)	21	a	5	5	9	5	2	26								
	b	5	6	2	6	3	22		b	5	8	8	7	4	32								
11	a	4	6	7	3	d	(20)	22	a	5	6	4	4	1	20								
	b	4	5	6	5	3	23		b	3	7	9	7	4	30								

TABLE 29 (CONTINUED)
Split pairs (conjugation not consummated)

SPLIT PAIR	INDIVIDUAL	WEEK					TOTAL	SPLIT PAIR	INDIVIDUAL	WEEK					TOTAL	SPLIT PAIR	INDIVIDUAL	WEEK					TOTAL
		1	2	3	4	5				1	2	3	4	5				1	2	3	4	5	
1	a	6	4	7	6	5	28	11	a	8	6	10	7	4	35	21	a	7	6	5	3	4	25
	b	6	6	9	7	3	31		b	8	6	6	5	4	29		b	8	7	6	1	1	23
2	a	8	6	6	6	2	28	12	a	7	6	3	4	1	21	22	a	6	7	7	2	3	25
	b	7	7	9	6	3	32		b	7	7	3	5	2	24		b	7	6	2	4	3	22
3	a	7	6	7	5	2	27	13	a	4	7	8	7	4	30	23	a	8	7	10	7	2	34
	b	7	5	6	6	2	26		b	7	7	7	5	1	27		b	7	7	5	7	3	29
4	a	7	4	6	4	1	22	14	a	7	8	2	4	2	23	24	a	7	7	6	4	3	27
	b	7	6	8	7	5	33		b	5	7	6	5	3	26		b	6	6	5	4	3	24
5	a	7	6	6	6	4	29	15	a	5	7	8	5	4	29	25		7	5	10	5	1	28
	b	8	4	5	5	5	27		b	5	7	3	6	2	23	26		4	3	3	3	1	14
6	a	7	5	9	6	3	30	16	a	6	4	5	3	2	20	27		7	6	8	3	3	27
	b	8	6	10	7	3	34		b	7	6	4	3	3	23	28		9	5	8	8	2	32
7	a	7	5	9	5	0	26	17	a	7	6	7	4	0	24	29		7	6	5	5	4	27
	b	7	6	8	6	1	28		b	7	6	9	6	2	30	30		6	6	5	5	3	25
8	a	7	6	7	5	2	27	18	a	8	6	9	6	1	30	31		8	7	8	6	4	33
	b	7	5	7	5	2	26		b	7	5	8	4	2	26	32		6	7	1	5	3	22
9	a	7	5	9	5	1	27	19	a	6	7	6	7	4	30	33		5	4	4	2	2	17
	b	8	6	8	6	3	31		b	5	8	9	7	3	32	34		3	4	8	5	3	23
10	a	6	6	7	5	2	26	20	a	7	6	7	4	6	30	35		8	6	7	5	3	29
	b	8	7	12	8	3	38		b	6	5	6	4	0	21								

TABLE 30

Experiment 2. Paramecium caudatum. April 10 to June 4, 1909. Number of fissions, first two weeks, for those that have conjugated (pairs) and those that have not ('split pairs' and 'free'). (d = died.) Numbers in parenthesis give total fissions for those that died before the end of the period.

Pairs

PAIR	INDIVIDUAL	WEEK		TOTAL	PAIR	INDIVIDUAL	WEEK		TOTAL	PAIR	INDIVIDUAL	WEEK		TOTAL	PAIR	INDIVIDUAL	WEEK		TOTAL
		1	2				1	2				1	2				1	2	
1	a	4	2	6	9	a	2	1	3	17	a	3	1	4	25	a	4	2d	(6)
	b	0	0	0		b	3	2	5		b	6	1d	(7)		b	5	2	7
2	a	6	3	9	10	a	5	1	6	18	a	4	3	7	26	a	d	(0)	
	b	6	3	9		b	4	1	5		b	4	3d	(7)		b	3	2d	(5)
3	a	d		(0)	11	a	5	d	(5)	19	a	d		(0)	27	a	1	d	(1)
	b	3	4	7		b	5	2	7		b	3	2	5		b	4	1d	(5)
4	a	3	3	6	12	a	4	2	6	20	a	3	2	5	28	a	6	d	(6)
	b	4	2	6		b	5	2	7		b	4	0	4		b	4	1d	(5)
5	a	4	1	5	13	a	1	d	(1)	21	a	3	1d	(4)	29	a	8	1d	(9)
	b	4	2	6		b	3	1	4		b	0	d	(0)		b	lost		
6	a	4	1	5	14	a	6	2	8	22	a	6	5	11					
	b	3	2d	(5)		b	d		(0)		b	6	2d	(8)					
7	a	3	2	5	15	a	5	2d	(7)	23	a	6	2	8					
	b	d		(0)		b	4	3	7		b	5	4	9					
8	a	1d		(1)	16	a	d		(0)	24	a	5	2	7					
	b	5	2	7		b	5	2	7		b	5	2	7					

TABLE 30 (CONTINUED)

Split pairs

SPLIT PAIR	INDIVIDUAL	WEEK		TOTAL	SPLIT PAIR	INDIVIDUAL	WEEK		TOTAL	SPLIT PAIR	INDIVIDUAL	WEEK		TOTAL	SPLIT PAIR	INDIVIDUAL	WEEK		TOTAL
		1	2				1	2				1	2				1	2	
1	a	6	4	10	6	a	8	4	12	11	a	6	2d	(8)	16	a	8	2	10
	b	8	4	12		b	9	5	14		b	7	1	8		b	8	4	12
2	a	9	2d	(11)	7	a	4	3	7	12	a	6	2	8	17	a	7	2	9
	b	8	2	10		b	7	1d	(8)		b	8	d	(8)		b	7	1d	(8)
3	a	11	4	15	8	a	d	(0)	(8)	13	a	7	1d	(8)	18	a	4	d	(4)
	b	6	3	9		b	9	4	13		b	8	2	10		b	4	d	(4)
4	a	7	4	11	9	a	8	d	(8)	14	a	8	d	(8)	19	a	6	2d	(8)
	b	9	1	10		b	7	2d	(9)		b	7	2	9		b	9	d	(9)
5	a	8	3	11	10	a	9	d	(9)	15	a	7	1	8	20	a	8	d	(8)
	b	6d		(6)		b	7	d	(7)		b	8	2	10		b	ost		

Free

(a and b have no relation, but were arbitrarily designated at the beginning)

1	a	8	2d	(10)	8	a	6	2	8	15	a	7	d	(7)	22	a	7	1d	(8)
	b	7	4	11		b	9	d	(9)		b	7	2	9		b	7	d	(7)
2	a	6	1d	(7)	9	a	8	d	(8)	16	a	6	1	7	23	a	4	0	4
	b	8	1	9		b	7	1	8		b	9	2	11		b	7	2	9
3	a	8	1d	(9)	10	a	9	1	10	17	a	8	3	11	24	a	8	3	11
	b	10	4	14		b	7	1	8		b	6	1d	(7)		b	9	2	11
4	a	11	4	15	11	a	7	2d	(9)	18	a	8	2d	(10)	25	a	6	3	9
	b	10	2	12		b	5	2	7		b	9	1d	(10)		b	9	d	(9)
5	a	7	2	9	12	a	10	3	13	19	a	10	4	14	26	a	d	(0)	(0)
	b	7	2	9		b	8	2d	(10)		b	d		(0)		b	7	3	10
6	a	6	2d	(8)	13	a	9	2	11	20	a	9	1d	(10)	27	a	9	1d	(10)
	b	9	3	12		b	9	1	10		b	8	2	10		b	7	d	(7)
7	a	9	3	12	14	a	7	2	9	21	a	d		(0)	28	a	9	2d	(11)
	b	7	1d	(8)		b	6	d	(6)		b	6	1d	(7)		b	d	d	(9)
															29	a	6	d	(6)
																b	7	d	(7)

Experiment 2. Number of fissions, last 6 weeks of the period of eight weeks, for those that have conjugated ('pairs') and for those that have not ('split pairs' and 'free'). (d = dead.) Numbers in parentheses give total fissions for those that died before the end.

	PAIR	INDIVIDUAL	WEEK						TOTAL 8 WEEKS	PAIR	INDIVIDUAL	WEEK						TOTAL 8 WEEKS
			3	4	5	6	7	8				3	4	5	6	7	8	
Pairs.....	1	a	6	7	10	6	2	d		8	b	6	5	6	5	2	6	37
		b	0	d						9	b	3	2	10	7	1	6	34
	2	a	4	4	7	5	0	2	31	10	b	5	3	6	1d			
		b	4	4	10	4	1	5	37	11	b	4	1	0		d		
	3	b	4	5	10	5	1	5	37	12	a	3	0	d				
	4	a	6	5	10	4	2	d			b	2	0	d				
		b	5	5	8	7	2	5	38	13	b	2	d					
Split pairs...	5	a	4	3	2d					14	a	3	2	d				
		b	3	4	10	3	1	3	30	15	b	4	0	0	d			
	6	a	4	5	5	4	2	0	25	17	a	0	0	d				
		b	5	6	8	7	2	5	38	19	b	2	d					
	1	a	4	3	11	7	2	5	42	4	b	5	2d					
		b	3	3	9	2	0	d		5	a	6	5	9	8	1	5	45
	2	b	5	6	9	6	1	d		6	a	5	4	d				
Free.....	3	a	6	5	8	2	2	6	44		b	5	5	9	7	1	6	47
		a	6	5	9	4	0	5	40	7	a	3	6	9	5	2	5	37
	1	b	2	4	11	5	1	4	38	10	a	6	5	9	6	2	3	41
		b	5	5	11	6	1	d			b	5	5	10	6	1	5	40
	3	b	3	4	10	3	1	3	38	11	b	6	5	9	6	2	5	40
	4	a	5	5	10	6	2	4	47	12	a	6	2	9	6	1	5	42
		a	4	4	9	6	1	6	39	13	a	4	3	8	7	2	d	
		b	4	d							b	4	2d					
	6	b	7	4	10	6	1	3	43	14	a	6	4	d				
	7	a	7	5	9	6	2	7	48	15	b	4	6	9	3d			
	8	a	5	6	8	6	2	6	41	16		5	3	d				
	9	b	6	6	8	5	1	d			a							

TABLE 32

Experiment 12. Paramecium aurelia. Number of individuals present on certain days of the experiment, with the reductions made. Such entries as '14-4' signify that 14 were present on the day in question, and that a sufficient number were removed to leave but 4 for future multiplication. No individuals were removed except in the numbers and on the dates shown. Each line started with a single individual, August 11.

Set K. Progeny of the conjugants of June 3. Cultivated in watch glasses since May 15.

LINE	AUGUST						SEPTEMBER						TOTAL FISSIONS
	14	16	20	25	27	29	1	2	3	5	6	7	
1	6-2	8-2	16-4	8-4	8-4	12-4	16-4	6-4	13-4	14-4	7	13	21
2	8-2	6-2	16-4	20-4	8-4	16-4	21-4	6-4	8-4	30-4	8	19	23
3	8-2	8-2	16-4	13-4	8-4	16-4	32-4	3	discontinued				(15)
4	2	12-2	21-4	12-4	8-4	lost							
5	9-2	8-2	12-4	10-4	14-4	13-4	19-4	4	discontinued				(16)
6	8-2	8-2	12-4	11-4	7-4	18-4	29-4	6-4	8-4	19-4	8	14	22
7	12-2	4-2	8-4	15-4	6-4	10-4	12-4	8	discontinued				(14)
8	7-2	4-2	5-4	14-4	11-4	12-4	13-4	6-4	4	8-4	6	8	15
9	5-2	7-2	32-4	12-4	8-4	16-4	14-4	6	discontinued				(16)
10	14-2	4-2	13-4	15-4	16-4	16-4	20-4	7-4	8-4	16-4	7	12	22

Set D 1. Conjugants of August 12, from the same culture as the non-conjugants of set D 2. Cultivated on slides from March 4 till June 7; from that time in watch glasses. (1 a and 1 b constitute the two members of pair 1, etc.)

1 a	{	8-2	2	3-2	23-4	6	8	8	5	5	5	2	d	8
		-2	10	22	46-10	9	12	discontinued						(8)
		-2	8-2	8	3	d								7
		-2	8-2	10	d									8
1 b		4	2	d										2
2 a		d												0
2 b	{	8-2	2	7	77-10	19	67	discontinued						(13)
		-2	2	11	8-4	2	d							6
		-2	2	10	14-4	5	13	26	22-4	6	8	8	8	11
									-4	6	10	10	10	12
3 a	{								-4	6	11	10	12	12
									-4	5	9	10	11	12
		1	d											0
		8-2	4-2	3	d									5
3 b	{	-2	2	d										3
		-2	8-2	6	9-4	6	7	26	26-4	4	14	13	13	12
									-4	7	13	11	14	12
									-4	4	12	13	13	12
	{								-4	4	9	11	11	12
									-4	4	14	15	15	12

TABLE 32 (CONTINUED)

Set D 2. Non-conjugants of August 12, from same culture as the conjugants of set D 1. Cultivated on slides from March 4, till June 7; from that time in watch glasses.

LINE	AUGUST						SEPTEMBER						TOTAL FISSIONS
	14	16	20	25	27	29	1	2	3	5	6	7	
1	8-2	6-2	4	1	1	d							6
2	8-2	3-2	4	7-4	4	4	3	1	d				6
3	d												0
4	8-2	2	5	13-4	4	d							6
	-2	4-2	4	9-4	6	1	1	d					7
5	4-2	4-2	5	d									6
	4-2	8-2	d										6
6	d												0
7	6-2	4-2	4	d									5
8	4-2	8-2	4	d									5
9	7-2	4-2	4	d									5
10	2	8-2	8	2	4	8	7	6	{ 4 2	2	d 1	d	7 7

TABLE 33

Experiment 13 b. *Paramecium aurelia*. Comparative number of fissions in the selected conjugant and non-conjugant lines, for five periods, between December 8 and February 27. (d = died out.)

LINE	DESCENDANTS OF CONJUGANTS						LINE	DESCENDANTS OF NON-CONJUGANTS					
	December 8-26	January 2-14	January 15-29	January 30-February 12	February 13-27	First 3 periods		December 8-26	January 2-14	January 15-29	January 30-February 12	February 13-27	First 3 periods
1	21	11	14	9	13	46	1	20	12	18	14	12	50
2	19	12	13	9	3	44	2	21	11	14	12	9	46
3	20	9	14	12	7	43	3	15	12	15	12	12	42
4	18	10	13	14	9	41	4	17	11	14	12	d	42
5	14	11	15	13	10	40	5	17	8	16	12	11	41
6	13	11	13	15	d	37	6	15	11	15	13	11	41
7	12	10	13	15	11	35	7	19	10	11	9	9	40
8	14	8	13	6	5	35	8	16	11	13	11	6	40
9	14	8	11	d		33	9	15	9	15	13	10	39
10	11	9	11	10	8	31	10	16	11	12	d		39
11	14	7	9	7	2	30	11	12	10	14	12	8	36
12	11	10	8	d		29	12	13	9	13	12	12	35
13	5	10	13	12	3	28	13	15	9	4	d		28
14	16	7	4	5	d	27	14	10	8	6	5	3	24
15	4	8	10	3	3	22							
16	9	7	5	7	0	21							
17	0	d											
18	d												
19	d												
20	d												
21	d												

TABLE 34

Experiment 15. Pure strain E; conjugants. Record of number of fissions by two-day periods, for each of the 88 lines descended from 28 conjugating pairs, throughout the twenty-four days of the experiment. For full explanation, see 'Explanation of tables 34 and 35,' page 346.

LINE	AUGUST			SEPTEMBER										TOTAL 24 DAYS
	27	29	31	2	4	6	8	10	12	14	16	18		
1 a x.....	0	2	2	2	3	4	4	4	3	3	3	3	33	
y.....	0	2	1	2	4	1	2	4	3	3	3	2	27	
b x.....	0	3	1	2	4	3	3	4	3	3	3	2	31	
y.....	0	3	1	0	3	2	2	3	3	2	3	1	23	
2 a x.....	2	1	2	1	3	3	3	3	3	3	3	2	29	
y.....	2	2	2	1	4	2	4	3	3	3	3	2	31	
b x.....	3	3	1	1	3	3	4	3	3	3		3		
y.....	3	2	1	2	3	3	2	3	3	3	2	3	30	
3 a x.....	2	3	1	3	4	3	2	4	3	3	3	3	34	
y.....	2	3	1	2	4	3	4	3	2	3	4	3	34	
b x.....	2	2	2	2	4	3	3	4	2	3	3	2	32	
y.....	2	3	0	2	4	3	4	4	2	3	3	3	33	
4 a x.....	3	2	2	2	1	1	0	1	0	1	0	0	13	
y.....	3	2	2	2	2	0	0	1	0	0	0	0	12	
b x.....	2	2	2	2	2	1	0	1	0	0	0	0	12	
y.....	2	2	2	2	2	1	1	1	0	0	0	0	13	
5 a x.....	3	3	2	1	0	1	0	1	0	0	0	0	11	
y.....	3	2	2	1	0	0		1	0	0	0	0		
b x.....	0	2	2	2	2	1	0	1	0	0	0	0	10	
y.....	1	2	2		2	0	1	1	0	0	1	0		
6 a x.....	0	2	1	1	1	1	1	2	2	2	1	2	16	
y.....	0	2	1	1	1	1	1	2	2	2	1	2	1	
b x.....	0	1	2	1	2	2	2	2	2		1	2		
y.....	0	1	2	2	2	1	2	2	1	2	2	0	17	
7 a x.....	0		1	2	3	4	3	3	3	3	3	3	(30)	
y.....	0	2	2	2	4	4	3	3	3	3	3	3	32	
b x.....	0		2	2	4	3	3	4	3	3	3	3	(33)	
y.....	1	2	1	2	4	3	4	3	3	3	3	3	32	
8 a x.....		2	0		3	3	3	3	3	3	3	3		
y.....	1	2	1	2	3	3	3	4	2	2	3	3	29	
b x.....	0	2	2	1	2	3	3	3	3	2	3	3	27	
y.....			2		3	3	2	4	2	3	3	3		
9 a x.....	1	2	2	3	4	4	3	3	3	3	3	4	35	
y.....	1	2	2	2	3	4	4	4	3	3	3	3	34	
b x.....	2	2	2	1	3	3	4	3	3	3	3	3	32	
y.....	1	2	0	0		4		4	3	3	3	4		
10 a x.....	2	2	2	2	3	2	3	3	3	3	3	1	29	
y.....	1	3	1		4	3	3	4	2	3	3	2		

TABLE 34 (CONTINUED)

LINE	AUGUST			SEPTEMBER										TOTAL 24 DAYS
	27	29	31	2	4	6	8	10	12	14	16	18		
10 b x.....	2	2	2	1	3	3	4	3	3	2	3	3	31	
y.....	2	3	0	2	3	2	3	3	3	3	3	3	30	
11 a x.....	2	2	2	2	4	3	4	3	2	3	3	2	32	
y.....	2	3	0	3		3	3	3	1	1	3	2		
b x.....	2	2	1	2	3	3	4	3	3	3	3	2	31	
y.....	2	2	1	2	3	2	4	2	3	2	2	3	28	
12 a x.....	2	2	2	2	4	3	3	3	3	2	4	3	33	
y.....	2	2	2	2	3	3	3	3	3	3	3	3	32	
b x.....	3	1	1	2	3	2	3	2	3	3	3	3	29	
y.....	3	2	1	0	2	3	1	3	3	2	3	2	25	
13 a x.....	2	3	1	2	3	2	3	3	3	2	3	2	29	
y.....	2	2	1		3	2	3	3	3	2	3	2		
b x.....	2	2	1	1	3	2	2	3	3	2	3	2	26	
y.....	2	2	0		2	1	3	3	2		2	3		
14 a x.....	2	2	2	2	3	3	3	3	3	3	2	4	32	
y.....	2	2	1	2	3	2	3	4	3	2	3	2	29	
b x.....	2	2	2	2	3	3	3	3	3	3	2	3	31	
y.....	2	2	1	2	3	3	3	3	3	3	2	3	30	
15 a x.....	2	2	2	2	1	2	0	1	0	1	0	0	13	
y.....	0		2	1	2	0	0	1	0	0	0	0	(10)	
b x.....	2	3	2	0	1	1	1	1	0	0	0	0	11	
y.....	2	3	2	2	2	1	1	1	0	1	0	0	15	
16 a x.....	2	3	1	2	3	3	4	4	3	2	3	3	33	
y.....	2	3	1	2	4	1	4	4	3	0		3		
b x.....	2	2	2	1	5	3	3	4	2	3	3	3	33	
y.....	2	2	1	2	4	3	3	3	3	3	0	1	27	
17 a x.....	0	0	2	3	3	2	4	3	3	3	3	2	28	
y.....	0	0	2	3	3	4	3	3	3	3	3	3	30	
18 a x.....	1	2	1	2	4	3	3	4	3	3	3	3	32	
y.....	0	2	2	1	3	3	2	4	2	2	3	3	27	
19 a x.....	2	2	2	1	3	3	2	4	3	2	3	3	30	
y.....	2	2	2	0	2	1	3	3	3	2	3	3	26	
20 a x.....	1	2	1	2	3		3	3	3	2	3	3		
y.....	0	2	1	2	3	3	1	3	3	2	3	3	26	
21 a x.....	2	2	1	1		1	2	3	3	3	3	3		
y.....	2	3	1	2	4	2	4	4	3	3	3	3	32	
22 a x.....	2	1	2	1	4		3	3	1	3	3	3		
y.....	2	2	2	2	2	3	3	3	1	3	3	2	28	
23 a x.....	2	3	2	2	4	3	3	4	3	2	3	3	34	
y.....	2	3	2	2	4		3	3	3	3	3	4		
24 a x.....	2	2	1	1	4	2	3	3	3		3	3		
y.....	2	2	2	1	4	1	2	3	2	2	3	3	27	
25 a x.....	2	2	2	2	4	3		4	3		4	2		
y.....	2	2	2	3	4	2	4	4	3	2	3	3	34	

TABLE 34 (CONTINUED)

LINE	AUGUST			SEPTEMBER										TOTAL 24 DAYS
	27	29	31	2	4	6	8	10	12	14	16	18		
26 a x.....	2	2	2	3	2	1	1	1	1	0	1	0	16	
y.....	2	3	1	2	0	1	1	0	1	0	0	0	11	
27 a x.....	1	3	1	1	2	2	2	2	2	2	3	1	22	
y.....	1	3	0		1		1	2	2	2	2	2		
28 a x.....	2	3	0	0	2	2	2	3	3	3	2	3	25	
y.....	2	3	0	2	4	2	2	3	3	2	3	3	29	

TABLE 35

Experiment 15. Pure strain E; non-conjugants. Record of number of fissions by two-day periods, for each of the 174 lines descended from 65 split pairs, throughout the 21 days of the experiment. For full explanation, see 'Explanation of tables 34 and 35' page 346.

LINE	AUGUST		SEPTEMBER										TOTAL 21 DAYS
	28	30	1	3	6	8	10	12	14	16			
1 a x.....	3	1	1	2	4	4	2	3	2	3	25		
y.....	3	0	2	2	4	4	2	3	2	3			
b x.....	1	1	2	3	4	3	2	3	1	3	23		
y.....	1	1		3	4	2	3	2	2	3			
2 a x.....	3	1	2	2	4	3	2	2	3	2	24		
y.....	3	1	2	3	4	3	2	3	2	2			
b x.....	2	1	1	2	3	4	2	3	2	3	23		
y.....	2	1	2	2	4	3	3	3	2	3			
3 a x.....	3	0	2	2	3	1	1	2	2	2	18		
y.....	3	2	2	2	4	2	2	2	2	3			
b x.....	2	1	2	3	4	3	2	2	2	3	24		
y.....	1	1	1	2	4	3	3	2	2	3			
4 a x.....	2	2	2	2	4	2	3	2	2	3	24		
y.....	3	2	1	2	3	2	2	2	2	3			
b x.....	2	3	2	2	3	3	3	2	3	2	25		
y.....	2	2		2	4	3	2	3	0				
5 a x.....	3	2	1	2	3	3	2	3	2	3	23		
y.....	3	2	2	2	1	3	3	2	3	2			
b x.....	2	2	2	3	4	2	2	2	2	3	24		
y.....	2	1	2	3	4	4	2	2	2	3			
6 a x.....	1	1		3	3	3	2	3	2	1	22		
y.....	2	2	1	3	2	3	2	2	2	3			
b x.....	1	3	2	2	4	3	2	3	2	3	25		
y.....	1	2	3	2	4	4	3	2	3	3			
7 a x.....	2	1	2	2	3	3	3	2	2	2	22		
y.....		0	2	1	3	2	2	2	2	2			

TABLE 35 (CONTINUED)

LINE	AUGUST		SEPTEMBER										TOTAL 21 DAYS
	28	30	1	3	6	8	10	12	14	16			
7 b x.....	3	1	2	3	3	4	3	2	3	2	26		
y.....	3	2	2		3	4	3	3	2	3			
8 a x.....	2	2	0	1	3	3	3	2	2	3	21		
y.....	2	2		3	3	2	3	2	2	3			
b x.....	3	1	2	2	4	3	2	3	2	3	25		
y.....	3	0	1	2	4	3	3	3	2	3			
9 a x.....	1		2	2	3	4	3	2	3	2	24		
y.....	2	2	2	2	4	3	2	3	2	2			
b x.....	2	2	2	3	3	2	3	2	3	3	25		
y.....	2	2	2	2	4	3	3	2	3	2			
10 a x.....	2	1	1	2	3	4	2	3	2	2	22		
y.....	2	2	2	2	3	3	3	2	3	2			
b x.....	2	2	3	2	5	2	3	2	2	3	26		
y.....	3	2	2	2	4	3	3	2	3	2			
11 a x.....	3	2	0	2	3	3	3	2	2	3	23		
y.....	2	2	1	2	3	3	2	2	2	3			
b x.....	2	2	2	3	3	3	3	1	2	3	24		
y.....	2	2	1	3	3	4	2	3	2	3			
12 a x.....	0	3	2	2	4	3	3	3	2	3	25		
y.....	2	2	1	2	3	3	3	3	2	2			
b x.....	2	1	2	3	4	2	3	3	2	2	24		
y.....	2	0	2	3	3	3	2	2	2	3			
13 a x.....	2	2	2	2	3	2	3	2	2	3	23		
y.....	2	1	2	2	3	3	3	2	1	1			
b x.....	2	2	0	1	4	3	2	3	2	2	21		
y.....	2	2	2	2	4	3	2	3	2	2			
14 a x.....	2	3	2	3	3	3	2	3	2	3	26		
y.....	3		1	2	3	2	2	3	2	3			
b x.....	3	2		1	3	2	3	2	2	3			
y.....	3	1	3	2	3	3	2	3	1	3			
15 a x.....	2	2	2	2	3	3	3	2	2	3	24		
y.....	2	3	2	1	4	2	2	2	2	3			
b x.....	0	2	0	2	4	4	3	3	2	3	23		
y.....	0	0		3	4	3	3	2	2	3			
16 a x.....	3	2	1	2	4	3	3	3	2	3	26		
y.....	2	2	2	3	4	3	2	3	2	2			
b x.....	0		2	2	4	3	3	2	3	2	23		
y.....	0	3	1	2	4	3	3	2	2	3			
17 a x.....	2	3	2	2	4	3	3	2	2	2	25		
y.....	3	1	1	2	4	3	2	2	2	3			
b x.....	0	3	2	2	4	3	3	3	2	2	24		
y.....	0		2	2	4	3	3	2	2	2			
18 a x.....	3	2	2	3	3	3	3	2	2	3	26		
y.....	2	3	1	3	4		3	2	2	3			

TABLE 35 (CONTINUED)

LINE	AUGUST				SEPTEMBER						TOTAL 21 DAYS
	28	30	1	3	6	8	10	12	14	16	
18 b x.....	0	2	2	2	4	3	3	2	3	3	24
y.....	0		1	2	4	3	3	3	2	3	
19 a x.....	2	2	2	2	4	3	2	3	2	3	25
y.....	2	2	1	3	3	2	3	2	3	2	23
b x.....	1	2	1	3	4	3	3	3	2	3	25
y.....	1	2		3	4	1	3	2	3	3	
20 a x.....	2	3	2	2	3	3	3	3	2	2	25
y.....	3	1	3	2		3	3	2	2	3	
b x.....	0	1		2	4	3	4	2	2	3	
y.....	1	1	1	2	4	3	3	2	2	3	22
21 a x.....	2	2	3	2	4	2	3	3	2	3	26
y.....	2	2	2	2	4	3	3	2	3	3	26
b x.....	0	2	1	3	4	3	2	3	2	3	23
y.....	0	1	2	2	4	3	3	1	2	3	21
22 a x.....	2	3	3	2	4	3	3	3	2	3	28
y.....	2	3	2	3	3	3	2	3	2	3	26
b x.....	0	2	2	3	4	4	2	3	3	3	26
y.....	0		1	3	4	3	2	3	2	3	
23 a x.....	3	2	2	2	4	1	2	2	2	3	23
y.....	2	1	3	2	3	2	2	2	3	3	23
24 a x.....	1	2	1	2	3	4	2	3	2	1	21
y.....	3	1	1	2	4	3	2	3	2	3	24
25 a x.....	2	2	2	2	3	4	2	3	2	3	25
y.....	2	3	1	3	4	2	2	3	2	3	25
26 a x.....	2	2	1	1	3	3	2	3	2	3	22
y.....	3	0		2	3	3	3	2	2	3	
27 a x.....	3	2	2	2	4	2	3	3	1	3	25
y.....	3	2	1	2	3	3	3	2	2	2	23
28 a x.....	3	1	2	2	3	3	2	3	2	3	24
y.....	3		2	2	4	3	2	3	2	3	
29 a x.....	3	2	2	2	3	3	3	3	2	2	24
y.....	2	2	2	3	3	3	2	2	2	3	24
30 a x.....	3	1	2	2	3	3	3	2	3	2	24
y.....	2	2	2	2	4	3	3	3	2	3	26
31 a x.....	3	2	2	2	4	2	3	2	2	1	23
y.....	4	0	2	2	4	3	2	2	2	3	24
32 a x.....	2	1	1	2	4	3	2	2	2	2	21
y.....	2	2	2	2	4	3	3	2	2	3	25
33 a x.....	2	2	2	2	4	3	2	2	2	3	24
y.....	3	2	2	2	4	3	2	3	2	3	26
34 a x.....	3	0	2	1	3	3	2	2	2	2	20
y.....	2	2	1	2	4	3	3	2	2	3	24
35 a x.....	3	2	2	2	4	3	2	3	2	3	26
y.....	3	0	1	3	3	3	3	2	2	3	23

TABLE 35 (CONTINUED)

LINE	AUGUST		SEPTEMBER									TOTAL 21 DAYS
	28	30	1	3	6	8	10	12	14	16		
36 a x.....	3	2	2	2	4	2	2	2	2	2	23	
y.....	3		1	3	3	3	2	3	2	3		
37 a x.....	2	2	1	3	3	4	2	3	2	3	25	
y.....	2	2	2	3	4	2	3	2	3	3	26	
38 a x.....	2	1	2	3	3	3	2	3	2	3	24	
y.....	2	1	1	3	4		2	3	2	2		
39 a x.....	1	3	2	1	3	3	3	2	2	3	23	
y.....	2	2	2	2	4	3	2	3	2	2	24	
40 a x.....	2	2	2	3	4	3	3	2	2	3	26	
y.....	2	2	3	2	4	3	3	3	2	3	27	
41 a x.....	2	2		2	5		2	3	2	3		
y.....	2	2	2	2	5	3	3	2	2	2	25	
42 a x.....	1	2	1	2	4	3	2	3	2	2	22	
y.....	1	2	3	2	4	3	3	3	2	2	24	
43 a x.....	2	2	2	2	4	3	3	2	2	2	24	
y.....	3	2	2	2	4	3	3	3	1	2	25	
44 a x.....	2	3	2	3	4	3	3	2	2	3	27	
y.....	2		1	3	4	2	3	2	2	2		
45 a x.....	1	1	1	2	4	2	2	2	2	2	19	
y.....	2	2	1	3	4	3	2	2	2	2	23	
46 a x.....	2	2		2	3	3	3	2	2	2		
y.....	2	2	2	2	4	2	2	3	2	2	23	
47 a x.....	2	1	2	2	4	3	2	2	2	3	23	
y.....	3	2	2	2	4	3	3	2	2	3	26	
48 a x.....	2	2	2	2	4	3	3	2	2	3	25	
y.....	2	2	2	2	4	3	2	2	2	2	23	
49 a x.....	3	0	2	3	4	3	2	3	2	2	24	
y.....	3	2	2	2	4	2	2	2	2	3	24	
50 a x.....	2	2	2	3	4	3	3	2	2	2	25	
y.....	2	1	0	3	4	3	3	1	2	3	22	
51 a x.....	3	2	3	2	4	3	3	2	2	3	27	
y.....	3	1	3	2	3	3	3	2	2	3	25	
52 a x.....	3	2	1	2	3	2	3	2	3	3	24	
y.....	2	3	1	2	4	3	3	2	2	3	25	
53 a x.....	3	2	2	2	3	3	3	2	3	3	26	
y.....	3	1	3	3	3	3	3	2	2	3	26	
54 a x.....	1	2	2	2	3	3	3	2	2	3	23	
y.....	2	2	2	2	3	1	2	2	2	3	21	
55 a x.....	2	2	2	2	3	2	3	2	2	3	23	
y.....	2	3	2	2	4	3	3	2	2	3	26	
56 a x.....	2	1	2		3	3	3	2	2	2		
y.....	2	2	2	3	2	2	3	2	2	3	23	
57 a x.....	2	1	2	3	3	2	3	2	2	2	22	
y.....	3	2	2	3	3	3	3	2	2	3	26	

TABLE 35 (CONTINUED)

LINE	AUGUST		SEPTEMBER									TOTAL 21 DAYS
	28	30	1	3	6	8	10	12	14	16		
58 a x.....	1	3	2	3	4	2	3	2	2	3	25	
y.....	3	2	2	3	4	3	3	2	2			
59 a x.....	2	1	2	2	3	3	3	2	2	3	23	
y.....	2	2	3	2	3	3	3	2	2	2		
60 a x.....	2	2	2	3	4	3	2	3	2	3	26	
y.....	3	1	2	3	3	3	2	2	2	3		
61 a x.....	2	2	2	2	4	4	3	2	2	3	26	
y.....	2	2	2	2	3	4	3	2	2	3		
62 a x.....	2	2	2	2	3	2	3	2	2	3	23	
y.....	2	2	1	3	3	3	3	2	3	3		
63 a x.....	1	2	1	3	3	2	3	2	2	3	22	
y.....	2	2	2	3	3	4	3	2	3	2		
64 a x.....	2	3	2	2	3	4	2	3	2	3	26	
y.....	2	1	2	2	4	3	3	2	3	3		
65 a x.....	2	2	2	3	3	3	3	2	2	2	24	
y.....	2	2	2	2	3	3	3	2	2	3		