

INHERITANCE IN HYDATINA SENTA

II. CHARACTERS OF THE FEMALES AND THEIR PARTHENOGENETIC EGGS¹

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INTRODUCTION

During the past few years, while working with the rotifer *Hydatina senta*, I have been constantly seeking some feature in regard to which the various parthenogenetic lines obviously differ. Owing to the rapidity of reproduction of this rotifer, and the comparative ease with which matings may be made

¹ Contribution from the Zoölogical Laboratory of the University of Michigan.

whenever males and sexual females are present, such a characteristic would furnish good material for the study of inheritance in what are probably the genetic equivalents of pure lines.

Such a character was first found in the autumn of 1912. At that time I received, through the kindness of Mr. C. F. Rousselet, some fertilized eggs in dried mud scraped from the bottom of a duck pond in England. Almost simultaneously, Prof. J. H. Powers sent some living females collected on the campus of the University of Nebraska. It was at once apparent, when these rotifers were examined, that the eggs of the English line were smaller than those of the Nebraska line. Other characters in which the two lines differed were later discovered. The inheritance of these characters through a number of generations is described in the following pages.

I am much indebted to the gentlemen named above for the material received from them, as well as to Prof. T. H. Morgan for rotifers which were sent at the same time, but which are not involved in this investigation.

DIFFERENTIATING CHARACTERS OF THE TWO LINES

Difference in size of egg

The parthenogenetic eggs of the English line were distinctly smaller than those of the Nebraska line. Although the larger eggs of the former were larger than the smaller eggs of the latter line, each line varied about its own mode, and the difference between the modes was great enough to leave no doubt of its significance. Proof of this will be given presently.

The parthenogenetic eggs of *Hydatina* are approximately ellipsoids. Length and breadth were therefore the measurements required. Measurements were made with a microscope, by means of an ocular micrometer attached to a screw with a graduated head. The screw was used to bring one of the marks of the micrometer tangent to one side or one end of the egg, and the measurement was read at the other side. Fractions of the finest divisions on the micrometer were estimated to tenths. Owing to the personal equation in determining when one of the

lines was tangent to the egg, the estimate of fractions was found to be more accurate (that is, more uniform) than the use of the graduated screw head. All measurements were made with the same microscope and the same combination of lenses. Since only relative sizes are of importance here, the measurements have been left in terms of the divisions of the micrometer. Absolute measurements, if desired, may be obtained, in millimeters, by multiplying the measure here given by 0.0082.

Measurements were usually made without difficulty. The eggs are as a rule cemented to some object, with their long axes parallel to the surface of the object. When they were attached to the bottom of the dish, they were in position to have both length and breadth measured. Practically all measurements were made of eggs on the bottom, though enough were taken at the surface film to make it certain that these did not differ in size from those at the bottom. If an egg did not lie in a horizontal position, that fact should be detected by the difference in focus of the two ends. In case of doubt as to position, the egg was moved with a needle, or measurement was not made.

A necessary precaution regarding measurements of eggs must be mentioned. The late eggs of a female are a little larger than the early eggs, on the average. This is indicated in the summary given in table 1. All the eggs laid by 11 females were measured and recorded in the order in which they were laid. Each family of eggs was then divided into tenths as nearly as possible, and the mean dimensions of the eggs in each tenth computed. As each family comprised 30 to 50 eggs, each tenth included 3 to 5 eggs. In table 1, it is shown that in family 1, for example, the mean dimensions of the eggs of the first tenth of the family were 17.2×14.2 ; in like manner, in family 6, the mean dimensions of the eggs in the eighth tenth were 16.3×14.4 . The mean of these means is then computed for each tenth of the family. For the sake of more ready comparison, a volume coefficient has been calculated as $(\text{Length}) \times (\text{Breadth})^2$. This does not give actual volume, even in units corresponding with the divisions of the micrometer, but it gives a volume coefficient, which is sufficient

TABLE 1

Showing relative size of eggs in different parts of the same family. The unit of measurement is the finest division of an ocular micrometer

FAMILY	MEAN DIMENSIONS OF EGGS IN EACH TENTH OF FAMILY—TENTHS				
	1	2	3	4	5
1	17.2×14.2	16.3×13.8	15.7×13.7	15.9×13.7	16.3×14.1
2	17.6×14.1	17.4×14.6	16.9×14.1	18.2×14.9	18.5×15.0
3	15.6×13.3	16.1×13.2	15.8×13.3	16.1×13.9	16.3×13.8
4	17.1×14.2	17.1×14.1	17.1×14.2	17.6×14.7	17.5×14.4
5	16.4×13.3	15.4×13.5	15.7×13.4	15.6×13.1	16.2×13.0
6	15.8×13.2	15.6×13.5	15.9×13.5	16.1×13.7	16.0×13.6
7	15.3×13.5	15.6×13.4	15.8×13.2	15.9×13.3	16.3×13.5
8	15.4×13.9	15.7×13.1	15.9×13.3	16.2×13.6	16.6×13.5
9	16.0×13.2	15.8×12.9	16.1×13.4	16.4×13.4	16.1×13.8
10	17.2×14.6	17.4×14.7	17.4×14.9	17.6×14.8	17.3×14.8
11	15.8×13.5	15.3×13.6	16.0×13.3	15.8×13.9	16.0×13.2
Mean.....	16.3×13.7	16.2×13.7	16.2×13.7	16.5×13.9	16.6×13.9
Volume coefficient.....	3059	3041	3041	3188	3207

FAMILY	MEAN DIMENSIONS OF EGGS IN EACH TENTH OF FAMILY—TENTHS				
	6	7	8	9	10
1	16.5×14.0	16.4×14.0	16.5×13.9	16.5×14.0	16.8×14.0
2	18.5×15.0	18.4×14.9	18.8×14.8	18.3×15.1	18.5×14.7
3	16.2×13.8	16.2×13.6	17.2×14.3	16.6×14.3	17.1×13.9
4	17.9×14.9	18.1×14.7	18.3×14.5	17.5×14.5	19.5×15.0
5	16.5×13.0	16.3×13.2	17.0×13.0	17.1×14.0	16.8×14.0
6	16.2×14.0	16.2×14.0	16.3×14.4	16.8×14.2	17.4×14.3
7	16.1×13.7	16.2×14.0	16.7×14.2	16.9×14.2	17.1×14.0
8	16.5×13.7	16.9×13.8	16.3×13.8	16.8×13.6	16.9×14.0
9	16.3×13.8	16.5×13.9	16.2×13.7	16.4×14.0	16.9×13.3
10	17.6×15.2	17.1×14.7	17.5×14.9	17.3×14.7	17.6×14.7
11	16.0×13.5	15.6×13.4	16.1×13.4	15.4×13.3	16.0×13.4
Mean.....	16.8×14.1	16.7×14.0	17.0×14.1	16.9×14.2	17.3×14.1
Volume coefficient.....	3340	3273	3380	3408	3439

where only relative volumes are of interest. Actual volume, in cubical units corresponding to the linear units of the micrometer, may be found by multiplying the coefficient of volume by $\frac{\pi}{6}$.

Barring certain fluctuations, there is a gradual increase in the size of the eggs with increasing age of the mother. For this reason, all measurements mentioned below were made upon eggs of the first day's laying in their respective families.

The distribution of the measurements of the eggs of the two original lines is shown in tables 2 and 3. In these tables, in

TABLE 2

Showing the distribution of the eggs of the Nebraska line of Hydatina senta, with respect to their length and breadth. The unit of measurement is the finest division of an ocular micrometer

NUMBER OF EGGS OF BREADTHS GIVEN BELOW	NUMBER OF EGGS OF LENGTHS GIVEN BELOW								
	16.0	16.5	17.0	17.5	18.0	18.5	19.0	19.5	20.0
13.5	1	2					1		
14.0	2	1	12	5	8	8	1		
14.5		3	12	13	10	11	5		1
15.0	1		8	13	24	7	14	3	
15.5				2	6	1	4		
16.0					3	2		1	

Number of eggs measured, 185; mean length, 17.90 ± 0.058 ; mean breadth, 14.69 ± 0.043 ; $\sigma_L = 0.786 \pm 0.041$; $\sigma_B = 0.590 \pm 0.030$.

TABLE 3

Showing the distribution of the eggs of the English line of Hydatina senta, with respect to their length and breadth. The unit of measurement is the finest division of an ocular micrometer

NUMBER OF EGGS OF BREADTHS GIVEN BELOW	NUMBER OF EGGS OF LENGTHS GIVEN BELOW							
	14.5	15.0	15.5	16.0	16.5	17.0	17.5	18.0
13.0		2	4	13	8	3	1	
13.5		3	8	17	10	2		
14.0	3	1	7	18	16	28	6	3
14.5			2	4	6	5	4	4
15.0					1	2		1

Number of eggs measured, 182; mean length, 16.40 ± 0.060 ; mean breadth, 13.81 ± 0.039 ; $\sigma_L = 0.804 \pm 0.042$; $\sigma_B = 0.522 \pm 0.028$.

order to increase the number of eggs in each size class, the class interval is taken as one-half the finest division of the ocular micrometer. All eggs recorded in my notes as measuring 16.3 to 16.7, inclusive, appear in these tables as measuring 16.5; all first recorded 16.8 to 17.2, inclusive, are here given as 17.

The mean dimensions of the English eggs are $(16.40 \pm 0.060) \times (13.81 \pm 0.039)$. Those of the Nebraska line are $(17.90 \pm 0.058) \times (14.69 \pm 0.043)$. The difference between the mean lengths is 1.50 ± 0.083 ; the difference between the mean breadths is 0.88 ± 0.058 . The difference is 18 times its mean error in the former case, 15 times its error in the latter; hence there can be no doubt that the differences are significant.

Difference in time of egg development

When two lots of eggs laid at the same time, one by the English line, the other by the Nebraska line, were reared to hatching, it was noted that those of the Nebraska line invariably began to hatch first. The time of development was more accurately determined for each line in the following manner. A number of females were placed together in a dish, and allowed to remain twenty minutes to an hour and a half. At the end of that time the females were removed. The eggs laid in the dish were preserved, and after a period of about twelve hours were examined every half hour, or thereabouts. As the young females hatched, they were removed and counted. Record was kept of the time in which the eggs were laid, and the time at which each lot of young females was later removed. It is assumed that the eggs were laid uniformly throughout the period of laying, and that, for the purpose of computing the mean time of development, the middle of the laying period may be taken as the time of laying of all the eggs. Likewise, the females removed at each later examination were reckoned as having hatched at the middle of the period elapsing since the last preceding examination. This method of determining the time of egg development introduces some errors, but when large numbers of eggs were used the errors could not have affected the mean to any great extent. It does

affect the variability of the time of development, as is explained below.

Records of the time of development were kept at four different times during the whole investigation. The mean time of development, as might be expected, is not the same in all these periods. The four sets of records are therefore kept separate. Tables 4 and 5 show the results obtained. The time of development is given in table 4 to the nearest hour.

In July, the mean time of development of the eggs of the English line was 14.98 ± 0.21 hours; of the Nebraska line, 13.33 ± 0.08 hours. The difference between the two means, as shown in the

TABLE 4

Showing the distribution of the eggs of the English and Nebraska lines of Hydatina senta, with respect to the time required for development

LINE	DATE	TIME OF EGG DEVELOPMENT, IN HOURS											
		12	13	14	15	16	17	18	19	20	21	22	23
Eng.	July.....		21	23	15	10	6	4	2	3	1		
	Nov.....			3	6	9	16	8	5	0	4		
	Dec.....			1	3	7	6	8	4	2	3	1	
	Mar.-Apr.....				2	6	4	10	11	9	4	1	1
Neb.	July.....	18	75	21	19	1							
	Nov.....		2	10	28	14							
	Dec.....		1	16	38	19	9						
	Mar.-Apr.....			1	20	18	15	8					

TABLE 5

Showing mean time of development, and variability of time of development, of the eggs of the English and Nebraska lines of Hydatina senta, as computed from table 4

LINE	DATE	NUMBER OF EGGS	MEAN TIME OF DEVELOPMENT, IN HOURS	STANDARD DEVIATION OF TIME OF DEVELOPMENT	EXCESS OF MEAN OVER THAT OF ENGLISH LINE	EXCESS OF STANDARD DEVIATION OVER THAT OF ENGLISH LINE
Eng.	July.....	85	14.98 ± 0.21	1.94 ± 0.15		
	Nov.....	51	17.08 ± 0.24	1.72 ± 0.17		
	Dec.....	35	17.66 ± 0.33	1.94 ± 0.23		
	Mar.-Apr.	44	18.55 ± 0.28	1.87 ± 0.20		
Neb.	July.....	34	13.33 ± 0.08	0.90 ± 0.06	-1.65 ± 0.22	-1.04 ± 0.16
	Nov.....	54	15.00 ± 0.10	0.77 ± 0.07	-2.08 ± 0.26	-0.95 ± 0.18
	Dec.....	83	15.23 ± 0.10	0.92 ± 0.07	-2.43 ± 0.34	-1.02 ± 0.24
	Mar.-Apr...	62	16.15 ± 0.14	1.06 ± 0.10	-2.40 ± 0.31	-0.81 ± 0.22

sixth column of table 5, is nearly eight times its mean error, hence there can be no doubt of its significance. In November, the time of development was greater for both lines, but again that of the English line was greater than that of the Nebraska eggs. December and March to April gave similar results. The reasons for the greater time required for development in the later determinations have not been fully determined, though temperature is a potent factor.

Another striking fact shown in tables 4 and 5 is that the variability of the time of development is greater in the English line than in the Nebraska line. This was a matter of frequent observation throughout the experiments. A lot of Nebraska eggs laid in a period of twenty minutes were practically all hatched within a period of an hour; English eggs, on the other hand, if laid in a period of twenty minutes, hatched irregularly over a period of four or five hours. The contrast between the two degrees of variability was much more striking than the standard deviation here given would indicate. There are two reasons for this. First, when the eggs of a given lot were laid during a period of an hour and a half, the period of hatching was necessarily greater than when the eggs were laid in twenty minutes. As this erroneous increase was as great for the Nebraska line as for the English line, the ratio of the standard deviation of the latter to that of the former was diminished (because $\frac{y+m}{x+m}$ is less than $\frac{y}{x}$, where y is greater than x and m is a positive quantity). Second, within the month of July, for example, the time of development was not the same on all days. In order that the results might be handled statistically, all these daily records have been combined. There is thus created an appearance of variability that would be diminished if all the determinations had been made on one day, or under one set of conditions.

The difference between the standard deviations of the time of development of the two lines is, according to table 5, four to six times its mean error. Since, for the two reasons given above, both standard deviations are too high, and their mean errors are

therefore also too high, the difference between the two standard deviations should exceed its own mean error more than table 5 indicates. It seems certain, therefore, that the difference is significant.

Difference in rate of egg production

The rate of egg production was found at once to be unequal in the two lines, when they were reared side by side under the same conditions. Every day more young females were isolated from the dishes containing the English females than from those of the Nebraska line. The difference in the rate of egg laying was determined as follows. A given small number of females, all of which were known to have begun laying, were put into a

TABLE 6

Showing rate of egg laying of the English and Nebraska lines of Hydatina senta

LINE	NUMBER OF FEMALES LAYING	NUMBER OF DAYS LAYING	NUMBER OF INDIVIDUAL DAYS OF LAYING	NUMBER OF EGGS LAID	NUMBER OF EGGS PER DAY PER FEMALE	VOLUME COEFFICIENT OF EGG OF MEAN DIMENSIONS	VOLUME COEFFICIENT OF TOTAL EGGS OF ONE FEMALE IN ONE DAY
English.....	14	1.99	27.86	418	15.0	3,123	46,845
Nebraska.....	22	1.57	34.54	420	12.2	3,868	47,189

dish, and removed a day or two later. The number of eggs laid in that time was determined by counting the females that hatched from them later. If, as happened several times, any of the adult females whose egg laying was being tested, died before removal, or showed by their condition that they had not been laying eggs recently, the dish containing them was rejected. The dishes usually contained only two or three females, in order that the conditions might remain at the optimum for a longer period. The results are summarized in table 6.

In table 6, the number in column four is the product of those in columns two and three. The number in the sixth column is obtained by dividing the number of eggs in the fifth column by the number of days in the fourth column. The volume coefficient in the seventh column is computed from the mean dimen-

sions of the eggs of the respective lines, as given in tables 2 and 3, and is calculated in the manner described for table 1. The number in the last column is the product of the numbers in the two next preceding columns. Other determinations of the rate of egg production in the same lines were made at a later date, and used as controls, as shown in table 13; but because no eggs of these lines were then being measured, the counts are not included in table 6, where egg volumes are concerned.

From the last column of table 6 it appears that the aggregate volume of egg substance produced in a day by an English female was nearly as much as that produced by a Nebraska female. The smaller size of the English eggs is not due, therefore, to a slower rate of metabolism involved in egg production, but to a tendency to put up the same quantity of substance in smaller packages.

Difference in place of egg laying

While measurements of eggs were being made, it was noted that almost invariably most of the Nebraska eggs were cemented to the bottom of the dish, whereas a large proportion of the English eggs were held at the surface film of the water. Measurement of this feature of the lines was made as follows. Into each of two dishes, and in equal quantities of water, were put approximately equal numbers of females of the two lines, respectively. They were left in the dishes during the same period of time. An effort was made to provide food that would not float at the surface film nor adhere to it, so that eggs might be laid elsewhere.

Table 7 shows the number of eggs found at the bottom of these dishes, and at the surface of the water. Counts were made in both lines on the same days, at intervals from July to December, 1913, and again in March and June, 1914, partly on the same days.

The determinations made from July to December show a much higher percentage of eggs at the surface in the English line than in the Nebraska line. I know of no satisfactory way of handling statistically the records of the location of eggs, in order to prove that the difference between the lines is significant. Had all the counts been made on one day, or under one set of

conditions, statistical treatment would be simple. Simultaneous tests were made in both lines, as shown in table 7, on 16 different days. If, as seems practically certain, the location of the eggs is affected by external conditions, there should be, from day to day, a fluctuation of the percentage of the eggs at the surface. Such a fluctuation does, in fact, appear in the records. The count for each day might serve, therefore, as a unit. But 16 units are too few for statistical treatment; and there is the further objection that, though the percentage of eggs fluctuated, it usually shifted in the same direction in both lines. When an unusually large number of eggs were at the surface in the English line, the Nebraska line also showed in most cases more than the

TABLE 7

Showing number of eggs laid on bottom of dish and at surface film of water, by the English and Nebraska lines of Hydatina senta

DATE	ENGLISH LINE			NEBRASKA LINE		
	Number of eggs at surface	Number of eggs at bottom	Per cent at surface	Number of eggs at surface	Number of eggs at bottom	Per cent at surface
July 19, 1913.....	29	8	54.1	15	34	9.9
20	60	135		0	142	
21	48	0		12	33	
22	68	10		10	47	
23	53	64		9	108	
25	154	66		22	110	
27	68	3		21	66	
28	26	82		0	42	
29	97	0		18	71	
30	15	74		0	89	
Aug. 1	99	88		6	75	
2	67	81		0	76	
Oct. 5	10	28		0	27	
Nov. 16	21	34	3	67		
Dec. 8	16	33	2	80		
Mar. 16, 1914.....			23	74	21.4	
			30	62		
			15	82		
June 9	84	6	32	146		
	86	9	20	75		
	132	21	88.9			
12	68	10				

average number at the surface (see July 19, 21, 22, 25, 27, and 29). Fewer than the average number at the surface also appeared in both lines on the same days (see July 20, 28, 30, August 2, October 5, November 16, and December 8). If this correspondence in the fluctuation of the two lines were ignored, and each day's count used as a unit for statistical treatment, there might be thereby created an appearance of fluctuation so great that the mean error would cast doubt upon the significance of the difference of the means of the two lines. In view of the fact that both lines usually shifted in the same direction, and every day showed a difference of the same sign, such a doubt can scarcely be entertained.

What physiological factors cause the eggs of one line to be laid at the surface, those of the other at the bottom, have not been determined. It may be that the fundamental cause is the demand for oxygen. I owe to Dr. O. C. Glaser the suggestion that the difference in the location of the eggs may be due to a difference in the permeability of the body of the females. Some brief experiments to test this supposition do not support the view, though the tests can not be regarded as conclusive. Healthy females of both lines, all of about the same age, were placed simultaneously in a $\frac{N}{10}$ solution of lithium chloride, and examined at intervals of one minute until all muscular movement ceased. Ciliary movement continued longer, but as it was hard to decide when it stopped, that movement was ignored. On different days, the time required to kill the rotifers varied from 19 to 26 minutes; but on the same day, the time required to stop muscular activity was almost exactly the same, to the minute, in both lines. Only about a dozen females of each line were tested with lithium chloride. A few similar tests with 0.5 per cent solution of sodium chloride gave essentially the same results, but the time was longer. Further discussion of permeability as affecting other characters than the location of the eggs is given beyond.

Some brief preliminary experiments indicate that, of external agents, temperature modifies the proportion of the eggs laid at the surface.

Difference in contractility of the foot muscles

A series of muscle bands extends from the sides of the body to the foot, enabling the animal to telescope the foot more or less completely within the rest of the body. When a number of females were killed in Bouin's fluid, it was found that the foot of the Nebraska females was retracted, on the average, much more fully than that of the English females. I thought at first that this might be due to the mechanical effect of a difference in the size of the body. The Nebraska females were noticeably larger than the English rotifers, a difference which can not well be measured in the living females, and which is not therefore discussed under a separate caption. Since, before killing the rotifers in Bouin's fluid, as much water as possible was first drawn off, it seemed possible that the larger bodies of the Nebraska females were caught more firmly between the surface film of water and the bottom of the dish, and that this mechanical stimulus resulted in the greater contraction of the muscles of the one line. This was shown not to be the case, however, by comparing young Nebraska females with old English females, the latter being the larger. The Nebraska females again showed the greater degree of foot retraction. Further proof that the retraction was not due to the mechanical stimulus of the film of water was found in the behavior of the rotifers when subjected to various salts. The rotifers were, in these tests, in an abundance of water, hence there was no disturbance by the surface film. The Nebraska females responded to all these chemical stimuli, if of the proper strength, by first retracting the foot wholly within the body, and resting for a time on the bottom of the dish. After they resumed swimming, they squirmed at frequent intervals and partly or wholly retracted the foot for a short time. The English rotifers, on the other hand, while performing moderate squirming movements, did not usually retract the foot within the body. It seems necessary to assume, therefore, that the two lines differed in the degree to which their foot muscles responded to stimuli.

To measure this quantitative character, in the absence of any natural units of measurement, the following classes or degrees of contraction were arbitrarily established. 1. Fully extended, sides of foot smooth. 2. Slightly contracted, sides of foot somewhat wrinkled. 3. Moderately contracted, but with toes wholly protruding from the body. 4. Greatly contracted, toes partly or wholly concealed within the body. All tests recorded were made by killing the animals in Bouin's fluid, because specimens were being preserved for cytological study.

TABLE 8

Showing distribution of the females of the English and Nebraska lines of Hydatina senta, with respect to the degree of contraction of the foot muscles (see text for further description of class numbers)

LINE	NUMBER OF FEMALES SHOWING FOLLOWING DEGREES OF CONTRACTION				TOTAL NUMBER OF ♀♀	MEAN CLASS NUMBER	STANDARD DEVIATION	EXCESS OF MEAN OVER THAT OF ENGLISH LINE
	1 Fully ex- tended	2 Slightly contracted	3 Moderately contracted	4 Greatly contracted				
Eng.....	34	13	3	1	51	1.39 ± 0.101	0.72 ± 0.071	
Neb.....	1	5	25	81	112	3.66 ± 0.057	0.61 ± 0.040	+2.27 ± 0.116

Table 8 shows the number of these fixed specimens falling within each of the above four classes. The mean class number of the English line is 1.39 ± 0.101 , that of the Nebraska line, 3.66 ± 0.057 . The difference between these means, as shown in the last column of table 8, is nearly 20 times its mean error, which leaves little doubt of the significance of the difference.

INHERITANCE OF DIFFERENTIATING CHARACTERS

Reciprocal crosses between the English and Nebraska lines were effected with some difficulty. Mating was not infrequently observed, but the number of sexual females, and hence also of males, was quite small in both lines, so that the simultaneous occurrence of males in one line and sexual females in the other was not common. Furthermore, many matings were failures; the female of a pair often proved to be sexual, but her eggs had

not been fertilized. The difficulty with which fertilization was brought about was new to me, and may indicate some degree of incompatibility between the lines.

A more serious obstacle to crossing was the fact that only a small percentage of the fertilized eggs hatched. The proportion of viable eggs is, as I have shown elsewhere (Shull '13), a heritable character, and varies in different lines. In the lines here described and in all their progeny, the proportion of viable eggs was small. Many ways have been tried to cause the eggs to hatch, but so far without success. Hatching may be prevented, and it may be hastened, but no way of inducing it has been discovered.

Notwithstanding these difficulties, enough results have been obtained to be of some theoretical interest. Four successive generations in the direct line have been obtained, and two generations from a back cross. No viable eggs were obtained from the last of these generations. Table 9 presents in concise form the relationship of all the lines studied. All lines except the original parent lines are designated by numbers. It is seen that in F_1 six viable lines were obtained, out of about 60 fertilized eggs. Fortunately, these six lines include representatives of both reciprocal crosses. In F_2 , six viable lines were secured from about 48 fertilized eggs. In F_3 , 15 lines from about 80 fertilized eggs, and so on. By F_3 back cross I mean the progeny obtained by crossing an F_2 with one of its grandparental lines, as is explained in the table. For convenience, the inheritance of all the differentiating characters of the original lines is shown in tabular form, in the same order as in table 9.

Each differentiating trait will now be taken up in turn, and its inheritance through the various lines presented.

Inheritance of size of egg

Table 10 gives, in summary form, the results of the measurements of the eggs in all the lines studied. By including in this table the standard deviation of both length and breadth, it is believed that the distribution of the eggs of each line, with regard to their dimensions, is sufficiently described. It is not necessary, therefore, to tabulate each line separately, as was done for the

English and Nebraska lines. With the aid of table 9, the relationship of each line to all the rest may be easily discovered, and the significance of the results here presented readily understood. Lines 95 to 99, inclusive, died out because of unfavorable conditions before their eggs were measured. Line 48, from the fertilized inbred eggs of the Nebraska line, comprised only one adult female, which laid five eggs. None of the females hatching from these eggs reached maturity. The mean dimensions of the five eggs were 17.9×14.2 , or almost as large as the Nebraska eggs. In line 104, only seven eggs were measured, the mean dimensions of these being 18.8×15.7 . In the remaining lines an adequate number of eggs was measured.

Attention should be directed to the following points, most readily shown in the last two columns of table 10. Lines 80 and 81, from inbred eggs of the English line, laid eggs that did not differ materially from those of the English line; certainly they were not any larger. The three lines derived from crosses between the English and Nebraska lines (44, 47, and 49) were all practically the same, in egg size, as the English line, as if small egg size were dominant. These three lines as shown in table 9, represent both reciprocal crosses.

All F_2 lines laid eggs at least as small as the English eggs. There is no evidence of inheritance from the large-egged Nebraska line. There is no appearance of segregation, with respect to egg size, in F_2 .

In all F_3 lines except line 61, the eggs are of practically the same size as the English eggs. Line 61 laid eggs somewhat longer. The difference between line 61 and the English line is more than eight times its mean error, and is probably significant, especially in view of the results in F_4 . In F_3 , then, the only evidence of inheritance of size of egg from the Nebraska line is in line 61.

In F_4 , the lines are probably not significantly different from the English line, except line 75, and perhaps line 79. In the former, the excess of the length of the eggs over that of the English eggs was nearly six times its mean error; in the latter the difference in breadth was five times its mean error. I believe these results

TABLE 9

Showing origin and relationship of all parthenogenetic lines of Hydatina senta whose inheritance is described in the text. All lines except the first parental lines are designated by numbers

GENERATION	NUMBER OF FERTILIZED EGGS	NUMBERS OF LINES	ORIGIN OF LINES
P ₁		English Nebraska	
F ₁	60	48..... 80, 81..... 44..... 47, 49.....	From fertilized egg of inbred ♀ of Neb. line From fertilized eggs of inbred ♀♀ of Eng. line From fertilized egg of ♀ of Neb. line, crossed with ♂ of Eng. line From fertilized eggs of ♀♀ of Eng. line crossed with ♂♂ of Neb. line
F ₂	48	50..... 51, 54..... 55, 56, 57.....	From fertilized egg of inbred ♀ of line 49 From fertilized eggs of inbred ♀♀ of line 44 From fertilized eggs of inbred ♀♀ of line 47
F ₃	80	58, 59, 60, 61, 62, 63, 66, 67, 68, 69, 70, 71, 72... 64..... 65.....	From fertilized eggs of inbred ♀♀ of line 56 From fertilized egg of inbred ♀ of line 57 From fertilized egg of inbred ♀ of line 50
F ₄	45	73..... 74..... 75, 76, 77, 78, 79.....	From fertilized egg of inbred ♀ of line 64 From fertilized egg of inbred ♀ of line 59 From fertilized eggs of inbred ♀♀ of line 61
F ₃ back-cross	56	82, 83, 84, 85, 86.....	From fertilized eggs of ♀♀ of line 56, crossed with ♂♂ of Neb. line
F ₄ from back-cross	114	87, 88, 89, 108.. 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 102, 103, 104, 105, 106, 107. 101.....	From fertilized eggs of inbred ♀♀ of line 84 From fertilized eggs of inbred ♀♀ of line 85 From fertilized egg of inbred ♀ of line 82

TABLE 10

Showing size of egg and variability of size of egg, of the lines of *Hydatina senta* described in this paper

GENERATION	LINE	NO. OF EGGS MEASURED	MEAN LENGTH	MEAN BREADTH	STANDARD DEVIATION OF LENGTH	STANDARD DEVIATION OF BREADTH	EXCESS OF MEAN LENGTH OVER THAT OF ENGLISH LINE	EXCESS OF MEAN BREADTH OVER THAT OF ENGLISH LINE
P ₁	Eng. Neb.	182	16.40±0.060	13.81±0.039	0.80±0.042	0.52±0.028		
		185	17.90±0.058	14.69±0.043	0.79±0.041	0.59±0.030	+1.50±0.083	+0.88±0.058
F ₁	48	5	(see text)					
	80	57	16.09±0.062	13.62±0.042	0.47±0.044	0.32±0.030	-0.31±0.086	-0.19±0.057
	81	37	16.19±0.092	13.58±0.060	0.57±0.065	0.37±0.042	-0.21±0.109	-0.23±0.071
	44	140	16.41±0.060	13.84±0.040	0.71±0.042	0.48±0.028	+0.01±0.084	+0.03±0.056
	47	144	16.36±0.052	13.87±0.037	0.52±0.037	0.45±0.026	-0.04±0.079	+0.06±0.054
	49	38	16.20±0.154	13.71±0.092	0.95±0.109	0.57±0.065	-0.20±0.165	-0.10±0.100
F ₂	50	258	16.28±0.047	13.62±0.030	0.75±0.033	0.49±0.021	-0.12±0.076	-0.19±0.049
	51	64	16.11±0.070	13.56±0.052	0.56±0.049	0.41±0.037	-0.29±0.092	-0.25±0.065
	54	147	15.96±0.050	13.50±0.034	0.61±0.035	0.41±0.024	-0.44±0.078	-0.31±0.052
	55	65	16.15±0.069	13.63±0.056	0.56±0.049	0.45±0.040	-0.25±0.091	-0.18±0.069
	56	59	16.36±0.079	13.76±0.065	0.61±0.056	0.50±0.046	-0.04±0.099	-0.05±0.076
	57	72	16.49±0.077	13.74±0.053	0.65±0.054	0.45±0.037	+0.09±0.097	-0.03±0.066
F ₃	58	93	16.51±0.077	13.88±0.058	0.74±0.054	0.56±0.041	+0.11±0.097	+0.07±0.070
	59	75	16.44±0.080	13.74±0.067	0.69±0.057	0.58±0.047	+0.04±0.100	-0.07±0.077
	60	77	16.41±0.070	13.77±0.052	0.61±0.049	0.46±0.036	+0.01±0.092	-0.04±0.065
	61	114	17.14±0.067	13.85±0.049	0.71±0.047	0.52±0.034	+0.74±0.090	+0.04±0.063
	62	110	16.37±0.066	13.75±0.048	0.70±0.047	0.50±0.034	-0.03±0.089	-0.06±0.061
	63	91	16.38±0.078	13.68±0.049	0.74±0.055	0.47±0.035	-0.02±0.098	-0.13±0.063
	66	127	16.38±0.059	13.70±0.042	0.66±0.042	0.48±0.030	-0.02±0.084	-0.11±0.057
	67	106	16.33±0.065	13.76±0.044	0.67±0.046	0.45±0.031	-0.07±0.088	-0.05±0.059
	68	55	16.53±0.106	13.59±0.060	0.78±0.075	0.44±0.042	+0.13±0.122	-0.22±0.071
	69	68	16.47±0.679	13.71±0.054	0.65±0.056	0.45±0.038	+0.07±0.099	-0.10±0.067
	70	82	16.45±0.083	13.65±0.052	0.75±0.059	0.47±0.037	+0.05±0.102	-0.16±0.065
	71	93	16.40±0.085	13.65±0.046	0.82±0.060	0.45±0.033	0.00±0.104	-0.16±0.066
	72	90	16.26±0.077	13.69±0.043	0.73±0.054	0.40±0.030	-0.14±0.097	-0.12±0.058
	64	99	16.38±0.072	13.70±0.059	0.72±0.051	0.58±0.042	-0.02±0.094	-0.11±0.071
65	99	16.18±0.079	13.83±0.046	0.78±0.056	0.46±0.033	-0.22±0.099	+0.02±0.060	
F ₄	73	67	16.36±0.083	13.74±0.048	0.68±0.059	0.40±0.034	-0.04±0.102	-0.07±0.061
	74	91	16.62±0.086	13.87±0.046	0.82±0.061	0.44±0.033	+0.22±0.104	+0.06±0.060
	75	100	17.01±0.085	13.95±0.044	0.85±0.060	0.44±0.031	+0.61±0.104	+0.14±0.059
	76	118	16.38±0.065	14.07±0.046	0.71±0.046	0.50±0.033	-0.02±0.088	+0.26±0.060
	77	136	16.18±0.058	13.79±0.038	0.69±0.041	0.45±0.027	-0.22±0.083	-0.02±0.054
	78	60	16.52±0.090	13.87±0.052	0.70±0.064	0.40±0.037	+0.12±0.108	+0.06±0.065
	79	70	16.86±0.118	14.23±0.076	0.99±0.083	0.64±0.054	+0.46±0.132	+0.42±0.085
F ₂ back-cross	82	57	16.51±0.080	14.04±0.043	0.60±0.057	0.32±0.030	+0.11±0.100	+0.23±0.058
	83	59	15.95±0.059	13.81±0.062	0.45±0.042	0.48±0.044	-0.45±0.084	0.00±0.073
	84	59	16.00±0.063	13.87±0.048	0.49±0.045	0.37±0.034	-0.40±0.087	+0.06±0.061
	85	66	16.32±0.068	14.03±0.039	0.55±0.048	0.31±0.028	-0.03±0.091	+0.22±0.055
	86	62	16.43±0.059	13.92±0.039	0.46±0.042	0.31±0.028	+0.03±0.084	+0.11±0.055
F ₄ from back-cross	87	81	16.29±0.054	13.68±0.038	0.49±0.038	0.35±0.027	-0.11±0.081	-0.13±0.054
	88	81	16.27±0.054	13.51±0.040	0.49±0.038	0.36±0.028	-0.13±0.081	-0.30±0.056
	89	67	16.29±0.054	13.71±0.046	0.44±0.038	0.38±0.033	-0.11±0.081	-0.10±0.060
	108	64	16.80±0.070	13.76±0.045	0.56±0.049	0.36±0.032	+0.40±0.092	-0.05±0.059

TABLE 10—Continued

GENERATION	LINE	NO. OF EGGS MEASURED	MEAN LENGTH	MEAN BREADTH	STANDARD DEVIATION OF LENGTH	STANDARD DEVIATION OF BREADTH	EXCESS OF MEAN LENGTH OVER THAT OF ENGLISH LINE	EXCESS OF MEAN BREADTH OVER THAT OF ENGLISH LINE
F ₄ from back- cross	90	64	18.23±0.098	14.68±0.069	0.77±0.068	0.55±0.049	+1.83±0.113	+0.87±0.079
	91	70	16.36±0.060	13.61±0.045	0.50±0.042	0.38±0.032	-0.04±0.084	-0.20±0.059
	92	49	16.27±0.076	13.51±0.047	0.53±0.054	0.33±0.033	-0.13±0.097	-0.30±0.061
	93	49	18.61±0.131	14.91±0.076	0.92±0.093	0.53±0.054	+2.21±0.144	+1.10±0.085
	94	49	18.51±0.084	14.79±0.061	0.59±0.059	0.43±0.043	+2.11±0.103	+0.98±0.072
	100	64	16.80±0.071	14.03±0.029	0.57±0.050	0.23±0.021	+0.40±0.093	+0.22±0.048
	102	36	16.24±0.087	13.67±0.075	0.52±0.062	0.45±0.053	-0.16±0.105	-0.14±0.084
	103	49	17.98±0.123	14.61±0.099	0.86±0.087	0.69±0.070	+1.58±0.136	+0.80±0.106
	104	7	(see text)					
	105	36	16.33±0.098	13.81±0.077	0.59±0.069	0.46±0.054	-0.07±0.115	0.00±0.086
	106	36	18.35±0.138	14.85±0.088	0.83±0.098	0.53±0.062	+1.95±0.150	+1.04±0.096
	107	36	18.08±0.140	14.67±0.087	0.84±0.099	0.52±0.062	+1.68±0.152	+0.86±0.095
	101	36	16.26±0.123	13.83±0.087	0.74±0.087	0.52±0.062	-0.14±0.136	+0.02±0.095

to be significant. Both of these lines, as shown in table 9, were derived from line 61, which itself laid eggs larger than the English eggs.

An F₃ generation could not be obtained to test further the inheritance of egg size in lines 75 and 79, for the few fertilized eggs that were laid in these lines did not hatch. It is believed, however, that the larger eggs of line 61, and its daughter lines 75 and 79, are the effects of inheritance from the Nebraska parent line. For further tests of this inheritance, recourse was had at this point to a back cross between an F₂ line and the Nebraska line. Line 56 was chosen from F₂ because it was producing many sexual females, so that crossing was not difficult.

In the back crosses, the eggs of no line are significantly larger than those of the English greatgrandparent. The appearance is again that of simple dominance of small egg size.

In the F₄ series of lines derived from the back cross, however, several possessed eggs unmistakably larger than the English eggs. The eggs of lines 103 and 107 were as large as Nebraska eggs, while those of lines 90, 93, 94, and 106 were even larger. In lines 108 and 100, the mean length is somewhat greater than that of the English eggs, though the difference is of very doubtful significance. If line 108 be not included, all the lines producing large eggs were derived from line 85, whose eggs were not significantly larger than those of the English line.

To summarize, all F_1 lines, all F_2 lines, 14 out of 15 F_3 lines, at least 5 out of 7 F_4 lines, all the back cross lines, and at least 8 out of 16 F_4 lines from the back cross, laid eggs as small as the English line. Of the remaining lines, the one in F_3 , and one in F_4 were intermediate between English and Nebraska; one in F_4 and two in F_4 from the back cross laid eggs that were not certainly significantly larger than English eggs; while two in F_4 from the back cross laid eggs as large as Nebraska eggs, and four in F_4 from the back cross laid eggs even larger than the Nebraska eggs.

Large size of eggs appears much less frequently than would be expected if egg size were a simple Mendelian character, especially in the first three filial generations.

Inheritance of time of egg development

Tables 11 and 12 give the results of the determinations of the time of development of the eggs of all lines described in this paper, except lines 48 and 49. These two lines were no longer in existence when work on the time of development began. Table 11 gives the number of hours required for development, table 12 the means, standard deviations, differences of means, etc. The last two columns of table 12 show the significance of the results. The English and Nebraska lines are repeated from tables 4 and 5 for comparison. All lines are given, as before, in the same order as in table 9.

The following points are worthy of attention. With respect to mean time of development, no line in F_1 , F_2 , or F_3 , differs significantly from the English line. In F_4 , the difference of line 76 may be significant, but is of the wrong sign to be attributed to inheritance from the Nebraska line. The F_3 back cross includes no line approaching significantly the time of development of the Nebraska eggs, while in F_4 from this back cross, only the eggs of line 94 appear to have developed in significantly less time than the English eggs. The time of egg development in line 94 is intermediate between the time of development of the English eggs and the Nebraska eggs.

To summarize, in all the experiments here recorded, only one line (94) shows any indication of an inheritance of the short time of development of the Nebraska line.

With respect to the variability of the time of development, the results are less definite. The Nebraska line showed much less variability than the English line, as was shown in tables 4 and 5, and the difference was shown to be even greater than those tables indicated. In the filial generations most lines showed less variability than the English line, as is indicated by the preponderance of *minus* signs in the last column of table 12. But the mean error of the standard deviation is so high that the difference is always of doubtful significance. In line 54 in F_2 and line 103 in F_4 from the back cross, the differences are greatest, and are here perhaps significant.

Inheritance of rate of egg production

The possible inheritance of the rate of egg production was tested through three filial generations, and in one line of F_4 . In December, 1913, it became necessary either to abandon some part of the work undertaken or to do all of it less completely. As the results of the study of the inheritance of the rate of egg production up to that time were less definite than any other results, and as enormous fluctuations in the rate of egg production by the same individual at different times, and by different individuals of one line at the same time, required that large numbers of records be obtained in order to make the results trustworthy, this part of the investigations was dropped. The records, as far as obtained, are given for what they are worth, in table 13.

The F_2 lines are partly intermediate between the English and Nebraska lines, while in one egg production was more rapid than in the English line, in others slower than in the Nebraska line.

In F_3 , many lines laid eggs more slowly than the Nebraska line, none as rapidly as the English line. The great variation in the results does not permit of any significant classification of the various lines. The fact that the mean number of eggs per

TABLE 11

Showing the distribution of the eggs of all lines of *Hydatina senta* described in this paper, with respect to the time of development of their eggs

GENERATION	LINE	DATE	TIME OF EGG DEVELOPMENT, IN HOURS															
			12	13	14	15	16	17	18	19	20	21	22	23	24			
P ₁	Eng.	July.....		21	23	15	10	6	4	2	3	1						
		Nov.....			3	6	9	16	8	5	0	4						
		Dec.....			1	3	7	6	8	4	2	3	1					
	Neb.	Mar.-Apr.....				2	6	4	10	11	9	4	1	1				
		July.....	18	75	21	19	1											
		Nov.....		2	10	28	14											
		Dec.....		1	16	38	19	9										
Mar.-Apr.....			1	20	18	15	8											
F ₁	80	Dec.....					4	14	7	7	1	0	1					
	81	Mar.-Apr.....					6	14	14	7	9	6	3	1				
	44	July.....	1	19	27	24	19	1	3	2	0	4						
	47	July.....		16	20	35	14	4	0	2	1	1						
F ₂	50	July.....		14	14	18	17	8	2	0	1							
	51	July.....		7	7	23	11	3	6	2	0	1						
	54	July.....		11	52	42	20	10	3	1								
	55	July.....			12	10	1	1	2	4								
	56	July.....		7	9	7	1	5	2	0	2	1						
	57	July.....		3	8	9	13	3	1	1								
F ₃	58	Nov.....				1	9	14	2	2	0	2						
	59	Nov.....			1	2	5	11	7	0	1							
	60	Nov.....				8	10	8	3	5	4	0	1					
	61	Nov.....			1	1	12	16	4	4	0	2						
	62	Nov.....			2	1	10	8	3	6	4	1						
	63	Nov.....			1	3	16	6	4	6	0	1	2					
	66	Nov.....			2	1	10	8	7	3	3	0	1					
	67	Nov.....			1	0	9	6	6	3	0	1						
	68	Nov.....				3	12	6	6	7	1	0	2					
	69	Nov.....			1	2	12	11	1	0	1	1						
	70	Nov.....					18	20	12	1	1	0	3					
	71	Nov.....			1	4	16	10	0	3	1	3						
	72	Nov.....					6	10	6	2	0	2	1	1				
64	Nov.....					1	14	4	4	3	0	1						
65	Nov.....					1	0	1	2	1								
F ₄	73	Nov.....			1	0	6	10	6	1	0	2						
	74	Dec.....				2	9	13	7	2	4	0	1	2	2			
	75	Dec.....					6	14	8	1	3	1	2	0	1			
	76	Dec.....				2	2	8	5	4	2	1	2	1	3			
	77	Dec.....			1	4	32	12	7	4	1	0	1					
	78	Dec.....						5	2	0	1	1						
	79	Dec.....					3	11	9	7	0	3	0	1				

TABLE 11--Continued

GENERATION	LINE	DATE	TIME OF EGG DEVELOPMENT, IN HOURS												
			12	13	14	15	16	17	18	19	20	21	22	23	24
F ₃ back-cross	82	Mar.-Apr.....				1	2	7	8	3	4	2	1	1	
	83	Mar.-Apr.....					3	5	9	8	6	6	1	0	1
	84	Mar.-Apr.....					6	8	10	6	5	2	2	2	
	85	Mar.-Apr.....							3	0	3	2	0	1	1
	86	Mar.-Apr.....					1	1	10	2	6	3	4	3	2
F ₄ from back-cross	87	Mar.-Apr.....				1	2	7	7	5	7	3	1	2	
	88	Mar.-Apr.....					4	3	9	13	9	5	1		
	89	Mar.-Apr.....					4	14	14	6	12	6	3	2	
	108	Mar.-Apr.....				1	2	4	4	3	2	2	3	1	
	90	Mar.-Apr.....					8	11	10	10	10	5	2		
	91	Mar.-Apr.....					3	9	14	3	5	4	3	1	
	92	Mar.-Apr.....					2	8	16	14	11	2	2		
	93	Mar.-Apr.....					2	6	9	13	14	11	5	4	
	94	Mar.-Apr.....			1	10	13	14	9	6	4	3			
	95	Mar.-Apr.....					3	6	12	12	7	1	2	1	
	96	Mar.-Apr.....				1	3	4	8	7	8	4	1	1	
	97	Mar.-Apr.....						2	4	3	2	0	1		
	98	Mar.-Apr.....					3	2	4	9	3	5	4	2	
	99	Mar.-Apr.....					2	1	7	8	5	7	1		
	100	Mar.-Apr.....					2	5	6	11	5	0	1	0	1
	102	Mar.-Apr.....				1	2	5	20	14	10	5	1		
	103	Mar.-Apr.....							16	28	43	19	10		
104	Mar.-Apr.....						1	1	2	2	0	2	1		
105	Mar.-Apr.....				1	3	6	10	13	5	6	1	1		
106	Mar.-Apr.....					2	9	7	9	6	6	1	1		
107	Mar.-Apr.....				1	3	2	5	5	4	0	3			
101	Mar.-Apr.....					1	3	19	12	8	0	2	1		

day for all the F₃ lines is a trifle less than the number per day for the Nebraska line in November, may be due to a loss of vigor resulting from inbreeding. I have shown elsewhere (Shull '12) that inbreeding results in a decrease of vigor in these rotifers. Lines 58 and 65, in particular, showed other evidences of weakness and were lost before any of the other F₃ lines. As shown in table 13, they were among the slowest egg producers.

No safe conclusions regarding inheritance of the rate of egg production can be drawn from the data obtained.

TABLE 12

Showing mean time of development, and the variability of time of development, of the lines of *Hydatina senta* described in this paper, as computed from data in table 11

GENERATION	LINE	DATE	NO. OF EGGS	MEAN TIME OF DEVELOPMENT IN HOURS	STANDARD DEVIATION OF TIME OF DEVELOPMENT	EXCESS OF MEAN OVER THAT OF ENGLISH LINE AT SAME DATE	EXCESS OF STANDARD DEVIATION OVER THAT OF ENGLISH LINE AT SAME DATE
P ₁	Eng.	July.....	85	14.98±0.21	1.94±0.15		
		Nov.....	51	17.08±0.24	1.72±0.17		
		Dec.....	35	17.66±0.33	1.94±0.23		
	Neb.	Mar.-Apr.	44	18.55±0.28	1.87±0.20		
		July.....	134	13.33±0.08	0.90±0.06	-1.65±0.22	-1.04±0.16
		Nov.....	54	15.00±0.10	0.77±0.07	-2.08±0.26	-0.95±0.18
		Dec.....	83	15.23±0.10	0.92±0.07	-2.43±0.34	-1.02±0.24
		Mar.-Apr.	62	16.15±1.14	1.06±0.10	-2.40±0.31	-0.81±0.22
F ₁	80	Dec.....	34	17.74±0.22	1.27±0.15	+0.08±0.40	-0.67±0.27
	81	Mar.-Apr.	60	18.57±0.27	2.09±0.19	+0.02±0.39	+0.22±0.28
	44	July.....	100	14.72±0.18	1.82±0.13	-0.26±0.28	-0.12±0.20
	47	July.....	93	14.88±0.15	1.49±0.11	-0.10±0.26	-0.45±0.19
F ₂	50	July.....	75	15.03±0.17	1.47±0.12	+0.05±0.27	-0.47±0.19
	51	July.....	60	15.47±0.22	1.67±0.15	+0.49±0.30	-0.27±0.21
	54	July.....	139	14.85±0.10	1.20±0.07	-0.13±0.23	-0.74±0.17
	55	July.....	30	15.43±0.32	1.76±0.23	+0.45±0.38	-0.18±0.27
	56	July.....	34	15.29±0.37	2.16±0.26	+0.31±0.43	+0.22±0.30
	57	July.....	38	15.32±0.21	1.32±0.15	+0.34±0.30	-0.62±0.21
F ₃	58	Nov.....	30	17.10±0.25	1.35±0.17	+0.02±0.35	-0.37±0.24
	59	Nov.....	27	16.93±0.23	1.18±0.16	-0.15±0.33	-0.54±0.25
	60	Nov.....	39	17.10±0.29	1.79±0.20	+0.02±0.38	+0.07±0.26
	61	Nov.....	40	17.08±0.22	1.39±0.16	-0.00±0.33	-0.33±0.25
	62	Nov.....	35	17.37±0.24	1.43±0.17	+0.29±0.34	-0.29±0.24
	63	Nov.....	39	17.13±0.29	1.83±0.20	+0.05±0.38	+0.11±0.27
	66	Nov.....	35	17.26±0.29	1.71±0.20	+0.18±0.38	-0.01±0.26
	67	Nov.....	26	17.15±0.28	1.41±0.20	+0.07±0.37	-0.31±0.26
	68	Nov.....	37	17.41±0.28	1.73±0.20	+0.33±0.37	+0.01±0.26
	69	Nov.....	29	16.97±0.26	1.38±0.18	-0.11±0.35	-0.34±0.25
	70	Nov.....	55	17.25±0.19	1.42±0.14	+0.17±0.31	-0.30±0.22
	71	Nov.....	38	16.84±0.27	1.69±0.19	-0.24±0.36	-0.03±0.25
	72	Nov.....	32	16.84±0.03	1.71±0.21	-0.24±0.38	-0.01±0.27
	64	Nov.....	27	16.93±0.26	1.36±0.19	-0.15±0.35	-0.36±0.25
65	Nov.....	5	17.40±0.61	1.36±0.43	+0.32±0.65	-0.36±0.46	
F ₄	73	Nov.....	26	17.27±0.29	1.46±0.20	+0.19±0.38	-0.26±0.26
	74	Dec.....	42	17.98±0.35	2.30±0.25	+0.32±0.48	+0.36±0.34
	75	Dec.....	36	17.94±0.31	1.88±0.22	+0.28±0.45	-0.06±0.32
	76	Dec.....	30	18.80±0.47	2.59±0.33	+1.14±0.57	+0.65±0.40
	77	Dec.....	62	17.68±0.17	1.32±0.12	+0.02±0.37	-0.62±0.26
	78	Dec.....	9	18.00±0.47	1.41±0.33	+0.34±0.57	-0.53±0.40
	79	Dec.....	34	18.12±0.27	1.55±0.19	+0.46±0.43	-0.30±0.30

TABLE 12—Continued

GENERATION	LINE	DATE	NO. OF EGGS	MEAN TIME OF DEVELOPMENT, IN HOURS	STANDARD DEVIATION OF TIME OF DEVELOPMENT	EXCESS OF MEAN OVER THAT OF ENGLISH LINE AT SAME DATE	EXCESS OF STANDARD DEVIATION OVER THAT OF ENGLISH LINE AT SAME DATE
F ₃ back cross	82	Mar.-Apr..	29	18.41±0.34	1.83±0.24	-0.14±0.44	-0.04±0.31
	83	Mar.-Apr..	39	18.95±0.28	1.75±0.20	+0.40±0.40	-0.12±0.28
	84	Mar.-Apr..	41	18.49±0.30	1.90±0.21	-0.06±0.41	+0.03±0.29
	85	Mar.-Apr..	10	19.30±0.62	1.95±0.50	+0.75±0.68	+0.08±0.54
	86	Mar.-Apr..	32	18.97±0.38	2.13±0.27	+0.42±0.47	+0.26±0.34
F ₄ from back cross	87	Mar.-Apr..	35	18.80±0.34	1.91±0.24	+0.25±0.44	+0.04±0.31
	88	Mar.-Apr..	44	18.89±0.22	1.47±0.16	+0.34±0.36	-0.40±0.26
	89	Mar.-Apr..	61	18.79±0.23	1.80±0.17	+0.24±0.36	-0.07±0.26
	108	Mar.-Apr..	22	18.86±0.50	2.20±0.35	+0.31±0.57	+0.33±0.40
	90	Mar.-Apr..	56	18.46±0.22	1.68±0.16	-0.09±0.36	-0.19±0.26
	91	Mar.-Apr..	42	18.64±0.28	1.80±0.20	+0.09±0.40	-0.07±0.28
	92	Mar.-Apr..	55	18.69±0.18	1.33±0.13	+0.14±0.33	-0.54±0.24
	93	Mar.-Apr..	64	18.63±0.22	1.74±0.16	+0.08±0.36	-0.13±0.26
	94	Mar.-Apr..	60	17.15±0.22	1.72±0.16	-1.40±0.36	-0.15±0.26
	95	Mar.-Apr..	44	18.68±0.23	1.53±0.17	+0.13±0.36	-0.34±0.26
	96	Mar.-Apr..	37	18.87±0.29	1.77±0.21	+0.32±0.40	-0.10±0.29
	97	Mar.-Apr..	12	18.75±0.39	1.36±0.28	+0.20±0.48	-0.51±0.34
	98	Mar.-Apr..	32	18.50±0.34	1.95±0.24	-0.05±0.44	+0.08±0.31
	99	Mar.-Apr..	31	18.23±0.27	1.50±0.19	-0.32±0.39	-0.37±0.28
	100	Mar.-Apr..	31	18.71±0.29	1.61±0.21	+0.16±0.40	-0.26±0.29
	102	Mar.-Apr..	58	18.71±0.18	1.37±0.13	+0.16±0.33	-0.50±0.24
103	Mar.-Apr..	116	18.82±0.10	1.13±0.07	+0.27±0.30	-0.74±0.21	
104	Mar.-Apr..	9	19.00±0.63	1.89±0.45	+0.45±0.69	+0.02±0.49	
105	Mar.-Apr..	46	18.76±0.25	1.67±0.18	+0.21±0.38	-0.20±0.27	
106	Mar.-Apr..	41	18.85±0.26	1.68±0.18	+0.30±0.38	-0.19±0.27	
107	Mar.-Apr..	23	18.61±0.40	1.91±0.28	+0.06±0.49	+0.04±0.34	
101	Mar.-Apr..	46	18.78±0.19	1.32±0.13	+0.23±0.34	-0.55±0.24	

Inheritance of place of egg laying

The hereditary relations of the various lines, with regard to the place of egg laying, appear to be rather definite. Table 14 gives the results obtained from all lines except 48, 49, 95 to 99, and 101, which were lost before the location of the eggs could be determined. Notice should be taken of the different results in the original English and Nebraska lines in different months, when the results in the filial lines are judged.

From July to December, all filial lines except 65, 71, 73, 75, 76, and 79, laid from 50 to 60 per cent of their eggs at the surface film; and the six lines just named varied so little above or below these limits that the difference is fairly chargeable to fluctuation. All the lines of the first four filial generations, therefore, are closely similar to the English line. In the period March to June, the

TABLE 13

Showing the number of eggs laid per day by the females of the lines of Hydatina senta described in this paper, through the F₃ generation and part of F₄

GENERATION	LINE	DATE	NUMBER OF MALES AYING	NUMBER OF INDIVIDUAL DAYS OF LAYING	NUMBER OF EGGS LAID	NUMBER OF EGGS PER DAY PER FEMALE
P ₁	Eng.	ly-Aug.....	14	27.86	418	15.0
		ov.....	12	13.80	141	10.2
	Neb	ly-Aug.....	22	34.54	420	12.2
		ov.....	23	23.40	160	6.8
F ₁	44	ly.....	4	10.10	158	15.6
	47	ly.....	4	12.60	167	13.2
F ₂	50	ly.....	4	11.10	178	16.0
	51	ly.....	4	10.60	123	11.6
	54	ly.....	4	12.60	188	14.9
	55	lg.....	6	13.60	151	11.1
	56	lg.....	6	7.40	95	12.8
	57	lg.....	6	15.50	224	14.4
F ₃	58	ov.....	16	16.00	70	4.4
	59	ov.....	17	17.20	112	6.5
	60	ov.....	16	14.80	125	8.4
	61	ov.....	20	21.40	143	6.6
	62	ov.....	18	18.80	139	7.4
	63	ov.....	16	17.40	129	7.4
	66	ov.....	10	10.80	97	9.0
	67	ov.....	15	14.80	136	9.2
	68	ov.....	9	8.50	32	3.8
	69	ov.....	15	14.60	66	4.5
	70	ov.....	17	17.70	147	8.3
	71	ov.....	13	13.60	126	9.3
	72	ov.....	17	17.50	127	7.3
	64	ov.....	14	14.90	70	4.7
	65	ov.....	10	9.80	32	3.3
F ₄	73	ov.....	13	13.4	91	6.8

numbers of eggs at the surface were higher in all lines, including the parental (English and Nebraska) lines. While none of the back cross lines, nor those of the F_4 generation derived from the back cross, laid quite as many eggs at the surface as did the English line during the same period, the difference is not certainly significant except in line 103. In line 103, only 35.5 per cent of the eggs were at the surface film. For reasons given (pp. 154-156), I know of no way to determine how small a difference, in the present case, is to be regarded as significant. But in view of the large number of eggs counted in line 103, a difference of more than 50 per cent (88.9 to 35.5) can hardly be considered meaningless. At the same time, the number of eggs at the surface is not as low as in the Nebraska line.

To summarize: line 103, of the F_4 generation from the back cross, is the only line showing any strong indication of inheritance from the Nebraska line, with regard to the place of egg laying.

Inheritance of contractility of foot muscles

Contractility was in all cases determined by killing the females within 24 hours after egg laying began, in Bouin's fluid. The method was in all particulars the same as given on page 157 for determining the difference between the English and Nebraska lines. Lines 48, 49, 91 and 95 to 99, inclusive, were lost before the contractility of the foot muscles could be tested.

The results, as shown in table 15, are briefly stated as follows: Of all the filial lines, only five showed greater contractility of the foot muscles than the English line. These five are the ones to which the *plus* sign is prefixed in the last column of table 15. In four of the five, the excess of the degree of contractility (mean class number) over that of the English line is less than its mean error. The remaining one, line 93, shows a degree of foot retraction that must be regarded as significant. Line 93, therefore, is the only filial line that gives certain indication of inheritance from the Nebraska line, with regard to the contraction of the foot muscles.

The fact that nearly all mean class numbers in the filial lines are slightly less than the mean of the English line may be due

TABLE 14

Showing number of eggs laid at bottom of dish and at surface film of water, by the lines of *Hydatina senta* described in this paper

GENERATION	LINE	DATE	NUMBER OF EGGS AT SURFACE	NUMBER OF EGGS AT BOTTOM	PERCENTAGE OF EGGS AT SURFACE
P ₁	Eng.	July-Dec.....	831	706	54.1
		June.....	370	46	88.9
	Neb.	July-Dec.....	118	1067	9.9
		Mar.-June.....	120	439	21.4
F ₁	80	July-Dec.....	436	340	56.1
	81	July-Dec.....	380	341	52.7
	44	July-Dec.....	913	703	56.1
	47	July-Dec.....	781	665	54.0
F ₂	50	July-Dec.....	898	675	57.0
	51	July-Dec.....	942	817	53.4
	54	July-Dec.....	978	682	58.9
	55	July-Dec.....	571	382	59.9
	56	July-Dec.....	926	703	56.8
	57	July-Dec.....	872	697	55.5
F ₃	58	July-Dec.....	201	194	50.8
	59	July-Dec.....	248	217	53.3
	60	July-Dec.....	204	166	55.1
	61	July-Dec.....	214	190	52.9
	62	July-Dec.....	238	189	55.7
	63	July-Dec.....	182	122	59.8
	66	July-Dec.....	176	144	55.0
	67	July-Dec.....	178	160	52.6
	68	July-Dec.....	219	194	53.0
	69	July-Dec.....	153	124	55.2
	70	July-Dec.....	129	103	55.6
	71	July-Dec.....	276	177	60.9
	72	July-Dec.....	231	196	54.0
	64	July-Dec.....	191	179	51.6
65	July-Dec.....	78	85	47.8	
F ₄	73	July-Dec.....	123	54	69.4
	74	July-Dec.....	490	425	53.5
	75	July-Dec.....	317	347	47.7
	76	July-Dec.....	341	349	49.9
	77	July-Dec.....	378	340	52.6
	78	July-Dec.....	143	107	57.2
	79	July-Dec.....	260	284	47.7

TABLE 14—Continued

GENERATION	LINE	DATE	NUMBER OF EGGS AT SURFACE	NUMBER OF EGGS AT BOTTOM	PERCENTAGE OF EGGS AT SURFACE
F ₃ back cross	82	Mar.-Apr.....	355	106	77.0
	83	Mar.-Apr.....	363	100	78.4
	84	Mar.-Apr.....	450	154	74.5
	85	Mar.-Apr.....	234	41	85.0
	86	Mar.-Apr.....	490	87	84.9
F ₄ from back cross	87	Mar.-Apr.....	267	54	83.2
	88	Mar.-Apr.....	422	110	79.3
	89	Mar.-Apr.....	216	50	81.2
	108	June.....	215	76	73.9
	90	Mar.-Apr.....	323	71	81.9
	91	Mar.-Apr.....	275	69	79.9
	92	Mar.-Apr.....	310	82	79.1
	93	Mar.-Apr.....	225	79	74.0
	94	Mar.-Apr.....	371	115	76.3
	100	June.....	204	64	76.1
	102	June.....	357	125	74.1
	103	June.....	468	852	35.5
	104	June.....	236	90	72.4
	105	June.....	301	111	73.1
	106	June.....	251	98	68.7
107	June.....	326	148	68.7	

to a change in judgment regarding the limits of the four arbitrarily selected degrees of contraction, as the investigation proceeded. The differences are so small, compared with their mean errors, that I believe them to be insignificant.

DISCUSSION

Review of results

The results of the experiments above described present certain unexpected features that need to be explained. In table 16 the results are summarized. Variability of the time of egg development, and the rate of egg production, are omitted from this table because of the irregularity of their inheritance. The following remarks pertain only to the four remaining characteristics, which are included in table 16.

TABLE 15

Showing distribution of the females of the lines of *Hydatina senta* described in this paper, with respect to the degree of contraction of the foot muscles (see text for further description of class numbers)

GENERATION	LINE	NUMBER OF FEMALES SHOWING FOLLOWING DEGREES OF CONTRACTION				TOTAL NO. OF ♀♀	MEAN CLASS NUMBER	STANDARD DEVIATION	EXCESS OF MEAN OVER THAT OF ENGLISH LINE
		1 Fully extended	2 Slightly contracted	3 Moderately contracted	4 Greatly contracted				
P ₁	Eng.	34	13	3	1	51	1.39±0.101	0.72±0.071	
	Neb.	1	5	25	81	112	3.66±0.057	0.61±0.040	+2.27±0.166
F ₁	80	100	17	14	2	133	1.38±0.063	0.73±0.045	-0.01±0.119
	81	95	18	6	2	121	1.30±0.058	0.64±0.041	-0.09±0.116
	44	36	16	5	1	58	1.50±0.096	0.73±0.068	+0.11±0.139
	47	70	26	0	5	101	1.41±0.072	0.73±0.051	+0.02±0.124
F ₂	50	43	9	0	2	54	1.28±0.088	0.65±0.062	-0.11±0.134
	51	38	10	2	2	52	1.38±0.102	0.74±0.072	-0.01±0.144
	54	45	7	1	3	55	1.33±0.102	0.76±0.072	-0.06±0.144
	55	43	12	1	0	56	1.25±0.063	0.47±0.045	-0.14±0.119
	56	33	7	1	0	41	1.22±0.073	0.47±0.052	-0.17±0.125
	57	37	9	1	1	48	1.29±0.088	0.61±0.062	-0.10±0.134
F ₃	58	35	11	3	1	50	1.40±0.097	0.69±0.069	+0.01±0.140
	59	45	9	3	2	59	1.36±0.095	0.73±0.067	-0.03±0.139
	60	38	7	1	2	48	1.31±0.102	0.71±0.072	-0.06±0.144
	61	107	21	3	1	132	1.23±0.045	0.52±0.032	-0.16±0.112
	62	70	11	3	2	86	1.27±0.069	0.64±0.049	-0.12±0.122
	63	71	11	1	3	86	1.26±0.070	0.65±0.049	-0.13±0.123
	66	83	16	10	4	113	1.42±0.075	0.80±0.053	+0.03±0.126
	67	85	17	5	0	107	1.25±0.051	0.53±0.036	-0.14±0.113
	68	114	15	7	1	137	1.23±0.048	0.57±0.034	-0.16±0.112
	69	67	15	3	1	86	1.28±0.063	0.58±0.045	-0.11±0.119
	70	49	11	1	0	61	1.21±0.058	0.45±0.041	-0.18±0.117
	71	88	14	1	2	105	1.21±0.054	0.55±0.038	-0.18±0.115
	72	34	16	0	0	50	1.32±0.066	0.47±0.047	-0.07±0.121
	64	71	7	4	2	84	1.25±0.070	0.64±0.049	-0.14±0.123
	65	48	3	2	2	55	1.24±0.093	0.69±0.066	-0.15±0.137

TABLE 15—Continued

GENERATION	LINE	NUMBER OF FEMALES SHOWING FOLLOWING DEGREES OF CONTRACTION				TOTAL NO. OF ♀♀	MEAN CLASS NUMBER	STANDARD DEVIATION	EXCESS OF MEAN OVER THAT OF ENGLISH LINE
		1 Fully extended	2 Slightly contracted	3 Moderately contracted	4 Greatly contracted				
F ₄	73	65	4	1	2	72	1.17±0.069	0.58±0.049	-0.22±0.122
	74	71	16	5	1	93	1.31±0.064	0.62±0.045	-0.08±0.120
	75	65	8	4	3	80	1.31±0.082	0.74±0.058	-0.08±0.130
	76	83	15	0	1	99	1.18±0.046	0.46±0.033	-0.21±0.111
	77	72	10	0	0	82	1.12±0.036	0.33±0.025	-0.27±0.107
	78	87	20	3	1	110	1.26±0.052	0.55±0.037	-0.13±0.113
	79	65	11	4	0	80	1.24±0.059	0.53±0.042	-0.15±0.117
F ₃ back cross	82	83	11	5	1	100	1.24±0.059	0.59±0.042	-0.15±0.117
	83	90	5	3	2	100	1.22±0.055	0.55±0.039	-0.17±0.115
	84	79	16	5	0	100	1.26±0.054	0.54±0.038	-0.13±0.115
	85	53	8	3	0	64	1.22±0.064	0.51±0.045	-0.17±0.119
	86	50	8	4	2	64	1.34±0.093	0.74±0.066	-0.05±0.137
F ₄ from back cross	87	85	10	4	1	100	1.21±0.055	0.55±0.039	-0.18±0.115
	88	65	10	3	3	81	1.31±0.079	0.71±0.056	-0.08±0.128
	89	79	19	2	0	100	1.23±0.047	0.47±0.033	-0.16±0.111
	108	79	18	2	1	100	1.25±0.054	0.54±0.038	-0.14±0.115
	90	84	10	4	2	100	1.24±0.062	0.62±0.044	-0.15±0.119
	92	81	12	4	3	100	1.29±0.068	0.68±0.048	-0.10±0.122
	93	76	52	40	28	196	2.10±0.076	1.07±0.054	+0.71±0.126
	94	78	28	2	0	108	1.30±0.047	0.49±0.033	-0.09±0.111
	100	86	10	3	1	100	1.19±0.052	0.52±0.037	-0.20±0.114
	102	79	13	6	2	100	1.31±0.067	0.67±0.047	-0.08±0.121
	103	81	12	5	2	100	1.28±0.068	0.68±0.048	-0.11±0.122
	104	83	15	2	0	100	1.21±0.043	0.43±0.030	-0.18±0.110
	105	76	20	4	0	100	1.28±0.053	0.53±0.037	-0.11±0.114
	106	80	14	4	2	100	1.28±0.063	0.63±0.045	-0.11±0.119
107	75	24	1	0	100	1.26±0.046	0.46±0.033	-0.13±0.111	
101	80	14	5	1	100	1.27±0.060	0.60±0.042	-0.12±0.117	

TABLE 16

Summary of the results of the experiments, showing in particular the similarity of the filial lines to the English parent line

CHARACTER	GENERATION					
	F ₁	F ₂	F ₃	F ₄	F ₅ back-cross	F ₄ from back-cross
Size of egg	All 3 like Eng.	All 6 like Eng.	14 lines like Eng., 1 (61) intermediate	5 like Eng., 1 (75) intermediate 1 (79) perhaps a little larger than Eng.	All 5 like Eng.	8 lines like Eng. 2 (103, 107) like Neb. 4 (90, 93, 94, 106) perhaps larger than Neb. 2 (108, 100) doubtful
Time of egg development	Both like Eng.	All 6 like Eng.	All 15 like Eng.	All 7 like Eng.	All 5 like Eng.	21 lines like Eng. 1 (94) intermediate
Place of egg laying	Both like Eng.	All 6 like Eng.	All 15 like Eng.	All 7 like Eng.	All 5 like Eng.	15 lines like Eng. 1 (103) intermediate
Contractility of foot muscles	Both like Eng.	All 6 like Eng.	All 15 like Eng.	All 7 like Eng.	All 5 like Eng.	15 lines like Eng. 1 (93) intermediate

In the several crosses obtained in F₁, each line possessed the four characteristics of the English line. This statement applies to both reciprocal crosses. It is rather curious, though not impossible, that all the dominant characteristics should have belonged to one of the original lines.

In F₂, where segregation and the reappearance of the characteristics of the Nebraska line might be expected, all of the lines again showed only the traits of the English rotifers.

In F₃, with a single exception, all the lines again repeated the four characters of the English line. The one exception is line 61, which, in egg size, was intermediate between the English and Nebraska lines, but like the English rotifers in other respects. It is important to note, as shown in table 9, that line 61 is descended from the same source (line 56) as twelve other lines in F₃. The fact that none of the other lines in F₃ showed any evidence of segregation can not, therefore, be attributed to the possibility that all the other lines descended from a parent line that was homozygous for each dominant character. If line 56 could transmit greater size of egg to line 61, there were twelve other F₃ lines in which larger eggs would have been possible.

In F_4 , the seven lines were again all like the English line, with the exception of one line (75), and perhaps a second (79). These two lines differed from the English line in size of egg, though perhaps not significantly in line 79. These two exceptional lines are descended, as table 9 shows, from line 61, which was itself the only exceptional line in F_3 .

In the back crosses there was no line that differed significantly from the English line in any of the four characters; and this was true, notwithstanding that line 56, ancestor of lines 61, 75, and 79, with their larger eggs, was used in making the back cross.

In the F_4 generation obtained by inbreeding the lines of the back cross, there was more evidence of segregation, though less than would be expected if the characters studied behaved in regular Mendelian fashion (see table 16).

Viewing the results as a whole, there was much less indication of segregation than might be expected. Furthermore, the four characters appear on the whole to have been transmitted as a group. Where segregation failed in one character, it usually failed in the rest. The departures from this rule are few in number, except in the last generation obtained. The importance of these exceptions is pointed out in what follows.

Explanations of results

Did crossing actually occur? The results in the early filial generations, in which all the lines were like the English parent line in all the characters studied, were what would have been expected if the eggs from which they originated had been parthenogenetic eggs or inbred fertilized eggs, of the English line, instead of cross fertilized eggs. It is not impossible that eggs more or less similar to the fertilized eggs may nevertheless be unfertilized. The fertilized eggs are, on the average, larger than the parthenogenetic eggs, and have a thicker shell set with pilose projections. I have shown elsewhere (Shull '10, p. 343) that parthenogenetic eggs may have thicker shells than usual, following the impregnation of the female that lays them. In the case described in my earlier paper, just mentioned, a sexual female had been

impregnated, and laid two large fertilized eggs, one of which hatched as a female. The same impregnated sexual female then laid numerous small eggs, all of which had somewhat thicker shells than male eggs, and required a somewhat longer time to develop, but which yielded males, showing them to have been parthenogenetic. If the thickening of the shell were carried further, the eggs might readily be classed as fertilized, and yet be unfertilized.

In view of the possibility that the eggs in these experiments were not truly fertilized, it seems best to state some facts which appear to me to exclude both parthenogenesis and inadvertent inbreeding.

In making the crosses, males of the one line were placed in a dish with young females and unhatched eggs of the other line. Before the females were half grown, and hence *before they could have produced any males of their own line*, each female was isolated in a dish and reared to maturity. This method, if carefully followed, insures that whatever fertilized eggs these females lay are cross fertilized eggs.

Lest, however, some error might have crept in, we may examine the internal evidence of crossing.

Line 44, derived from a cross between a Nebraska female and an English male, should, in case it was produced *either* parthenogenetically or by an inbred Nebraska female, have been like the Nebraska line. Instead, it was like the English line. And lines 51 and 54, descendants from line 44 by inbreeding, were likewise similar to the English line in all four characters.

Line 47 (an F_1) yielded many more male-producers than either of the original lines. Had it been produced parthenogenetically, the proportion of male-producers should have been the same as in the line that produced it.

The rate of egg laying and general vigor of the females varied in the several F_1 lines. Had they been produced parthenogenetically they should all have been alike.

In view of these internal evidences of crossing, and the method of making the crosses, there can be no doubt, it seems to me, that the lines described in this paper were produced by true crosses

between the English and Nebraska lines. This conclusion is strongly supported by the results in the F_4 generation, and F_4 from the back cross, results which can be best explained as due to inheritance from the Nebraska line.

Typical Mendelian behavior a possibility. The curious results summarized at the beginning of the discussion may, with the aid of an assumption regarding physiological facts, have a truly Mendelian explanation. Suppose each of the four characters in question were a simple Mendelian character. The original English and Nebraska lines were probably homozygous with respect to all four characters; the few results obtained by inbreeding them (see lines 48, 80, 81) are what would be expected if they were homozygous, and all other lines collected with them, in England and Nebraska, respectively, were like them at least in egg size. Moreover, there is no internal evidence in the experiments to show that they were other than homozygous.

If the original lines were homozygous, the three hybrid lines in F_1 were heterozygous in all four characters. Females of such lines should produce sixteen kinds of gametes, and there should be 256 different combinations in F_2 . One of the 256 should be homozygous for all the characters of the English line, one homozygous for all the characters of the Nebraska line. Of the remainder, 80, or over 31 per cent, should have received each of the characters of the English line from at least one parent. If—and here is the physiological assumption—only those fertilized eggs containing factors for all the characters of the English line could hatch and produce viable lines, the results may be explained. As may be seen from table 9, in no generation was the viability of the fertilized eggs as high as 30 per cent.

The chief objection to this view is found in the fact that a few of the lines did not possess all of the characters of the English line. Lines 90, 106, and 107 were like the English rotifers in only three of the four characteristics; lines 93, 94 and 103 were unlike the English line in two of the four characters; yet these lines were healthy.

The Mendelian explanation becomes easier if we assume that the characters studied were not simple, but dependent upon, to

use the terminology of G. H. Shull ('14), plural determiners. The proportion of combinations in F_2 , for example, which would include all the characters of the English line, would be increased by the presence of plural determiners for each character, the amount of increase depending on the number of plural factors. The fertilized eggs that hatched might well have given rise, under these circumstances, to lines like the English line in all four characters, purely as a matter of chance, without the assumption that mortality was selective.

The probability that the eggs that hatched would produce lines wholly like the English line would be still greater if the plural determiners for each character were also, again employing the terminology of G. H. Shull, duplicate determiners (that is, factors, each of which alone produces a character indistinguishable from that produced by any of the remaining duplicate determiners) and fully dominant. I do not think this condition is probable in the case of the four characters here studied, partly because, as Shull (loc. cit) has pointed out, very few cases of duplicate genes have yet been demonstrated, and partly because the unequal intermediate characters appearing in several lines (see those of F_3 , F_4 , and F_4 from the back cross) are better explained as due to (perhaps) unequal and cumulative determiners.

The four characters perhaps identical. At the end of the F_2 generation, and before the data in F_3 were all collected, the fact that the four characters seemed to go together in the same lines suggested the possibility that the four characters were but different manifestations of a single character. At first I tried to relate all of them to size of body. As stated on page 157, the bodies of the Nebraska females were somewhat larger than those of the English females. Naturally, I supposed, their eggs should be larger, and perhaps the size influenced the time of development. Larger bodies might have induced the females to remain mostly at the bottom, and so lay their eggs there. And lastly, it seemed possible, as explained on page 157, that the contraction of the foot muscles might be due to mechanical stimulus dependent on large bodies.

This hypothesis was soon abandoned, because, as recounted on page 157, the contraction of the foot muscles was proven not to be due to size of body. Dr. O. C. Glaser suggested that the single difference at the basis of the four described differences between the two lines, might be a difference in permeability; that, in some way, larger eggs, quicker development of eggs, laying eggs at the bottom of the water, and greater contractility of the foot muscles, might be due to a greater or less degree of permeability. Some brief experiments to test this possibility have been described on page 157. They resulted negatively, that is, both lines appeared to be equal in permeability; but I hope further tests may be made.

Serious obstacles to any explanation by which the four apparently distinct characters are combined into one, are found in those lines in F_3 , in F_4 and especially in F_4 from the back cross, in which the four characters *do not go together*. Egg size is repeatedly shown to be more or less independent of the other three characters (lines 61, 75, 90, 106, 107). Short time of development of the eggs may separate, to some extent, from egg laying at the bottom and high contractility of the foot muscles (line 94). Egg laying at the bottom may, to a degree, part company with great contractility of muscles and short time of egg development (line 103). Great contractility of muscles need not occur in the same line with egg laying at the bottom and short time of egg development (line 93).

If the four visible characters are in reality but one, the exceptions just noted will require subsidiary hypotheses.

Association of factors. Any hypothesis that reduces the number of characters which differentiated the original lines from four or more to one, makes simpler an explanation that is essentially Mendelian. In F_2 , for example, where only one fertilized egg in eight yielded a line of rotifers that could be tested (see table 9), those few lines might by chance easily possess the dominant (single) character. The force of the preceding explanation (identity of the four characters) depended on the fact that it substituted one character for four; the weakness of that explanation

was that in certain lines the four characters were evidently *not* one.

Association of factors has all the advantage, it seems to me, of uniting the four characters into one, under most circumstances, and has the further advantage that the union of the determiners is not an ironclad one. We have been made familiar with association of genes through the work of Morgan and his students (Morgan '10, '11 a, '11 b, '14; Bridges '13; Dexter '12; Sturtevant '13 a, '13 b; and numerous other papers) on the fly *Drosophila*. They assume a form of association that may be occasionally broken. The same kind of association may be present in *Hydatina*; the four characters being ordinarily associated, but with the possibility that one or more of them may sometimes part company with the rest.

What the mechanism of association may be, in *Hydatina*, if association exists, is a question I have not attempted to answer. In *Drosophila*, Morgan and his pupils have held it probable that the genes for all associated characters reside in the same chromosome. The chromosomes in *Drosophila* are few in number, and three—or four (Muller '14)—of them have been designated as the seat of different determiners. In *Hydatina*, on the other hand, the chromosomes are numerous (12 to 15 in the haploid groups) and small, as shown by Whitney ('09). I would hesitate to locate in one of these chromosomes all of the heritable characters of the parthenogenetic lines that have been discovered.

The fact that crossing between the English and Nebraska rotifers was more difficult, as stated on page 158, than has been found true of other lines, may be due to some deep-seated disturbance of the normal process of fertilization in these rotifers, a disturbance which carried with it the association of the characters of the English line.

While the hypothesis of association is not to be stated in detail, and while it must not be strongly maintained, it appears to me at the present time quite possible, and not open to most of the objections which tend to disprove the other possible explanations mentioned above.

SUMMARY

Two parthenogenetic lines of the rotifer *Hydatina senta*, obtained from England and Nebraska, respectively, were found to differ in the following respects:

1. The eggs of the Nebraska line were larger than those of the English line. Measurements to demonstrate this difference were made only upon eggs laid in the first 24 hours of egg laying of any female, because it was shown that the eggs of one female gradually increased in size with increasing age of the mother.

2. The Nebraska eggs developed in about two hours less time, on the average, than did the English eggs. Moreover, the time of development of the Nebraska eggs was much more uniform than that of the English eggs; eggs of the Nebraska females laid at the same time and reared under identical conditions seldom differed from one another, in the time of development, more than an hour, while the extreme times of development of English eggs under like conditions differed by four or five hours.

3. The rate of egg production in the Nebraska line was lower than in the English line, being a little over 12 per day per female in summer in the former line, 15 per day in the latter. Inasmuch as the Nebraska eggs were the larger, the volume of egg substance produced in a given time by a single female was approximately equal in the two lines.

4. More than 50 per cent of the eggs of the English rotifers were laid at the surface film of the water, during the summer; less than 10 per cent of the Nebraska eggs were laid at the surface, the remainder being cemented at the bottom of the dish. The reason for this difference in the location of the eggs is not known; it may be due to a difference in the demand for oxygen. Brief experiments tend to show that the difference in place of egg laying is not due to a difference in permeability. Temperature appears to modify the percentage of eggs laid at the surface.

5. The foot muscles of the Nebraska females responded more vigorously to chemical stimuli than did those of the English

females, so that when the animals were killed in a fixing fluid the foot of the Nebraska females was often retracted completely within the body, that of the English females being much more extended.

Reciprocal crosses were made between the two lines, and the inheritance of the above mentioned characters was traced through six filial generations. The inheritance of the rate of egg production, and of the uniformity of the time of egg development, was too irregular to summarize. No conclusions regarding these two characters are drawn, except that the rate of egg production in the filial lines decreases, probably owing partly to loss of vigor attendant upon inbreeding.

Regarding the four remaining characters (size of egg, time of egg development, place of egg laying, and contractility of the foot muscles) only generalized statements can be made here. Details must be obtained from the discussion and from the description of the experiments.

In F_1 every characteristic of the English line appears to be dominant. In F_2 , there is no evidence of segregation; all the lines show all of the characteristics of the English line. In F_3 , with one exception, all lines are like the English line in each of the four characteristics; the one exception is a line laying eggs of intermediate size, but like the English line in other respects. In F_4 , there is one line (perhaps also a second) that is exceptional in laying eggs intermediate in size, while the other characters of this line, and all four characters of the other lines, were like those of the English line. In a back cross between an F_2 and the Nebraska line, all characters of all lines are again English. In a generation descended by inbreeding from the back cross, there is evidence of segregation of egg size, less evidence of segregation of the factors for the other characters. The results just stated are summarized in tabular form in table 16.

Taking the experiments as a whole, they show much less segregation than was to be expected. The characters of the English line appear to hang together, the transmission of one being the same, with a few exceptions, as that of all the others.

Evidence is given to show that the results of the experiments must be regarded as valid, and several possible explanations of the peculiarities of those results are discussed. The explanation may be typically Mendelian, if selective mortality of the fertilized eggs be assumed, or if each character be represented by plural factors. The view that all four visible differentiating characters are but different manifestations of a single character, is held to be improbable. The hypothesis that the genes for the four characters are associated, but that the associations may be broken, is not strongly advocated; but the objections to it seem less formidable than the objections to the other explanations offered. No mechanism of association is suggested, though it may depend in some way upon an abnormal process of fertilization.

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