THE CONTROL OF SEX BY FOOD IN FIVE SPECIES OF ROTIFERS

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SIX FIGURES

It has been shown in the American and English rotifer, Hydatina senta, that food conditions are the controlling factors in regulating the parthenogenetic production of the two sexes. When the parthenogenetic females were fed upon a diet of the colorless flagellate, Polytoma, they produced female-producing daughters exclusively even through a period of twenty-two months and extending through many scores of generations. However, when the parthenogenetic females were suddenly transferred from the Polytoma diet to a diet of the green flagellate, Chlamydomonas pulvisculus, they produced in many instances as high as 80 per cent, or higher, of male-producing daughters within a few hours. In a few selected experiments the percentage of male-producing daughters reached 100 per cent when the diet of colorless Polytoma was suddenly changed to a diet of the green Chlamydomonas.

If the production of the sexes can be regulated in this rotifer by the diet it is of considerable interest to know whether the diet can regulate the production of the sexes in other species of rotifers. Furthermore, it is quite important to determine whether it was the stimulus produced by the change of food from the Polytoma to the Chlamydomonas diet that caused the male-producing daughters to be suddenly produced or whether it was a sufficient quantity of more easily assimilated food that changed the mechanism of the daughters from female to male-producers, or perhaps some other factor.

The colorless Polytoma was reared in stable tea (horse manure) solution in a subdued light while the green Chlamydomonas was reared in bouillon solution in direct sunlight. It is very probable that their values as foods would be considerably different, in as much as Polytoma lacks chlorophyl and consequently cannot carry on photosynthesis while Chlamydomonas possesses chlorophyl and manufactures and stores starch in its cell. Thus the colorless flagellate would contain neither starch nor sugars while the green flagellate might have more or less of each.

In order to get new data on these two problems the former experiments upon Hydatina senta were carefully reviewed and new experiments carried out upon the following rotifers: from the order of Ploima the species, Branchionus pala, Diaschiza sterea, Diglena catellina, and from the order of Scirtopoda the species Pedalion mirum. These species were identified by Harry K. Harring, Custodian of the Rotatoria in the United States National Museum, to whom I am greatly indebted for the favor.

Hydatina senta

The results obtained from numerous experiments carried out upon this species from New Jersey and from England have already been published in detail and need not be repeated again. However, as no diagrams have been published of the American form, diagrams have been drawn to the same scale of a female and a male individual, a parthenogenetic female-producing egg, a parthenogenetic male-producing egg, and of a fertilized egg. These are shown in figure 1. A general review of the results of the former experiments have been put into a plotting in diagram 1, showing that a continuous diet of Polytoma caused only female-producing females to be produced for nearly two years but when the food was suddenly changed to the green Chlamydomonas a high percentage of male-producing females appeared within a few days. An important feature of these experiments, which does not show in the diagram, is the place where the stimulus is effective in changing the females from female-pro-

ducers to male-producers. The mother of the female-producing or male-producing daughters is the individual influenced by the diet. If the mother is fed the colorless diet all of her daughters

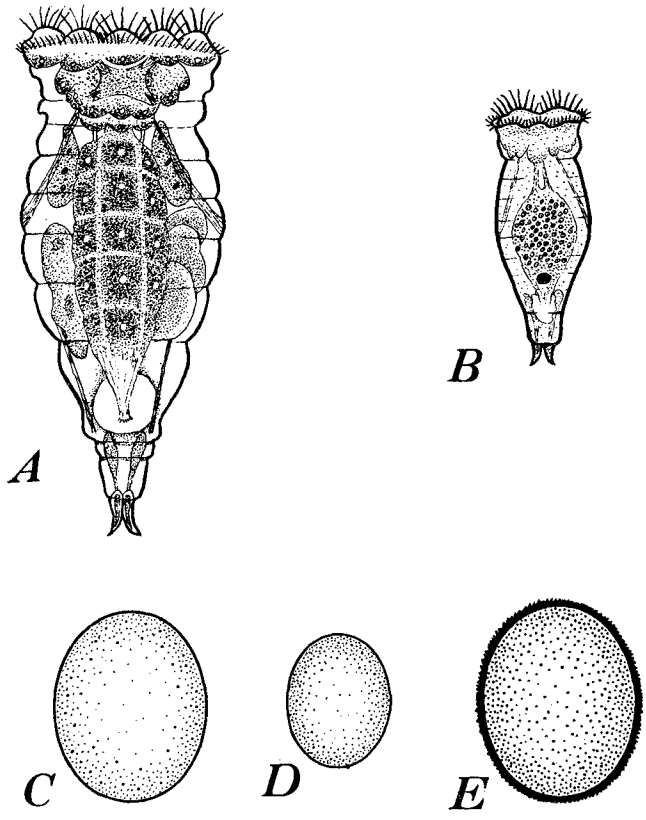


Fig. 1 Hydatina senta. *A*, female; *B*, male; *C*, parthenogenetic egg which develops into a female; *D*, smaller parthogenetic egg which develops into a male; *E*, fertilized egg, which is often called resting or winter egg, and always develops into a female. This egg if unfertilized would have formed a small parthenogenetic egg which would have developed into a male.

will produce females but if the diet of the mother is changed from the colorless flagellates to the green flagellates she will begin soon to produce daughters which will produce males.

Brachionus pala

In August of 1908 a small pond was found at Cold Spring Harbor, Long Island, New York that contained great numbers of this species of rotifer. The pond was located in a small sunny pig pasture in which there were kept a few pigs. These animals lay in the pond a large part of the time during the hot days and consequently the water became somewhat foul and furnished an ideal culture medium for the rotifers and the va-

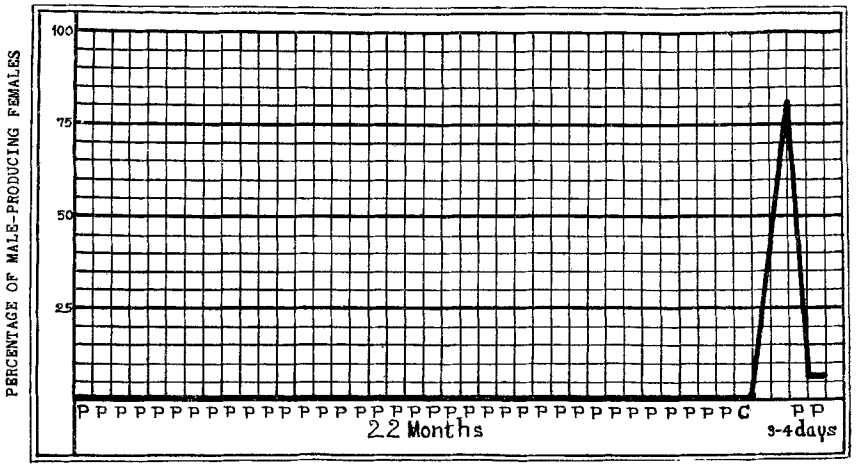


Diagram 1 Hydatina senta. Showing that a continuous diet of Polytoma through a long period of time yielded only female-producing females but when the diet was suddenly changed to Chlamydomonas male-producing females appeared at once. *P* indicates a Polytoma diet, *C* indicates a Chlamydomonas diet.

rious micro-organisms on which they fed. No experiments were carried out at that time but some mud from the bottom of the pond was taken, dried, put into a paper bag, and stored in an ordinary laboratory room.

In February of 1915 some of this dried mud was put into a solution of 10 cc. of bouillon and 140 cc. of tap water and placed near a window. Within a few days various protozoa were numerous in the jar and a few females of the rotifer, Brachionus pala, were found. These females undoubtedly had hatched

from the winter eggs which are the fertilized eggs. Such eggs of Hydatina senta have been found to live six years in old culture water and three years in a dried state. When dried they have withstood for several days the extreme low temperature of liquid air which is about −191°C. and also they have withstood as high a temperature as +110°C. for a few hours. Moreover, it has been found that species of rotifers that produce fertilized eggs cannot themselves be dried and later be revived by placing in water as is true of those rotifers which do not produce fertilized eggs.

These females of Brachionus pala continued to live and to reproduce rather slowly in the culture jar of bouillon and water. From this stock jar females were taken and many preliminary experiments were carried out in attempts to find optimum food conditions. Finally it was found that when bouillon cultures, inoculated with the miscellaneous green flagellates that developed from the same mud from which the rotifers developed, were placed in direct sunlight they developed very fine food cultures for the rotifers. In order to prevent the temperature of the culture jars from rising too high in the direct sunlight and thereby killing all the flagellates as well as the rotifers that might be in them, the jars were placed in a large pan through which tap water flowed. In this way the temperature could be regulated at will and was not allowed to rise above 35°C. to 37°C. The green flagellates seem to be the most active at this temperature and as the rotifers could eat them only in an active state all the cultures were usually kept in this pan. At night and on cloudy days the temperature would be the same as the room temperature of about 20°C.

The stock solution of bouillon was made by boiling one Armour's beef bouillon cube in 400 cc. of tap water.

The bouillon culture of green flagellates was allowed usually to stand from several days to several weeks in direct sunlight with occasional additions of fresh bouillon. During this time several inoculations with the rotifers were made and usually at the end of a few days there would be a good culture of both the green flagellates and the rotifers. After some time if no

fresh bouillon was added the whole culture of the flagellates and rotifers became balanced, that is the flagellates reproduced at such a rate as to keep the rotifers moderately supplied with food. When they were thus supplied moderately with food only females were found in the culture jar but when fresh bouillon was added to the general culture it furnished additional food for the flagellates causing them to multiply very rapidly and to produce enormous numbers of themselves in the jar. With this great increase of food the rotifers also began to eat ravenously and soon produced a great number of females which to a large proportion produced male offspring. In this manner of increasing the food by adding fresh bouillon males could be produced in enormous numbers within a short time. These males copulated with many of the young females and within a few days the majority of the adult females were carrying fertilized eggs. Soon the cultures were depleted of food by the great increase of rotifers and then nearly all the rotifers died of starvation but left thousands of fertilized resting eggs.

It has been shown by Lauterborn that if the male egg is fertilized in Asplanchna it produces the winter or resting egg. The same fact has been shown by Whitney and Shull to be true of Hydatina senta and recently it has been shown by Moro to be true also of Brachionus pala.

These experiments were done in mass cultures many of which contained many thousands of individuals and countings were made of the female-producing and male-producing females at various intervals. These two kinds of females were easily distinguished from each other because after they had extruded the eggs they carried around the eggs attached to their bodies. The male-producing females carried small eggs and the female-producing females carried large eggs. The details of the experiments are in table 1. Drawings of the females carrying the different eggs and also of a male are in figure 2. In diagram 2 a plotting is seen of experiment 1, and in diagram 3 a plotting is seen of a part of experiment 3.

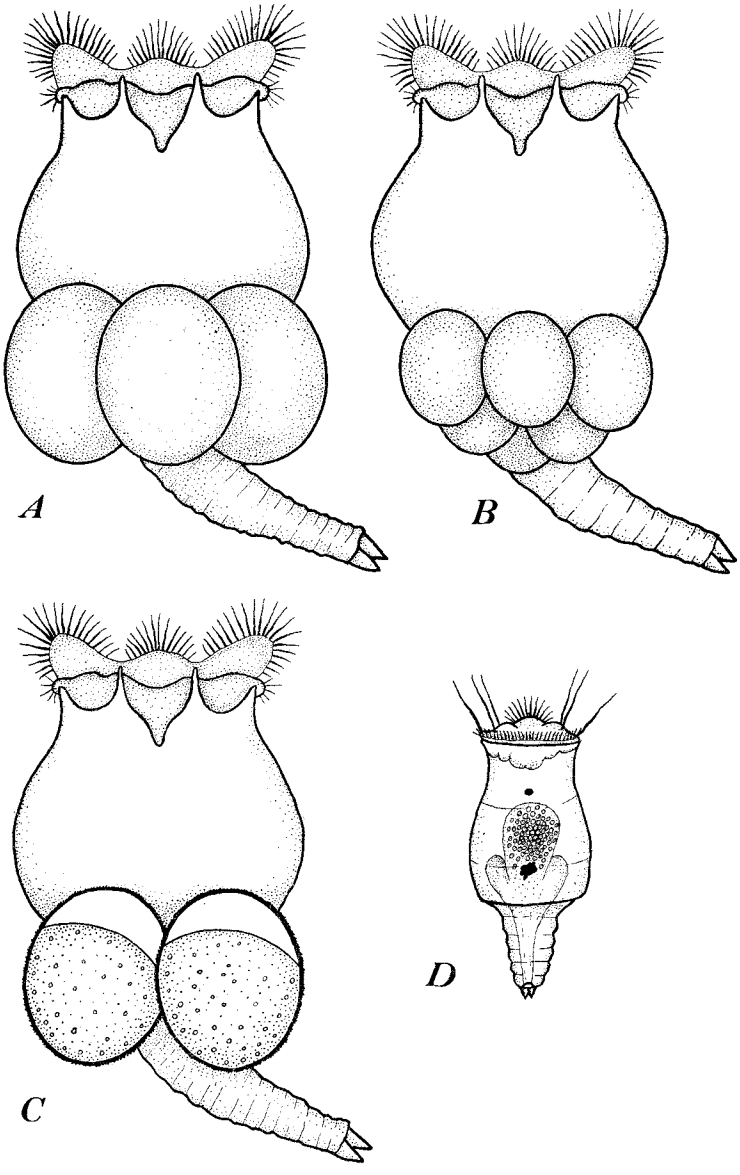


Fig. 2 Brachionus pala. *A*, female with attached parthenogenetic female eggs; *B*, female with attached parthenogenetic male eggs; *C*, female with attached fertilized eggs; *D*, male.

TABLE 1

Experiments with Brachionus pala and a mixed diet of miscellaneous green flagellates showing that when the flagellates were caused to be very abundant by the addition of fresh culture media the percentage of the male-producing female rotifers was very high and the percentage of the female-producing female rotifers was very low but when no fresh culture media was added and the flagellates occurred only in moderate numbers the percentage of male-producing females was very low and the percentage of the female-producing females was very high.

	1915		CULTURE WATER	♀♀	♂♀	% ♂♀
Experiment 1	April	6	15 cc. bouillon 135 cc. tap water mixed green protozoa			
	April	14	15 cc. bouillon added.....			
	April	17	.....	20	0	0
	April	18	15 cc. bouillon added.....	60	0	0
	April	19	.....	18	2	10
	April	20	15 cc. bouillon added.....	28	12	30
	April	21	.....	70	30	30
	April	23	.....	95	5	5
	April	25	.....	95	5	5
	April	28	.....	200	1	0.5
	May	1	15 cc. bouillon added.....	200	1	0.5
	May	3	15 cc. bouillon added.....			
	May	7	.....	65	35	35
	May	11	.....	97	3	3
	May	19	15 cc. bouillon added.....	100	0	0
	May	21	15 cc. bouillon added.....			
	May	22	.....	70	30	30
	May	24	.....	100	100	50
	June	1	.....	100	0	0
			Total.....	1218	224	
Experiment 2	March	8	15 cc. bouillon 135 cc. tap water mixed green protozoa			
	April	19	15 cc. bouillon added.....	200	0	0
	April	24	15 cc. bouillon added			
	April	26	.....	200	200	50
	April	30	.....	200	2	1
	May	3	15 cc. bouillon added.....			
	May	5	.....	80	20	20
	May	6	15 cc. bouillon added.....			
	May	7	.....	70	30	30
	May	11	.....	99	1	1
			Total.....	849	253	

TABLE 1—Continued

	1915		CULTURE WATER	♀♀	♂♀	% ♂♀
Experiment 3	April	19	15 cc. bouillon 135 cc. tap water mixed green protozoa			
	April	22	15 cc. bouillon added.....			
	April	24	15 cc. bouillon added.....			
	April	26	15 cc. bouillon added.....			
	May	25	.....	95	5	5
	May	28	15 cc. bouillon added.....			
	June	1	15 cc. bouillon added.....			
	June	2	.....	5	95	95
	June	9	.....	50	0	0
			Total	150	100	
Experiment 4	April	19	20 cc. bouillon 130 cc. tap water mixed green protozoa			
	April	22	20 cc. bouillon added.....			
	April	24	20 cc. bouillon added			
	April	26	20 cc. bouillon added.....			
	June	1	20 cc. bouillon added.....	100	0	0
	June	3	20 cc. bouillon added.....			
	June	6	.....	50	50	50
			Total.....	150	50	
Experiment 5	April	19	20 cc. bouillon 130 cc. tap water mixed green protozoa			
	April	22	20 cc. bouillon added.....			
	April	24	20 cc. bouillon added.....			
	April	26	20 cc. bouillon added.....			
	April	28	20 cc. bouillon added.....			
	June	1	20 cc. bouillon added.....			
	August	20	.....	200	0	0
	August	21	10 cc. bouillon added.....			
	August	27	.....	100	100	50
			Total.....	300	100	

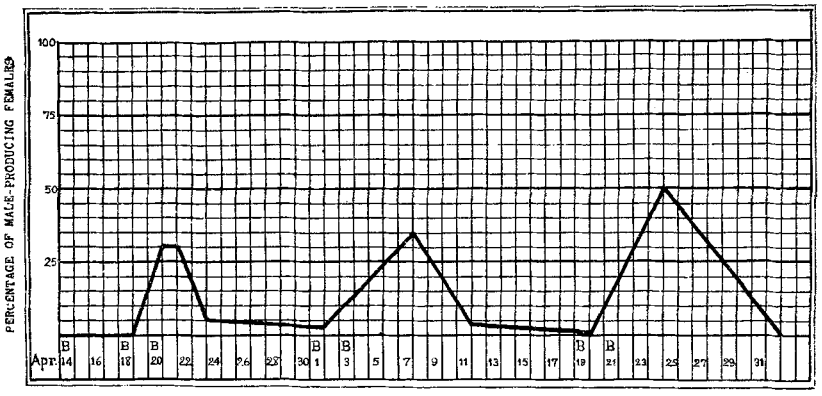


Diagram 2 Brachionus pala. Experiment 1 of table 1. Showing the production of a high percentage of male-producing females when the food conditions were changed by the additions of bouillon. *B* indicates the addition of bouillon.

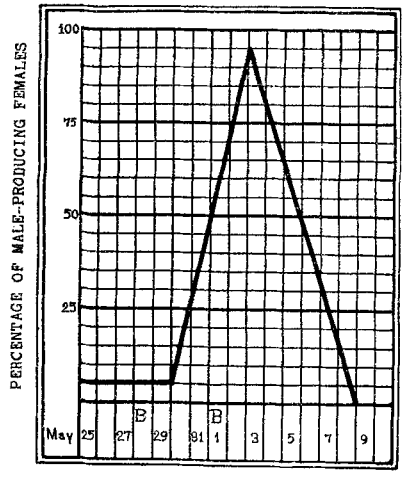


Diagram 3 Brachionus pala. Experiment 3 of table 1. Showing the production of a high percentage of male-producing females when the food conditions were changed by the additions of bouillon. *B* indicates the addition of bouillon.

Diaschiza sterea

In March of 1915 four fertilized eggs of Diaschiza sterea were isolated from a battery jar containing miscellaneous varieties of animals growing in a stable tea (horse manure) solution. This jar was made in September of 1914 and had been left standing since that time without a renewal of stable tea. These fertilized eggs were put into fresh tap water for a few hours and then later a little stable tea was added to the water. Within a short time the eggs hatched and produced females. These females were fed upon the colorless flagellate, Polytoma, and reproducing readily produced a culture of many females but no males.

The Polytoma was reared in a solution of stable tea as follows: 800 grams of fresh horse manure mixed with 1200 cc. of tap water, was cooked in an autoclave at 15 lbs. steam pressure for one hour and then 1000 cc. of the liquid was pressed out of the cooked manure. This solution, hereafter called stable tea, was used as a stock supply. One part of this stable tea and three parts of sterilized water were mixed and inoculated with Polytoma. Every day one-half of this culture was poured off and replaced by a fresh solution of one part stable tea to three parts water. In this manner a vigorous growing pure culture of Polytoma was daily maintained.

The Diaschiza sterea is a small rotifer, no larger than a small paramoecium, and on account of their small size it was found impossible to make individual pedigreed cultures of them. Consequently all the experiments were made in mass cultures either in small watch crystals or in depression slides. In a weak solution of the Polytoma culture (1:2) the female rotifers grew very readily but produced entirely female offspring. However, when these females were transferred to a weak bouillon solution (2 cc. or 5 cc. of the stock bouillon solution to 148 cc. sterilized water) to which pure cultures of Chlorogonium[1] had been added males appeared within a few days varying in pro-

[1] This green flagellate formally has been known as Chlorogonium euchlorum but recently Wille has reclassified it as Chlamydomonas euchlorum. Engler u. Prantl, Pflanzenfam. Nachtrage I Teil, 2d Abt., 1911, p. 18.

portion to the females from 10 per cent to 60 per cent. Diagrams of a male and a female and the different eggs are in figure 3. The details of the experiments are in table 2 and a plotting of experiments 10 to 12, and 16 and 17, are in diagram 4.

The Diaschiza sterea are bottom feeders eating only microorganisms that settle and remain on the bottom of the culture dishes. As both Polytoma and Chlorogonium did this to a considerable extent they furnished very good food material. Chlamydomonas was tried as a food but the rotifers soon died, apparently from starvation.

At this time another series of experiments was made to determine whether the males were caused to appear by the stimulus of the new food or by the stimulus of the bouillon in which the new food and the rotifers were placed. In order to determine this point some culture water from the Polytoma cultures in which the rotifers were thriving was filtered and the female rotifers put back into it. Then the Chlorogonium which had been cultivated in a bouillon solution (1 part of the stock bouillon solution to 1 part water) were thoroughly washed by placing them in test tubes filled with sterilized water and centrifuging on a large centrifuging machine. This process would, of course, collect the Chlorogonium in a small mass at the end of the tube. This washing process was repeated three or four times until the Euglena were undoubtedly thoroughly freed from any bouillon on their external parts. These washed Chlorogonium were then fed to the rotifers that had been put back into the filtered Polytoma culture water. Males soon appeared and in some experiments as many as 40 per cent were found. These experiments demonstrate that the stimulus which caused males to be produced was in the Chlorogonium itself and not in the bouillon. The details of the experiments are in table 3. All the experiments with this species of rotifer were done at room temperature on a table in subdued light.

Diglena catellina

In July of 1915 a few females of the rotifer, Diglena catellina, were found in some general mixed culture jars which were probably inoculated by a collection of various organisms collected

in a towing from a small pond in Middletown. These rotifers are also very small and only mass culture experiments were made.

TABLE 2

Experiments with Diaschiza sterea and two diets of pure cultures of Polytoma and Chlorogonium showing that only females were produced in the colorless diet of Polytoma but when the female rotifers were transferred to a diet of Chlorogonium males appeared soon in the cultures.

EXPERIMENTS	TIME	CONTROL REARED AND CONTINUED IN STABLE TEA CULTURE CONTAINING POLYTOMA				FEMALES REARED IN STABLE TEA CULTURE CONTAINING POLYTOMA TRANSFERRED TO BOUILLON CULTURE CONTAINING CHLOROGONIUM			
	Hours	Females isolated	Estimated number of offspring			Females isolated	Estimated number of offspring		
			♀	♂	% ♂		♀	♂	% ♂
1	60	40	400	0	0	20	140	60	30
2	60	40	400	0	0	20	140	60	30
3	60	40	400	0	0	20	160	40	20
4	60	40	400	0	0	20	160	40	20
5	60	40	400	0	0	20	180	20	10
6	90	40	500	0	0	20	180	120	40
7	90	40	500	0	0	20	210	90	30
8	90	40	500	0	0	20	225	75	25
9	90	40	500	0	0	20	210	90	30
10	90	40	500	0	0	20	150	150	50
11	90	40	400	0	0	20	90	60	40
12	90	40	400	0	0	20	120	80	40
13	90	40	400	0	0	20	90	60	40
14	90	40	400	0	0	20	130	70	35
15	90	40	400	0	0	20	140	60	30
16	90	40	400	0	0	20	80	120	60
17	90	40	400	0	0	20	80	120	60
18	90	40	400	0	0	20	120	30	20
19	90	40	396	4	1	20	105	45	30
20	90	40	396	4	1	20	96	54	36
21	90	40	396	4	1	20	135	15	10
22	90	40	396	4	1	20	90	60	40
23	90	40	396	4	1	20	111	39	26
24	90	40	396	4	1	20	81	69	46
25	90	40	396	4	1	20	105	45	30
26	90	40	396	4	1	20	111	39	26
27	90	40	396	4	1	20	126	24	16
Total		160	1696	4		540	3565	1735	32

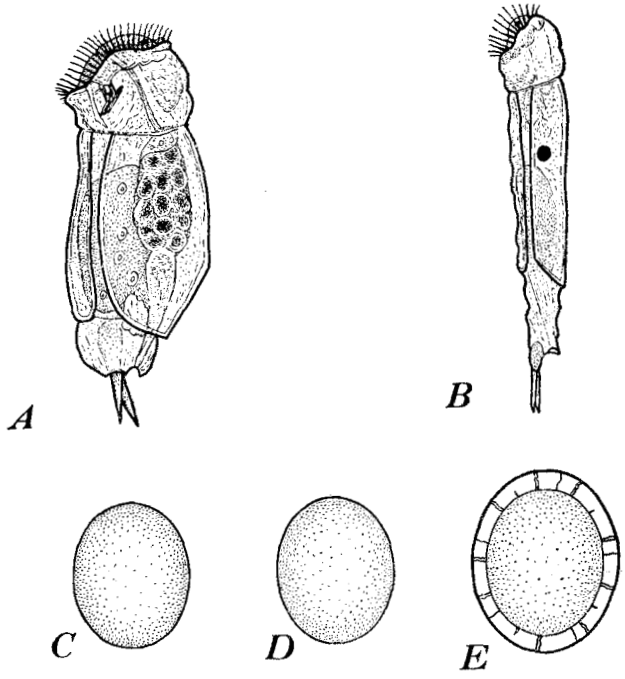


Fig. 3 Diaschiza sterea. *A*, female; *B*, male; *C*, parthenogenetic female egg; *D*, parthenogenetic male egg; *E*, fertilized egg.

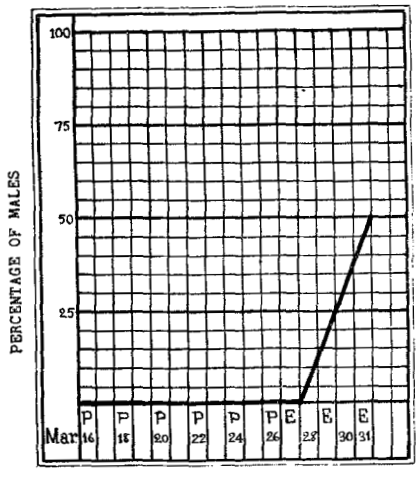


Diagram 4 Diaschiza sterea. Experiments 10, 11, 12, 16, and 17 of table 2. Showing the percentage of males produced when the diets were changed. *P* indicates the feeding of a Polytoma diet, *G* indicates the feeding of a Chlorogonium diet.

The female rotifers were placed in a weak solution of Polytoma culture water which contained many Polytoma. Here

TABLE 3

Experiments with Diaschiza sterea showing that it was the food and not the culture media that caused the males to be produced

EXPERIMENTS	REARED AND CONTINUED IN STABLE TEA CULTURE CONTAINING POLYTOMA					FEMALES REARED IN STABLE TEA CULTURE CONTAINING POLYTOMA TRANSFERRED TO FILTERED STABLE TEA CULTURE WATER TO WHICH WAS ADDED BOUILLON FED CHLOROGONIUM THAT HAD BEEN WASHED				
	TIME		Estimated number of offspring			TIME		Estimated number of offspring		
	Days	Females isolated	♀	♂	% ♂	Days	Females isolated	♀	♂	% ♂
1	4	10	125	1	1	4	12	80	20	20
2	4	10	125	1	1	4	12	100	25	20
3	4	10	125	1	1	4	12	100	25	20
4	4	10	125	1	1	4	12	106	18	15
5	4	10	125	1	1	4	12	87	37	30
6	4	10	125	1	1	4	12	81	43	35
7	4	10	125	1	1	4	12	112	12	10
8	4	10	125	1	1	4	12	112	12	10
9	4	10	125	1	1	4	12	93	31	25
10	14	20	2000	0	0	4	20	210	90	30
11	14	20	2000	0	0	4	20	162	87	35
12	14	20	2000	0	0	4	20	175	75	30
13	14	20	2000	0	0	4	20	120	60	30
14	14	20	2000	0	0	4	20	130	70	35
15	14	20	2000	0	0	4	20	175	75	30
16	14	20	2000	0	0	4	20	120	80	40
17	14	20	2000	0	0	4	20	140	60	30
18	14	20	2000	0	0	4	20	130	70	35
19	14	20	2000	0	0	4	20	120	80	40
20	14	20	2000	0	0	4	20	140	60	30
21	14	20	2000	0	0	4	20	160	40	20
22	14	20	2000	0	0	4	20	140	60	30
23	14	20	2000	0	0	4	20	150	50	25
24	14	20	2000	0	0	4	20	160	40	20
25	14	20	2000	0	0	4	20	150	50	25
26	14	20	2000	0	0	4	20	160	40	20
27	14	20	2000	0	0	4	20	180	20	10
28	14	20	2000	0	0	4	20	170	30	15
29	14	20	2000	0	0	4	20	150	50	25

they lived and produced many daughters which produced in their turn daughters. This was continued for several weeks and many hundred females were produced but not a single male

TABLE 4

Experiments with Diglena catellina showing that when the rotifers were fed the colorless Polytoma diet no males were produced but when the females were transferred to a diet of miscellaneous green flagellates males soon appeared.

EXPERIMENTS	TIME	CONTROL REARED AND CONTINUED IN STABLE TEA CULTURE CONTAINING POLYTOMA				FEMALES REARED IN STABLE TEA CULTURE CONTAINING POLYTOMA TRANSFERRED TO A MIXED STABLE TEA AND BOUILLON CULTURE CONTAINING MISCELLANEOUS GREEN FLAGELLATES			
	Hours	Females isolated	Estimated number of offspring			Females isolated	Estimated number of offspring		
			♀	♂	% ♂		♀	♂	% ♂
1	72	20-30	200	0	0	20-30	160	40	20
2	72	20-30	200	0	0	20-30	170	30	15
3	72	20-30	200	0	0	20-30	180	20	10
4	72	20-30	200	0	0	20-30	160	40	20
5	72	20-30	200	0	0	20-30	170	30	15
6	72	20-30	200	0	0	20-30	180	20	10
7	72	20-30	200	0	0	20-30	170	30	15
8	72	20-30	200	0	0	20-30	160	40	20
9	48	15-20	150	0	0	15-20	105	45	30
10	48	15-20	150	0	0	15-20	105	45	30
11	48	15-20	150	0	0	15-20	120	30	20
12	48	15-20	150	0	0	15-20	135	15	10
13	48	15-20	150	0	0	15-20	120	30	20
14	48	15-20	150	0	0	15-20	120	30	20
15	48	15-20	150	0	0	15-20	120	30	20
16	90	50	500	0	0	15-20	160	40	20
17	90	50	500	0	0	15-20	170	30	15
18	90	50	500	0	0	15-20	180	20	10
19	90	50	500	0	0	15-20	160	40	20
20	90	50	500	0	0	15-20	140	60	30
21	90	50	500	0	0	15-20	170	30	15

individual appeared during this time. However, when some of these females were taken from the Polytoma culture and placed in a weak culture water of bouillon and stable tea (10

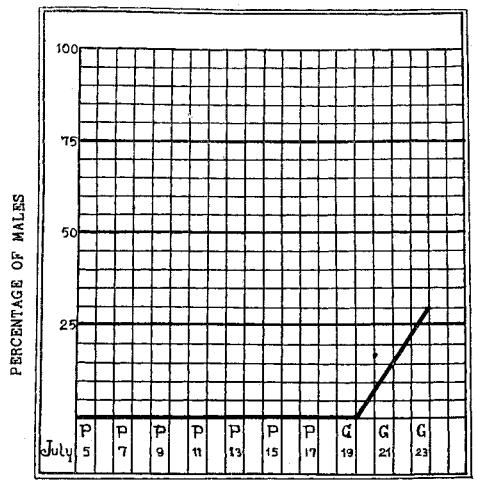


Diagram 5. Diglena catellina. Experiment 20 of table 4. Showing the percentage of males produced when the diets were changed. *P* indicates the feeding of a Polytoma diet, *G* indicates the feeding of a mixed green flagellate diet.

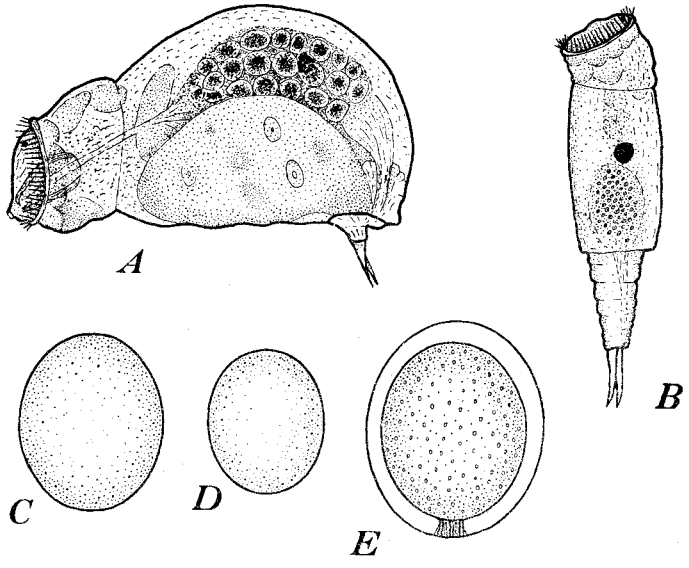


Fig. 4 Diglena catellina. *A*, female; *B*, male; *C*, parthenogenetic female egg; *D*, parthenogenetic male egg; *E*, fertilized egg.

cc. bouillon, 2 cc. stable tea, and 138 cc. tap water) in which there were growing a mixed culture of various green flagellates many male individuals appeared within a few days.

The average percentage of males produced in this species is lower than it is in the preceding species. This may have been due to the inferior quality of these flagellates as a food for this particular species of rotifer, or as these rotifers are also bottom feeders perhaps they could not obtain a superabundance of food as many of the flagellates were more or less free-swimming.

These experiments were all done at room temperature on a table in subdued light. Diagrams of a female and a male rotifer and the different eggs are in figure 4, a plotting of experiment 20 is in diagram 5, and the details and the results of the experiments are in table 4.

Pedalion mirum

In February of 1915 some of the same dried mud that was collected at Cold Spring Harbor in 1908 and produced Brachionus pala in February of 1915 was put into weak stable tea water (3 cc. stable tea added to 147 cc. water) and after a few days several females of the jumping rotifer, Pedallion mirum were found. After considerable experimenting a suitable method was found by which large numbers of these rotifers could be readily reared.

In some experiments weak solutions of stable tea alone were used and in others bouillon was added to the stable tea solution. All the cultures were inoculated with a miscellaneous collection of green flagellates and then kept in direct sunlight as much as possible. The temperature was not allowed to rise above 37°C. This was rendered possible by placing the culture jars in a large pan through which water flowed from the tap. At night, however, the temperature was that of the room, 20°C.

After the cultures had been progressing for a few days the flagellates, mostly Chlamydomonas, and the rotifers became more or less balanced and only female rotifers occurred. How-

ever, when new stable tea or bouillon was added to the balanced jars a rapid increase in the number of flagellates took place. This furnished a superabundance of food for the rotifers and they became loaded with eggs. This superabundance of food could be maintained for several days and in the meantime a great number of the rotifers were seen to be carrying small male eggs. Some females had as many as eighteen of these eggs attached to the outside of their bodies. A little

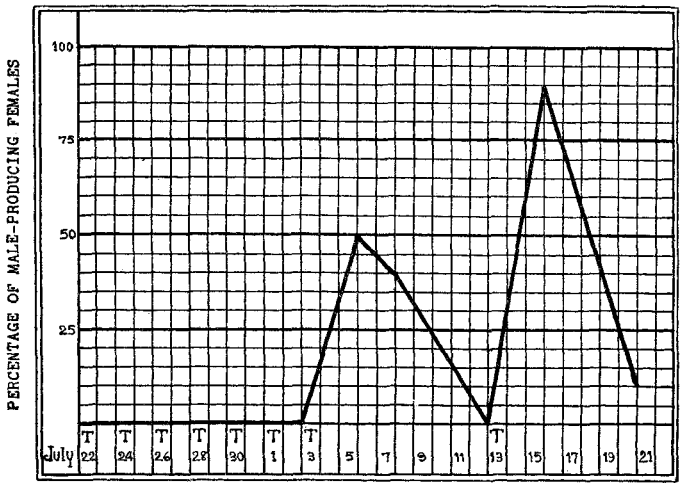


Diagram 6 Pedalion mirum. Experiment 1 of table 5. Showing the production of a high percentage of male-producing females when the food conditions were changed by the additions of stable tea. *T* indicates the additions of stable tea.

later there would be found a very few females carrying female eggs, a few carrying male eggs, and a large number carrying one fertilized egg on the inside of the body. In some jars as many as 90 per cent of the females bore male or fertilized eggs.

After a time nearly all the flagellates were eaten and nearly all the rotifers would die but usually a few survived and as the flagellates would begin to increase again in a few days there would be produced again a balanced culture. In such cul-

tures only female rotifers were usually found. If new stable tea or bouillon was added another epidemic of males was produced. Diagrams of the different females carrying eggs and also a diagram of a male are in figure 5. A plotting of experiment 1 is in diagram 6 and the details of the experiments are in table 5.

TABLE 5

Experiments with Pedalion mirum and a mixed diet of miscellaneous green flagellates showing that when the flagellates were caused to be very abundant by the addition of fresh culture media the percentage of the male-producing female rotifers was very high and the percentage of the female-producing female rotifers was very low, but when no fresh culture media was added, thus causing the flagellates to occur in moderate numbers, the percentage of male-producing females was very low and the percentage of the female-producing females was very high.

	1915		CULTURE WATER	♀♀	♂♀	% ♂♀
Experiment 1	July	22	2 cc. stable tea 150 cc. tap water Miscellaneous green flagellates			
	July	24	2 cc. stable tea added....			
	July	26	2 cc. stable tea added....			
	July	28	2 cc. stable tea added....			
	July	30	2 cc. stable tea added....			
	August	2	.....	200	0	0
	August	5	.....	100	100	50
	August	7	.....	120	80	40
	August	8	.....	160	40	20
	August	10	.....	270	30	10
	August	12	.....	398	2	0.5
	August	13	2 cc. stable tea added....			
	August	14	.....	70	30	30
	August	15	.....	10	90	90
	August	18	.....	60	40	40
	August	20	.....	90	10	10
			Total.....	1558	342	

TABLE 5—Continued

	1915		CULTURE WATER	♀♀	♂♀	% ♂♀
Experiment 2	July	24	2 cc. stable tea 150 cc. tap water Miscellaneous green flagellates			
	July	24	2 cc. stable tea added ...			
	July	26	2 cc. stable tea added ...			
	July	28	2 cc. stable tea added ...			
	July	30	2 cc. stable tea added....			
	August	2	5 cc. bouillon added.....	200	0	0
	August	5	5 cc. bouillon added.....	200	0	0
	August	7	.....	200	0	0
	August	8	.....	160	40	20
	August	10	.....	291	9	3
	August	11	2 cc. stable tea added....			
	August	13	2 cc. stable tea added....			
	August	14	.....	90	10	10
	August	17	.....	60	40	40
	August	18	.....	50	50	50
	August	20	.....	90	10	10
			Total.....	1341	159	
Experiment 3	July	28	75 cc. old stable tea culture 75 cc. tap water 5 cc. bouillon Miscellaneous green flagellates			
	August	5	2 cc. stable tea added....			
	August	7	2 cc. stable tea added....	70	30	30
	August	8	.....	100	0	0
	August	10	.....	100	0	0
	August	11	2 cc. stable tea added....			
	August	13	2 cc. stable tea added....			
	August	14	.....	50	50	50
	August	16	.....	60	40	40
	August	18	.....	400	1	0.25
			Total.....	780	121	

TABLE 5—Continued

	1915		CULTURE WATER	♀♀	♂♀	% ♂♀
Experiment 4	July	27	75 cc. old stable tea culture 75 cc. tap water 5 cc. bouillon Miscellaneous green flagellates			
	August	2	5 cc. bouillon added.....			
	August	3	5 cc. bouillon added..... 2 cc. stable tea added....			
	August	5	2 cc. stable tea added....			
	August	7	2 cc. stable tea added....	40	160	80
	August	10	.....	396	4	1
	August	11	2 cc. stable tea added....			
	August	13	2 cc. stable tea added....			
	August	14	.....	140	60	30
	August	15	2 cc. stable tea added....			
	August	16	.....	100	100	50
	August	18	.....	200	0	0
			Total.....	876	320	
Experiment 5	July	28	40 cc. old stable tea culture 110 cc. tap water 5 cc. bouillon Miscellaneous green flagellates			
	August	5	2 cc. stable tea added....			
	August	7	2 cc. stable tea added....	96	4	4
	August	8	.....	285	15	5
	August	11	2 cc. stable tea added....			
	August	13	2 cc. stable tea added....			
	August	14	.....	160	40	20
	August	15	2 cc. stable tea added....			
	August	16	.....	150	50	25
	August	18	.....	90	10	10
	August	20	.....	200	0	0
			Total.....	1081	119	

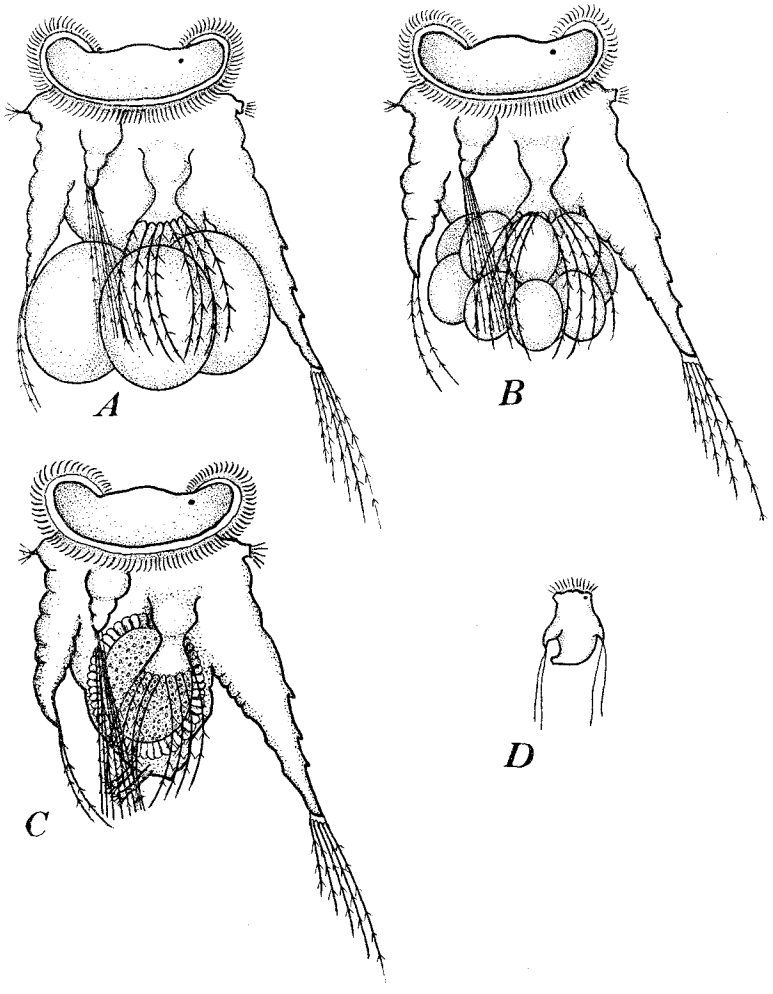


Fig. 5 Pedalion mirum. *A*, female with attached parthenogenetic female eggs; *B*, female with attached parthenogenetic male eggs; *C*, female with the fertilized egg inside her body; *D*, male.

THE INFLUENCE OF A SINGLE DIET

In all of the preceding experiments there were used either two diets, one of colorless flagellates alternated with one of green flagellates or a mixed diet containing miscellaneous species of protozoa, principally green flagellates. During the experiments with the mixed diets when at times they consisted of nearly all of one species of flagellate, Chlamydomonas, it was suspected that one favorable diet could be so manipulated as to yield either female-producing or male-producing rotifers. In order to test this hypothesis pure cultures of the green Chlamydomonas were made in mixtures of bouillon and stable tea solutions. These cultures were inoculated with the rotifer, Brachionus pala, and several experiments were started and carried through to completion.

The flagellate, Chlamydomonas, passes through several stages in its life cycle, some of which are to the advantage of the rotifers and others of which are distinctly to the disadvantage of the rotifers as far as being of food value. In favorable culture solutions the individuals of Chlamydomonas are very active during the sunny part of the day but do not reproduce very much. However, at night, having reached their full size, many of them become motionless and begin to divide inside of their outer envelope. Here each divides three times resulting in eight small individuals. Sometime in the morning hours the parent envelope breaks and the eight young come out and soon become separated from each other. These young individuals are active during the day while increasing in size and at night having reached their full size they become quiescent, begin to divide, and before morning break into eight small individuals. There is considerable variation in this life cycle. Some may be in the eight-cell stage and break out in the early evening while others may be in such stages in the late morning but the large majority go through the cycle as indicated.

When the Chlamydomonas are small in size they are the most readily eaten by the rotifers. As they reach full size, although they may be very active, they are not eaten at all by the rotifers.

In fact in some old cultures where there were great numbers of these full sized flagellates in an active state but not dividing the rotifers died from starvation. The rotifers are transparent and one can see the food in their stomachs after they have eaten.

By adding fresh stable tea or bouillon or both to the cultures enormous numbers of the small sized Chlamydomonas were produced and in a short time the jars were swarming with female rotifers each of which was carrying many small male eggs. Then if no fresh stable tea or bouillon was added nearly all the flagellates were soon eaten, and consequently nearly all the rotifers died. After a short time the few flagellates that had escaped would increase their numbers considerably and the few rotifers that had escaped starvation could thus get a moderate amount of food. Soon the flagellates and the rotifers would form a balanced culture and continue thus for many days. In this balanced condition nearly all female-producing females were produced. However, if fresh stable tea or bouillon were added again to the culture an enormous increase in the numbers of the small sized Chlamydomonas took place accompanied by a rapid increase of the female rotifers. Soon a very high percentage of these females would be carrying male eggs. Thus it is shown that a moderate amount of the diet Chlamydomonas, will cause only female-producing females to be produced but that an abundant diet or a superabundant diet of the same Chlamydomonas will produce in some experiments as high as 90 per cent of male-producing females.

The details of these experiments with a pure culture of Chlamydomonas are seen in table 6 and a semi-diagrammatic plotting of the average results of the nine experiments are seen in diagram 7. Diagrams of the different stages in the life cycle of Chlamydomonas is seen in figure 6.

TABLE 6

Experiments with Brachionus pala and a pure diet of Chlamydomonas showing that when the Chlamydomonas were caused to be very abundant by the addition of fresh culture media the percentage of the male-producing female rotifers was very high and the percentage of the female-producing female rotifers was very low but when no fresh culture media was added, and thereby causing the Chlamydomonas to occur in moderate numbers the percentage of male-producing females was very low and the percentage of the female-producing females was very high.

	1915	CULTURE WATER	♀♀	♂♀	% ♂♀
Experiment 1	September 22	10 cc. bouillon 140 cc. tap water Chlamydomonas			
	September 23	10 cc. bouillon added.....			
	September 25	10 cc. bouillon added.....			
	September 27	10 cc. bouillon added.....			
	September 28	3 cc. stable tea added....			
	October 2	10 cc. bouillon added.....			
	October 11	.....	20	80	80
	October 13	.....	20	180	90
	October 20	10 cc. bouillon added.....	95	5	5
	October 28	10 cc. bouillon added.....			
	October 31	.....	80	120	60
	November 1	.....	50	150	75
	November 5	.....	200	0	0
		Total.....	465	535	
Experiment 2	October 2	3 cc. stable tea 10 cc. bouillon 137 cc. tap water Chlamydomonas			
	October 4	10 cc. bouillon added.....			
	October 6	10 cc. bouillon added.....			
	October 11	10 cc. bouillon added.....			
	October 21	.....	40	160	80
	October 28	10 cc. bouillon added.....	95	5	5
	November 1	10 cc. bouillon added.....	200	0	0
	November 8	.....	20	80	80
	November 16	.....	98	2	2
		Total.....	453	247	

TABLE 6—Continued

	1915	CULTURE WATER	♀♀	♂♀	%♂♀
Experiment 3	October 2	3 cc. stable tea 10 cc. bouillon 137 cc. tap water Chlamydomonas			
	October 4	10 cc. bouillon added.....			
	October 6	10 cc. bouillon added.....			
	October 11	10 cc. bouillon added.....			
	October 21	.....	40	160	80
	October 22	.....	40	360	90
	October 28	10 cc. bouillon added.....	97	3	3
	November 1	10 cc. bouillon added.....			
	November 8	.....	20	80	80
	November 16	.....	100	0	0
		Total	297	603	
Experiment 4	October 2	3 cc. stable tea 10 cc. bouillon added 137 cc. tap water Chlamydomonas			
	October 4	10 cc. bouillon added.....			
	October 6	10 cc. bouillon added.....			
	October 11	10 cc. bouillon added.....			
	October 22	10 cc. bouillon added.....			
	October 23	.....	20	180	90
	November 8	10 cc. bouillon added..... 3 cc. stable tea added....	95	5	5
	November 10	10 cc. bouillon added.....			
	November 14	.....	25	75	75
	November 20	.....	50	0	0
		Total.....	190	260	
Experiment 5	October 2	3 cc. stable tea 10 cc. bouillon 137 cc. tap water Chlamydomonas			
	October 4	10 cc. bouillon added.....			
	October 6	10 cc. bouillon added.....			
	October 11	10 cc. bouillon added.....			
	October 22	10 cc. bouillon added.....			
	October 23	.....	40	160	80
	October 24	.....	40	160	80
	November 8	10 cc. bouillon added..... 3 cc. stable tea added....	95	5	5
	November 10	10 cc. bouillon added.....			
	November 12	.....	20	80	80
	November 18	.....	50	1	2
		Total.....	245	406	

TABLE 6—Continued

	1915	CULTURE WATER	♀♀	♂♀	% ♂♀
Experiment 6	October 2	10 cc. bouillon, 3 cc. stable tea, 137 cc. tap water, Chlamydomonas			
	October 4	10 cc. bouillon added.....			
	October 6	10 cc. bouillon added.....			
	October 11	10 cc. bouillon added.....			
	October 22	10 cc. bouillon added.....			
	October 23	.....	40	160	80
	October 24	.....	20	180	90
	November 8	10 cc. bouillon added..... 3 cc. stable tea added...	97	3	3
	November 10	10 cc. bouillon added.....			
	November 12	.....	20	80	80
	November 20	.....	100	0	0
		Total.....	277	423	
Experiment 7	October 2	10 cc. bouillon, 3 cc. stable tea, 137 cc. tap water, Chlamydomonas			
	October 4	10 cc. bouillon added.....			
	October 6	10 cc. bouillon added.....			
	October 11	10 cc. bouillon added ...			
	October 22	10 cc. bouillon added.....			
	October 26	.....	30	170	85
	November 8	10 cc. bouillon added..... 3 cc. stable tea added...	98	2	2
	November 10	10 cc. bouillon added.....			
	November 12	.....	20	80	80
	November 14	.....	15	85	85
	November 20	.....	98	2	2
		Total.....	261	339	
Experiment 8	October 2	10 cc. bouillon, 3 cc. stable tea, 137 cc. tap water, Chlamydomonas			
	October 4	10 cc. bouillon added.....			
	October 6	10 cc. bouillon added.....			
	October 11	10 cc. bouillon added.....			
	October 22	10 cc. bouillon added.....			
	October 26	.....	20	180	90
	November 8	10 cc. bouillon added..... 3 cc. stable tea added...	98	2	2
	November 10	10 cc. bouillon added.....			
	November 12	.....	50	50	50
	November 16	.....	10	90	90
	November 20	.....	100	0	0
		Total.....	278	322	

TABLE 6—Continued

	1915	CULTURE WATER	♀♀	♂♀	% ♂♀
Experiment 9	October 2	10 cc. bouillon 3 cc. stable tea 137 cc. tap water Chlamydomonas			
	October 4	10 cc. bouillon added.....			
	October 6	10 cc. bouillon added.....			
	October 11	10 cc. bouillon added.....			
	October 22	10 cc. bouillon added.....			
	November 1	.....	40	160	80
	November 8	10 cc. bouillon added.... 3 cc. stable tea added...	95	5	5
	November 10	10 cc. bouillon added.....			
	November 12	.....	40	60	60
	November 13	.....	15	85	85
	November 20	.....	99	1	1
		Total.....	289	311	

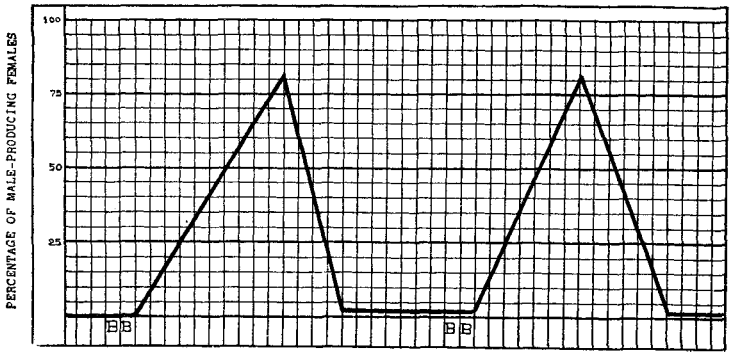


Diagram 7 Brachionus pala. Experiments 1 to 9 of table 6. Showing the production of a high percentage of male-producing females when the food of pure cultures of Chlamydomonas was made superabundant by the additions of bouillon. *B* indicates the addition of bouillon.

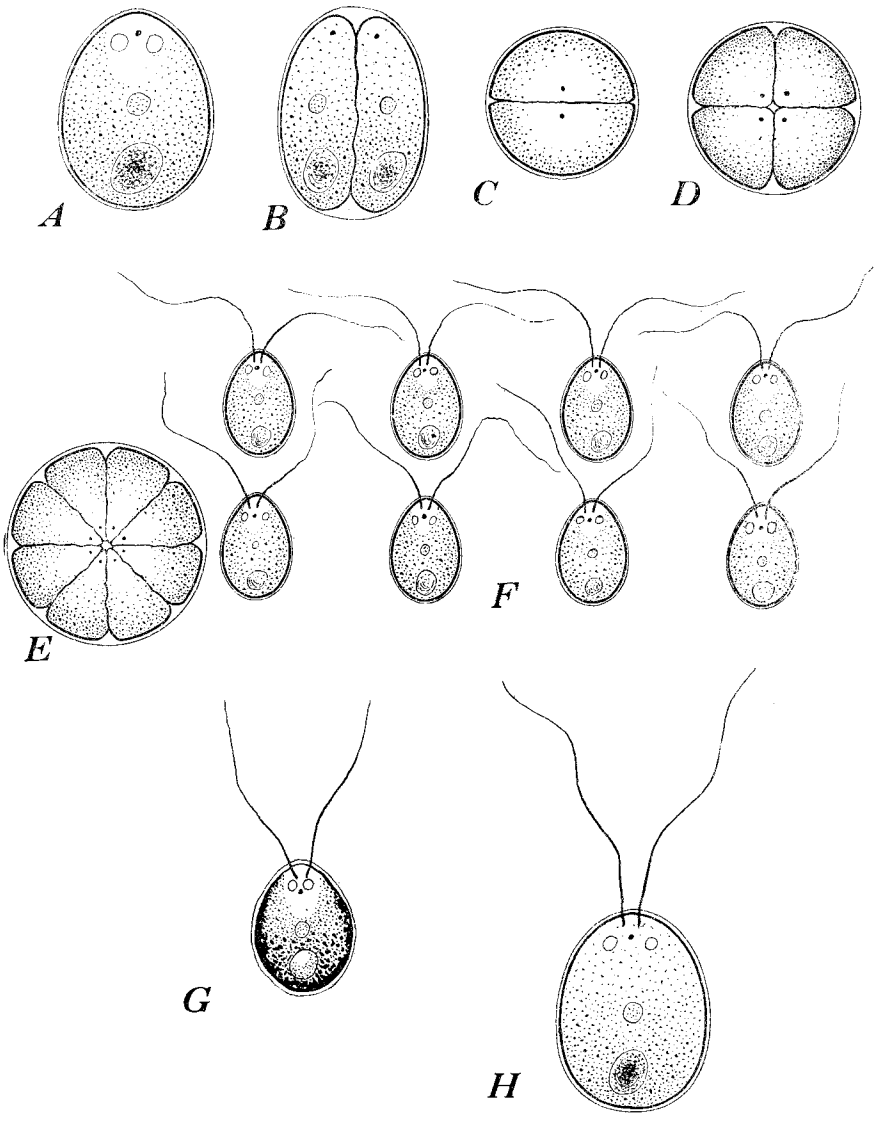


Fig. 6 Chlamydomonas pulvisculus. *A*, mature individual in quiescent stage; *B*, two cell stage, side view; *C*, two cell stage, end view; *D*, four cell stage, end view; *E*, eight cell stage, end view; *F*, the eight active cells after they have broken out of the parental envelope. This is the stage in which the rotifers can eat the Chlamydomonas the most readily; *G*, a later stage; *H*, mature active stage which the rotifers seem unable to eat.

GENERAL DISCUSSION

Probably the production of the male-producing females in all the experiments with the five different species of rotifers can be explained by the quality of the food in the diet. In the experiments with Hydatina senta, Diaschiza sterea, and Diglena catellina the colorless flagellate, Polytoma, very likely furnished a moderate amount of nutrition so that the rotifers lived and reproduced at a moderate rate. When the rotifers were transferred to the green diet they probably ate about the same amount of food but might have digested and assimilated it more easily and faster, and consequently the formation of eggs was more rapid. However, in some way, as yet unknown, the green diet changed the mechanism of the eggs in such a manner that instead of developing into female-producing females they developed into male-producing females. Moro has shown in her chemical experiments with Brachionus pala that a mother forms and extrudes the eggs that develop into male-producing daughters very much faster than a mother that is forming and extruding eggs which develop into female-producing daughters. The ratio was 4 to 1.

In my experiments with Brachionus pala and a pure Chlamydomonas diet it has been found that a mother produces very few eggs that develop into male-producing daughters when she is kept on a scanty diet but when the diet of the same flagellates in the same culture water is made abundant or superabundant the mother produces many eggs which develop into male-producing daughters. Thus showing that there is something in an abundant diet that causes a mother to produce male-producing daughters.

Moro concludes that sudden changes of temperature, either the raising or the lowering of it, causes mothers to produce male-producing daughters. In Hydatina senta Punnett, Whitney, and Shull have found no evidence to support this contention. In the recent experiments with Diaschiza sterea and Diglena catellina the temperature was that of the room at all times. These rotifers in the Polytoma diet produced only fe-

males but when they were put upon the green diet at the same temperature they produced many males. In the experiments with Pedalion mirum and Branchionus pala which were carried on partly in direct sunlight the temperature varied from 20°C. to 37°C. on nearly every day because both sets of experiments were carried on, luckily, in periods of many successive days of sunshine. When the green flagellates were scanty or in the mature stages, regardless of the daily changes in temperature from room temperature to 37°C. during the mornings and from 37°C. to room temperature during the evenings, very few male-producing females were produced. However, when fresh Chlamydomonas culture media was added to the jars in which the rotifers were living an enormous increase in the numbers of Chlamydomonas occurred and soon, in some jars, as high as 95 per cent of male producing females were found. In former observations upon Hydatina senta it has been noted that at the higher temperatures of about 25°C. to 28°C. its green food was very active and could be readily obtained by the rotifers and as many as 80 per cent produced were male-producers but at the lower temperatures of 9°C. to 12°C. the greater part of its green food became quiescent in which stage it was impossible for the rotifers to eat it and all the females produced were female-producers. The general conclusion can be drawn that the changes in temperature unless accompanied by changes in the amount of the diet are not potent factors in regulating the production of female and male-producing female rotifers.

SUMMARY

1. In pedigreed cultures of Hydatina senta a diet of the colorless flagellate, Polytoma, which is probably a poor diet, causes only female-producing daughters to be produced, whereas a diet of the green flagellate, Chlamydomonas pulvisculus, which is probably an optimum food, causes nearly all male-producing daughters to be produced.

2. In mass cultures of Brachionus pala a scanty diet of miscellaneous green flagellates caused nearly all female-producing fe-

males to be produced whereas a superabundance of this same green diet, produced in the jars by the addition of fresh culture media, caused in some experiments as high as 95 per cent of male-producing females to appear.

3. In mass cultures of Diaschiza sterea living in stable tea water at room temperature the diet of Polytoma caused only females to be produced but when the rotifers were transferred to bouillon water containing an abundant diet of Chlorogonium many males were produced.

4. In mass cultures of Diaschiza sterea living in stable tea water at room temperature the diet of stable tea reared Polytoma caused only females to be produced but a diet of a bouillon reared Chlorogonium which had been washed and was added to the filtered stable tea water caused many males to be produced, thus showing that it was the diet and not the change of culture water that caused males to be produced.

5. In mass cultures of Diglena catellina at room temperature the diet of Polytoma caused only females to be produced but a diet of miscellaneous green flagellates caused many males to be produced.

6. In mass cultures of Pedalion mirum a scanty diet of miscellaneous green flagellates caused nearly all female-producing females to be produced whereas a superabundant diet of the same green flagellates, produced in the jars by the addition of fresh culture media, caused in some experiments as high as 90 per cent of male-producing females to be produced.

7. In mass cultures of Brachionus pala a scanty diet of pure cultures of Chlamydomonas pulvisculus caused nearly all female-producing females to be produced whereas a superabundance of this same Chlamydomonas diet, produced in the jars by adding fresh culture media, caused in many experiments as high as 90 per cent of male-producing females to be produced.

8. The colorless diet of Polytoma causes female-producing females to be produced and the green diets of Chlamydomonas, Chlorogonium, or miscellaneous green flagellates causes male-producing females to be produced.

9. A scanty or poor diet of pure cultures of the green flagellate, Chlamydomonas pulvisculus, causes nearly all female-producing females to be produced whereas a copious or superabundant diet of the same pure cultures of the flagellates causes nearly all male-producing females to be produced.

10. A poor or scanty diet causes only female-producing females to be produced but a plentiful diet of the right kind causes nearly all male-producing females to be produced.

December 6, 1915

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