

The Life-Cycle of *Moina rectirostris*.

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IN the summer of 1904 the late Mr. G. H. Grosvenor began some experimental observations on the life-cycle of *Moina*, with the object of testing Weismann's hypothesis (1) that the succession of parthenogenetic and sexual individuals is controlled by an internal rhythm independently of external circumstances. The species of the genus *Moina*, as pointed out by Weismann, belong to the polycyclic group of Cladocera which inhabit small ponds and which produce sexual individuals with great frequency, and this fact, together with the ease with which they can be cultivated, renders them convenient for experimental purposes.

After cultivating the animals for some time, Grosvenor noticed that if the parthenogenetic females were kept isolated in separate vessels from the time of their birth to the period at which they produced young, they gave rise to a much smaller proportion of males than was the case when they were kept crowded together in the same vessel. His method of cultivation was to place the young newly hatched parthenogenetic females, either isolated or together in numbers of about five to fifteen, in ordinary tumblers three quarters full of tap-water, and to add to the water a small quantity of a stock infusion

made from dry horse-dung. The actual numbers obtained by him in these early experiments were as follows:

Moina rectirostris: Oxford, 1904. June, July and October.

Isolated.		Crowded.	
No. of ♂s.	No. of ♀s.	No. of ♂s.	No. of ♀s.
4	95	50	153
4.2% per cent. males.		24.6 per cent. males.	

Moina macrocopa: Oxford, 1904. June and July.

Isolated.		Crowded.	
No. of ♂s.	No. of ♀s.	No. of ♂s.	No. of ♀s.
25	224	41	92
10% per cent. males.		30.8% per cent. males.	

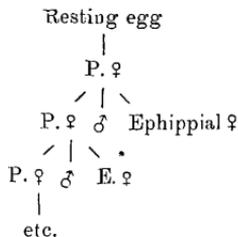
From these figures it will be seen that when the parthenogenetic females were kept isolated they produced 4.2 per cent. and 10 per cent. males, but that when they were kept crowded together but otherwise under identical conditions they produced 24.6 per cent. and 30.8 per cent. males.

In 1906 Grosvenor asked me to join him in repeating and extending this investigation, and during the summer and autumn of that year we bred *Moina rectirostris* on a large scale and subjected them not only to the conditions of isolation and crowding, but also to different temperatures. We obtained the *Moina* from a small pond at Sutton near Stalham on the Norfolk Broads, and most of the experiments were done in the fresh-water laboratory belonging to Sir Eustace and Mr. Robert Gurney, to whom we offer our thanks for giving us every accommodation in their laboratory.

The result of these experiments was to show that Grosvenor's idea as to the factor of isolation and crowding influencing the life-cycle was well founded, and also to prove that the influence of temperature, especially when combined with isolation, was of great importance. We were, in fact, able by keeping the parthenogenetic females isolated in an incubator at about 28° C. to inhibit absolutely and for an indefinite period the production of sexual forms. Although this latter result was of a far more definite kind than could be drawn from any experiments published at the time or since, we refrained from publishing them, as we hoped to find out what the exact nature of the factor of isolation and crowding might be, but the experiments which we made to settle this point were inconclusive. Owing to other duties and preoccupations the work lapsed, but Mr. Grosvenor's sudden death makes it desirable to publish the results as they stand.

Before referring to, and explaining, the tabular statement of our results, it is necessary to make some general observations on the life-cycle of *Moina rectirostris*.

The fertilised egg, after lying dormant, invariably hatches out as a female, which reproduces parthenogenetically. The parthenogenetically produced young may be either themselves parthenogenetic females, or they may be males or ehippial females. The parthenogenetic females of this generation, again, may produce all three kinds of individuals, and so on indefinitely. Schematically we may represent this type of life-history as follows :



In fact, as Weismann pointed out, there is only one generation which invariably and uniformly consists of parthenogenetic females, viz. the first generation which hatches out from the resting eggs. Any of the succeeding generations produced parthenogenetically may consist of males and ephippial females as well as of parthenogenetic females, but that they invariably are so constituted is negatived by our experiments.

The ephippial or sexual females and their relation to the parthenogenetic females demand a word of explanation. The two kinds of female do not differ from one another structurally at first, but a young female that is destined to produce a resting egg and ephippium can be soon recognised by the greater opacity of the ovary, and later by the presence of the large resting egg in the ovary. At most two such resting eggs are brought to maturity and deposited in the specially prepared brood-pouch or ephippium, where they may be seen as opaque dark-red bodies of very large size. The ordinary eggs of the parthenogenetic female are, on the other hand, very numerous, small, and of a transparent greenish colour. Now, as the result of our observations, it is found that an ephippial female, if it is not fertilised by a male, may reabsorb its resting eggs and turn into a parthenogenetic female later. But we did not find any case of a female that had produced eggs parthenogenetically turning into an ephippial female, and giving rise to resting eggs. This is in agreement with Weismann's observations on Cladocera in general, Issakowitsch (2) being the only observer who records the contrary in the case of *Simocephalus vetulus*.

In the tables given on p. 521, 522, no mention is made of the occurrence of ephippial females, because it was impracticable to include them by the methods necessary for dealing with such large numbers of individuals, owing to the necessity of keeping the females alive for some time in order to determine whether they were ephippial or not. Nevertheless this was done in a large number of cases, and it was found that ephippial females occurred in the same broods in

which males were being produced, so that it appears that the conditions which call forth the production of males are the same as those which determine the production of ehippial as opposed to parthenogenetic females.

A good deal of confusion has arisen in regard to the life-cycle of Cladocera by the tacit assumption that the conditions controlling the production of males are sex-determining conditions—that alterations in the food supply, temperature, etc., condition the production of males as opposed to females.

This is an altogether mistaken view. Alterations in the external conditions do not alter the sex-ratio, except in so far as they may change the method of reproduction from that by means of parthenogenetic females to that by means of sexual males and females. For instance, in our own experiments isolation in the warmth led to the entire suppression, not only of males, but also of sexual females, while crowding at room temperature or in the cold led to the increase of males and ehippial females as opposed to parthenogenetic females. Thus, what is really shown to be influenced by the external conditions is not the sex-ratio, but the production or suppression of the sexual forms of both sexes.

The same stricture applies to all the experimental work on the life-history of the Cladocera, which is sometimes loosely termed work on sex-determination, but which is really calculated to throw light, not on the determination of sex, but on the alternation of asexual with the sexual mode of reproduction. Since in our experiments the same conditions called forth the production of males and sexual females, we can only conclude that these conditions were not specific sex-determining conditions, but simply determined that sexual males and females should be produced as opposed to parthenogenetic females. It is, of course, possible that external conditions, besides influencing the occurrence of sexual as opposed to asexual reproduction, may also influence the proportions of males to sexual females in any brood, but our experiments do not throw any light on this point, and the experiments of other observers appear to be beset by the

same limitation. The main results of our experiments are given in tabular form on p. 521, 522.

Table I gives the actual numbers of the males and females produced by parthenogenetic females when isolated in separate vessels and when crowded together in the incubator between 25° and 30° C. (column H); at room temperature, about 14° C, (R); and in an ice-chest between roughly 8° and 1° C. (C). In all, ten experiments are included, Nos. 2 to 12 in the first vertical column. These numbers refer to the particular females which were used as the original parents to start the lines of descent which were employed in the experiments. In the course of the experiments the actual pedigrees of every female used was known, but in Table I all the offspring of succeeding generations are summed and entered together according to whether they were produced from an isolated or "crowded" parent at room temperature, in the incubator or ice-chest. The offspring of the ten different lines are, however, kept separate in the horizontal lines. The first total given in the first horizontal line below the table gives the grand total of males and females produced by all the different lines.

Thus the isolated females in the incubator gave rise to 1167 individuals, all of which were females, no males being produced. The isolated females at room temperature gave 323 males to 1385 females, or 19·1 per cent. males, those in the cold chamber 10 males to 174 females, or 5·4 per cent. males.

The crowded females in the incubator gave 286 males to 657 females, or 30·3 per cent. males; those at room temperature gave 1631 males to 1487 females, or 52·3 per cent. males; and those in the cold chamber gave 167 males to 226 or 42·5 per cent. males.

The most striking of these results is the entire absence of males in the broods of females kept isolated in the incubator. This occurred in six different lines of descent in which parallel cultures at other temperatures, or when the parents were crowded, gave a large proportion of males, so that it cannot be argued that special female-producing lines were by

chance chosen for these experiments.¹ The suppression of the sexual forms must therefore be certainly attributed to the condition of high temperature and isolation of the parents.

In line 5 eight successive generations, containing 413 individuals, were propagated without the appearance of sexual forms.

The effect of isolation is also seen in the fact that the isolated parents at room temperature and in the cold chamber gave a smaller proportion of males than when the parents were crowded under the same conditions, viz. 19.1 per cent. and 5.4 per cent. males as against 52.3 per cent. and 42.5 per cent.

The effect of temperature by itself is seen in the fact that when the parents were isolated in the incubator they gave 0 per cent. males, while isolation at room temperature gave 19.1 per cent. males; also crowding in the incubator gave 30.3 per cent. males, while crowding at room temperature gave 52.3 per cent.

The effect of low temperature, i. e. in the cold chamber at about 5° C., gave actually a lower percentage of males than at room temperature both when the parents were isolated and when they were crowded. The numbers of broods and of individuals produced under these conditions were small, as it was found that the extremely low temperature inhibited growth and reproduction. It might be expected *a priori* that since more males are produced at room temperature than in the incubator, a still greater proportion of males would be produced in the cold, but it appears that the factor of extreme cold introduces another element—possibly that of very slow growth—into the conditions, which acts in an opposite direction.

The influence of isolation and of crowding can be shown by another method of arranging the results. In Table II are given

¹ Prof. R. C. Punnett, in his work on "Hydatina," has suggested that such female-producing lines exist. Owing to the way in which our experiments were performed, viz. by subjecting the individual members of each brood to different conditions, it is impossible to account for our results in this way. We are, however, not in a position to deny that it may be possible to segregate out a pure female-producing line in *Moina* in accordance with Punnett's suggestion.

the proportions of males and females produced at room temperature according to the number of parents present in the vessel. The number of parents kept in the same vessel varied from one to thirty-four. It cannot be said that the proportion of males to females steadily rises as the degree of crowding of the parents increases, as the numbers fluctuate rather erratically, but by grouping the numbers together in various ways it can be shown that on the whole the proportion of males does increase with an increased intensity of crowding. For instance, to take the largest grouping, when one parent was present 19.1 per cent. males were produced, when two to seven parents 37.3 per cent. males, when eight to thirteen parents 60.9 per cent. males, and when fourteen to thirty-four 65.2 per cent. males. We may take it, therefore, as established that the two factors of crowding and temperature profoundly influence the production of the sexual forms, and that by isolation of the parents at a temperature of about 28°C. it is possible to suppress entirely this production.

The inquiry as to how these factors of crowding and temperature brought about this effect was found to be beset with great difficulties. It seemed probable at first that the effect of crowding was due to the accumulation of excretory matter in the glasses, but experiments in which isolated females were placed in culture-water which had previously contained numerous individuals of *Moina* gave negative results, the isolated females in the incubator in such culture-water producing invariably females. Culture-water which had contained great numbers of *Moina* was also evaporated down to dryness and the residue dissolved in fresh culture-water, but again with negative results. It is, of course, possible that the excretory matter is of an unstable character, and quickly oxidised or destroyed as soon as it is formed. There is, however, another possibility, viz. that the effect of crowding is to lessen the food supply for each individual. The food of *Moina* consists of the organisms in the infusion, but it is uncertain whether the Cladocera can feed indifferently on all the bacteria and infusoria found in such infusions, or

whether they are confined to some particular kind of food. If they are omnivorous it is inconceivable that the presence of eight or nine individuals in our culture-glasses should cause any shortage of food, but if they really confine themselves to some particular organism in the infusion as food it is very probable that there is a limited supply of this organism, and that the presence of several *Moina* is sufficient to upset the balance. From the behaviour of *Moina* in the culture-medium it would seem that they are actively hunting some special prey, and the peculiarly local occurrence of the various kinds of Cladocera points to their being in general dependent on special organisms for food. There can be no doubt that an important step forward will be taken when we can settle this question and cultivate these Cladocera on relatively pure cultures of their appropriate food. When the organisms are cultivated on the mixed and complicated fauna of an infusion, as was the case in our experiments, it is impossible to regulate the food supply, as there is no means of controlling the relative abundance of the particular constituent which alone may be serving as food.

For the present we must be content to say that under certain optimum conditions, which can be attained by isolation and a moderately high temperature, it is possible to propagate *Moina* entirely by parthenogenesis without any production of sexual forms, but whether this result is due to the absence of excretory matters or to an abundant food supply it is impossible to decide.

Although our results are incomplete in this direction, yet we are able to furnish a definitely negative answer as to the truth of Weismann's contention that the life-cycle is an hereditarily fixed process which runs its course independently of external conditions. It is, however, by no means certain that the life-cycles of other Cladocera are as sensitive to external conditions as *Moina*. It may be found possible to inhibit the production of sexual forms indefinitely in other species of Cladocera, but it seems probable that the artificial production of sexual forms in any particular generation of those forms

which normally produce only one or two epidemics of sexual forms in the year may be beyond the power of the experimenter, and may be dependent on an internal rhythm such as Weisman suggests. Probably for each species of Cladocera this internal rhythm exists, by which the parthenogenetic or sexual nature of the successive generations is determined, but it is now certain that this "determination" is of a plastic nature, and subject to radical modification in response to changing conditions. The degree of plasticity, and the extent to which the normal life-cycle may be modified, probably differs very greatly in different species. Scharfenberg (3), who has recently worked with *Daphnia magna*, admits the influence of external conditions, but holds that the life-cycle is fairly rigidly determined in Weismann's sense. Issakowitsch, on the contrary, holds that nutritive conditions have a preponderating influence on the life-cycle, and the earlier experiments of Kerhevé point in the same direction.

A critical discussion of the literature (see Scharfenberg [3]), is, however, better postponed until further experiments have been performed, and especially the nature of the food which the various kinds of Cladocera are dependent on has been thoroughly investigated.

SUMMARY.

(1) According to Weismann, in the life-cycle of *Moina* sexual forms should be produced in every parthenogenetic generation independently of external conditions.

(2) By isolating the parthenogenetic females at birth until the production of the brood at a temperature of 25° to 30° C. the production of sexual forms is entirely suppressed, 1167 parthenogenetic females and no sexual forms having been obtained by this means.

(3) Parallel cultures with related females crowded together in the culture-glasses at a temperature of 25° to 30° C. gave 30.3 per cent. males, and at about 14° C. gave 52.3 per cent. males. Isolated females at 14° C. gave 19.1 per cent. males.

Isolated females in an ice chest at about 5°C. gave 5.4 per cent. males, when crowded together 42.5 per cent.

(4) The intensity of crowding, measured by the number of parents kept together in the same glass, is shown to have a not very constant effect on the proportions of males produced, on the whole the proportion of males increasing with the intensity of crowding.

(5) The influence of isolation and of a high temperature on the suppression of the sexual forms may be ascribed either to the comparative absence of excretory matter under these conditions, or else to the nutritive conditions being under these circumstances highly favourable.

TABLE I.—Number of Males and Females in Broods produced at Different Temperatures in Individual lines.

Line.	Isolated.						Crowded.					
	H.		R.		C.		H.		R.		C.	
	♂ s.	♀ s.	♂ s.	♀ s.	♂ s.	♀ s.	♂ s.	♀ s.	♂ s.	♀ s.	♂ s.	♀ s.
2.	—	215	58	—	174	—	63	291	282	—	290	28
	0%		25%				17.7%		49.3%		0%	
3.	—	—	28	—	25	—	—	—	12	—	22	—
			52.8%						35.2%			
4.	—	—	8	—	57	—	—	—	122	—	127	13
			12.3%						48.9%		46.4%	
5.	—	419	—	24	1	153	2	73	36	—	35	—
	0%		0%		5%		2.6%		50.7%		0%	
6.	—	—	78	—	567	—	4	—	592	—	405	41
			12.1%		50%				59.3%		45%	
7.	—	98	58	—	145	—	—	—	151	—	191	28
	0%		28.5%						44.1%		63.3%	
9.	—	62	8	—	54	—	86	—	63	—	160	—
	0%		12.9%				100%		28.2%			
10.	—	87	65	—	130	—	—	—	313	—	134	—
	0%		33.3%						70%			
11.	—	286	2	—	159	—	41	199	54	—	123	76
	0%		1.2%				17.1%		30.5%		49%	
12.	—	—	18	—	50	5	17	94	6	—	9	—
			26.5%		21.7%		50%		100%		36%	
Total	0	1167	323	1385	10	174	286	637	1631	1487	167	226
	0%		19.1%		5.4%		30.3%		52.3%		42.5%	

TABLE II.—Number of Males and Females among Offspring according to Number of Parents in Tumbler. Temperature R.

No. of parents in number.	♂s.	♂s. o.	♂s.	♂s. o.	♂s.	♂s. o.	♂s.	♂s. o.	♂s.	♂s. o.	♂s.	♂s. o.	♂s.	♂s. o.	♂s.	♂s. o.	♂s.	♂s. o.	
1	328	1385	19.1																
2	29	70	26.6	83	135	38.1	19.1												
3	54	56	49.0					213	419	33.7									
4	42	95	30.6	129	284	31.2													
5	87	187	31.7																
6	235	214	52.3	380	492	40.1													
7	95	278	25.4																
8	229	147	60.9	371	270	57.8													
9	142	93	86.0																
10	76	56	57.5	106	78	57.6													
11	30	22	57																
12	58	43	58	206	190	52.0													
13	148	147	50																
14	89	8	91.7	289	39	88.1													
15	200	31	86																
16	18	23	36.1	30	101	22.9													
17	17	78	17.8																
23	0	28	0																
34	15	10	60																

LITERATURE.

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3. Scharfenberg.—'Internat. Revue der Gesamten Hydrobiologie und Hydrographie,' Bd. iii, Biolog. Suppl. (See this paper for literature up to 1911.)
4. Punnett.—'Proc. Roy. Soc.,' vol. lxxviii, B., 1906, p. 223.