PARTHENOGENETIC AND SEXUAL REPRODUCTION IN *SIMOCEPHALUS VETULUS* AND OTHER CLADOCERA.

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In 1879 Weismann formulated the theory that in the Cladocera "der Eintritt der Geschlechtsperiode wird überhaupt nicht durch direct wirkende äussere Momente veranlasst, sondern lediglich durch innere in der Natur des Organismus begründete." There can be no doubt that this total denial of the influence of environment can no longer be maintained. It is as certain as experiment can ever make it, that under some conditions parthenogenetic reproduction will proceed longer than under others, and that to some degree the appearance of sexual forms can be controlled by controlling the environment. The latest and probably the most conclusive evidence of this is given by Grosvenor and Smith for *Moina.* It is true that many experimenters have failed to find any evidence of the direct effect of environment, but this negative evidence cannot be put against the quite satisfactory positive results of other workers. On the other hand, even those who admit the influence of environment do not give up the idea of an underlying "cycle" as well (Issakowitsch, Papanicolau, Woltereck, R. Hertwig).

The object of this note is to present some new evidence and point out some general indications that the change from parthenogenetic to sexual reproduction, and the degeneration which so often accompanies it under experimental conditions, is determined by environment only, and that the number of preceding parthenogenetic generations or the lapse of time since the last sexual act are not, as such, relevant to the matter at all.

My own experiments have dealt chiefly with *Simocephalus vetulus,* and were not undertaken in relation to the reproductive cycle problem, but in connection with various problems of heredity. As, however, the experiments have been extensive, and as one line in particular was bred for 46 successive generations, they are able incidentally to afford very instructive information upon the sexuality question.

It would be useless to review completely the bulky literature dealing with reproduction in Cladocera, and for this I refer the reader to the discussions and literature lists in the works of Papanicolau, Woltereck and Hertwig. It is well, however, to mention a few researches dealing specially with *S. vetulus.*

This species was one of those dealt with by Weismann in 1879, and on which his theory quoted above was based.

Issakowitsch (1908), who experimented with this among other species, comes to the conclusion that it has an inherent reproductive cycle, the duration of which is not, however, independent of external conditions. For the "cycle" is due to an increasing "depression" of the germ cells (in the nucleo-cytoplasm relation sense) which in the early stages can be alleviated by various external factors, but at last gets to a stage when nothing but conjugation will save it from death.

Kuttner (1909) supported Weismann's extreme view. *S. vetulus* was one of the species experimented with.

We must consider Papanicolau's experiment (1911) rather more closely as it was carried out in great detail and thoroughness, and as the conclusions have been accepted by R. Hertwig and used by him in support of his theory of "depression" periods leading to sexual conjugation.

Papanicolau's main experiment dealt with a line bred from an *ex-ephippio* female at room temperature, and gave the following chief results.

1. The line bred parthenogenetically for twenty generations, sexual and degenerate forms getting more and more numerous, the twentieth generation consisting entirely of males and degenerated animals, and parthenogenesis then ceased altogether.

2. Sexual individuals began to appear in late broods of early generations (e.g. in the tenth brood produced by generation 2, in the eighth brood produced by generation 3) and appeared in earlier and earlier broods in later generations, till they occurred in the second brood of generation 17 and first of generation 20.

3. A great many weak and degenerate forms appeared, often abnormal and dying before birth. The appearance of these degenerate forms accompanied that of the sexual ones, and the same phenomenon

of their earlier and earlier appearance in succeeding generations was observed.

4. There was a gradual decrease in the number and an increase in the size of the eggs from generation to generation, and also, as regards size, from brood to brood of the same generation.

He therefore concludes that there is an inherent "sexual cycle" of the orthodox kind in *S. vetulus,* beginning with vigorous parthenogenetic reproduction which gradually gives place to increasing sexuality and degeneracy, ending in total cessation of asexual reproduction. He is, however, prepared to admit that external conditions influence the duration of the cycle to a certain extent.

The line of *S. vetulus* whose sexual condition I wish to describe originated from a parthenogenetic female taken from one of the laboratory tanks in September, 1911. I have of course no knowledge of the number of generations which intervened between it and the last ephippial egg.

Some of the descendants of this individual formed the material on which I did some experiments recently published on the transmission of environmental effects, and also an experiment on parthenogenetic inheritance, not yet published. The method of breeding employed for the great majority of the individuals was that which I described as "normal" or "control" in my former paper. They were bred in cylindrical glass tubes 10 × 3 cms., which when corked (as they always were) contained about 50 c.c. of water and 15 c.c. of air. With rare exceptions one specimen only was kept in each tube, and when it had produced a brood, this was removed within 36 hours. The culture medium was the water from a tank in which were living a number of *Lepidosiren paradoxa,* fed daily with *Anodonta.* The water in this tank was changed weekly (except for that held in a thick layer of mud at the bottom of the tank) and was thick with Infusoria and organic detritus. A jar of water was taken from this tank on alternate days, strained through linen to remove Rotifers, Lynceids, etc. which swarmed in it, and used to renew the water every alternate day in all the breeding tubes. Each *Simocephalus* was picked out of its tube with a pipette, the tube emptied and refilled with the new water and the specimen returned.

The sexual conditions of this line are shown in Table I.

The sex of a new-born *S. vetulus* is very difficult to determine, and I did not attempt to do so, as the experiment was not directed towards this question. The sex of every individual which was kept to maturity

TABLE I.

Number of Broods and Individuals tested for Sexuality in each Generation.

Generation	1st Broods	2nd Broods	3rd Broods	4th Broods	5th Broods	6th Broods	7th Broods	8th Broods	9th Broods	10th Broods	11th Brood
1	1—1	—	—	—	—	—	—	—	—	—	—
2	1—1	1—2	×	×	—	—	—	—	—	—	—
3	×	3—4	1—1	1—4	—	—	—	—	—	—	—
4	4—13	×	×	×	—	—	—	—	—	—	—
5	7—21	5—13	2—13	1—6	1—1	×	×	—	—	—	—
6	*8—11	4—5	4—10	2—2	2—2	×	1—1	—	—	—	—
7	*11—25	5—10	2—2	×	×	—	—	—	—	—	—
8	11—11	×	2—2	4—10	×	—	—	—	—	—	—
9	*8—15	1—9	×	×	×	×	×	—	—	—	—
10	10—26	×	1—2	×	—	—	—	—	—	—	—
11	9—20	9—17	5—11	1—2	×	×	—	—	—	—	—
12	18—36	×	×	×	×	—	—	—	—	—	—
13	24—82	3—12	1—3	1—2	×	—	—	—	—	—	—
14	38—72	1—3	3—9	1—2	×	—	—	—	—	—	—
15	31—62	×	×	×	×	—	—	—	—	—	—
16	28—28	×	—	—	—	—	—	—	—	—	—
17	26—26	—	—	—	—	—	—	—	—	—	—
18	24—48	×	×	×	×	×	×	×	8—8	—	—
19	39—44	×	×	×	×	×	×	×	1—2	1—2	—
20	×	6—29	×	×	×	×	×	×	×	×	1—3
21	11—37	×	×	×	×	×	×	×	×	1—2	—
22	6—9	×	×	×	×	2—4	—	—	—	—	—
23	2—4	—	—	—	—	—	—	—	—	—	—
24	2—4	—	—	—	—	—	—	—	—	—	—
25	2—3	—	—	—	—	—	—	—	—	—	—
26	×	3—12	—	—	—	—	—	—	—	—	—
27	9—31	—	—	—	—	—	—	—	—	—	—
28	13—17	—	—	—	—	—	—	—	—	—	—
29	6—7	—	—	—	—	—	—	—	—	—	—
30	2—4	3—3	—	—	—	—	—	—	—	—	—
31	3—3	—	—	—	—	—	—	—	—	—	—
32	3—10	—	—	—	—	—	—	—	—	—	—
33	5—7	—	—	—	—	—	—	—	—	—	—
34	5—9	—	—	—	—	—	—	—	—	—	—
35	3—4	—	—	—	—	—	—	—	—	—	—
36	1—6	—	—	—	—	—	—	—	—	—	—
37	3—7	—	—	—	—	—	—	—	—	—	—
38	3—4	—	—	—	—	—	—	—	—	—	—
39	4—4	—	—	—	—	—	—	—	—	—	—
40	3—3	—	—	—	—	—	—	—	—	—	—
41	3—3	—	—	—	—	—	—	—	—	—	—
42	3—3	—	—	—	—	—	—	—	—	—	—
43	3—3	—	—	—	—	—	—	—	—	—	—
44	3—3	—	—	—	—	—	—	—	—	—	—
45	3—3	—	—	—	—	—	—	—	—	—	—
46	×	—	—	—	—	—	—	—	—	—	—

In each column the number of broods (families) tested is given first, and then the number of individuals from these broods which were allowed to come to maturity (see text).

×, Brood recorded, but not tested for sexuality.

* Males occurred in these broods (see text).

was however recorded, and these form the material available for the table. One example of how the table is to be read will explain it at once. Take generation 10[1] for example. In this generation ten first broods were tested for sex—i.e. the first broods from ten individuals of the preceding generation. By "tested for sex" I mean that one or more of the young were brought to maturity and the sex recorded. In this case altogether 26 individuals were taken from the ten broods. In the same generation no individuals belonging to second broods were kept to maturity, but one third brood was tested by the isolation of two members of it. As the members of a brood in *Simocephalus* (as in *Daphnia*) generally all, or mainly all, belong to the same sex, it is important to state how many broods were tested, and not merely the number of individuals without reference to the number of families from which they were taken.

The result of the experiment was as follows:—*No* sexual (ephippial) females appeared during the whole of the experiment. Males appeared in three generations only, where the asterisks are placed in the table. In generation 6 there was one male from one of the first broods, in generation 7 two of the first broods furnished between them five males, and in generation 9 there were three males from one of the first broods. All the other broods and all the other generations afforded parthenogenetic females only.

Thus so far from a progressive increase in sexuality, the very few sexual forms that did appear were in the early generations of the experiment.

These 46 generations were passed through in twenty-one months, at room temperature. The average duration of each generation would however have been far greater at the mean annual temperature in their natural ponds. Papanicolau estimates the maximum number of parthenogenetic generations in a natural "cycle" at twelve, and this agrees roughly with my estimate of the greatest number that could be produced in a year under the climatic conditions of Glasgow. Even during the time in which the line was bred in my experiment, parthenogenesis lasted therefore for nearly four times as many generations as may be expected in nature (though of course the natural "cycle" is by no means a rigid one), and at the end of the experiment no trace of loss of vigour was detectable.

According to Papanicolau, not only should sexual forms appear with

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[1] The original parthenogenetic female from which the line was started being counted as generation 1.

increasing frequency in later generations, but also in later broods of earlier generations. Now all the four broods which gave males in my experiment were first broods. Yet broods of a much higher order were tested and furnished parthenogenetic females only.

Also according to Papanicolau, a potent factor in producing sexual forms is not only the order of the brood, but the order of the brood to which the parent belonged. The greater part, but far from all, of each generation in my experiment was descended from first broods in the preceding generation. This however was also the case in Papanicolau's main experiment (Tafel I), in which the line ended altogether in sexual and degenerate forms in the twentieth generation. A direct test of the influence of the order of the brood was also made. It will be seen that in generation 22 two sixth broods were tested for sex. Now the parents of these sixth broods were members of the tenth brood of a specimen of generation 21 which was itself a member of the eleventh brood of an individual of generation 20, itself descended from the tenth brood of generation 19, the parent of which was a member of the ninth brood of generation 18. This is a far severer test than any which Papanicolau describes, and yet all were parthenogenetic females. (Breeding from late broods like this makes each generation fall far behind, in point of time, the generations bred chiefly from first broods. The sixth broods of generation 22 were contemporary with the first broods of generation 36 of the main part of the line.)

The third of our list of Papanicolau's conclusions has been no more my experience in this experiment than were his others. He found degenerate broods extremely common, increasing in frequency *pari passu* with the sexual forms. I have had very few of these degenerates, and they certainly showed no tendency to increase as the line grew older.

As mentioned above, the great majority of the individuals of this line were bred under the conditions which I have for convenience termed "normal" as distinct from the intentionally abnormal conditions under which a part of the line was bred. The "normal" and abnormal environments resulted in similar sexual conditions however. The abnormal environments included feeding with a protophyte culture which produced certain specific abnormal characters (described in my paper of 1913), cultivation in chemical solutions, and at high and low temperatures. In the case of the high temperature, part of the line was subjected to the abnormal condition for four or five successive generations. Naturally abnormalities and a higher death rate were produced by these conditions, but in the main part of the line, under the normal conditions, "degenerates" have been extremely rare.

On two occasions however there was an epidemic of deaths among young individuals and embryos in the part of the line under normal conditions. The first of these occurred in generation 4, the second in generation 30. The second one was very instructive as at that time I had under identical conditions parallel cultures of two other lines started from specimens obtained from different localities to that from which the parent of the principal line was obtained. The two lines in question were started from parthenogenetic females from Cambridge and Beith respectively, and generation 13 of the Cambridge line and generation 14 of the Beith line were contemporary with generation 30 of the main line, and all of them showed simultaneously the same "degeneration." In the main line four females laid altogether 19 eggs, from which only two living young were obtained. In the Cambridge line, six females laid together 31 eggs, and only 12 living young were born, while from the Beith line no living young were produced from 29 eggs laid by six females. These figures all refer to the first broods of the parents. All three lines had completely recovered by the second broods of the same generation, the second batches of eggs laid by all these sixteen females developing perfectly normally into normal healthy young. Moreover, except for occasional cases here and there, no more "degenerate forms" appeared in any of the three lines. In fact in the main line (Table I), in the last eleven generations not a single one of the new-born young isolated failed to arrive at maturity, and to produce perfectly normal young. Clearly therefore the temporary and simultaneous disturbance in the three lines was due to some temporary unfavourable factor in the environment, although there was no reason to suppose that the water in the Lepidosiren tank varied in any way from the normal at that time; while all the other factors of the environment were, so far as could be ascertained, unaltered.

The last of the visible accompaniments of degeneracy observed by Papanicolau which we need consider is the diminution in number and the increase in size of the parthenogenetic eggs as the line grew older. These are really largely the same phenomenon, there being a strong organic correlation between the number and size of the eggs, the larger the number the smaller being their size. This is the case, that is to say, if we judge the size of the eggs by that of the new-born young hatched from them. I have found it very difficult to get a satisfactory series of measurements of the eggs owing to their rapid increase in size as they develop. The young however do not grow between ecdyses to any extent detectable by the micrometer. An actual determination of the coefficient of correlation between size of young and number of eggs for *S. vetulus* gave – ·74 ± ·04. The value for *S. exspinosus*, worked out in larger numbers, gave as an average – ·43. The values were computed for groups for individuals belonging to the same generation and all giving birth to their young nearly at the same time, and refer to first broods only.

As these experiments were primarily directed to the inheritance of certain dimensions of the young, I have a large number of measurements available. Table II gives the mean length of the new-born young

TABLE II.

Mean Length of the New-Born Young (in first Broods only).

Unit of Measurement = 0·018 mm.

Generation	Mean Length of New-Born Young	Number of Broods	Number of Individuals	Generation	Mean Length of New-Born Young	Number of Broods	Number of Individuals
3	43·717	3	23	*25*	43·667	4	30
4	44·448	4	29	*26*	44·654	3	13
5	43·455	2	11	*27*	43·954	12	76
6	43·450	4	20	*28*	45·277	23	195
7	45·643	2	7	*29*	44·629	11	58
8	43·911	5	28	*30*	45·000	1	1
9	43·333	6	24	*31*	45·133	3	15
10	43·276	5	29	*32*	46·500	2	4
11	43·191	9	44	*33*	43·714	3	28
12	41·628	7	39	*34*	42·071	3	14
13	43·952	15	104	*35*	44·050	3	10
14	43·616	62	468	*36*	47·083	1	6
15	41·736	64	639	*37*	43·547	3	21
16	42·194	62	474	*38*	45·864	3	11
17	44·896	28	203	*39*	not measured		
18	44·279	26	231	*40*	not measured		
19	43·384	43	241	*41*	43·115	3	13
20	43·263	42	302	*42*	44·017	3	30
21	41·536	29	223	*43*	44·692	3	26
22	41·310	37	179	*44*	43·667	3	12
23	44·000	8	47	*45*	43·278	3	27
24	44·350	4	20	*46*	44·848	3	23

Note. The column "Number of Individuals" gives the number of young measured in each generation, not the total number of young born. When any brood contained less than ten individuals all of them were measured, but when there were more than ten, only the first ten, taken at random. Thus the mean number of young per brood cannot be obtained from this table by dividing the number of individuals in each generation by the number of broods.

(i.e. in their first instar), for the last 44 generations, the first two not being available for this purpose. Only *first* broods are dealt with in the table, and those portions of the line living under the abnormal conditions described above are omitted, as these conditions directly affected the size of the new-born young.

It will be seen that the size fluctuates greatly, but there is no real tendency to a progressive increase. If the forty-four generations are divided into four groups of eleven each, we find:

Mean length	for generations	3—13	... ...	43·637
„	„	14—24	... ...	43·017
„	„	25—35	... ...	44·423
„	„	36—46	... ...	44·457

It will be seen that the second set of eleven generations average smaller than the first. It is true that the last period gives the highest average, thanks to generation 36 which was represented by only a single brood of exceptionally large individuals. The mean of the last six generations however is 43·936, almost exactly equal to the total mean (43·889) and so is the mean for the first six, 44·104. There is thus no justification for supposing that the size of the young is increasing, although by taking twenty successive generations (Papanicolau's number) a good case might be made out for either increasing or decreasing size according to the portion of the table included by them.

I can fully confirm Papanicolau's statement that the size of the egg (or new-born young) increases from brood to brood—at any rate up to about the fifth. Exact measurements of this increase were given in my former paper. This increase however is in no sense due to a cyclical or cumulative action of the increasing lapse of time since the last sexual act. It is a phenomenon which begins afresh in each generation, and seems to be a common occurrence throughout the animal kingdom. The increasing size of the eggs of successive ovipositions has been shown in detail by Halban for *Salamandra maculosa* and *S. atra*, and also for *Anura* and *Chelonia*. It is the experience of every poultry keeper that the young hens lay smaller eggs than the older ones, and it is well known to be the case with man that the size of the new-born infant tends to increase in successive parturitions.

Whilst the line described above was in progress I also bred numerous others, the most important being two started respectively from a parthenogenetic female kindly sent me from Cambridge by Mr F. A. Potts and from one from Beith, 16 miles from Glasgow, kindly sent to me by

Mr John Ritchie. From the Beith specimen 17 generations were taken and from the Cambridge specimen 16 generations, giving with the original females 18 in the Beith and 17 in the Cambridge lines. They were kept under exactly the same conditions as the main line, and in neither of them were any sexual forms found at all, nor any appearance of "degeneracy," though as described above a very large proportion of the eggs of the first broods of the contemporary thirteenth generation of the Cambridge, and fourteenth of the Beith lines failed to develop.

It again happens that if the period during which these lines were bred is divided into two portions, the mean size of the young in the earlier generations is less than in the later ones. Combining the contemporary generations of the two lines together we find that the mean length of the first brood young of the first period (seven generations measured) is 45·256, and of the second period (also seven generations measured) is 45·904. A closer analysis shows however that it would be again quite unjustifiable to conclude that a progressive increase was taking place. If we arrange the generations in order of the magnitude of the new-born young, we find the order, beginning with the smallest, is (generations 1 and 9 not being available)

8, 5, 16, 2, 11, 17, 4, 3, 10, 14, 6, 12, 15, 7.

In a fairly extensive experience of breeding Cladocera I have of course often experienced the phenomena of bursts of sexuality and of "degeneracy"—or better, of high mortality. I have had both these phenomena appearing in *D. pulex*, and the sexuality in *S. exspinosus* kept under the conditions described as normal above, and also in *S. vetulus* kept under other conditions.

It is obvious therefore from this and from the work of others that the same species may under certain conditions exhibit an increasing tendency to sexuality and degeneration ending in total cessation of parthenogenetic reproduction, and under others may continue the asexual reproduction apparently indefinitely. My main line was to all appearance as vigorous at the 46th generation as at the first, and so were the Cambridge and Beith lines as long as they were bred—and it must be remembered that the parthenogenetic females which originated the three lines may themselves have had a long parthenogenetic ancestry. There is no justification for the supposition that if the experiment had been continued "long enough" the lines would have at last been unable to maintain themselves without sexual reproduction.

Three conditions of the environment seem to be specially significant in interpreting the almost uniform lack of sexuality, and the total lack of any tendency to degeneration observed in these three lines under long continued parthenogenesis.

Firstly, with the few exceptions of those individuals fed with the protophyte culture, they were all fed with the same food, and this food supply, though it may have fluctuated from day to day, probably did not do so over long periods, as the water in the Lepidosiren tank (which had been established more than three years before the experiment began) was changed weekly. Hence any cyclical change in the food supply was probably a weekly one, and there was little chance of a progressive deterioration in the culture medium lasting over weeks and months which might have caused sexuality and degeneration to set in.

Secondly, practically every individual was isolated in a separate tube within 48 hours of birth. The only exceptions were so rare as to be negligible, and moreover in only one case where more than one individual was kept in the same tube was such a specimen used as the ancestor of any considerable number of generations. In the light of Grosvenor and Smith's results as to increased sexuality in overcrowded animals, this is a significant point.

Thirdly, the water was changed regularly in all the tubes every second day—with again an insignificant number of exceptions.

General considerations.

It is obvious that it is no longer necessary in the present state of our knowledge to discuss the extreme Weismannian hypothesis that the parthenogenetic or sexual mode of reproduction is determined entirely by internal changes which are an integral part of the physiology of the animal and independent of environment. The idea of an internal cycle or rhythm still persists however, and it is undoubtedly the most generally accepted view to-day among workers on Cladocera that the change from parthenogenetic to sexual reproduction is determined by such a cycle, with the limitation that this change can be accelerated or delayed by particular conditions of the environment. This is expressed by Hertwig in the following sentence (p. 29): "Fortgesetzte Parthenogenese führe schon *als solche*[1] in der Beschaffenheit der Tiere zu Veränderungen, welche die Entwickelung der Geschlechtsgeneration veranlassen. Diese Umformung der Zellen könne durch einen entgegengesetzt wirkenden Faktor, wie die Wärme, vielleicht dauernd zurückgedrängt werden."

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[1] My italics.

Amongst other upholders of the view of an inherent cyclical change which can be influenced to a greater or less extent by environment are Issakowitsch, Papanicolau, Woltereck. The kind of cycle maintained by Woltereck is however quite different from that supposed by Hertwig. The last-named worker correlates it with his nucleo-cytoplasm relation theory and considers that the lapse of parthenogenetic generations and of time leads to a state of depression in the germ cells that eventually under normal conditions ends in sexual conjugation or death. Woltereck justly points out that the theory would have to be strained to breaking point to cover his own experiments with *Hyalodaphnia*, etc. For instance, Woltereck bred a line of *H. cucullata* parthenogenetically for four years, during which time it fluctuated between pure parthenogenesis and pronounced sexuality. Hence we should have to suppose that the line recovered from pronounced sexual depression periods without sexual conjugation.

Woltereck's own view is that there are two antagonistic substances in the egg, the predominance of one resulting in parthenogenetic individuals, and of the other in sexual forms. These substances are supposed to wax and wane alternately and autonomously. At such times (labile periods) as they are nearly equally balanced, external conditions are able to turn the scale one way or the other, and thus at times environment influences sex. "Mann kann deshalb die *innere zyklische Periodizität der Valenz* (of these two substances) mit Recht als das Kernproblem der Cladocerenfortpflanzung bezeichnen" (p. 123).

Although Woltereck's cycle is far more compatible with the observed facts than is the theory of a depression caused by continued parthenogenesis as such, nevertheless there does not seem to be sufficient evidence for the existence of any sort of cycle at all, while there is strong evidence against it.

The evidence for the existence of such cycles consists mainly of:

1. Experiments where the conditions were supposed to be constant, such as (*a*) Papanicolau's work with *Simocephalus* and *Moina*, where an increasing sexuality and degeneration are observed, and (*b*) Woltereck's with *Hyalodaphnia*, where the line fluctuated between pure parthenogenesis and a high degree of sexuality.

2. Experiments where abnormal conditions of various kinds are found to have a determining influence in some cases and not in others. When such conditions are effective it is supposed that it is because the cycle has reached the point where the tendencies to parthenogenesis and sexuality are nearly balanced. When they are ineffective, it is ascribed

to the fact that the cycle is too near one end or the other for the opposite condition to be evoked.

Now it is quite possible to account for these observations without invoking an internal cycle. Firstly, it is extraordinarily difficult to keep all the conditions of the environment constant, and especially the great difficulty of keeping food cultures constant for long periods imposes upon the experimenter, who assumes that physiological changes in his animals were not correlated with changes in the environment, the obligation of stating very fully what precautions and tests he took to ensure that the conditions really were constant.

Secondly, even where the conditions are constant throughout the whole experiment, an increased tendency to sexuality in, say, the twentieth generation as compared with the tenth may be due to the fact that the line has been living for a longer period in an unsuitable environment—perhaps, for example, one deficient in some essential constituent or "vitamine." As both Papanicolau and Woltereck point out, not only the number of generations but also the length of time during which a line has been subjected to the experimental conditions is of importance in determining sexuality, for sexual forms appeared in late broods of early generations and early broods of late generations. In my experience with *S. vetulus* an individual of any generation produces its fourth brood about the same time as the members of its first broods produce their first brood of offspring. That is, the fourth broods of the nth generation are contemporary with the first broods of the $n+1$th generation. If this held true for Papanicolau's strain of *S. vetulus,* it is easy to calculate from his Tafel I that sexual and degenerate forms appearing in the later broods of the earlier generations actually appeared earlier, in point of time, than those of later generations.

I have described experiments (1913) where the effects of a peculiar environment produced on a given generation of *S. vetulus* were still detectable in their great-grandchildren, and Woltereck has produced evidence to show that an environment acting on an individual may determine the sexuality of its grandchildren. It is plain, therefore, both that environmental effects may persist for some generations after they were produced—in other words that it may act in cumulative fashion—and also that the length of time and not only the number of generations during which the line has been in the conditions of the experiment, is of importance in determining sexuality. Taking this in conjunction with the fact that under some environmental conditions no tendency to sexuality or degeneration appears even after an enormous number of parthenogenetic generations, it seems necessary, on the principle of accepting the simplest hypothesis which will fit the facts, to conclude that the sexual cycle (obligatory parthenogenesis—labile period—obligatory or preponderating sexuality, often accompanied under experimental conditions by "degeneration") is, when present, due entirely to the cumulative effect of an unfavourable environment or to an actual though often unsuspected change from a favourable to an unfavourable environment.

This view does not, of course, diminish the physiological interest of the change from the parthenogenetic to the sexual mode of reproduction. Indeed the tendency to replace asexual by sexual reproduction under certain conditions (often unfavourable ones) is a phenomenon of the deepest significance, but it seems to be no more due to an inherent life cycle than the increasing hunger, ending in degeneration and death unless the conditions are changed, which accompanies the withholding of food from an organism, is due to an inherent physiological cycle.

The ascertainment of the exact conditions under which asexual is replaced by sexual reproduction, and the precise advantage conferred by the latter, is indeed a most important task. The external conditions under which parthenogenesis will continue indefinitely are certainly different for different species. They may be rarely realised in nature, and still more rarely for any prolonged period, natural conditions being subject to seasonal and other changes. For many species of the related Ostracoda however these conditions do seem to obtain in nature. On the other hand the other extreme is not impossible, that for some species in which asexual reproduction is one of the normal modes of reproduction, there are no conditions under which asexual reproduction can go on for more than a limited time. In other words, that there is no environment which does not act in a way prejudicial to parthenogenetic reproduction. If such species do exist, it might be legitimate to speak of their sexual cycle, but in that case it would be necessary to remember that the "cycle" is not due to anything of general import but merely to the peculiar relations of that particular species to the environment.

It appears that a similar change of view is taking place in regard to the life cycle of the Infusoria. For many years the orthodox view has been that originated by Maupas as the result of his classical experiments. According to this view the life history is a cyclical one, the near descendants of the exconjugate being in a state of immaturity, which gradually yields, as asexual multiplication proceeds, to puberty, ending in senility and to the necessary death, through internal causes, of the asexual colony. As better and better methods of cultivation have been evolved the duration of this "cycle" has been gradually lengthened, and artificial stimuli substituted for the "rejuvenescing" conjugation, till at last we have Woodruffe, after breeding *Paramecium aurelia* for 3340 asexual generations without evoking any signs of "degeneration," coming to the conclusion "dass das Protoplasma einer einzigen Zelle unter günstigen äusseren Umständen ohne Hilfe von Konjugation oder einer künstlicher Reizung imstande ist, sich unbegrenzt fortzupflanzen und zeigt ferner in klarer Weise, dass das Altern und das Befruchtungsbedürfnis nicht Grundeigenschaften der lebendigen Substanz sind" (p. 36).

Similar conclusions have been reached by Jennings as the result of extensive experiments on individuals of Paramecium which had conjugated, and others which had been prevented from conjugating though ready to do so.

Conclusion.

The following conclusions, though referring particularly to *S. vetulus*, may probably be safely extended to a wide range of the Cladocera.

1. Certain not yet fully elucidated factors in the environment influence the onset of sexuality.

2. Certain factors likewise bring about "degeneration" or high rate of mortality.

3. - Certain factors of the environment may act cumulatively over a number of generations.

4. Therefore the increasing sexuality and "degeneration" (or high mortality) observed under certain supposedly constant experimental conditions receive a ready explanation in the supposition that the environment is one favourable to the development of these phenomena.

5. This explanation is made much more probable when we find that under other experimental conditions there is no tendency to increasing sexuality or degeneration.

6. Many species exhibit the phenomenon of specially labile periods, when sexuality is easily influenced by certain factors of the environment. This labile condition is usually ascribed to the fact that the line is in about the middle of the reproductive cycle, the diminishing tendency to parthenogenesis being about equally balanced by the increasing tendency to sexual reproduction. Such a balanced condition must however be passed through equally whether the tendency to sexuality

is being increased by the progress of the "cycle" or by the cumulative effect of an unfavourable environment. Hence the existence of labile periods is as readily explained on the one hypothesis as on the other.

7. There is no justification for retaining the hypothesis of an inherent reproductive cycle—that is to say, the hypothesis that the number of generations or lapse of time since the last fertilised egg influences, as such, the production of sexual or degenerate forms. For the production of these forms is under certain conditions not influenced even by the lapse of an enormous number of parthenogenetic generations, while their production certainly is influenced by environment in other cases. The residuum of cases being equally well explicable on either hypothesis (cycle or environment) it is most reasonable to suppose that the factor that was effective in the one case (environment) was the one that was effective in the other, and conversely, that the ineffective factor of the one case ("reproductive cycle") was ineffective in the other.

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