

SEX OF ADULT CECIDOMYIDÆ (OLIGARCES sp.)  
ARISING FROM LARVÆ PRODUCED  
BY PÆDOGENESIS.

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The question of the sex of adult forms arising from parthenogenetically and pædogenetically produced individuals has called forth some debate and speculation, often in the absence of suitable date. Sturtevant (1923) summarized the evidence then available concerning some of the Chironomidæ and Cecidomyidæ in the following statements.

"Parthenogenesis has been described in the Chironomid genera *Chironomus*, *Corynoneura*, and *Tanytarsus* by Grimm (1870) Johannsen (1912), Goetghebuer (1913), Edwards (1919) and others. Eggs are produced in some cases by the larvæ, in others by the pupæ, and in still others by the imagines. In all cases in which imagines have been produced by parthenogenetic (including pædogenetetic) lines, these have been females and have bred parthenogenetically if at all. Males are known to occur in these genera, and in one case even in a species that reproduces parthenogenetically; but in no case are males reported as arising from larvæ known to have been produced by parthenogenesis.

"The first case of parthenogenesis recorded among the Diptera was that of the Cécidomyiid, *Miastor*, discovered by Wagner (1863). In this case it is the larvæ that reproduce parthenogenetically. Imagines are often produced, but when they do appear both sexes are found (Meinert 1864, Wagner 1865, Kahle 1908). Kahle states that there is a significant excess of females, and Felt (1911) describes only the female, though he does not state that males were absent. It is not known whether the imagines breed at all, or not; Kahle states that he did not

observe copulation. It does not appear to have been entirely proven that the males arise from larvæ that have been produced by pædogenesis, though most students of *Miastor* have apparently taken this for granted without making cultures from isolated larvæ."

This latter objection has been removed by the writer, who, in using *Miastor* and *Oligarces* as material for experimental work, has, for some time, made cultures from isolated larvæ, and maintained them on artificial culture media. The methods, and some of the results of this work have been reported already. A suitable method of cultivation of *Miastor metraloas* on an artificial culture medium was found (Harris 1923, 1, 2). This method was improved and simplified in later work on *Oligarces* (Harris 1923, 3). Both of these methods remove the possibility of contamination of the culture by other larvæ than those under observation. The data contained in this report are taken largely from the work on *Oligarces*.

Cultures of pædogenetic larvæ of *Oligarces*, if kept in darkness at a suitable temperature, are easily maintained in Petri dishes in the laboratory, on the following culture medium; 0.5 per cent malt extract, 2 per cent agar agar, 97.5 per cent water. Thus the difficulty of making cultures from isolated larvæ is greatly lessened. It may be said, however, that in making cultures from isolated pædogenetic larvæ it is advisable to take a mother larva containing nearly full term embryos, as cultures made from an individual larva are more liable to be unsuccessful than those in which several larvæ are present. By using a mother larva containing embryos about to be born, one is assured of starting a culture from an isolated larva, while, at the same time, providing the new colony, almost immediately, with several young larvæ with which to carry on the strain. Similar results may be obtained by freeing full term embryos from the skin of the mother larva by dissection in water on a microscope slide, and then transferring these young of an isolated mother to a new culture. Both of the foregoing methods are recommended as time-saving, and as productive of well established colonies.

I have previously reported (Harris 1923, 3, 1924, 1) that pupæ and adults can be produced at will in the laboratory by maintaining a crowded condition in the previous generation. Pupæ and adults produced in this way were used as material for the present study. These pupæ and adults arose in all cases from pædogenetic stock, taken in nature, and maintained on artificial culture medium in the laboratory. In no case are data given on cultures which did not pass at least one pædogenetic generation in the laboratory previous to the appearance of pupæ-larvæ. In some cases the data concern mass cultures, in other cases cultures which originated from an isolated pædogenetic mother larva.

It was found that the pupæ and adults which appeared in mass cultures, made from pædogenetic larvæ of pædogenetic origin, were as likely to be males as females. Thus in cultures B<sub>12</sub> and C<sub>12</sub> of 28 pupæ, chosen at random and sexed, 18 were males and 10 were females. In culture M79, of 2 adults, 1 was a male, 1 a female. A similar sex ratio was observed in culture MNO 340.

All of these cultures were originally made of varying numbers of isolated pædogenetic larvæ of known pædogenetic origin. Thus it is clear that male as well as female pupæ and adults arise from larvæ which have been produced by pædogenesis. Male pupæ, when dissected, were found to contain large numbers of sperms in the gonads, while in the female usually about four or five large eggs are visible in living specimens. It seems likely then that both males and females are functionally as well as morphologically different. Up to the present time, however, I have not attempted to obtain progeny from adults, and so cannot state whether or not copulation can be easily obtained in the laboratory.

Whether the number of males will be equal to or greater than the number of females, or *vice versa*, in a given culture, was shown to depend upon the type of stock which was selected in making the cultures, for it appears that in the same colony in nature certain pædogenetic larvæ belong to male producing strains, while other larvæ constitute female producing strains.

Thus in the eighth generation of material originally taken from a single colony in nature, and maintained in the Laboratory by the means already described, individual 4XX was segregated and transferred to a fresh culture made of the standard medium. In the third filial generation pupæ and adults appeared as a result of crowding in the previous generation ( $F_2$ ). All of these pupæ and adults, 38 in number, which were sexed were females. In the fifth filial generation of the same strain, pupæ and adults again appeared under similar conditions, in three separate cultures. In culture M222, 26 pupæ and adults were sexed. All were females. This was equally true for the 23 individuals sexed in culture M223, and for the 59 individuals sexed in culture M224. Thus all of the 108  $F_5$  pupæ and adults which were sexed were females. It is apparent that the progenitor of this strain, individual 4XX, must have belonged to a female-producing strain, since all of the pupæ and adults produced by her offspring, both in the  $F_3$  and  $F_5$  generations, were females.

Conversely, at the same time, and under similar conditions, individuals of other strains of known pædogenetic origin, gave rise to male pupæ and imagines only. Thus in culture MN320 5 pupæ appeared; all were males. Similarly in culture CO<sub>2</sub>3 five adults arose from an isolated pædogenetic mother of known pædogenetic origin. These five adults were males.

Male pupæ of *Miastor metraloas* appeared in a colony, maintained in decayed birch, wood in the laboratory in Paris (Prof. Maurice Caullery, Director) under crowded conditions in a tin box. Though these did not arise from larvæ all of which had been carefully segregated and examined, the fact that they appeared some two months after the material had been transferred to the laboratory makes it more than probable that they were produced by larvæ which had arisen pædogenetically from pædogenetic larvæ originally present in the material. This conclusion is further supported by the fact that all pædogenetic larvæ which were examined and segregated reproduced pædogenetically under similar conditions, about 2 weeks after birth. This would allow for four laboratory pædogenetic generations before the appearance of the male pupæ.

Table 1.

Sex of Imagines and Pupae produced by Paedogenetic Larvae of Known Paedogenetic Origin.

Culture	Pupae arose by	No. of ♀ ♀	No. of ♂ ♂	Total	Per Cent of ♂ ♂
A.					
B <sub>12</sub> , C <sub>12</sub>	crowding	10	18	28	64.3
M79	crowding	1	1	2	50.0
MNO 340	crowding	1	1	2	50.0
B. Female-producing strain.					
4XXF <sub>3</sub>	crowding	38	0	38	0.0
4XXF <sub>5</sub> (M222)	crowding	26	0	26	0.0
4XXF <sub>5</sub> (M223)	crowding	23	0	23	0.0
4XXF <sub>5</sub> (M224)	crowding	59	0	59	0.0
X8F <sub>2</sub>	crowding	1	0	1	0.0
C. Male-producing strains.					
MN320	crowding	0	5	5	100.0
CO <sub>2</sub> 3	CO <sub>2</sub>	0	5	5	100.0

All cultures were maintained on the standard artificial culture medium, described earlier in this paper, under as nearly identical external conditions as possible, save culture CO<sub>2</sub>3. The mother of the five imagines which appeared in this culture was treated with carbon dioxide at birth, and for some time subsequently, after which she was transferred to the standard medium employed in these studies. In all cases cultures were maintained in closed boxes in the same incubator, thus being exposed to similar conditions in respect to temperature, humidity, and light, as well as food. Thus the conclusion seems warranted that the observed sex-ratios are the expression of an internal mechanism, which, in the present instance in all probability, has not been greatly affected by external conditions (save possibly in the case of the strain produced by the paedogenetic mother larva which was treated with carbon dioxide.) Sufficient data are not yet at hand to warrant a statement concerning whether or not the carbon dioxide treatment changes the sex-ratio as in *Cladocera* (Banta 1923).

#### Summary.

Males as well as females arise from mass cultures of larvae that have been produced by paedogenesis in *Oligarces* sp.

But males and females are not produced by the same individual, under normal conditions.

In the descendants of members of a colony taken from nature there were found to be two types of pædogenetic larvæ in respect to the sex of pupæ and adults which they produced, (1) male-producing, and (2) female-producing.

These two types of pædogenetic larvæ are not morphologically distinguishable.

The evidence indicates that the distinction is genetic, since it holds not only in the case of a given individual, but also, without observed exception, in its offspring through five generations of pædogenetic reproduction in two of which certain numbers of adult forms were produced.

Thus the potentiality for producing only males or only females seems to be inherited; there existing in the colony male-producing and female-producing strains.

#### LITERATURE.

- Banta, A. M. and Brown L. A., 1923. Further control of sex in a species of Cladocera. *Anat. Rec.* Vol. 24, Abs. 144 p. 420.
- Edwards, F. W. 1919. Some Parthenogenetic Chironomidæ. *Ann. Mag. Nat. Hist.* (9) 3; 222-228.
- Felt, E. P. 1911. *Miastor americana*. An account of pedogenesis. *New York State Mus. Bull.* 147; 82-104.
- Goetghebuer, M. 1913. Un cas de parthénogenèse observé chez un Diptère Tendipédide (*Corynoneura celeripes*). *Bull. Acad. Roy. Belgique.* 1913, 231-233.
- Grimm, O. 1870. Die ungeschlechtliche Fortpflanzung einer Chironomus-Art und deren Entwicklung aus dem unfruchteten Ei. *Mem. Acad. Imp. Sci. St. Pétersbourg sér.* 7, tome 15.
- Harris, R. G. 1923, 1. Sur la culture des larves de Cecidomyides pædogenétiques (*Miastor*) en milieu artificiel. *Comptes Rendus Soc. de Biol. Paris*, Tome 88 p. 255-7.
- Harris, R. G. 1923, 2. Occurrence, Life-cycle, and Maintenance under Artificial Conditions of *Miastor*. *Psyche* Vol XXX. p. 95-101.

- Harris, R. G. 1923, 3. Control of the Appearance of Pupa-larvæ in Pædogenetic Diptera. Proc. Nat. Acad. Sci. Washington, Vol. 9-407-413.
- Harris, R. G. 1924, 1. Further Data on the Control of the Appearance of Pupa-larvæ in Pædogenetic Cecidomyidæ. (*Oligarces* sp.) in press.
- Harris, R. G. 1924, 2. Reversal of Function in a Species of *Oligarces* (in press).
- Johannsen, O. A. 1912. Parthenogenesis and Pædogenesis in *Tanytarsus*. Maine Agr. Exp. Sta. Bull. 187; 3-4.
- Kahle, W. 1908. Die Padogenesis der Cecidomyiden. Zoologica, Bd. 21. Heft. 55. 80 pp., 6 plates.
- Meinert, F. 1864. Weitere Erläuterungen über die von Prof. Nic. Wagner beschriebene Insectenlarven, welche sich durch Sprossenbildung vermehrt. Zeits. wiss. Zool. 14; 394-399.
- Sturtevant, A. H. 1923. The Probable Occurence of Parthenogenesis in *Ochthiphila polstigmata*. (Diptera). Psyche vol. XXX 22-27.
- Wagner, N. 1863. Beitrag zur Lehre von der Fortpflanzung der Insecten-larven. Zeits. wiss. Zool. 13. 513-527.
- Wagner, N. 1865. Ueber die viviparen Gallmückenlarven. Zeits. wiss. Zool. 15, 106-107.

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