ARTIFICIAL FERTILIZATION OF AQUATIC INSECTS

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INTRODUCTION

In the following paragraphs are recorded a few attempts of artificial fertilization of several species of aquatic insects. Artificial fertilization as used in this paper does not mean the stimulating of the eggs with artificial, chemical or physical stimuli; it merely means the artificial mixing of the eggs and the sperm outside of the insect's body.

The experiments described here were carried out at the Ohio State University Lake Laboratory at Put-in-Bay, Ohio, during the summer of 1924. I wish to take this opportunity to thank Dr. C. H. Kennedy, of the Laboratory Staff, for suggesting this work and for aid received in the identification of specimens. Dr. P. W. Claassen, of Cornell University, was kind enough to identify the stoneflies that were sent to him.

Extensive experiments were carried out with the spotted mayfly, *Ephemera simulans* Walk; the Canadian soldier (mayfly) *Hexagenia bilineata* Say; the fish fly, *Sialis infumata* Newn; the large stone fly, *Acroneuria evoluta* Klapalek; and the large midge, *Chironomus plumosus* L.

METHOD.

As indicated in the introduction, artificial fertilization as used in this paper means the artificial mixing of the ova and spermatozoa outside of the insect's body. The mixing of the ova and sperms was carried out in the following media: (1) Normal physiological salt solution; (2) Two-thirds normal physiological salt solution; (3) One-third normal physiological salt solution; (4) Sterile lake water. In each case after the eggs had been treated with spermatazoa in one of the above media, they were incubated in sterile lake water, because all the insects used in these experiments with the exception of *Sialis infumata*, deposit their eggs on the water.

The reason for using different media was to determine whether the medium in which the eggs and sperms were mixed

would have any effect upon the process of fertilization. It is a well known fact that animal tissues in general retain their power of irritability longer when kept in a normal physiological salt solution. The physiological salt solution may, therefore, be taken to represent as nearly as possible the conditions as they exist in the insect's body. Then by modifying this solution—by diluting in this case—it would be possible to determine how far conditions could vary without making fertilization impossible.

The first step in the solution of the problem was the collecting of mature insects, and the separation of males and females to prevent mating. Since as a rule an insect is mature as soon as it has wings, it is easy to obtain mature individuals. In the case of the mayflies, however, special care has to be taken to avoid sub-imagos. This difficulty can be overcome by collecting the insects in the morning or early afternoon and then keeping them until evening or the next morning. By that time they have passed through the final moult and are ready for the experiment.

The next step is the removal of the ovaries and the testes. The ovaries can easily be removed under the low power of a binocular. The testes, however, are often so small that it is rather difficult to remove them alone, and the best thing, therefore, is to remove the entire abdominal contents. In this way one is always certain to get the testes. In the larger species of mayflies the removal of the eggs is especially easy; all that is necessary is to cut off the abdomen, immediately behind the thorax, and then force the eggs out by gently compressing the abdomen. It might be of interest to note that when a female mayfly is placed in tobacco extract, she extrudes her eggs. This observation was first made by Mr. Crosly, of Put-in-Bay, Ohio.

After a female has been selected for the experiment, the ovaries are removed as indicated above. Part of the ovarian contents is then placed on a slide in a few drops of one of the different media and mixed with the abdominal contents of the male. The latter material is teased to pieces with a pair of needles to insure the liberation of the spermatazoa. The eggs are stirred at intervals for thirty minutes to insure thorough mixing with the spermatazoa. At the end of thirty minutes the eggs are removed from the liquid in which they were mixed with the sperms and incubated in lake water. The remainder

of the ovarian contents is incubated in lake water without being mixed with spermatazoa. This serves as a check to detect females that might have been already fertilized.

Since *Sialis infumata* does not deposit its eggs in the water, but near the water, so that the young larvæ drop in the water, it was necessary to modify the process of incubation. So instead of placing the eggs—both those treated with sperms and those used as a check—in lake water, they were incubated in cheese cloth placed in dishes containing lake water. This provided a moist atmosphere without actually submerging the eggs in water.

RESULTS.

Although many trials were made with *Sialis infumata*, *Acroneuria evoluta* and *Chironomus plumosus*, no positive results were obtained. Likewise reciprocal crosses between *Hexagenia bilineata* and *Ephemera simulans* gave negative results.

Tables I and II give the results for *Ephemera simulans* and *Hexagenia bilineata* respectively.

TABLE I. EPHEMERA SIMULANS WALK.

Date of Incubation	Date Hatched	Period of Incubation	% Hatched	% Hatched In Check	Medium
6–26	7–4	8 days	75%	None	Normal salt solution
6–26	7–4	8 days	65%	None	2–3 normal salt solution
6–26	7–4	8 days	10%	None	1-3 normal salt solution
6–26	7–4	8 days	1%	None	Lake water

TABLE II. HEXAGENIA BILINEATA SAY.

Date of Incubation	Date Hatched	Period of Incubation	% Hatched	% Hatched In Check	Medium
6–30	7–10	10 days	20%	None	Normal salt solution
6–30	7–13	13 days	One egg	None	2–3 normal salt solution
6–30	7–13	13 days	One egg	None	1-3 normal salt solution
6–30	7–13	13 days	1-10%	None	Lake water

When the dishes with *Hexagenia bilineata* were re-examined on the 14th of July, approximately fifty percent of the eggs fertilized in the normal physiological salt solution had hatched;

of those fertilized in two-thirds normal physiological salt solution, two eggs had hatched; one percent of those fertilized in the one-third normal physiological salt solution had hatched; and two percent of those that were fertilized in lake water. The last examination of these dishes was made on the 24th of July. It was found that approximately fifty percent had hatched in each dish containing fertilized eggs. The dishes containing the eggs used as checks were examined on the same day. It was found that not a single one of these eggs had hatched.

CONCLUSIONS.

(1) The results for *Ephemera simulans* and *Hexagenia blineata* show that artificial fertilization is possible in these two species.

(2) Whether the medium in which the eggs and sperms are mixed has any effect upon the process of fertilization as shown by the number of eggs actually hatched is uncertain. Table I would seem to show that the medium did have an effect upon the process of artificially fertilizing mayfly eggs. It shows that there is a definite relation between the number of eggs hatched and the kind of medium used. Table II, however, shows no such relation between the number of eggs hatched and the kind of medium used.

(3) It would also be premature to conclude that artificial fertilization in *Sialis infumata*, *Acroneura evoluta*, and in *Chironomus plumosus* is impossible. The same is probably also true for crosses between *Hexagenia bilineata* and *Ephemera simulans*. More extensive experiments will be necessary before these questions can be settled definitely.