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## Some Observations on the Embryology of Chronomus

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## SOME OBSERVATIONS ON THE EMBRYOLOGY OF CHIRONOMUS.

BY W. N. CRAVEN.

## A. INTRODUCTION—TIME AND PLACE OF LAYING.

All the eggs used in the following investigation were collected from a small pond in the eastern edge of the city of Indianola. The eggs were gathered from time to time at intervals of from one to three days, between June 20th and September 30th, 1902. They were found in abundance along the bank, in water from one to six inches deep. Usually they were attached to a piece of wood, or some plant growing in the edge of the water. Many were also found attached to the bottom. A few were found floating in the water, but as by far the greater number were attached, it is probable that those found floating had broken from their attachment, or by some accident had not been fastened.

It was found that the eggs collected in the early morning were always considerably advanced in their development; so that some of them must have been deposited the previous day. Those collected in the early afternoon were always at least in the early stages of development, while the most freshly laid eggs were collected about ten o'clock A. M. As the insects were always present about the edges of the pond in the early morning, it is probable that some little time, perhaps two or three hours, were consumed in depositing and fastening an egg mass. It is probable that the greater number were laid in the early morning—before ten A. M. As all the eggs in which the first stages of development had been reached were found about noon, by far the greater number must have been deposited in the early morning.

## B. THE EGG MASS.

The eggs are laid in a clear jelly like mass, which is about three-eighths of an inch in diameter and varying from one-fourth to three-fourths, or sometimes nearly an inch in length. The egg mass was always attached by one end, and since its specific gravity is less than that of water, it assumes a nearly vertical position in the water. The eggs are arranged in the mass in the form of a spiral, which runs the length of the mass. The eggs are placed with their long axes in the line of the spiral, each egg lying slightly above and its end projecting over its predecessor. Hence in the normal position of the eggs in the water the embryo lies with its long axis nearly horizontal.

## C. TIME OF INCUBATION.

The time of incubation varied considerably for different egg-masses, although those of the same egg mass always hatched within two or three hours of the same time.

The shortest period in which any hatched was about seventy-five hours; the longest period was about 110 hours. The time of incubation seemed to depend almost entirely upon the temperature and sunshine. Those kept at a temperature above 80 degrees F. and in the sunshine developed most rapidly, while those which were away from the light and cooler developed more slowly.

In those egg masses which were kept in conditions such that they developed more slowly there were found more eggs which did not hatch. This would seem to indicate that any considerable reduction of temperature or deprivation of sunlight would entirely prevent development. No accurate observations were made, however, upon this point.

#### D. THE EGG.

The eggs are elongated-oval appearing slightly larger at the extremity which is to become the anterior, when viewed dorso-ventrally. When viewed from the side they appear more convex on the side which is to become the ventral, the dorsal side in the majority of cases appearing slightly concave, through part of its length. (See fig.)

The eggs vary somewhat in size. From a number of measurements, the average size was found to be very close to 1.21 inch in length by 1.50 inch in thickness. From these measurements individual eggs varied as much as 1.240 inch in length and 1.500 inch in thickness, or very nearly 10 per cent.

The chorion is perfectly transparent, and somewhat flexible, allowing the egg to be distorted by pressure from the cover glass, without breaking. After hatching the empty shell was quite flexible. No micropile was observed.

The vitelline membrane does not fit against the chorion at all points, but is drawn away at the ends, usually more at the posterior end.

The egg contents are composed almost entirely of the nutritive yolk substance. There is very little of the outer protoplasmic layer present (keimhautblasten of Weisman). The yolk has a yellowish green color, is very granular, and highly refractive. Numerous oil globules of varying size can be seen scattered through it. In some of the eggs collected late in the season the yolk had a more brownish color. They seemed to be identical with those previously obtained in other respects. Only a few of these were obtained and it was not determined whether they belonged to different species, or the change in color was due to the lateness of the season.

#### E. EARLY STAGES IN DEVELOPMENT.

The first change noticeable was a slight drawing in of the vitelline membrane at each end. Shortly afterward there appeared at the anterior end four rather large, quite granular cells (Pl. 1, fig. 1-a). About an hour later other cells not so large began to appear on the surface of the yolk. These form a layer (Pl. 1, fig. 2) which increases in thickness and number of cells by addition from within, until an irregular layer of cells, the blastoderm, of comparatively considerable thickness, covers the entire surface (Pl. I, fig 3.) The cells then arrange themselves regularly and form a layer of columnar cells, the blastoderm covering the yolk. During these changes the large cells are at all times distinctly visible at the anterior end of the egg (Pl. 1, figs. 2, 3 and 4-a). When the formation of the blastoderm begins, the egg contents crowd out to the end, entirely filling the cavity of the shell.

There next appears at the dorsal, anterior end, a thickening of the cell layer, by addition of new cells beneath the surface layer (Pl. I, fig. 5-p). This is the beginning of the primitive band. At the same time the blastoderm begins to fold in above this point, and as the cells underneath increase in number the fold pushes them downward and backward (Pl. I, figs. 7 and 8.)

While the above changes are taking place the cells of the blastoderm crowd away from the sides, so that now it forms a band completely surrounding the egg. A dorsal view of this stage is seen in Plate II, figure 3.

The fold noticed above makes the first segment of the embryo, the procephalic lobes. It is pushed around to the ventral side of the embryo (Pl. II, figs. 4 and 5) and at the same time the ventral part of the fold becomes constricted laterally. Plate II, figures 1 and 2, are diagrams showing the relations of the parts of the fold, at this time, as viewed from the dorsal and ventral aspects respectively. The sides are next folded in in the line of the groove so that in ventral and dorsal views the procephalic lobe is plainly visible (Pl. II, figs. 6 and 7). A little later the infolding of the primitive band to form the stomodoeum begins, and at the same time a longitudinal groove, the gastrula invagination, extends along its ventral side. Plate II, figure 6-g, represents the first beginning of the gastrula infolding.

#### F. FORMATION OF THE SEGMENTS.

Following the gastrula invagination, and the formation of the procephalic lobes, the primitive band grows rapidly backward. The yolk is pushed up to the dorsal side, and the embryo lies as a band extending about the egg but not meeting on the dorsal side. As the primitive band extends backward from the procephalic lobes it becomes constricted by transverse furrows which divide it into segments. The mandibular segment is the first to appear, then the first and second maxillary segments. Following these, the segments appear in a general way in succession from before backward. Frequently, however, two or three segments seem to be formed almost simultaneously.

#### G. THE PROCEPHALIC LOBES.

In the first place the procephalic lobes are represented by a somewhat globular fold at the anterior end of the primitive band (Pl. II, figs. 6 and 7). There soon appears on the ventral median surface on the fold a depression (Pl. II, fig. 8-a). As this deepens the anterior wall of the lobes is thickened and the lateral portions are pushed backward, forming a slight fold so that the procephalic lobes proper become smaller in size. The mandibular segment crowds forward on each side apparently absorbing this fold.

#### II. THE APPENDAGES.

##### (a) *The antennae.*

The antennæ appear as buds from the base of the procephalic lobes just in front of the mandibular segment. They are first noticeable as small projections near the dorsal part of the head (Pl. III, fig. 2-a). This takes place about the 30th hour of incubation. The antennal bud grows downward along

the side of the head fold and in front of the mandibular segment, becoming somewhat thicker at the same time (Pl. III, a). The segments do not appear in the antennæ until near the close of embryonic life. They then appear as constrictions of the already formed antennæ, not by the addition of segments from behind. At the close of embryonic life the antennæ are composed of three segments (Pl. III, figs. 10 and 12-a).

(b) *The mandibles.*

The mandibles appear as appendages of the second segment. This segment is divided laterally into two parts, by the appearance of the gastrula invagination and the stomodoeum, almost as soon as it appears (Pl. II, fig. 8). Almost immediately the two lobes move forward to the sides of the antennal segment (Pl. II, fig. 10-m). As the embryo develops these rudimentary mandibles become narrower and longer (Pl. III, figs. 5 and 7-md.) About six or eight hours before the time of hatching they have grown downward and are visible in a ventral view of the embryo (Pl. III, figs. 5 and 7 md.) A short time before hatching they become divided into two segments by a very slight constriction, and the distal segment develops into a sharp pointed organ. This is the larval mandible.

(c) *The maxillæ.*

The first maxillæ develop as appendages of the third segment. This segment, like the mandibular segment, becomes divided by a dorso-ventral constriction almost as soon as it is formed, and begins to move forward as two lobes, which constitute the rudimentary appendages and their attachment. The anterior edge of this segment moves forward until it is slightly in front of and outside the posterior edge of the procephalic lobes. (Pl. III, figs. 2, 3 and 6, 1st mx.) The maxillæ grow downward and inward (Pl. III, fig. 11, 1st mx.), and just before the close of embryonic life a small segment is formed at the end. In the newly hatched larva there is visible a third, very small segment at the extremity of the maxillæ.

The second maxillæ are very little developed. They appear as slight protuberances of the fourth segment. They are noticeable just before hatching (Pl. II, fig. 11-m).

(d) *The thoracic appendages.*

No appendages are visible upon the thoracic segments until near the close of embryonic life. About eight to ten hours before hatching there appear upon the first thoracic segment a pair of appendages. These are not as yet segmented and lie close together on the ventral surface of the embryo (Pl. III, figs. 8, 10, 11 f.) These develop into blunt rounded appendages, upon the surface of which there appear, just before hatching, a number of setæ. These are noticeable in figures 8, 10, 11 and 12, Plate III. They serve as legs during the larval life of the insect.

(e) *Abdominal appendages.*

No abdominal legs were observed at any time. On the ventral surface of the last segment, however, appear a pair of large tubular appendages, fringed with

a circle of cilia about their external opening. These are in connection with the tracheal system, a tube from which extends along their entire length, opening at the distal extremity. They serve as larval respiratory organs. They may be seen in the embryo in figure 7, Plate III, r., and in the newly hatched larva.

## DESCRIPTION OF PLATE I.

- Fig. 1. The egg 2 or 3 hrs. after laying. (a) Large cells which later develop into the reproductive organs.
- Fig. 2. The eggs at about 2 or 4 hrs. showing the layer of blastodermal cells. (a) Large reproductive cells.
- Fig. 3. The egg at about an hour later than in fig. 2, showing the thickened layer of blastodermal cells, and the large reproductive cells still visible at a.
- Fig. 4. The egg at about the sixth hr. of incubation.  
(a) reproductive cells.  
(b) cells arranged in a columnar layer forming the blastoderm.
- Fig. 5. The reproductive cells have retreated to the interior and are no longer visible. (p) The beginning of the primitive band.
- Figs. 6 and 7. A little later than 5, showing the first infolding of the blastoderm. (v) Vitelline membrane.
- Fig. 8. Egg at about hr. of incubation. (v) Vitelline membrane. (p) Beginning of stomodoeum.

## DECRPTION OF PLATE II.

- Figs. 1 and 2. Diagram showing the form of the first fold at the anterior end. In fig. 1 as viewed from the dorsal and fig. 2 as viewed from the ventral sides.
- Fig. 3. Dorsal view at the same stage showing the yolk mass crowded to the sides, the blastoderm forming a complete dorso-ventral band.
- Figs. 4 and 5. Showing the fold pushed to the ventral side and backwards.
- Fig. 6. Ventral view of embryo. (p) procephalic lobe. (g) beginning of gastula invagination. (s) beginning of proctodoeum.
- Fig. 7. Dorsal view of stage shown in fig. 6. (p) procephalic lobe. (s) proctodoeum.
- Fig. 8. Egg at about eighth hr. of incubation. (v) Vitelline membrane. (p) Beginning of mandibular segment. (g) Gastrula.
- Figs. 9 and 10. Two views about 4 and 7 hrs. later than fig. 8, showing the divided mandibular segment (m) pushing upward on each side of the procephalic lobes and carrying with it the antennal point (a).
- Fig. 11. Dorsal view of embryo at same stage as fig. 10.
- Fig. 12. Ventral view of embryo at same stage as fig. 10. (m) Divided mandibular segment.

## DESCRIPTION OF PLATE III.

- Figs. 1 and 2. Two views of the anterior part of the embryo at about 28 hrs-32 hrs. (a) antennae (md) mandibular segment. (1 mx). First maxillary segment. (2 mx) second maxillary segment.
- Fig. 3. Side view of the entire embryo a little later than in fig. 2, showing thoracic and abdominal segments. Lettering as in fig. 2.
- Figs. 4, 5, and 6. Dorsal and ventral views of the anterior portion of the embryo, and entire side view at about 40 to 45 hrs. Lettering as in fig. 2.
- Fig. 7. Ventral view of embryo at about 55 hrs. to 60 hrs., showing mandibles beginning to form (md) and respiratory appendages at r.
- Figs. 8 and 9. Side and ventral views about 5 hrs. later than fig. 7.
- Figs. 10 and 12. Side and ventral views of the anterior and of the embryo just before hatching. Lettering as in figs. above.
- Fig. 12. Side view of the entire embryo just before hatching.
- Fig. 13. Optical section of embryo just before hatching, showing proctodoeum, stomodoeum, nerve-cord, and the still prominent sexual cells.

# PLATE I

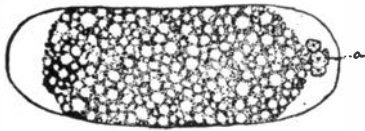


Fig. 1

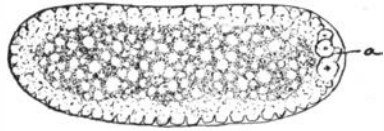


Fig. 2

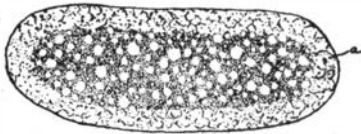


Fig. 3

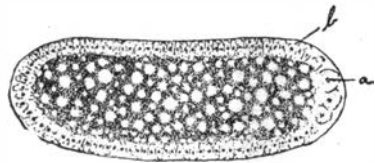


Fig. 4

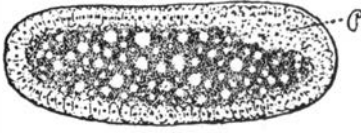


Fig. 5

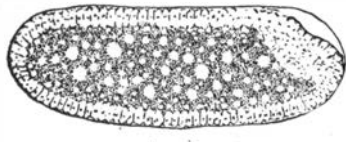


Fig. 6

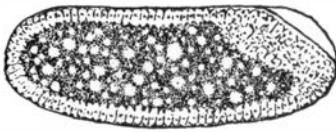


Fig. 7

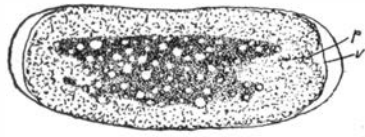
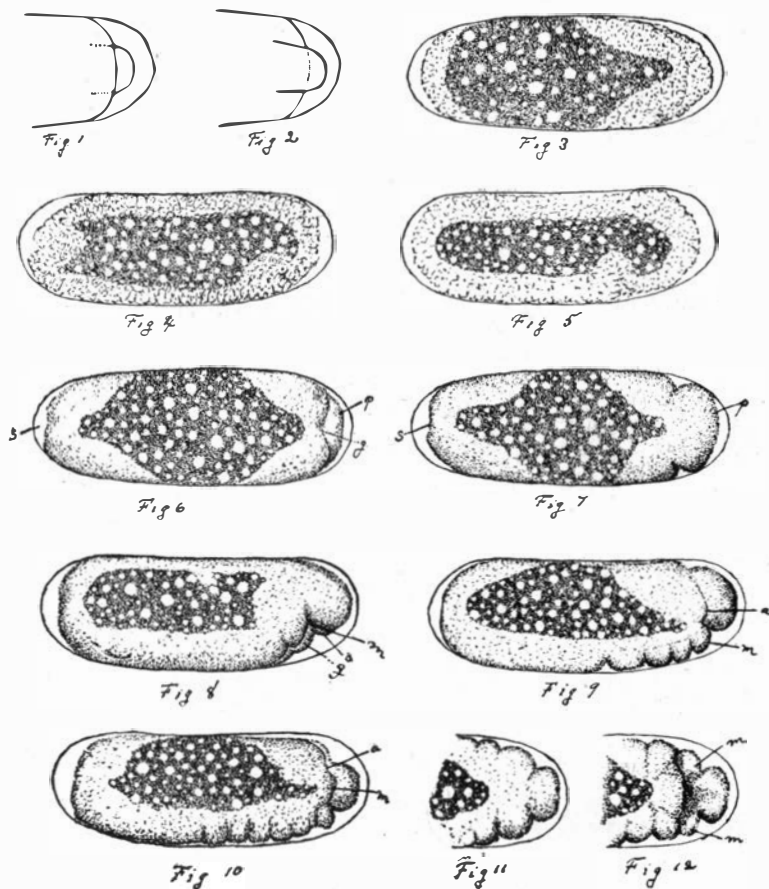


Fig. 8

(Craven. Chironomus.)

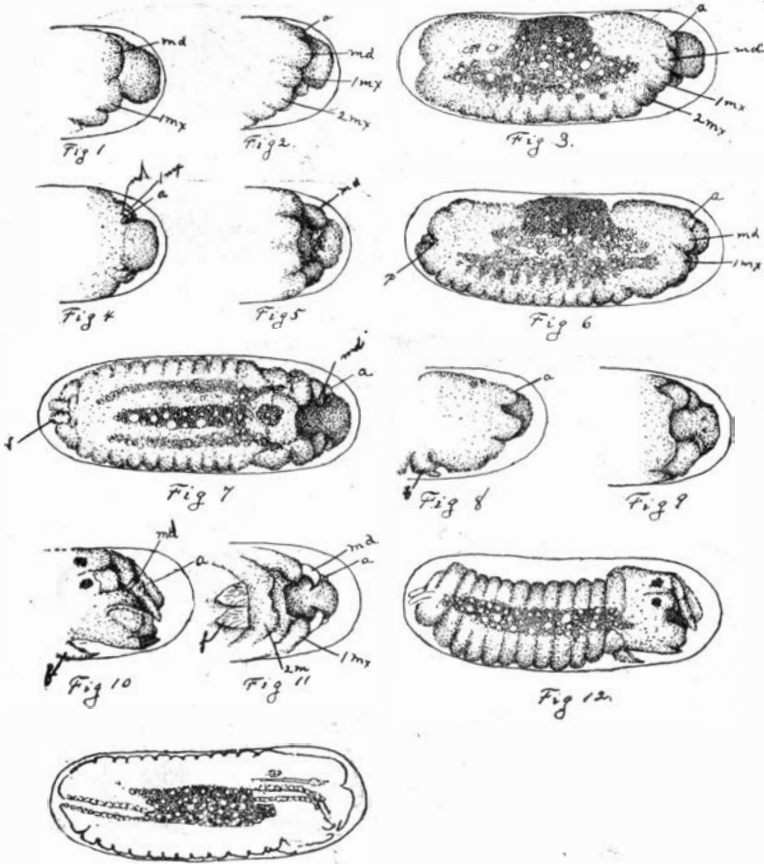
# PLATE II



(Craven. Chronomus.)



# PLATE III



(Craven. Chiromonus.)