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Studies on embryonic determination of the harlequin-fly, *Chironomus dorsalis*. II. Effects of partial irradiation of the egg by ultra-violet light

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WITH ONE PLATE

INTRODUCTION

IN an earlier study (Yajima, 1960) of centrifugation of the egg of *Chironomus dorsalis*, the author obtained various types of double malformation (double cephalon and double abdomen) which are correlated with the orientation of the egg at the time of centrifugation. As a result the author proposed (1) that the embryonic character of *Chironomus* was determined by two formative localities; one located in the anterior end of the egg and determining the cephalic structures, and the other located in the posterior end and having a tendency toward abdominal development, (2) that the thoracic structures are determined as the result of interaction between the two localities and, finally, (3) that such localities must be carried by a substratum not moved by centrifugal forces of less than 27,000 g.

By constriction experiments and their combination with translocation of the posterior pole material in the eggs of the leaf-hopper, *Euscelis plebejus*, Sander (1959, 1960) suggested that two 'Factorenbereiche' are present in the anterior and the posterior half respectively, and that by their interaction the segmentation of the embryo and its site are determined. He further showed that the posterior formative factor must be carried by posterior pole material. In other words, both Sander and the present author came to quite similar conclusions from independent work on two different insects.

Ultra-violet irradiation has often been used in the experimental embryology of insects, although it was mostly used to make defects in the presumptive embryonic area (Geigy, 1931*a*, *b*; Oelhafen, 1961), or to test the regulative power of uninjured parts, or to trace the movement of small areas of the embryo during embryogenesis (Nitschmann, 1959; Hathaway & Selman, 1961).

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However, Seidel (1934) employed ultra-violet irradiation to prevent the formative action of the yolk system of the egg of *Platycnemis*. In the *Habrobracon* egg von Borstel & Wolff (1955) and von Borstel (1957) studied ultra-violet action on the nucleus and the cytoplasm separately, and the photoreactivation capacity of each component.

In the present experiments ultra-violet irradiation was used to suppress the action of both formative localities. In the first place, egg masses containing several hundreds of eggs were exposed to ultra-violet light, varying the length of exposure and the developmental stage of irradiation. Secondly, single eggs freed from the jelly material of the egg mass were partially irradiated using a cover-glass screen.

MATERIAL AND METHODS

The eggs of *Chironomus dorsalis* were collected in the field. Since the eggs are enclosed in a jelly mass, the jelly was dissolved by sodium hypochlorite in the experiment involving partial exposure.

In most of the experiments of this study eggs were irradiated at the mid-cleavage stage because of the abundance of various types of abnormalities following ultra-violet treatment at this stage.

The following six stages were irradiated:

1. Early cleavage stage, soon after the formation of pole cells.
2. Mid-cleavage stage.
3. Syncytial blastoderm stage, soon after nuclear migration.
4. Syncytial blastoderm, 1 hr. after nuclear migration.
5. Syncytial blastoderm, 2 hr. after nuclear migration.
6. Syncytial blastoderm, 4 hr. after nuclear migration; at this stage cell boundaries are formed except at the yolky side.

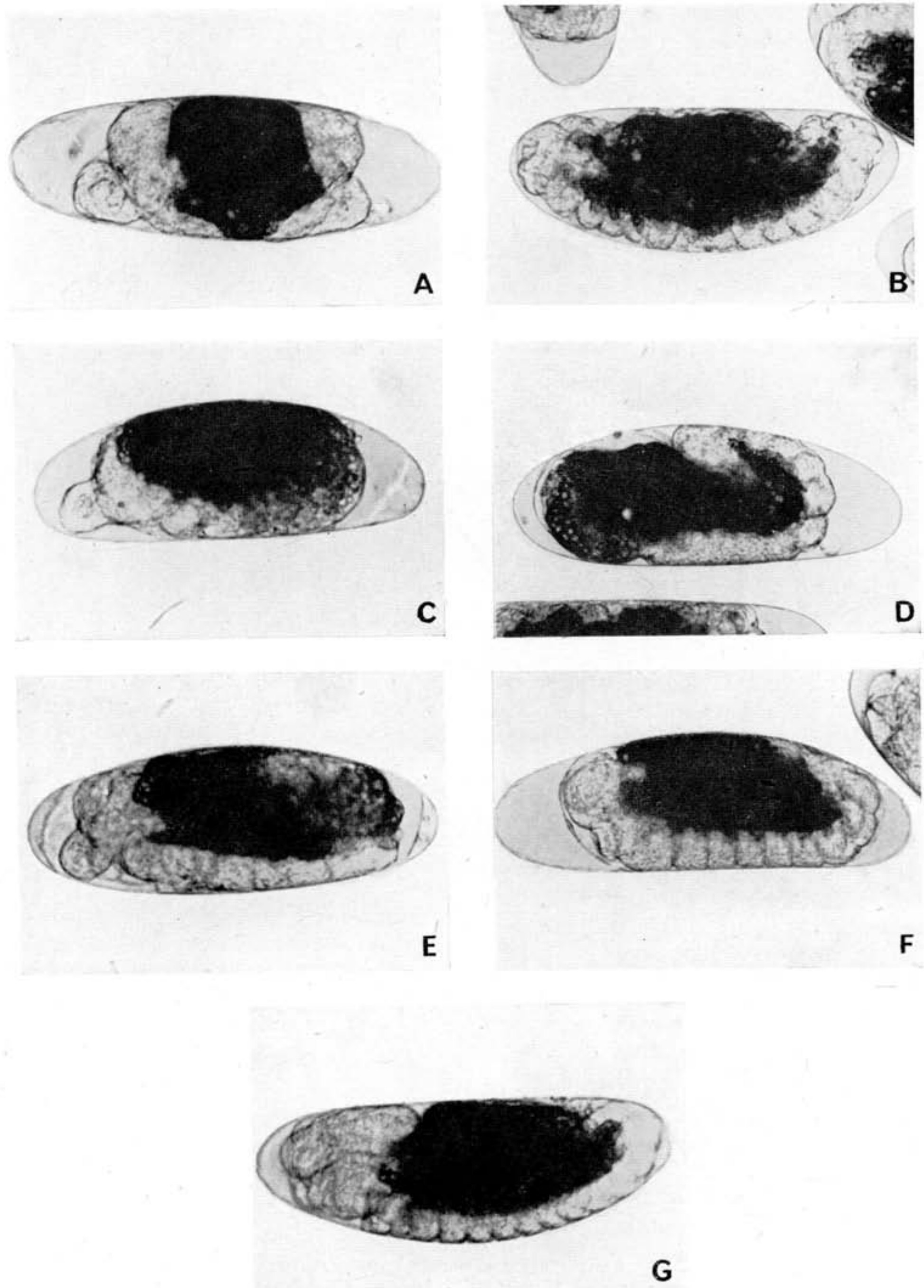
As the source of ultra-violet, a GL-lamp (15 W., low pressure mercury lamp, Toshiba) was used. Most of the energy emitted by the lamp is at a wave length of 2537 Å.

In all the experiments, the duration of exposure was within the range of 0·5–4 min.

The eggs were exposed at a constant distance of 7 cm. in 2·5–4 mm. of water. Most of incident energy, 1750 μ w/cm.^{2}, as measured by a germicidal intensity meter (General Electric, model UV-480, type Do-91), is not absorbed by water of this depth.

Ultra-violet transmittance of the whole egg and of the cytoplasm was measured by Beckmann's DU type spectrophotometer. Before measurement, the following preliminary procedures were applied to the materials.

The whole egg. Many eggs were put on a quartz cover-glass. While water around the eggs was soaked by filter paper, the eggs were collected by it. By such a treatment the eggs were stuck to the cover glass in a compact mono-layer.



EXPLANATION OF PLATE

Normal embryo and various types of malformation resulting from ultra-violet irradiation (see text).

FIG. A. Double cephalon.
FIG. B. Double abdomen.
FIGS. C, E. Posterior defect.
FIGS. D, F. Anterior defect.
FIG. G. Normal embryo.

The cytoplasm. Many eggs with surrounding water removed were crushed between two quartz cover-glasses to form a thin layer of the cytoplasm. Chorions contained in the cytoplasmic layer occupy only a few per cent of the breadth of the layer.

After these preliminary procedures, the preparation was set into the spectrophotometer.

Since chorions were difficult to collect in a large number, transmittance of the chorion was calculated from the data for the whole egg and the cytoplasm.

TABLE 1

Ultra-violet transmittance of whole egg, cytoplasm and chorion

	Whole egg	*Cytoplasm*	*Chorion**
Per cent. T	7·1	70·0 (15 μ) 44·3 (30 μ)†	87·4

* The value for the chorion was calculated from the data for the whole egg and the cytoplasm.
† Calculated value from the datum for 15 μ.

For partial irradiation of the egg, a glass vessel was used (see Text-fig. 2). After irradiation, eggs were transferred to tap water and cultured.

RESULTS

Description of abnormalities

By ultra-violet irradiation of *Chironomus* eggs the following four kinds of abnormalities were obtained.

(*a*) Double malformation: Two types of double malformation are obtained. One type is such that two sets of the head segments in front of the first maxilla are connected at their posterior sides with their heads pointing in opposite directions. The animal *lacks thorax and abdomen* (Plate 1, A); this abnormality is the same as 'double cephalon' obtained by centrifugation reported in the preceding paper (Yajima, 1960). The other type is such that two sets of the segments behind the second abdominal segment are connected at their anterior ends. *In this type, head and thorax are missing* (Plate 1, B); this is the same as the 'double abdomen' of the preceding paper. Consequently, the terminology 'double cephalon' and 'double abdomen' will be used in this paper.

(*b*) Single larva with defective parts: Abnormalities in which the cephalic or the abdominal end is missing (Plate 1, C–F).

(*c*) i. Undifferentiated cell mass; Mass of cells multiplied without differentiation on the sides or in the centre of the embryo; ii. Dead eggs.

Since (*c*) i and ii groups cannot be distinguished easily they are treated as one group for computing percentages.

Irradiation of egg in jelly mass

Duration of exposure and frequency of abnormalities

In this series only eggs at the mid-cleavage stage were used. In each experiment ten egg masses were irradiated, each contained about 350 to 450 eggs and the duration of exposure ranged between 0·5 and 3 min.

The results are shown in Table 2. Numbers refer to the percentage of abnormal and normal embryos found. The maximum frequency of double cephalon and

TABLE 2

Effect of ultra-violet irradiation of eggs within the jelly mass

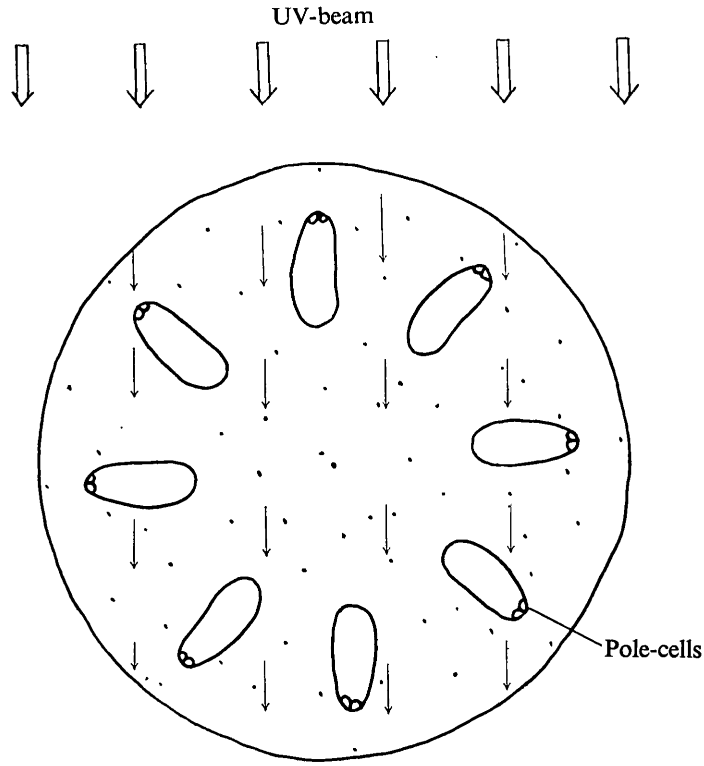
Duration of exposure (min.)	*Normal embryo (%)*	*Double cephalon (%)*	*Double abdomen (%)*	*Posterior defect (%)*	*Anterior defect (%)*	*Undifferentiated and dead eggs (%)*
0·5	100	0	0	0	0	0
1	80·2	2·9	1·5	8·4	3·4	5·6
1·5	55·9	*6·8*	2·4	17·5	5·1	12·0
2	37·6	1·0	4·5	16·7	6·1	33·8
2·5	32·0	0·1	*8·8*	21·1	7·9	29·9
3	21·7	0·04	5·4	15·5	7·7	49·3

of double abdomen are found after 1·5 and 2·5 min. of treatment respectively. This fact suggests that the sensitivity to ultra-violet light differs between the cephalic and the abdominal formative localities. This will be discussed later. Anterior and posterior defects steadily increase in number with the duration of exposure up to 2·5 min. and the frequency of anterior defects is lower than that of posterior ones. The frequency of (*c*) group also increased with the duration of exposure.

Relation between the orientation of the eggs in the jelly mass and the frequency of occurrence of double malformation

In addition to the difference in sensitivities of the formative localities to ultra-violet light, the orientation of the eggs in the jelly mass must be taken into consideration as possibly contributing to the difference in incidence of the two abnormalities.

The egg mass of *Chironomus dorsalis* has the shape of a banana in which the eggs are arranged in a loose spiral. The cross section of the egg mass, diagrammatically shown in Text-fig. 1, indicates that the anterior side of the eggs is directed toward the centre of the jelly mass. Double cephalons were found on the side of the egg mass nearer to the ultra-violet source, and double abdomens on the opposite side. If the ultra-violet light comes from the direction of the arrows in Text-fig. 1, the eggs on the nearer side will be irradiated from the posterior sides, while the eggs on the farther side will receive more ultra-violet energy on the anterior sides. Further comments will be made in later paragraphs on partial irradiation.



TEXT-FIG. 1. Cross-section of the egg mass (pole cells show the posterior side of the egg).

Developmental stages of irradiation and the frequency of double malformation

Changes in the frequency of double malformation with the developmental stage at the time of ultra-violet irradiation were found. Two durations of ultra-violet exposure, 1·5 and 2 min., were given to ten egg masses in each series of the experiment.

The results are given in Table 3 as the percentage of double malformations formed. As the table shows the frequency decreases gradually after stage 4. Until 1 hr. after nuclear migration malformations are still obtained in small numbers, but more than 1 hr. later the value is extremely small and, finally, at 4 hr. malformations are no longer produced. No difference is found between the stages in ease of production of double cephalon or double abdomen.

The centrifugation experiment (Yajima, 1960) showed that double malformations were determined at about the nuclear migration stage. But in the present study of ultra-violet irradiation, they are found up to the stage a few hours after nuclear migration. The reason for this difference must be that while the centrifuging procedure requires longer than ultra-violet irradiation, and the stratification it produces tends to be lost as soon as centrifugation is stopped, irradiation can be done much faster (in a few minutes) and its effect would last longer. In other words, irradiation may be more precise than centrifuging in defining the stage of determination. It is therefore considered that the stage of determination must actually be somewhat later than the stage of nuclear migration.

TABLE 3

Developmental stages of ultra-violet irradiation and the frequency of double malformation

Irradiated stage	Duration of exposure (min.) 1·5: Double cephalon malformation (%)	1·5: Double abdomen malformation (%)	2·0: Double cephalon malformation (%)	2·0: Double abdomen malformation (%)
(1) Early pre-migration stage, soon after the formation of pole cells	—	—	0·29	0·8
(2) Middle pre-migration stage	6·8	2·4	1·0	4·5
(3) Syncytial blastoderm, soon after nuclear migration	2·9	1·5	1·0	3·1
(4) Syncytial blastoderm, 1 hr. after nuclear migration	0·9	0·6	0·6	2·2
(5) Syncytial blastoderm, 2 hr. after nuclear migration	0·07	0·07	0·02	0·1
(6) Syncytial blastoderm, 4 hr. after nuclear migration	0	0	0	0

As a matter of fact, as the cellular blastoderm stage is approached, it becomes impossible to obtain double malformations by ultra-violet treatment. This fact, together with the results of the centrifugation experiment, suggests that the differentiation of the egg surface is completed before the cellular blastoderm stage.

Partial irradiation

As already stated in the previous section, in order to obtain double malformations irradiation of a part of the egg seems to suffice. Therefore, partial irradiation was applied to the egg using the vessel shown in Text-fig. 2. To vary the area of exposure a cover-glass is stuck to the vessel with vaseline and is slid by a needle. The cover-glass used in this experiment absorbs over 95 per cent. of the incident energy.

Six patterns of irradiation as shown in Text-fig. 3 were used and the results are shown in Table 4.

As to the relationship between the appearance of double malformation and the area of irradiation, double abdomens resulted from irradiation ranging between one-third to two-thirds of the egg from the anterior end (I, II, III of Text-fig. 3),

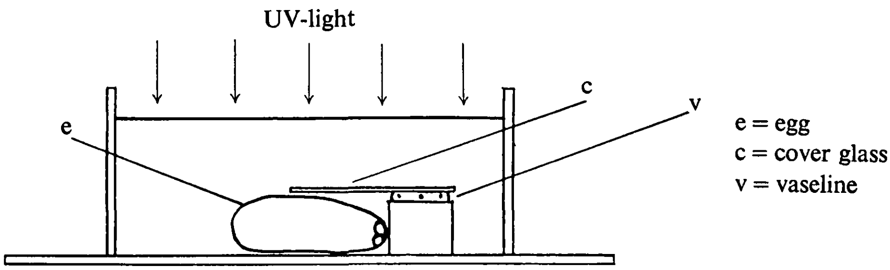
TABLE 4

Effect of partial irradiation of single egg by ultra-violet beam

Pattern of irradiation	*Duration of exposure (min.)*	*Normal embryo (%)*	*Double cephalon (%)*	*Double abdomen (%)*	*Anterior defect (%)*	*Posterior defect (%)*	*Undifferentiated and dead eggs (%)*
I. Irradiation of anterior third	1	100	0	0	0	0	0
	2	92·8	0	0	0	0	7·1
	3	90·0	0	0	0	0	10·0
	4	50·0	0	20·5	29·4	0	0
II. Irradiation of anterior half	1	100	0	0	0	0	0
	2	88·8	0	11·1	0	0	0
	3	55·5	0	22·2	0	0	22·2
	4	81·3	0	13·1	0	0	5·2
III. Irradiation of anterior two-thirds	1	100	0	0	0	0	0
	2	32·3	0	14·7	35·2	0	17·6
	3	7·6	0	7·6	84·6	0	0
	4	24·0	0	0	16·0	0	60·0
IV. Irradiation of posterior third	1	100	0	0	0	0	0
	2	100	0	0	0	0	0
	3	89·2	0	0	0	10·7	0
	4	76·7	0	0	0	23·2	0
V. Irradiation of posterior half	1	100	0	0	0	0	0
	2	100	0	0	0	0	0
	3	88·5	0	0	0	11·4	0
	4	66·6	0	0	0	27·2	12·1
VI. Irradiation of posterior two-thirds	1	93·3	3·3	0	0	3·3	0
	2	73·6	5·2	0	0	21·0	0
	3	42·3	0	0	0	26·9	30·7
	4	10·7	0	0	0	28·3	60·7

and double cephalons were obtained only by irradiation of the posterior two-thirds of the egg (VI of Text-fig. 3).

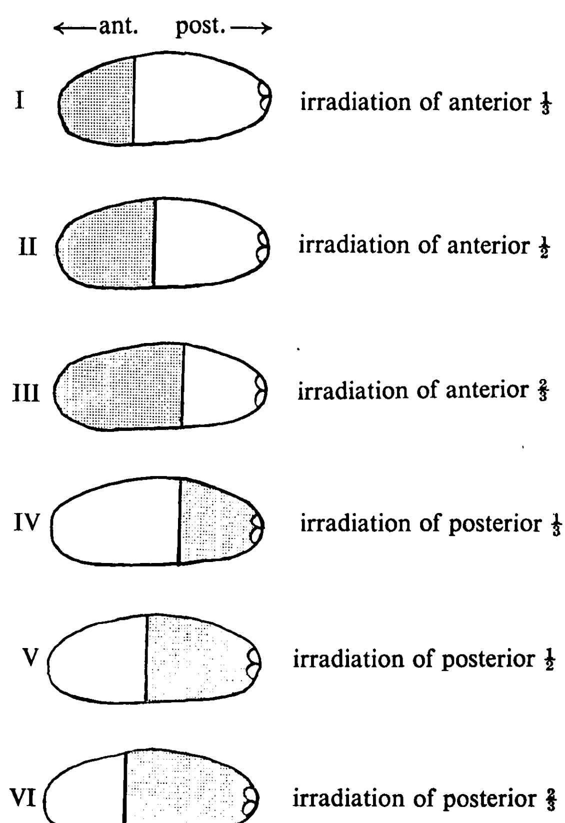
As to the duration of exposure, double abdomens were obtained by irradiations



TEXT-FIG. 2. The vessel for partial irradiation.

lasting from 1 to 4 min., and the greater the area irradiated the shorter becomes the duration required. However, as the area of irradiation is enlarged, occurrence of (*c*) groups becomes noticeable.

On the other hand, the appearance of double cephalons was limited to shorter



TEXT-FIG. 3. Pattern of partial irradiation. ant. = anterior side, post. = posterior side, shaded = area of irradiation.

irradiation lasting from 1 to 2 min. This means that the frequency of double cephalon is lower than that of double abdomen and the range of ultra-violet intensity is rather narrow, most of the treated eggs developing normally.

It is concluded from this that the sensitivities of the two formative localities to the ultra-violet light differ from each other.

DISCUSSION

Presence and the extent of the formative localities

From the ultra-violet irradiation of the *Chironomus* egg it is clear that double cephalon and double abdomen can be obtained separately by irradiating the posterior two-thirds of the egg and the anterior one-third of the egg, respectively. This may be interpreted to mean that the locality which determines the cephalic characters is localized in the anterior one-third of the egg, and the locality determining the abdominal characters lies in the posterior two-thirds of the egg. This may offer a concrete topographical basis to the idea of the formative locality (see Introduction) which was suggested previously (Yajima, 1960).

As Table 1 shows, the intensity of incident ultra-violet light is reduced to 87·4 per cent. of the original value by passing through the chorion. About 55 per cent. of the energy that does penetrate the chorion must be absorbed within 30 μ of the surface. From this fact, it is justifiable to think that the formative localities above described must be carried by the superficial layer of the egg. This also agrees with the author's previous supposition based on the centrifugation data (see Introduction).

Since ultra-violet light at a wave length of 2537 Å is effective in causing double malformations, we may assume that these arise as a result of interference with pyrimidine and purine bases.

Determination of intermediate characters

In an earlier paper (Yajima, 1960), the author tentatively proposed that the intermediate characters—a few posterior cephalic segments, thorax and a few anterior abdominal segments—must be formed by interaction of the anterior and posterior localities (see Introduction).

In the present study, when double abdomen was obtained by the irradiation of the anterior one-third of the egg, the more posterior region which presumably corresponds to the presumptive thorax in normal development was not directly irradiated. Nevertheless, the resulting malformation lacked the thorax. In other words, the intermediate characters never appear unless the two localities co-exist. In this connection Sander's opinion for the leaf-hopper referred to earlier is extremely important.

The time of action of the formative locality

As was made clear by the centrifugation experiment, the endoplasm and the nucleus are not determined until nuclear migration is over, because double malformations are obtained by centrifugation before this stage while centrifugation after this stage gives rise to embryos defective at one or other end.

On the other hand, as shown in the experiments involving irradiation of the egg in the jelly mass, a stage at which the double malformations can be obtained by ultra-violet treatment can be traced back till soon after the formation of the pole cells (see Table 2). Thus the double malformation by ultra-violet at the early stage must be related to injury of some part of the egg other than the endoplasm or the nuclei. Because of the shallow penetration of ultra-violet light, such a part of the egg determining double malformation must be in the superficial layer. The above analysis may further lead to the idea that the formative locality acts on the endoplasm and the nuclei to determine their potencies during nuclear migration and for a few hours following it.

SUMMARY

1. Irradiation by ultra-violet light of the egg mass as a whole and partial irradiation of single eggs were employed for the analysis of embryonic determination in the harlequin-fly, *Chironomus dorsalis*.

2. Three kinds of abnormalities were obtained; i.e. double malformations, single larvae with defective parts, and undifferentiated or dead eggs.

3. Relationships between the frequency of occurrence of double malformations and the duration of exposure and developmental stage of irradiation are as follows:

(*a*) For exposures lasting from 0·5 to 3 min., double cephalons—duplication of cephalic structures—were obtained most often by 1·5 min. exposure and double abdomens were produced most often by 2·5 min. exposure.

(*b*) The frequency of double malformations decreases gradually after nuclear migration. Up until 1 hr. after nuclear migration, malformations are still obtained in small numbers but at 4 hr. they are no longer obtained.

4. After partial irradiation, double cephalon was caused by posterior irradiation and double abdomen by anterior irradiation.

5. The topographic extent of the formative localities is discussed.

RÉSUMÉ

Étude du développement embryonnaire de la mouche 'arlequin',
Chironomus dorsalis *II. Effets d'une irradiation partielle de l'oeuf par les rayons ultraviolets*

1. Afin d'analyser de développement embryonnaire de la mouche 'arlequin', Chironomus dorsalis, on effectue des irradiations aux rayons ultra-violets en soumettant l'ensemble des oeufs à une irradiation totale ou les oeufs isolés à une irradiation localisée.

2. Trois types d'anomalies ont été obtenues: monstres doubles, larves uniques avec des territoires altérés et des oeufs non différenciés ou morts.

3. Le rapport entre la fréquence d'apparition des monstres doubles, la durée d'exposition et le stade du développement au moment de l'irradiation s'établit de la manière suivante:

(*a*) Dans le cas d'une durée d'irradiation de 0,5 à 3 min., on obtient des céphalons doubles—dédoublement des structures céphaliques—surtout après 1,5 min., et des abdomens doubles surtout après 2,5 min. d'exposition.

(*b*) La fréquence des malformations doubles décroit progressivement après la migration du noyau. Une heure après la migration du noyau, on obtient encore des malformations en petit nombre mais après 4 heures, on n'en observe plus.

4. Après irradiation de la région postérieure, il y a formation d'une tête double et après irradiation de la région antérieure, on observe un abdomen double.

5. L'extension topographique des territoires formateurs est discutée.

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