SCIENTIFIC APPARATUS AND LABORATORY METHODS

A METHOD FOR THE STUDY OF CHROMOSOMES IN ENTIRE INSECT EGGS

IN view of the numerous difficulties attending the study of chromosomes in insect eggs it is believed that the method outlined below may prove of value to other workers in the field, as it has to us. Its essential feature is the use of the recently developed Feulgen test for thymonucleic acid[1] by means of which chromatin alone may be stained, leaving the other components of the cell almost transparent. Applying this method to the entire egg it is possible, in suitable material, to reveal the chromosomes distinctly without staining the yolk or other egg constituents, with the result that the maturation and cleavage divisions may be studied with ease.

Not only does this method eliminate the laborious processes of embedding, sectioning and examining deeply stained serial sections, but it adds greatly to the reliability of the observations and also greatly extends the usefulness of each good preparation. Reliability is enhanced by the fact that in the entire egg, thus prepared, no mechanical disturbance of parts has occurred and the inter-relations of the components (polar bodies, egg nucleus, sperm, etc.) are obvious. It is not necessary to reconstruct as with sections. Usefulness of the individual preparation is increased by the fact that all the mitotic figures are intact; and both usefulness and reliability are increased by the fact that the egg may be moved about (rotated) in such a way as to permit bringing each figure into the most favorable position for study, and to permit examination of individual figures in different aspects (side view, polar view, etc.).

We have used the method only on the eggs of one species—the fungus gnat *Sciara coprophila* Lint. The eggs of this fly are approximately 0.2 mm in length and 0.1 mm in thickness.

In such eggs it is possible to examine the chromosomes in any part of the egg with a 2 mm oil immersion objective and in most cases with a 1.5 mm objective. From our experience it seems probable that the method could be used satisfactorily even with considerably larger eggs for a study of the maturation divisions and of such cleavage divisions as occurred near the periphery of the egg, because of the possibility of rolling the egg about until the desired part becomes uppermost. In the case of eggs with opaque or too resistant outer membranes such membranes would, of course, have to be removed mechanically, or punctured, as the case required.

[1] Lee, "Microtomist's Vade-mecum," 9th edition, pp. 437–438 and footnote, p. 438.

The procedure we have followed is given below: At suitable intervals after the eggs are laid they are fixed in a modification of Carnoy's solution (equal parts chloroform, absolute alcohol and glacial acetic acid) for from one half hour to two hours, and washed in several changes of absolute alcohol over a period of at least two hours. Then they are passed through the higher alcohols, 95 per cent., 85 per cent., 70 per cent. (one hour each), the lower alcohols, 50 per cent., 30 per cent., 15 per cent. (one half hour each) and tap water (several changes) in preparation for the Feulgen treatment. For our material the stain is most successfully obtained in the following manner:

(1) Place eggs for 15 minutes in cold normal HCl solution, (2) transfer for 8–10 minutes to a similar HCl solution heated to 60° C, (3) rinse in cold HCl solution, (4) place for 2–5 minutes in SO_2 water, (5) stain 1 hour in fuchsin sulfuric acid solution, (6) wash in two changes (fifteen minutes each) of the SO_2 water, (7) rinse in several changes of tap water. The HCl and SO_2 solutions must be fresh, and the fuchsin sulfuric acid solution an amber color without red precipitate. The formulae for the solutions and additional precautions as to their use may be found in Lee (*loc. cit.*).

The method requires some experience. Unless properly treated the chromosomes are apt to be pink or pale red, but in suitable preparations they are deep red in color, not unlike the color produced by safranin. The color is relatively permanent.

After staining, the eggs are dehydrated rapidly in alcohol (not more than 5 minutes in each change up to 85 per cent.), cleared in xylol and mounted in balsam in the usual manner on slides or between coverslips. If they are to be moved about and examined in different positions it is best to do the manipulating soon after mounting, although in our experience the balsam may readily be softened around the coverslip with xylol at any time within a few weeks after mounting. A green light filter aids materially in studying the figures.

In rolling the eggs during observation we have used the simple expedient of moving the coverslip to and fro. This usually suffices to turn the eggs. If more accurately controlled manipulation were desired the procedure could be modified as required—*e.g.*, the eggs could be examined in immersion oil without the use of a coverslip. When a coverslip is used it is necessary, of course, to adjust the amount of balsam to the size of the eggs in order to have as little as possible between the egg and the glass and at the same

time to avoid flattening the egg by pressure of the coverslip. In practice this presents little difficulty.

The fixative noted above is used simply because it appears to give the best fixation in our material. Others may be substituted if desired. Contrary to early reports the Feulgen method of staining appears to be applicable to material fixed in any of the ordinary fixatives. We have used it after Fleming, Gilson's mercuric nitric solution and Bouin, as well as Carnoy.

As an aid in transferring the eggs during dehydration, staining, etc., any of the standard methods of handling small objects may be used. We use the method of packing them in Drosophila pupa skins when they are in 70 per cent. alcohol after fixation[2] and then pushing them out of these cases either singly or in groups during the final process of mounting in balsam.

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RECONSTRUCTION WORK BY THE USE OF CELLOPHANE

IN 1930–31 the writer began investigations on the development of the jugular lymph sacs in turtles. In review of the literature on the vascular system, one observes that the only available extra-vitamin methods of studying the development of vascular elements, are by plastic reconstructions and by injections. Excellent as these methods are in studying vascular anatomy, they merely show the extent of the system at any stage and many questions as to the exact way in which growth takes place can not be determined by them. For that reason a new method for studying the development of the system was being sought. It seems hardly necessary to emphasize the advantage of using all possible methods in studying systems, if not always in one's own work, certainly through understanding the value of the observations of others and the necessity of comparing and testing the limitations of different methods.

Material was being sought which was thin and transparent, so depth could be observed when used in reconstructions. Cellophane answered the purpose, it being material of transparency, which is strong, durable and .0017″ in thickness.

Camera lucida drawings were made of serial sections on separate sheets of cellophane. A projection microscope was used for the drawings, so that a relatively large field with a high magnification could be obtained. This material made it possible to check each drawing carefully and quickly with the one preceding, before they were placed in reconstruction form. It was found to be the best method to get every vascular element in the reconstruction and to establish, thereby, a graded series of stages of embryos of different ages, complete in all details, in which the vascular elements could be studied and compared in their proper relations. In some cases it seemed highly desirable to use different colored inks to represent the arteries, veins, lymphatics and nerves.

It seems to the writer that where wax reconstructions are necessary, the use of cellophane marks a decided advancement in the preliminary steps for making the plastic reconstructions. The transparency of the material, making observation of depth possible, gives one an accurate course of the parts under observation in relation to other structures.

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SPECIAL ARTICLES

SIMULTANEITY IN THE ONSET OF POLIOMYELITIS

THE observation has often been made that when multiple cases of poliomyelitis occur in a family or a similar group of children, the onset of symptoms in all the individuals affected is simultaneous or nearly so. Sometimes the first symptoms are weakness or paralysis of muscles; at other times the symptoms are indefinite and mild, with weakness or paralysis of muscles following only after several days, or not at all.

These occurrences point to a common and coincidental exposure to the virus of the group of children affected. From these children, secondary cases of the disease may arise in other children, according to circumstances varying from group to group. The secondary cases will be separated from the primary ones by an interval of one, two or even more weeks, which interval is called the "incubation period."

A corresponding simultaneity is shown in a remarkable manner by groups of monkeys (*Macacus rhesus* and *cynomolgus*) inoculated experimentally with a potent virus by simple nasal instillation. The incubation period in these animals is regular and

[2] C. W. Metz, "A Simple Method of Handling Small Objects in Making Microscopic Preparations." *Anat. Rec.*, Vol. 21, No. 4, pp. 373–374, 1921.