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Transplantation of Nuclei in *Drosophila melanogaster*

(micromanipulation/mosaics/eggs)

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ABSTRACT **Nuclei surrounded by ooplasm of the syncytial stage of developing eggs of wild-type *Drosophila melanogaster* were implanted into freshly laid fertilized eggs of females of a *y w* stock. More than half of the recipient eggs produced larvae, but few of the larvae hatched or developed further. The best sets of experiments gave about twelve percent of imagos, mostly *y w* in appearance. Several larvae were mosaics with yellow Malpighian tubes, and two flies had part of the abdominal segments of the wild type. Half of the flies were fertile, but they produced only *y w* offspring, except for two males that had *y w* appearance, but wild-type gonads. When crossed with *y w* females, they gave wild-type females and *y w* males.**

Successful transplantation of nuclei in frog eggs (1) led to the hope that similar experiments could also be performed in animals in which genetic analysis of the transplanted nuclei could be more easily obtained. The obvious choice, *Drosophila*, has been used by only a few investigators because operations upon its eggs appeared to be too difficult. The first report of transplantation of nuclei in *Drosophila* was made by Geyer-Duszynska (2), who used unfertilized eggs as hosts and early developmental stages of a genetically different strain as a donor. The mortality was very high; out of 1426 operated eggs, only one developed into a larva. Illmensee (3) reported similar experiments with a somewhat better yield, namely several advanced embryos and one larva that hatched out of 118 eggs. Recently, he was able to culture tissues of such embryos in adult hosts, from which he transferred them into advanced larvae (4). After metamorphosis, these tissues gave various adult structures, thus proving that the transplanted nuclei could support further development. Essentially the same type of experiments have just been reported by Schubiger and Schneiderman (5). In no case did the operated eggs develop into adult flies containing tissues derived from injected nuclei.

I made transplantations into fertilized eggs in order to increase the likelihood of obtaining viable embryos that can continue to develop normally. The nuclei of donors and recipients differed genetically, so that the tissues contributed by each of them could be recognized as mosaic areas in the adults.

MATERIAL AND METHODS

Freshly laid eggs of the strain *y w* (*yellow* body, *white* eyes, colorless Malpighian tubes) of *D. melanogaster* served as hosts, while older eggs of the wild type, containing a syncytium of about 256 nuclei, were the donors. The nuclei were transplanted along with the small amount of surrounding ooplasm. The total amount of transplanted material was about 2% of the volume of the egg.

Operations were performed under a dissecting microscope, with the aid of a movable stage and of a mechanical micromanipulator. Injections were made with a thermally actuated micropipette pointed into a needle about 30 μm wide. The pipette was made from a 1.4-mm-wide Pyrex glass (mp) capillary that was pulled over a microflame into a needle of the desired diameter. The point of the needle was sharpened on a rapidly rotating grinding wheel with a fine abrasive powder. The tube was closed by melting 1 cm from the point and then half-filled with a fluorocarbon oil (Kel-F 3; 3 M Co.). The portion containing air was surrounded by a loop of thin resistance wire that could be heated electrically (Fig. 1). By these means it was possible to control the speed of injection and the injected volume accurately. The material was aspirated into a preheated capillary that was allowed to cool.

The host eggs were dechorionated and arranged on a piece of double-faced "Scotch" tape (3 M Co.) fastened to a microscope slide, so that the ends were in line with the injection needle. The donor and host eggs were aligned in two parallel rows, so that it was possible to take with a needle a certain amount of the ooplasm from one egg and inject it immediately into the nearest recipient egg. In all experiments reported here, the nuclei were taken from the middle region of the egg and injected into the posterior end.

When the egg membrane is pierced, part of the ooplasm leaks out because of the egg's turgor. This loss of ooplasm causes abnormal development, thus presenting a serious problem for the success of transplantation. The turgor and the resulting loss were eliminated by slightly drying the eggs over $CaCl_2$ before the operation. The operation was performed in the air so that the wound could dry after the needle was retracted. The wound was then closed with a solution of gum damar in heptane, applied with a fine capillary. To avoid desiccation or excessive rehydration, the operated eggs were allowed to develop in an atmosphere kept humid over a solution of 0.9% NaCl.

RESULTS

After the technique was perfected in all its details, a good rate of survival after operation was obtained. Table 1 shows

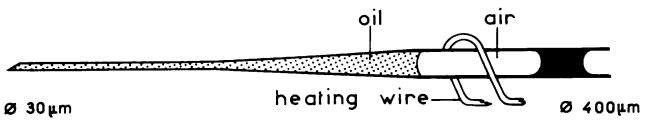

FIG. 1. Micropipette fabricated from a Pyrex melting-point capillary (the drawing is exaggerated in thickness). The wide end of the capillary is fastened to the micromanipulator.

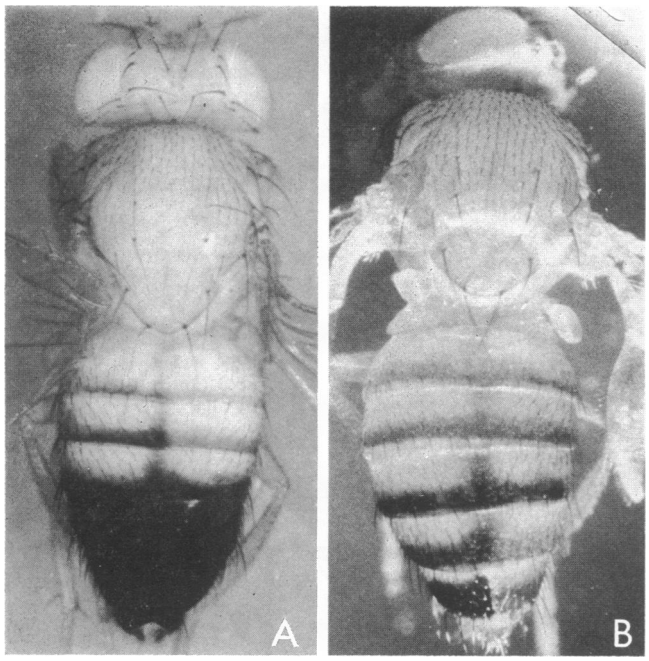

FIG. 2. Mosaic *Drosophila* flies formed after injection of wild-type nuclei into *y w* eggs. (*A*) In the male (D 208), the left halves of the second and third, the major part of the fourth, and the complete fifth, sixth, and last abdominal segments are wild type. (*B*) In the female (D 176), the fourth, and halves of the fifth and sixth abdominal segments are wild type.

the stages of development reached in several successful sets of operations. There was a high mortality in early development, due mainly to the excessive disturbance from injection. In many cases, embryogenesis proceeded to the larval stage, but the larvae were unable to hatch. They nearly always showed visible malformations, in particular defective mouth hooks. After hatching, many larvae died in the first instar, probably because of inability to feed properly. A few larvae died in the second and third instars, so that the number of pupae and adult flies formed was rather small.

In most cases, no tissues were found in the surviving larvae and adults that could be attributed to the implanted nuclei. It is possible that the small amount of ooplasm transferred from the donor contained no nuclei, or that the implanted nuclei did not survive the operation. However, in the series quoted in Table 1, nine surviving larvae had yellow Malpighian tubes, while their other tissues, as far as phenotypic differences could be observed, were not of the wild type. Eight of these larvae metamorphosed into flies externally yellow-white.

One of the larvae (D 208) gave a male fly that was distinctly mosaic (Fig. 2 *A*). Another mosaic fly, a female (D 176, Fig. 2*B*), had been obtained from an earlier set of operations, not included in the table, in which only a few larvae hatched. Both mosaics were predominantly *y w*, they had white eyes and a pale body, but darkly pigmented wild-type tissues in several abdominal segments. Their Malpighian tubes were yellow. Both of these flies were sterile.

Four of the flies obtained from larvae with yellow Malpighian tubes were also sterile, lacking gonads, but having normal external genitalia. Four other flies were fertile, but gave only *y w* descendants when mated with *y w* flies. The proportion of fertile and sterile flies was similar in the cases where there was no trace of mosaic tissues.

Two males in the experiment D 207 were of particular interest. As larvae, they showed no pigmentation in Malpighian tubes and they metamorphosed into flies externally completely yellow-white. When these males were mated with *y w* females, the progeny of one of them was composed of wild-type females and *y w* males, while that of the other included both wild and *y w* females, besides *y w* males. This result could occur only if the gonads of the first male were formed by the injected nuclei and those of the second were a mosaic of host and donor nuclei. Since the genes *y* and *w* are sex-linked, the female descendants with wild phenotype were heterozygous *y w*/+ +, while the male descendants were *y w*.

DISCUSSION

The fact that most of the mosaics obtained had a *y w* phenotype with colored Malpighian tubes indicated that the injected nuclei probably entered into the yolk zone and formed yolk cells in the embryo. These cells are later integrated into the midgut (6) and apparently also form, at least in part, the Malpighian tubes. The likelihood that the injected nuclei would enter the peripheral blastema, which gives origin to most of the adult, seems much smaller.

The sterility of many flies is not surprising, since the injections were made into the posterior end of the eggs, easily disrupting the polar plasm. The resulting defect was similar to the deficiency of flies formed from eggs with irradiated polar plasm (7). Injection into the posterior end, however, increased the chances for the migration of the injected nuclei into the polar plasm ahead of the host nuclei, which in fact occurred in the two cases mentioned.

TABLE 1. *Survival and development of fertilized eggs of* Drosophila melanogaster *after injection of nuclei-containing ooplasm*

Experiment*	D 181	D 182	D 184	D 207	D 208	Total	%
No. of operated eggs	40	31	54	63	46	234	100.0
Dead after operation or during early development	19	14	14	32	21	100	42.8
Unhatched fully formed, but defective larvae	7	9	24	15	3	58	24.8
Hatched larvae	14	8	16	16	22	76	32.4
No. of larvae pupated	6	4	4	8	8	30	12.8
No. of adults hatched	5	3	4	8	8	28	12.0
No. of mosaics (larvae or adults)	3	1	4	2	1	11	4.7

* Each experiment includes all the operations performed in one day.

These experiments demonstrate that it is possible to transplant nuclei surrounded by cytoplasm from one egg of *Drosophila* to another, and to obtain adult flies. Up to the stage of 256 nuclei, the nuclei seem to be equivalent and omnipotent, since they were able to give rise to various adult tissues and even formed functional gonads. With this technique it is now possible to make a genetic analysis of the injected nuclei, which would be of particular interest if nuclei of more advanced stages of development, or from differentiated cells, could be successfully implanted.

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