OVARY TRANSPLANTATION IN THE STUDY OF A MATERNAL EFFECT IN *DROSOPHILA MELANOGASTER*¹

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Received November 1, 1962

A MATERNAL effect was postulated to account for striking differences that were detected in the results of reciprocal crosses between flies from the tumorous head (*tu-h*) stock of *Drosophila melanogaster* and laboratory stocks (GARDNER and WOOLF 1949). The maternal effect hypothesis was supported by additional reciprocal crosses using flies from other laboratory stocks (GARDNER and STOTT 1951; GARDNER, STOTT and DEARDEN 1952) and from natural populations (GARDNER and GARDNER 1953). Further genetic data supporting the hypothesis were obtained from the results of a series of backcrosses (GARDNER 1959). These latter investigations indicated that the maternal effect was dependent on genetic transmission through the maternal line rather than on an independent cytoplasmic factor or substance.

Experiments designed to identify any chemical substance that might be involved and to explain the mechanism of the maternal effect have been conducted. GARDNER, TURNER and BERSETH (1960) injected crude extract prepared from *tu-h* females into wild females and obtained results suggesting that a transfer of the maternal effect had been accomplished. These results, however, could not be confirmed, and the more extensive injection experiments of GARDNER and HANSEN (1962) did not show a difference between progeny obtained from females injected with crude extract and those from controls.

The ovary-transplantation experiments reported here were undertaken to determine if the maternal effect is autonomous in the ovaries of female flies homozygous for the gene *tu-1* or if the ovaries are conditioned by some factor or substance in the maternal surroundings to produce the maternal effect.

METHODS AND MATERIALS

The method of ovary transplantation was a modification of that developed by EPHRUSSI and BEADLE (1936). It was designed to permit a single operator to carry out the entire procedure.

A modification of the apparatus designed for injections of liquids (GARDNER and HANSEN 1962) was used to transplant ovaries in these experiments. Pressure in the tubing assembly was controlled by movements of the tongue of the oper-

¹ Aided by Grant E-274D from the American Cancer Society, Grant DRG-178G from the Damon Runyon Memorial Fund, and Grant RG-9683 from the National Institutes of Health.

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ator over the end of a tube that branched from the main system. Transplantation needles were prepared from capillary tubing with an inside diameter of 0.4 mm. The tubing was heated in a bunsen burner flame and drawn to the appropriate diameter. A constriction made near the tip of the needle assured that the tissue of the donor would remain near the tip of the pipette. The constriction was created with a micro-burner made from a steel injection needle (size B-D26). The point of the steel needle was squared with a file and the base of the needle was connected to the gas supply with a rubber tube. In making the constrictions, glass needles were held vertically in the horizontal flame of the micro-burner. Needles with satisfactory constrictions were then sharpened by chipping a rough point and grinding to a sharp point on fine carborundum paper.

Ovary transplants were made mostly between third instar larvae of approximately the same age. Some were made between newly emerged adult virgin females. It had been shown that oviduct connections could be established when ovaries were transplanted into third instar larvae (BEADLE and EPHRUSSI 1935). Transplants between larvae, therefore, could be used to investigate the effects that resulted when ovaries carrying eggs with particular genetic markers were placed in strange maternal surroundings. BODENSTEIN (1947) had demonstrated that no oviduct connection could be established when adult ovaries were transplanted. Transplants of adult ovaries, therefore, could be used only to observe the possible influence on the host ovaries of a diffusible substance from the transplanted ovary.

In making larval transplants, donor larvae were washed in 95 percent ethyl alcohol, rinsed, and dissected in insect Ringer's solution. Host larvae were washed in a drop of 95 percent ethyl alcohol, etherized, and attached to a glass slide by means of two-sided Scotch tape. All injections were made free-hand under the dissection microscope with a magnification of 20 $\times$. Each transplant was made by drawing a single ovary dissected from the donor into the pipette and immediately inserting it posteriorly through the ventral surface of the host. A small amount of Ringer's solution was injected along with the ovary. An excess of Ringer's solution was then dropped over the entire host larva to release the larva from the tape. Larvae, each carrying a transplanted ovary, were placed in vials containing a small amount of the standard cornmeal-agar-molasses-live yeast medium, along with several layers of moist paper, and left to pupate.

Stocks from which the host larvae were taken had been prepared so that each larva carried a recessive gene in homozygous condition, thus providing a visible marker that could be observed in adult flies. These markers were used to confirm genetically the functional connection between transplanted ovaries and oviducts. Five inbred *D. melanogaster* stocks were used in these experiments: (1) a stock of white-eyed flies homozygous for both *w* and for the *tu-h* genes (*tu-1* and *tu-3*); (2) a white stock homozygous for wild-type genes *tu-1*⁺ and *tu-3*⁺; (3) a stock with sepia-eyes, homozygous both for *se* and for *tu-1*⁺ and *tu-3*⁺; (4) a wild-type Cockapontsett stock; and (5) a red-eyed *tu-h* stock homozygous for *tu-3* and *tu-1*. These stocks had been maintained for several years at Utah State University. The mutant gene sepia in (3) arose spontaneously in the wild Samarkand stock

and a line was maintained that carried *se* in homozygous condition. This stock with Samarkand background proved valuable in the transplantation experiments because it incorporated a visible marker and had been shown (GARDNER *et al.* 1960; GARDNER and HANSEN 1962) to be stable and comparatively resistant to the maternal effect. All cultures were maintained on the standard cornmeal-agar-molasses-live yeast medium in milk bottles at $25 \pm 1^\circ\text{C}$.

To determine whether the maternal effect is autonomous in the ovaries of *tu-h* females, ovaries from $w^+ w^+$, *tu-h* female larvae were transplanted into $w w$ (not *tu-h*) female larvae. Surviving adult females were mated to white males, and progeny were scored for eye color and tumorous head phenotype. Red-eyed progeny would be expected from functioning transplanted ovaries, whereas eggs from the ovaries of the host would be expected to produce white-eyed progeny. As a control, reciprocal crosses were made between white-eyed flies that were not tumorous head and red-eyed *tu-h* flies.

In a second experiment, ovaries from $se^+ se^+$, *tu-h* female larvae were transplanted to *se se* (Samarkand) female larvae. Surviving females were mated with sepia males, and progeny were scored for eye color and tumorous head phenotype. Eggs from transplanted ovaries would produce flies with red eyes whereas those from the ovaries of the host would result in sepia-eyed offspring. Females evidencing functional oviduct connections were dissected and the number of ovaries observed. Reciprocal crosses between *tu-h* and flies carrying the gene *sepia* in Samarkand background, were carried out as a check for the maternal effect.

Further larval transplants were made to determine whether wild-type ovaries were autonomous in the bodies of *tu-h* females. Ovaries from wild Cockaponsett larvae were transplanted into $w w$, *tu-h* female larvae. Surviving females were mated with white *tu-h* males. Red-eyed progeny would indicate that functional oviduct connections had been made; white-eyed progeny would result from eggs produced in the ovaries of the host. All progeny were scored for eye color and tumorous head phenotype. Reciprocal crosses were used to check the maternal effect. As in the preceding experiments, females evidencing functional oviduct connections were dissected and the number of ovaries observed.

For transplants between adults, ovaries dissected from newly-emerged flies in insect Ringer's solution were injected immediately into the abdomens of young virgin females. Four inbred *D. melanogaster* stocks were used in these transplants: (1) a stock of homozygous red-eyed, *tu-h* flies (w^+ , *tu-1*, *tu-3*); (2) a wild Stephenville stock homozygous for *tu-1^s*; (3) wild Samarkand; and (4) wild Cockaponsett. To test the hypothesis that a diffusible substance produced in the ovaries of adult flies was responsible for the maternal effect, ovaries from *tu-h* females were transplanted into Samarkand females. Females with transplanted ovaries were then mated with *tu-h* males and the progeny were scored for tumorous head phenotype.

To test the possibility that a deficiency of a diffusible substance in the ovaries causes the maternal effect, ovaries from Cockaponsett females were transplanted into Stephenville and *tu-h* females. Females with transplanted ovaries were mated with *tu-h* males. Before eclosion of the progeny, the parents were removed

from the culture bottles and the females were dissected to confirm the maturation of the transplanted ovaries. Only cultures involving flies in which the transplanted ovary was present and mature were retained. Progeny from these cultures were scored for tumorous head phenotype. Reciprocal crosses between flies of the stocks involved in the experiment and *tu-h* flies were used as controls for the maternal effect.

To check whether some substance in the crude extract of wild-type flies might influence the maternal effect, a previous experimental design (GARDNER and HANSEN 1962) was altered, and further injection experiments were conducted. Extracts were prepared by homogenizing 1 gram of female adult flies (Samarkand) in 4 ml of insect Ringer's solution. A test tube containing the extract was held in an ice bath during preparation and centrifuged (3,000 rpm) at 4°C for 20 minutes. The supernatant was held in an ice bath until it could be injected into the lateral thorax of virgin females. Control females were handled in the same way but injected with Ringer's solution instead of extract.

In the following tables, percentages are recorded to the nearest whole number. Female parents are always written first.

RESULTS

Four white-eyed females (A, B, C, D), into which larval ovaries from $w^+ w^+$, *tu-h* females had been transplanted, produced some red-eyed progeny after mating with white-eyed males. This indicated that functional oviduct connections had been made to the transplanted ovaries. The presence of three ovaries was later confirmed by observation in the dissected females.

Female A produced eggs for 35 days after mating. Of her 336 progeny, 129 were red-eyed, originating from eggs of the transplanted ovary, and 207 were white-eyed, from the host ovary. Of the 129, 70 (54 percent) showed the tumorous head phenotype. None of the 207 white-eyed progeny from Female A were tumorous head. Female B produced a total of 60 progeny, 20 from the transplanted ovary and 40 from the host ovary. Nine of the 20 progeny from the transplanted ovary showed the tumorous head phenotype. Female C produced eggs for 20 days after mating, and all 43 progeny were from the transplanted ovary. This indicated that only the transplanted ovary had a functional oviduct connection. On dissection, it was observed that three ovaries were present but only one was connected to the oviduct. Female D produced 38 progeny, of which two were from the transplanted ovary.

Data from this experiment are summarized in Table 1. All four females with functional oviduct connections to the transplanted ovaries produced some tumorous head progeny. On the average, about half of the progeny from implanted ovaries were tumorous head. None of the 283 white-eyed progeny from intact host ovaries showed the tumorous head phenotype.

Results expected on the basis of previous experiments were obtained from the reciprocal crosses included to check the maternal effect. Between one and two percent of the progeny from the cross between white females (that were other-

TABLE 1

Progeny from white-eyed females, into which larval ovaries of w⁺w⁺, tu-h flies had been transplanted, and white males. Reciprocal crosses between tu-h and white were included as a check for maternal effect

Implanted female	Red-eyed (transplanted ovary)			White-eyed (host ovary)		
	Abnormal	Normal	Percent abnormal	Abnormal	Normal	Percent abnormal
A	70	59	54	0	207	0
B	9	11	45	0	40	0
C	17	26	40	0	0	0
D	1	1	50	0	36	0
Totals	97	97	50	0	283	0
Control crosses for maternal effect						
White ♀ × tu-h ♂	18*	1236*	1			
tu-h ♀ × white ♂	206	372	36			

* Males white-eyed.

wise wild-type) and *tu-h* males were abnormal, compared with about 36 percent from the reciprocal cross between *tu-h* females and white males. A higher proportion (50 percent) of abnormal flies was obtained from the implanted ovaries (*tu-h* × white-eyed, wild-type) than from the direct cross (36 percent) between *tu-h* females and white-eyed (otherwise wild-type) males. This difference may have been at least partially due to the fewer flies and the lessened crowding in the cultures from females with transplanted ovaries. It has been observed (GARDNER and RATTY 1952; SIMMONS and GARDNER 1958) that the expression of tumorous head is higher under optimal environmental conditions.

In the second experiment, seven implanted females (A to G) produced red-eyed progeny, indicating that functional oviduct connections had been established to the transplanted ovaries. Two of these (B and G) produced no tumorous head offspring, but their combined total of progeny was only five. Tumorous head flies were observed in the progeny of the other five females with percentages ranging from 13 to 20. In this experiment, 230 progeny were produced from transplanted ovaries; 14 percent of these expressed the tumorous head phenotype. None of the sepia-eyed progeny were tumorous head. Females A, C, E, and G produced only red-eyed offspring, indicating that only the transplanted ovary had a functional oviduct connection. Two of these females (E and G) were each found on dissection to have three ovaries of which only one was connected. Females A and C died and were not detected in time to make dissections. Female E deposited eggs for 25 days and produced 187 red-eyed progeny of which 14 percent were tumorous head. Data from this experiment are summarized in Table 2. These results, like the results of the first experiment, indicate that the maternal effect is produced autonomously by the *tu-h* ovaries when they are removed at the third instar and transplanted to wild-type females.

The third experiment was designed to determine whether wild-type ovaries are autonomous when transplanted in the larval stage into *tu-h* females. Of the

TABLE 2

Progeny from sepia-eyed (Samarkand) females, into which larval ovaries of se⁺se⁺, tu-h flies had been transplanted, and sepia males. Reciprocal crosses between tu-h and sepia (Samarkand) were included as a check for maternal effect

Implanted female	Red-eyed (transplanted ovary)			Sepia-eyed (host ovary)		
	Abnormal	Normal	Percent abnormal	Abnormal	Normal	Percent abnormal
A	2	13	13	0	0	0
B	0	3	0	0	29	0
C	2	10	17	0	0	0
D	1	5	17	0	115	0
E	27	160	14	0	0	0
F	1	4	20	0	36	0
G	0	2	0	0	0	0
Totals	33	197	14	0	180	0
Control crosses for maternal effect						
Sepia ♀ × tu-h ♂	1	1541	<1			
tu-h ♀ × sepia ♂	58	578	9			

72 females that survived the transplantation, 38 produced progeny; 19 of these had some red-eyed progeny, indicating a functional oviduct connection with the transplanted ovary. The number of offspring from each transplanted ovary was small, ranging from one to 46. Of the 203 flies produced from the transplanted ovaries, none were tumorous head. This result is comparable with the control cross between Cockaponsett females and *w tu-h* males. Seven percent of the progeny from the reciprocal control cross between *w tu-h* females and Cockaponsett males were abnormal. The data are summarized in Table 3. These results indicate that wild-type ovaries transplanted in the third instar stage to *w tu-h* females were autonomous. The wild-type (with respect to tumorous head expression) was not influenced by the maternal surroundings in the *w tu-h* host.

No oviduct connections were established in adults, and therefore the next experiments could only be used to check on a possible diffusible substance released from transplanted adult ovaries. Wild-type (Samarkand) females, each carrying a transplanted *tu-h* (adult) ovary, were mated with *tu-h* males. A total of 721 progeny were produced, none of which showed the tumorous head phenotype. The tumorous head phenotype was evidenced by 94 percent of the progeny from Stephenville crosses and by 82 percent of the progeny from the *tu-h* crosses. These results were comparable with the results of control crosses between Stephenville females and *tu-h* males (94 percent) and *tu-h* × *tu-h* (80 percent). Data from these and additional control crosses are recorded in Table 4. The proportion of progeny expressing the tumorous head phenotype was not altered by the implanted adult ovaries.

Results of the fifth experiment in which extracts from the bodies of Samarkand females were injected into Stephenville and *tu-h* females are summarized in Table 5. No significant difference was detected among the progeny of the

TABLE 3

Progeny from white-eyed tu-h (w tu-h) females, into which ovaries of wild-type Cockaponsett females had been transplanted, and w tu-h males. Reciprocal crosses between Cockaponsett and w tu-h are included

Implanted female	Red-eyed (transplanted ovary)			White-eyed (host ovary)		
	Abnormal	Normal	Percent abnormal	Abnormal	Normal	Percent abnormal
A	0	8	0	0	0	0
B	0	15	0	20	9	69
C	0	11	0	18	8	69
D	0	4	0	0	0	0
E	0	6	0	35	23	60
F	0	3	0	41	45	48
G	0	6	0	11	17	39
H	0	2	0	0	0	0
I	0	19	0	41	61	40
J	0	10	0	34	35	49
K	0	4	0	41	23	64
L	0	12	0	0	0	0
M	0	46	0	0	0	0
N	0	20	0	58	25	70
O	0	25	0	41	45	48
P	0	8	0	30	26	54
Q	0	2	0	23	12	66
R	0	1	0	27	33	45
S	0	1	0	0	0	0
Totals	0	203	0	420	362	54

Control crosses for maternal effect						
Cockaponsett ♀ ×						
w tu-h ♂	0	694	0			
w tu-h ♀ ×						
Cockasponett ♂	79*	998*	7			

* Males white-eyed.

females injected with extract and those injected with Ringer's solution.

DISCUSSION

The results of these experiments show that the maternal effect, which had been postulated from genetic evidence to be associated with *tu-h* females, is already determined in third instar larvae. Ovaries from *tu-h* larvae at this stage of development behave autonomously when transplanted into wild-type hosts. Likewise, wild-type larval ovaries are autonomous when transplanted into *tu-h* female larvae. The evidence accumulated in these experiments did not substantiate the earlier hypothesis that a diffusible substance from either the ovary or other body tissue influences the maternal effect. Beyond the third instar stage of development, the mechanism of the maternal effect is apparently controlled within the ovary.

TABLE 4

*Results of crosses between females with adult ovary transplants and tu-h males.
Results of reciprocal control crosses included for comparison*

Treatment or cross	Progeny		
	Abnormal	Normal	Percent abnormal
Samarkand with <i>tu-h</i> ovary $\times$ <i>tu-h</i>	0	721	0
Stephenville with Cockaponsett ovary $\times$ <i>tu-h</i>	336	23	94
<i>tu-h</i> with Cockaponsett ovary $\times$ <i>tu-h</i>	139	31	82
Reciprocal control crosses			
<i>tu-h</i> $\varnothing$ $\times$ Samarkand δ	96	586	14
Samarkand $\varnothing$ $\times$ <i>tu-h</i> δ	0	1298	0
<i>tu-h</i> $\varnothing$ $\times$ Cockaponsett δ	137	648	17
Cockaponsett $\varnothing$ $\times$ <i>tu-h</i> δ	4	1109	<1
<i>tu-h</i> $\varnothing$ $\times$ Stephenville δ	89	551	14
Stephenville $\varnothing$ $\times$ <i>tu-h</i> δ	634	38	94
<i>tu-h</i> $\varnothing$ $\times$ <i>tu-h</i> δ	339	85	80

TABLE 5

Results of crosses between Stephenville and tu-h females that had been injected with Samarkand extract, and tu-h males. Control females were injected with Ringer's solution

Treatment or cross	Progeny		
	Abnormal	Normal	Percent abnormal
Stephenville injected with Samarkand extract $\times$ <i>tu-h</i>	1006	48	95
Stephenville injected with Ringer's $\times$ <i>tu-h</i>	777	46	94
<i>tu-h</i> injected with Samarkand extract $\times$ <i>tu-h</i>	512	92	85
<i>tu-h</i> injected with Ringer's $\times$ <i>tu-h</i>	176	40	81

The mechanism of the maternal effect in tumorous head differs from that associated with pigmentation in the meal moth *Ephestia kühniella* (KÜHN, CASPARI and PLAGGE 1935). When ovaries from nonpigmented moth larvae were transplanted into larvae of pigmented females that were later mated with nonpigmented males, the larvae produced from the transplanted ovary showed pigmentation. A diffusible substance (kynurenine) responsible for the pigmentation was taken up by the egg from the maternal surroundings.

Two related lines of investigation have been initiated because of the results of these experiments: (1) chemical comparisons between *tu-h* ovaries and those of wild-type flies at different stages of development using chromatography, and (2) reciprocal transplantation of wild-type and *tu-h* eye and antennal discs at different stages of development.

SUMMARY

Reciprocal ovary transplants were made between tumorous head and wild-type *Drosophila melanogaster* in the third instar larval and in the adult stages to determine whether a diffusible substance is associated with the maternal effect. Results showed that ovaries of third instar larvae are autonomous for the maternal effect. No evidence was found for a diffusible substance.

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