MATERNAL EFFECT INVOLVED IN THE INHERITANCE OF ABNORMAL GROWTHS IN THE HEAD REGION OF DROSOPHILA MELANOGASTER

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Received January 12, 1949

AN abnormality expressed as irregular growths in the head region of *Drosophila melanogaster* is under investigation in the genetics laboratory at the UNIVERSITY of UTAH. The experimental evidence indicates that a maternal effect controlled by a sex-linked gene is involved in the expression of the abnormality. In addition to the maternal effect an autosomal gene is required for the expression of the character.

MATERIALS AND METHODS

The study involves a stock of flies, carrying an inherited abnormality with a varied expression, characterized by growths in the surface of the head. Some examples of the growths are shown in semidiagrammatic drawings in figure 1. The growths are irregular in shape and size and occur in different regions. They are distributed mainly in the areas normally occupied by the eye, face, and antenna. A size gradation has been observed from very small irregularities involving only a few facets of the eye or a small area elsewhere on the head to massive abnormalities, in which both eyes are involved and most of the head is distorted by amorphous growths. A detailed description of the different expressions is being prepared by W. W. NEWBY. The trait first appeared in the spring of 1945 at the UNIVERSITY OF TEXAS in a sample from a wild population which had been collected at Acahuizotla, Mexico in 1941. Flies showing the abnormality were received at the UNIVERSITY OF UTAH in the fall of 1946 through the courtesy of DR. WILSON STONE. The abnormal flies have been inbred during the past two years at the UNIVERSITY OF UTAH. A slight increase has been observed in the penetrance and expressivity following selection. At present the inbred stock shows an average penetrance of about 76 percent at 22°C.

The name *tumorous head* suggested itself to the writers early in the study as a suitable descriptive term to identify the character. This name was used in preliminary reports (GARDNER, in press, WOOLF, in press) and is used here to designate the character and the stock which carries it. The word tumor is used in a broad sense to mean enlargement or growth and there is no intention to imply a relationship with any other character described in Drosophila or any other animal group for which the same name has been used. Other characters in Drosophila have been identified with the same word (STARK 1919; BRIDGES and BREHME 1944) but there seems to be no relationship between these and the trait discussed here. The symbol *tu-h* is used to symbolize the tumorous head stock. The symbols *tu-1* and *tu-3* are designated tentatively to symbolize the sex-linked gene which controls the maternal effect and the third

chromosome gene respectively. In diagrams of crosses where chromosomes are identified, arabic numbers are used to symbolize the chromosomes of the *tu-h* stock. In cases where the parents of a cross are symbolized, the female parent is always written first. The flies were raised in half-pint milk bottles on the standard cornmeal, agar, mollasses media at 22°C.

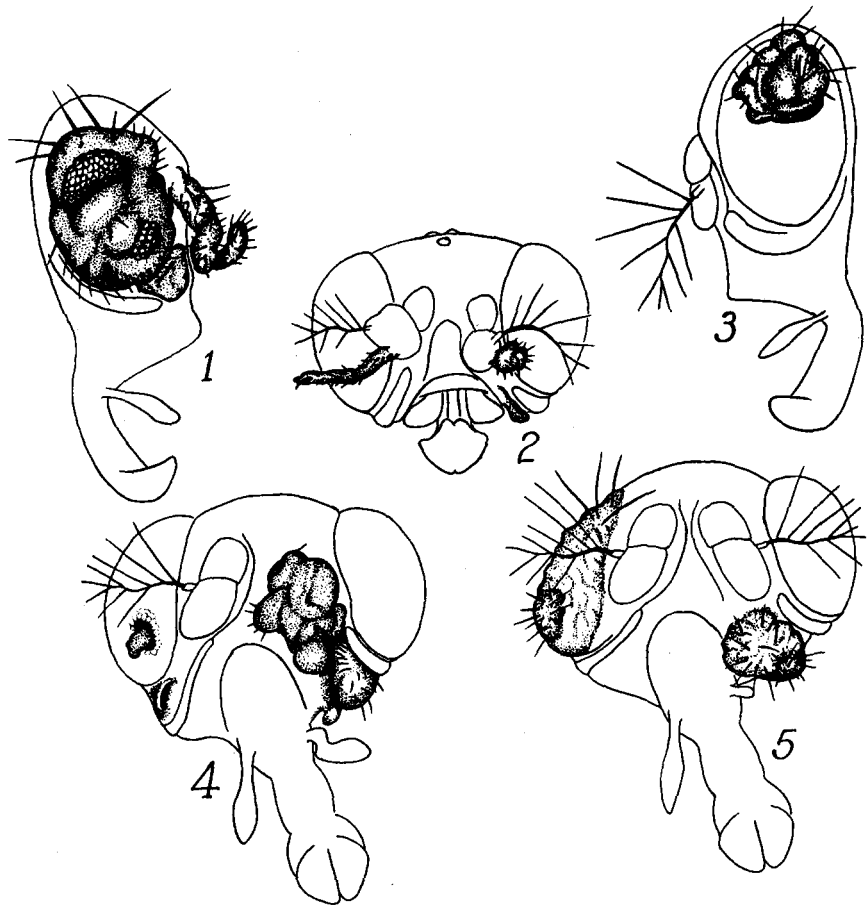

FIGURE 1.—Semidiagrammatic drawings of head growths in *tu-h* stock. The stippled areas represent the abnormalities. 1. Side view of head showing massive amorphous abnormality involving most of right eye, lower face and region of the antenna. 2. Face view showing three small growths. The one originating in the base of the right antenna is slightly leg-like. 3. Side view showing a single growth of medium size in upper part of left eye. 4. Face view showing three growths of different sizes. 5. Face view showing two growths. One covers the area normally occupied by the entire right eye.

EXPERIMENTAL RESULTS

Genetic Mechanism Involved

Results of reciprocal crosses involving *tu-h* and stocks with marked chromosomes indicate that two major loci are involved in the production of the abnormality. A sex-linked gene (*tu-1*) is responsible for the maternal effect and a third chromosome semidominant gene (*tu-3*) is also required for the expression

of the abnormality. These facts were demonstrated by the use of flies prepared from the *tu-h* stock by appropriate crosses which contain suitable dominant markers for the identification of the different chromosomes. The markers provided a means of eliminating the chromosomes from the *tu-h* stock one by one and thus determining which were necessary for the production of the abnormality. The essential crosses are reconstructed and the data are summarized in table 1.

Crosses 1–4 (table 1) were matings between flies prepared by crossing $\frac{ClB}{+}\ \frac{H}{Sb}$ females with *tu-h* males$\left(\frac{tu\text{-}1}{\rightarrow}\ \frac{tu\text{-}3}{tu\text{-}3}\right)$. The F_1 females $\left(\frac{ClB}{tu\text{-}1}\ \frac{H}{tu\text{-}3}\right.$ and $\left.\frac{ClB}{tu\text{-}1}\ \frac{Sb}{tu\text{-}3}\right)$ were backcrossed individually to *tuh* males.

From the resulting progenies flies were selected which were known to be homozygous for *tu-1* and were also known to carry one or two *tu-3* chromosomes as indicated by the markers $\left(\frac{tu\text{-}1}{tu\text{-}1}\ \frac{H}{tu\text{-}3}, \frac{tu\text{-}1}{tu\text{-}1}\ \frac{Sb}{tu\text{-}3}, \frac{tu\text{-}1}{tu\text{-}1}\ \frac{tu\text{-}3}{tu\text{-}3}\right.$ etc.$\left.\right)$

These were mated together as shown diagrammatically in crosses 1–3. From the progeny of cross 3 flies expressing Hairless and Stubble were selected and mated as shown in cross 4.

The results of these four crosses demonstrate the fact that *tu-3* is located in the third chromosome. From cross 1 an average of 74 percent of abnormal flies was obtained. This is approximately the same average percentage of abnormal flies as that produced by the inbred *tu-h* stock. Both major factors were present and the cross was essentially parallel with those involving flies from the inbred *tu-h* stock. Cross 2 was similar but carried only three *tu-h* third chromosomes compared with four in cross 1 and produced an average of 46 percent abnormality compared with 74 percent for cross 1. Cross 3 carried only two *tu-h* third chromosomes and an average of 32 percent of abnormal flies was obtained. Cross 4 had no *tu-h* third chromosomes and produced no abnormal flies. These results show that in the presence of the sex chromosomes from the *tu-h* stock the percentage of abnormal flies was roughly proportional to the number of *tu-h* third chromosomes present.

Further evidence for the location of *tu-3* in the third chromosome and for its behavior as a semidominant was obtained by classifying the progeny from crosses 2 and 3. Cross 2 carried only one marker (*Sb*) and three *tu-h* third chromosomes. Half of the progeny would be expected to carry only one *tu-3* and these would express the Stubble character. Half would be homozygous for *tu-3*. Only 21 percent of the Stubble flies were abnormal compared with 80 percent for those not Stubble. Two markers (Hairless and Stubble) were present in cross 3 and a larger number of progeny was obtained. The results showed that 18 percent of the flies expressing Hairless and 15 percent of those expressing Stubble were abnormal while 65 percent of those expressing neither

marker, and therefore homozygous for *tu-3*, were abnormal. No abnormal flies were found to express both markers (Hairless and Stubble). These flies would not have any *tu-3* genes and the results indicate that the expression does not occur in the absence of this gene. The results of crosses 1–4 prove that *tu-3* is semidominant and is located in the third chromosome. The results show further that *tu-3* is homozygous viable under the conditions of the experiment at 22°C.

TABLE 1

Crosses designed to demonstrate the location and action of genes involved in the expression of abnormal growths and the results of these crosses.

	Cross	Bar or Non-bar	Abn ♀	+♀	% Abn ♀	Abn ♂	+♂	% Abn ♂	Avg. % Abn
1.	$\frac{tu\text{-}1}{tu\text{-}1}\ \frac{tu\text{-}3}{tu\text{-}3} \times \frac{tu\text{-}1}{\rightarrow}\ \frac{tu\text{-}3}{tu\text{-}3}$		645	126	84.0	516	291	64.0	74.0
2.	$\frac{tu\text{-}1}{tu\text{-}1}\ \frac{tu\text{-}3}{tu\text{-}3} \times \frac{tu\text{-}1}{\rightarrow}\ \frac{tu\text{-}3}{Sb}$		81	62	57.0	70	112	38.0	46.0
3.	$\frac{tu\text{-}1}{tu\text{-}1}\ \frac{tu\text{-}3}{H} \times \frac{tu\text{-}1}{\rightarrow}\ \frac{tu\text{-}3}{Sb}$		275	378	42.0	161	530	23.0	32.0
4.	$\frac{tu\text{-}1}{tu\text{-}1}\ \frac{Sb}{H} \times \frac{tu\text{-}1}{\rightarrow}\ \frac{Sb}{H}$		0	149	0	0	147	0	0
5.	$\frac{ClB}{tu\text{-}1}\ \frac{tu\text{-}3}{tu\text{-}3} \times \frac{tu\text{-}1}{\rightarrow}\ \frac{tu\text{-}3}{tu\text{-}3}$	Bar Non-bar	19 29	656 641	2.8 4.3	10	666	1.5	2.9
6.	$\frac{ClB}{tu\text{-}1}\ \frac{Sb}{tu\text{-}3} \times \frac{tu\text{-}1}{\rightarrow}\ \frac{H}{tu\text{-}3}$	Bar Non-bar	6 10	407 338	1.5 2.9	6	321	1.8	2.0
7.	$\frac{ClB}{+}\ \frac{tu\text{-}3}{tu\text{-}3} \times \frac{tu\text{-}1}{\rightarrow}\ \frac{tu\text{-}3}{tu\text{-}3}$	Bar Non-bar	1 0	97 85	0 0	0	84	0	.4
8.	$\frac{tu\text{-}1}{tu\text{-}1}\ \frac{tu\text{-}3}{tu\text{-}3} \times \frac{+}{\rightarrow}\ \frac{tu\text{-}3}{tu\text{-}3}$		131	21	86.0	95	37	72.0	80.0
9.	Lausanne$\times \frac{+}{\rightarrow}\ \frac{tu\text{-}3}{tu\text{-}3}$		1	859	.12	1	835	.12	.12
10.	Lausanne$\times \frac{tu\text{-}1}{\rightarrow}\ \frac{Sb}{H}$		0	814	0	0	881	0	0
11.	$\frac{+}{+}\ \frac{H}{Sb} \times \frac{+}{\rightarrow}\ \frac{H}{Sb}$		0	145	0	0	123	0	0

A maternal effect was indicated by the differential results of preliminary reciprocal outcrosses and has been demonstrated by further studies (see table 3). Proof that a sex-linked gene is responsible for the maternal effect was obtained by making use of a stock carrying *tu-3* and a marker in the first chromosome. Flies used for crosses 5 and 6 (table 1) were prepared as described above

for crosses 1–3 but females carrying *ClB* instead of those without the marker were selected. Parents in cross 5 were both homozygous for *tu-3*. The female carried one sex chromosome from the *tu-h* stock and a *ClB* chromosome containing a marker, Bar eye, (*B*), a crossover suppressor (*C*) and a lethal (*l*). The results showed an average of 2.9 percent of abnormal flies. No Bar eyed males were obtained in the presence of the pseudodominant lethal (*l*). Cross 6 was comparable but carried only two *tu-3* genes instead of four and an average of 2 percent of abnormal flies was produced.

Results of crosses 5 and 6 compared with those of crosses 1 and 3 show that a sex-linked gene is involved. Crosses 1 and 5 differ only in the number of *tu-1* genes present in the female parent. The difference between two and one *tu-1* genes results in a reduction from 74 percent to 2.9 percent of abnormal flies. Likewise, crosses 3 and 6 are comparable. The difference between two and one *tu-1* genes in this combination results in a reduction from 46 percent to 2 percent of abnormal flies. Both Bar eyed and non-Bar eyed females expressed the abnormality in about equal proportion.

Parent flies used in cross 7 were prepared by crossing females carrying dominant markers with *tu-h* males as follows:

$$\frac{ClB}{+}\ \frac{H}{Sb} \times \frac{tu\text{-}1}{-}\ \frac{tu\text{-}3}{tu\text{-}3}.$$

F_1 females showing Stubble were then crossed with F_1 males showing Hairless·

$$\frac{ClB}{tu\text{-}1}\ \frac{Sb}{tu\text{-}3} \times \frac{+}{-}\ \frac{H}{tu\text{-}3}.$$

The parent flies used in cross 7 were selected from the F_2 progeny. It will be observed (table 1) that this cross is like cross 1 except for the complete absence of *tu-1* in the female parent. One abnormal fly was obtained in a total of 267.

Results of crosses 5, 6, and 7 compared with those of crosses 1, 2, and 3 demonstrate the fact that the maternal effect is controlled by a sex-linked gene (*tu-1*). Comparatively little effect can be attributed to *tu-1* in heterozygous condition. It is therefore recessive or nearly so. Comparison between the results of crosses 5 and 7, however, indicate that *tu-1* may not be completely recessive in cross 5. The parents in crosses 5 and 7 differed only in one respect. The female in cross 5 was heterozygous for *tu-1* and the female in cross 7 carried no *tu-1* genes. The difference between 2.9 percent and .4 percent suggests that *tu-1* may have a slight maternal effect in heterozygous condition. The absence of *tu-1* in the male makes no difference as shown by the results of cross 8. This cross differed from cross 1 only in one respect. The male from cross 8 (obtained from the progeny of cross 7) carried no *tu-1* gene. The percentage of abnormal flies (80 percent) was slightly above that from cross 1 (74 percent) and that from the inbred *tu-h* stock (76 percent) but the difference was not considered significant.

A small percentage (less than 1 percent) of abnormal flies was obtained

from cross 7 which carried no *tu-1* genes in the female parent. A few abnormal flies were also obtained from outcrosses between *tu-h* males and females from laboratory stocks (see table 3). No maternal effect would be expected in these crosses and the small percentage could not be explained on the basis of the interaction described above. It was presumed that either *tu-1* or *tu-3* must have a slight independent action when introduced by the male. Crosses 9, 10, and 11 were designed to test this possibility and indicate the source of this small expression. The Lausanne stock which had been shown to behave like most other laboratory stocks in crosses with *tu-h* was chosen for the experiment. Lausanne females were crossed with males homozygous for *tu-3* but not carrying a *tu-1* gene as shown in cross 9. The male parent in this cross was like the one used in cross 8 and was obtained from the same source. Lausanne females were also crossed with males carrying *tu-1* but not *tu-3* as shown in cross 10. The male in this cross was like the one used in cross 4 and was prepared in a similar way.

TABLE 2

Crosses designed to test the second chromosome and results of these crosses.

	CROSS	Abn ♀	+♀	Abn ♂	+♂	% Abn.
1.	$\frac{tu\text{-}1}{tu\text{-}1}\ \frac{2}{2}\ \frac{tu\text{-}3}{tu\text{-}3} \times \frac{tu\text{-}1}{\rightarrow}\ \frac{2}{2}\ \frac{tu\text{-}3}{tu\text{-}3}$	385	65	298	165	75
2.	$\frac{tu\text{-}1}{tu\text{-}1}\ \frac{2}{Cy}\ \frac{tu\text{-}3}{tu\text{-}3} \times \frac{tu\text{-}1}{\rightarrow}\ \frac{2}{Cy}\ \frac{tu\text{-}3}{tu\text{-}3}$	208	57	172	113	69
3.	$\frac{tu\text{-}1}{tu\text{-}1}\ \frac{Cy}{Pm}\ \frac{tu\text{-}3}{tu\text{-}3} \times \frac{tu\text{-}1}{\rightarrow}\ \frac{Cy}{Pm}\ \frac{tu\text{-}3}{tu\text{-}3}$	251	85	204	152	66

From a total of 1,696 flies resulting from cross 9, two were abnormal. The evidence is meager but does indicate that the small percentage of abnormality from crosses involving *tu-h* males and females not carrying *tu-1* was produced by the independent action of *tu-3*. The results of cross 10 show that no abnormal flies were produced in a total of 1,695. This indicates that *tu-1* has no independent action when introduced by the male. In cross 11 both *tu-1* and *tu-3* were absent and no abnormality was produced, as would be expected.

The above crosses prove that two major genes are involved and that they are located in the first and third chromosomes. Appropriate crosses were made to determine whether or not modifiers are located in chromosomes other than the first and third in the inbred *tu-h* stock. Crosses designed to test the second chromosome are given in table 2 along with the results obtained. Flies used in all three crosses were homozygous for both major genes and differed only in the number of *tu-h* second chromosomes present. Parents in cross 1 each carry two *tu-h* second chromosomes. The percentage of abnormal flies was comparable with that of the inbred *tu-h* stock as would be expected. Each parent in cross 2 carried only one *tu-h* second chromosome and 69 percent of the progeny were

abnormal. All second *tu-h* chromosomes were removed from cross 3 and 66 percent of abnormal flies was obtained. These results suggest that a second chromosome modifier is present in the *tu-h* stock and that it has a slight enhancing effect. Wide variation is observed in the results of single crosses even in the inbred *tu-h* stock and therefore the significance of this difference is not clear. Further study is necessary before a conclusion can be drawn. The average penetrance of the *tu-h* stock has increased about 10 percent during the two year period of inbreeding, suggesting that modifiers with a slight enhancing effect have been accumulated.

In all of the results cited in tables 1 and 2 it will be observed that a higher percentage of abnormality occurs among the females than among the males from the same cross. This is also true of the inbred stock and was observed in outcrosses as well. The degree of expression or extent of the abnormality in individual flies is also greater among the females than among the males. The possibility that *tu-1* has a direct action in addition to the maternal effect which is exerted in greater intensity in homozygous females than in simplex males has been considered as a possible explanation. Evidence against this explanation comes from the fact that the usual pattern expected from a sex-linked gene with direct action has not been observed in the results of out-crosses. Males and females are produced in a regular proportion regardless of the segregation of the sex chromosomes. This differential suggests that the expression is associated in some way with sex.

Maternal Effect Shown in Results of Reciprocal Outcrosses

Results of reciprocal outcrosses showed that a maternal effect is involved in the production of the head growths. A considerably greater percentage of abnormality occurred regularly in the F_1 of a cross between a *tu-h* female and a male from another stock than in the F_1 from the reciprocal cross. The results of reciprocal outcrosses with laboratory stocks are given in table 3. Only the total flies and average percentage of abnormality in the progeny from each cross are given, with accuracy to the nearest whole number. Four wild stocks and eleven other laboratory stocks carrying mutants in different chromosomes were included in the study. The F_1 results summarized in table 3 show that an average of about 30 percent of the F_1 progeny from the outcrosses involving females from the *tu-h* stock was abnormal. The females from the *tu-h* stock were expected to be homozygous for *tu-1*. This condition would be expected to produce the maternal effect. The F_1 progeny would be heterozygous for *tu-3* making a combination which might be expected on the basis of results shown in table 1 to produce about 30 percent of abnormal flies. These outcrosses would be essentially similar to cross 3 (table 1). In the outcrosses, however, two *tu-3* genes would enter through the female while in cross 3 one *tu-3* gene was introduced by each parent. A rather wide range, from 14 percent to 52 percent of abnormal flies, was observed, from single crosses, but a wide range of expression occurred also in the inbred *tu-h* stock. With one exception (Oregon) the reciprocal crosses involving males from the *tu-h* stock resulted in progeny less than 1 percent of which were abnormal. This indicates clearly that a

maternal effect is involved. Abnormal males and abnormal females were obtained in the results of both reciprocal crosses in all cases in which the ab-

TABLE 3

Average percentage of abnormality from reciprocal outcrosses between tu-h and laboratory stocks.

LABORATORY STOCK	F_1				F_2			
	tu-h× LAB. STOCK		RECIPROCAL CROSS		*tu-h*× LAB. STOCK		RECIPROCAL CROSS	
	*n	**%	n	%	n	%	n	%
Oregon R.	817	38	1,396	42	1,621	20	1,728	26
Canton	750	25	1,542	0	540	0	329	0
Florida	107	52	220	1	96	4	287	1
Wild (Turtox)	807	19	947	1	706	1	1,275	1
w	197	19	583	1	293	2	223	1
al dp d b c px sp	96	33	78	0	1,304	9	1,122	10
ss^a	167	49	598	0	720	0	497	0
e	255	20	96	0	197	1	351	0
Bd^G	157	20	53	0	328	2	167	5
In(3R)C l(3)e	216	19	42	0	236	4	341	3
Muller 1^{13}	361	37	289	0	531	1	537	1
Cy/Pm; H/Sb	90	39	88	1	1,580	1	320	1
D/G1	170	14	132	0	956	4	106	1
M(3) 124/In(3R)C e l(3)e	101	23	35	0	695	0	423	1
ey^D/ar	64	37	27	0	173	0	cross failed	

* n = total flies.

** % = percentage of abnormality with accuracy to nearest whole number. Less than .5% is recorded as 0.

normality was present but abnormal females were present in a higher proportion than abnormal males.

The F_2 results (table 3) were comparable for reciprocal crosses. Small percentages of abnormal flies not exceeding 5 percent, were observed in the results of all crosses except those involving Oregon and *al dp d b c px sp*. The

high percentages from the Oregon crosses are interpreted further on. About 10 percent of abnormal flies was obtained in the F_2 from both reciprocal crosses involving *al dp d b c px sp* suggesting the presence of a modifier. Since the F_1 results from this cross show the maternal effect and conform to the usual pattern the modifying factor in the *al dp d b c px sp* stock must influence the reaction dependent upon *tu-3* directly.

The comparable F_2 results from reciprocal crosses, showing only slight expression, indicate that little if any maternal effect occurs in the second generation. This would be expected since the maternal effect is dependent upon the genotype of the female parent in the immediate cross. The F_1 combination (*tu-1*/+) is incapable of producing the maternal effect in appreciable degree as indicated by crosses 5 and 6, table 1. The eggs destined to produce the F_2, therefore, would not carry much maternal effect, if any, and the low percentages of abnormality would be expected to result mostly from the independent action of *tu-3*. Some of the F_2 progenies produced as much as 5 percent of abnormal flies which is more than would be expected from the usual independent action of *tu-3*. This suggests the presence of modifiers in the stocks concerned. The F_2 results give further evidence for the maternal effect and the genetic mechanism described above.

The explanation is further borne out by F_3, F_4, F_5 and backcross results. It was observed, for example, that F_3 progeny from original crosses between *tu-h* females and males from laboratory stocks (other than Oregon) produced about 10 percent of abnormal flies while the F_3 from the original reciprocal cross (*tu-h* male $\times$ laboratory stock) produced less than 1 percent of abnormal flies. The F_4 from this latter cross was comparable with the F_3 of the former cross. The following explanation will account for this difference. The F_1 progeny from all *tu-h* females showed the maternal effect as described above. The F_1 females were heterozygous for *tu-1* and, therefore, were incapable of producing much maternal effect through their eggs. Some F_2 females received the *tu-1* gene in homozygous condition and produced eggs from which abnormal F_3 flies were produced. On the other hand the F_2 flies from the reciprocal P_1 cross could not be homozygous for *tu-1* and, therefore, would not show an appreciable expression in the F_3. Some F_3 females, however, could be homozygous for *tu-1* and would be expected to produce the substance in their eggs making it possible for the F_4 progeny to express the trait in about the same proportion as the F_3 in the cross above. F_4 and F_5 results from both crosses are comparable and give evidence of the segregation of *tu-1* and *tu-3*.

PRESENCE OF *tu-1* IN OREGON R STOCK

The result of the cross between Oregon R females and *tu-h* males was the only one which differed markedly from the F_1 pattern represented in table 3. In this case the reciprocal crosses gave a comparable percentage of abnormal flies. Therefore, no maternal effect was in evidence when this cross was considered alone. High percentages of abnormality were also recorded for the F_2 of both reciprocal crosses, in marked contrast with the usual F_2 pattern. These deviations suggested that Oregon females are like *tu-h* females in their

ability to influence the expression of the abnormality. This similarity was demonstrated by crossing Oregon flies reciprocally with *tu-h* flies carrying third chromosome markers. The crosses are reconstructed and the results are given in table 4. It will be observed that Oregon females behaved like *tu-h* females and produced a maternal effect in the presence of *tu-3* introduced by the male. When *tu-3* was not present in the male no abnormality was obtained. The reciprocal crosses showed the maternal effect from *tu-h* only in the presence of *tu-3* in the female. These results indicate that Oregon carries a potential maternal effect but lacks the other partner necessary for the expression of the abnormality. Therefore, the inbred Oregon stock does not express the phenotype expressed by *tu-h*. From this evidence it was postulated that Oregon carries *tu-1* or an allele capable of producing a comparable maternal effect but does not carry *tu-3*.

TABLE 4

Crosses designed to demonstrate the maternal effect from Oregon R stock and the results of these crosses.

CROSS	Abn. ♀	+♀	Abn. ♂	+♂	% Abn.
1. Ore × $\frac{tu\text{-}1}{\rightarrow}\ \frac{tu\text{-}3}{tu\text{-}3}$	17	21	19	22	46
2. Ore × $\frac{tu\text{-}1}{\rightarrow}\ \frac{H}{Sb}$	0	177	0	187	0
3. $\frac{tu\text{-}1}{tu\text{-}1}\ \frac{tu\text{-}3}{tu\text{-}3}$ × Ore	63	70	47	72	44
4. $\frac{tu\text{-}1}{tu\text{-}1}\ \frac{H}{Sb}$ × Ore	0	187	0	191	0

The possibility that the maternal effect in Oregon is controlled by *tu-1* or an allele of *tu-1* was examined further through a series of backcrosses summarized in table 5. It will be observed that the F_1 females from both reciprocal crosses between *tu-h* and Oregon behaved like females from the Oregon and *tu-h* inbred stocks. The differences in percentage of abnormality observed in the different backcross results can be explained on the basis of the number of *tu-3* genes present. Comparable backcrosses involving the Canton stock, which does not produce the maternal effect, are given in table 5 for comparison. Since the F_1 females having received one sex chromosome from Oregon and one from *tu-h* have the same general effect as the females from either parental stock it is concluded that *tu-1* or an allele of *tu-1* is present in Oregon. It should be pointed out here that the expression of the abnormality has never been observed in the Oregon stock. The maternal effect, therefore, has no independent action in producing the tumorous head phenotype but acts only in the presence of *tu-3*. It follows that the small percentage of abnormal flies resulting from crosses between *tu-h* males and females from

laboratory stock (table 3) likely results from the independent action of *tu-3* as described above.

Results in the F_3, F_4, and F_5 from original reciprocal crosses between *tu-h* and Oregon were also comparable. From one series of 26 F_3 and F_4 crosses originating from *tu-h* × Oregon and Oregon × *tu-h*, which were made at random (parents not selected for the abnormality), 20 progenies showed some expression (ranging from 5 percent to 70 percent of abnormal flies). All female parents were expected to produce the maternal effect and the difference in expression was explained on the basis of the segregation of *tu-3*. Similar

TABLE 5

Average percentage of abnormality from backcrosses involving Oregon and tu-h compared with those involving Canton and tu-h.

PARENTS (P_1)		BACKCROSS TO *tu-h*				BACKCROSS TO OTHER STOCK			
		*F_1 × *tu-h*		*tu-h* × F_1		F_1 × P_1		P_1 × F_1	
FEMALE	MALE	**n	***%	n	%	n	%	n	%
tu-h	Oregon	649	42	409	35	120	22	380	19
Oregon	*tu-h*	333	56	167	64	134	19	372	29
tu-h	Canton	212	0	897	36	224	0	262	0
Canton	*tu-h*	605	0	570	50	266	0	231	0

* Female parent is always written first.

** n = total flies.

*** % = percentage of abnormality with accuracy to nearest whole number. Less than 0.5% is recorded as 0.

evidence of segregation was observed in the F_3, F_4, and F_5 results from other series of crosses involving *tu-h* and laboratory stocks other than Oregon. However, in these series of crosses there was a lower percentage of individual progenies expressing the trait and a lower percentage of abnormality was obtained in most progenies. For example, in a series of 28 F_3 and F_4 crosses between *tu-h* and $\frac{M(3)\ 124}{In\ (3R)C\ e\ l(3)e}$ only 8 progenies showed any expression of the trait. Both *tu-1* and *tu-3* were segregating in these latter crosses.

DISCUSSION

The pattern observed in the present study is that of a genetic mechanism involving a sex-linked gene which is recessive, or nearly so, and a third chromosome semidominant gene. It is complicated by the fact that the sex-linked gene has no direct effect on the flies which carry it but acts only through the mother by creating a change in the egg before fertilization. The nature of the change is unknown but it seems likely that a substance of some kind is involved. The substance presumably is accumulated in the egg cytoplasm at an early stage while the egg is still in the ovary. The genotype of the mother, therefore, determines the condition of the egg which will reflect its influence

into the development of the phenotype of the immediate progeny regardless of the sex-linked *tu-h* genes actually present in the fly. In order to produce this substance in appreciable quantity the mother must carry the sex-linked gene in homozygous condition. The only stock other than *tu-h* found so far to be capable of producing the cytoplasmic substance is Oregon R.

The situation is further complicated by the fact that the cytoplasmic substance is ineffective when alone. The abnormality is produced only through the interaction between this substance and a third chromosome, semidominant gene. This autosomal semidominant is homozygous viable at 22°C. Thus the immediate genotype of the fly is responsible for one partner in the interaction and the genotype of the mother, for the other partner. The more extreme expression in the females from all crosses suggests that the sex of the fly has some influence on the expression of the character.

There are some examples in the literature which bear a resemblance to the present situation. TOYAMA (Review by DOBZHANSKY 1941) has described maternal effects in the silkworm which resembles the one discussed here but they are not as complex. Different races of moths produce eggs with different visible characteristics. The eggs of a given female are all alike and always conform to the pattern of the race or genotype to which the female belongs. The characteristics of the egg are thus determined by the genotype of the mother in whose body the eggs develop. The maternal effect observed in the present study also resembles the case of dextral and sinistral snails described by BOYCOTT *et al.* (1930) and STURTEVANT (1923) but again is more complex. The coiling of the shell of the snail is dependent upon a gene which acts through the cytoplasm of the egg. Again in this case the immediate phenotype is controlled not by the individual snail's own genes but by the genes of the mother.

DOBZHANSKY (1935) has described a maternal effect expressed in the hybrids between race A and race B of *Drosophila pseudoobscura*. When a female from race B is crossed with a male from race A the F_1 males are sterile and have small testes. From the reciprocal cross sterile males are also produced but they have testes of normal size. The development of the abnormally small testes was shown to be the result of an interaction between the cytoplasm of the eggs deposited by a race B mother and the chromosomes of race A introduced by the sperm. This example has a greater resemblance to the present case than those cited above but there is one striking difference. Race A and B represent taxonomically distinct populations which are well on the way toward species formation. All hybrid males from either of the reciprocal crosses are sterile. It is not difficult to visualize how incompatibility and abnormality might arise in hybrids of widely separated groups. GOLDSCHMIDT (1934) has observed that cytoplasmic influence in general seems to increase with taxonomic difference. Most cases of cytoplasmic influence (Review by CASPARI 1948) also follow crosses between members of widely separated taxonomic groups. Very few cases have been reported in which differential action of the cytoplasm is found by crossing different mutants of one species. In the present case the two genes involved appear to represent independent mutations which have come

together in the same stock and are being maintained together. In fact, it seems possible that the *tu-h* stock first originated at the UNIVERSITY of TEXAS through a spontaneous mutation which produced the third chromosome semidominant in the sample of the wild Mexican population which already carried *tu-1*.

SUMMARY

The inheritance of an abnormality characterized by irregular growths in the head of *Drosophila melanogaster* has been analyzed. Two major genes are involved. One is a sex-linked recessive or slightly dominant gene with a maternal effect. The other is semidominant, homozygous viable at 22°C, and is located in the third chromosome. The inbred (*tu-h*) stock at present has an average penetrance of 76 percent at 22°C. An average of about 30 percent of abnormal flies is produced in the F_1 when *tu-h* females are crossed with males from laboratory stocks. Less than 1 percent of abnormal flies result from the reciprocal cross. The F_2 results were comparable for reciprocal crosses and only small percentages of abnormality were obtained. The Oregon R stock carries the sex-linked gene which produces the maternal effect but lacks the third chromosome gene and, therefore, does not express the abnormality.

ACKNOWLEDGEMENT

The authors acknowledge with thanks the interest, advice, and criticism of PROFESSOR RICHARD GOLDSCHMIDT and PROFESSOR CURT STERN. They express appreciation to DR. D. M. HAMMOND, DR. HORACE DAVENPORT, DR. W. W. NEWBY, DR. F. E. STEPHENS, and MRS. DORTOHEA MULAIK who have read the manuscript and made valuable suggestions. DR. W. W. NEWBY prepared the drawings. The project was supported by a grant from the UNIVERSITY OF UTAH RESEARCH FUND.

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