MALE GENITAL DISC DEFECT IN *DROSOPHILA MELANOGASTER*¹

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DURING larval and early pupal stages, the testes of *Drosophila melanogaster* are ellipsoidal in form. Following attachment of the developing seminal vesicle to the testes, the latter elongate and coil. The seminal vesicles are derived from the genital disc. If attachment does not occur the testes do not develop in this manner, even though they may increase in size. A defect of the genital disc, resulting in an absence of the seminal vesicles, occurs in over 60% of the male progeny when females with specific attached-X chromosomes are backcrossed to males from the tumorous-head strain (symbolized by tu-h) for several generations (WOOLF 1966). Undeveloped (bean-shaped) testes are the most obvious manifestation of this genital disc defect. The genetic mechanism causing this defect is complex consisting of a third chromosome gene from flies of the tu-h strain, a maternal effect attributable to chromosome 1, and polygenic modifiers. Flies from the tu-h strain contain a set of these modifiers, primarily on chromosome 2. Seven of nine different attached-X chromosomes tested were shown to contain the gene producing the maternal effect (WOOLF 1966).

The tumorous-head strain is characterized by flies with amorphous head growths (NEWBY 1949). The abnormality results from a third chromosome gene (*tu-3*) showing reduced penetrance. The frequency of affected flies is increased by a sex-linked recessive gene (*tu-1*) responsible for a maternal effect and polygenic modifiers mainly on chromosome 2. When *tu-1* and *tu-3* are homozygous, and modifiers are present at a high frequency, over 80% of the flies of the strain show an amorphous head growth (GARDNER and WOOLF 1949; GARDNER, STOTT and DEARDEN 1952). The sex-linked gene *tu-1* occurs at a high frequency in some natural populations (JOHNSON and GARDNER 1965).

The objectives of this paper are to present evidence that (1) *tu-3* is the gene responsible for the male genital disc defect, (2) the sex-linked gene producing the maternal effect involved in the male genital disc defect is *tu-1*⁺, (3) modifiers have a profound influence on the frequency of males with the genital disc defect, and (4) even though the genital disc defect is sex-limited, it occurs in transformed females (XXY flies homozygous for *tra*).

PROCEDURE AND RESULTS

All progeny were reared at 25°C in half-pint bottles containing the standard agar-cornmeal-brewers yeast-molasses-propionic acid medium. Flies were dissected in insect saline solution.

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Because of ease of scoring, undeveloped (bean-shaped) testes were used as the only criterion for the genital disc defect, even though a defective disc may manifest itself in another way (WOOLF 1966). The tumorous-head strain used in this study is tu-h ASU (WOOLF 1965). Chromosomes 1, 2 and 3 from this strain are symbolized by 1, 2 and 3.

Experiments designed to test the hypothesis that tu-3 is responsible for both the male genital disc defect and the tumorous-head abnormality: The recessive genes *cu* (curled) and *sr* (stripe) are at positions 50 and 62, respectively, in the right arm of the third chromosome. Gene *tu-3* is between these two genes at about position 58 (GARDNER 1959). Females were synthesized with the following third chromosomes: *ru h th st cu sr e^s ca/In(3L)P st tu-3*. These females were mated with *ru h th st cu sr e^s ca* males. Male offspring were isolated of the type *ru h th st cu/ru h th st cu sr e^s ca*. Each male resulted from a crossover right of *cu*. Since the parental females were not homozygous for *tu-1*, there was no way to determine if these males carried *tu-3*. Each crossover type male was mated singly with *H^s/In(3R)C Sb e l(3)e* females. Stubble, non-ebony male and female offspring [*ru h th st su/In(3R)C Sb e l(3)e*] were collected and mated together. Selection was made for curled, non-Stubble male and female offspring. These flies were the beginning of new strains. Fifty strains were established. The flies in each strain were homozygous for *tu-3* or the wild-type allele *tu-3⁺*. Males from these strains were mated with Stephenville females. These wild-type females are homozygous for *tu-1* and show a profound maternal effect activity for the tumorous-head abnormality (GARDNER and STOTT 1951). The F₁ offspring were examined for the tumorous-head abnormality. Males from the 50 strains were also mated with females possessing *FMA3, γ²* attached-X chromosomes, and with the F₁ females resulting from this mating. These attached-X chromosomes contain the gene producing the maternal effect responsible for the male genital disc defect. Although this defect does not appear in F₁ males, it does occur in BC₁ (first generation backcross) males (WOOLF 1966). BC₁ males were dissected and examined for undeveloped testes.

Males from 37 of the strains produced F₁ flies with the tumorous-head abnormality (when crossed with Stephenville females) and BC₁ males with the genital disc defect (when mated with *FMA3, γ²* attached-X females). Males from 13 of the strains failed to produce either trait in these progeny (Table 1). These concordant results demonstrate that *tu-3* is responsible for both abnormalities. The conclusion is reached that *tu-3* affects the development of the imaginal disc in the head region causing the tumorous-head abnormality in the presence of one maternal effect (produced by Stephenville females), and also affects the development of the male genital disc in the presence of a different maternal effect (produced by *FMA3, γ²* attached-X females).

These data can be used to estimate the position of *tu-3* between *cu* (position 50) and *sr* (position 62). Interpolation gives: $50 + 37/50 (12) = 58.9$, which agrees well with the position of about 58 reported by GARDNER (1959).

Experiments designed to test the hypothesis that tu-1⁺ is responsible for the maternal effect involved in the male genital disc defect: The maternal effects involved in the occurrence of the tumorous-head abnormality and genital disc defect will be referred to here as maternal effects 1 and 2, respectively. Initial

TABLE 1

Frequency of backcross one males with the genital disc defect when FMA3,, y² attached-X females were backcrossed to males from derived strains, and frequency of F₁ males and females with the tumorous-head abnormality when males from these strains were mated with Stephenville females (see text for origin of derived strains).

Males from strain	Backcross 1 males Genital disc defect		F ₁ Progeny Tumorous-head abnormality	
	<i>n</i>	percent	<i>n</i>	percent
1	118	22.9	250	61.2
2	100	23.0	120	80.0
3	114	0.0	181	0.0
4	123	26.0	377	60.7
5	100	18.0	366	48.9
6	126	24.6	394	51.0
7	98	0.0	244	0.0
8	121	20.7	195	58.2
9	108	23.2	137	60.6
10	119	0.0	250	0.0
11	96	0.0	318	0.0
12	125	27.2	282	55.7
13	115	19.1	213	66.7
14	105	20.9	458	50.9
15	119	27.7	274	70.4
16	99	17.2	73	61.6
17	127	13.4	311	66.9
18	105	20.0	339	64.9
19	148	25.0	435	73.1
20	103	0.0	259	0.0
21	111	0.0	233	0.0
22	121	10.7	196	60.2
23	131	19.8	260	59.6
24	116	26.7	226	55.3
25	106	20.7	171	57.9
26	140	19.3	215	64.2
27	86	0.0	184	0.0
28	164	16.8	318	69.2
29	169	29.6	327	61.8
30	127	20.5	157	54.8
31	95	21.0	220	76.8
32	119	17.6	261	66.7
33	96	0.0	167	0.0
34	125	0.0	114	0.0
35	134	29.8	120	56.7
36	109	0.0	134	0.0
37	110	17.3	175	70.3
38	104	8.7	210	49.5
39	115	19.1	206	55.8
40	105	27.6	171	53.8

TABLE 1—Continued

Males from strain	Backcross 1 males Genital disc defect		F ₁ Progeny Tumorous-head abnormality	
	<i>n</i>	percent	<i>n</i>	percent
41	113	26.6	156	41.0
42	124	17.7	208	55.8
43	105	26.7	150	47.3
44	103	14.6	238	46.6
45	86	0.0	226	0.0
46	90	13.3	237	62.9
47	102	0.0	148	0.0
48	119	0.0	144	0.0
49	120	14.2	165	83.6
50	92	10.9	155	63.4

experiments (WOOLF 1966) showed that 2 of 9 different attached-X females tested produced maternal effect 1, ascribed to *tu-1*. The other 7 produced maternal effect 2. The first free-X chromosome tested in the homozygous condition was negative for maternal effect 1 and positive for maternal effect 2. This free-X chromosome contained the recessive gene for carnation eyes (*car*). These observations suggested that maternal effect 2 is caused by *tu-1*⁺.

The recessive gene (*tu-1*) responsible for maternal effect 1 is located in or near the heterochromatin at about position 64.5 (GARDNER 1959). Experiments were designed to determine if maternal effect 2 is present in the absence of homozygosity for *tu-1*. Females from the laboratory stock $\gamma^2 v f car$ were mated with *tu-h* males. The genes γ^2 (yellow body), *v* (vermillion eyes), *f* (forked bristles) and *car* (carnation eyes) are located at positions 0.0, 33.0, 56.7 and 62.5, respectively. F₁ females were backcrossed to *tu-h* males. Male offspring were selected that were *car*, $\gamma^2 v f car$, γ^2 , $\gamma^2 v$, and $\gamma^2 v f$. The chromosomes containing *car* and $\gamma^2 v f car$ should carry the gene responsible for maternal effect 2, if this gene is at position 64.5. The chromosomes containing γ^2 , $\gamma^2 v$, and $\gamma^2 v f$ should carry *tu-1*. Males of each type were mated singly with *M5; Cy/Pm; ru h D³ st ri ln(3R)C e l(3)e/ln(3L)P st tu-3* females. Appropriate backcrosses were made to *tu-h* flies to yield females with chromosomes 2 and 3 from the *tu-h* strain (therefore to possess modifiers and *tu-3*) and X chromosomes of the constitution *car/1*, $\gamma^2 v f car/1$, $\gamma^2/1$, $\gamma^2 v/1$ or $\gamma^2 v f/1$. These females were mated to *tu-h* males. The progeny were examined for the male genital disc defect and the tumorous-head abnormality.

The results of all matings are compatible with the hypothesis that *tu-1*⁺ is a semidominant gene responsible for maternal effect 2 (Table 2). Ten matings were made of the type ♀♀ *car/1* × ♂♂ *tu-h*. Each of the chromosomes with *car* was derived independently by a crossover between positions 56.7 and 62.5. Since the region right of the crossover point is from the $\gamma^2 v f car$ chromosome, these matings were *tu-1*⁺/*tu-1*. All females produced maternal effect 2. The frequency of male offspring with the genital disc defect ranged from 7.4% to 54.7% in the

TABLE 2

Frequency of the tumorous-head abnormality and genital disc defect when the parental females are $tu-1^+/tu-1$ and $tu-1/tu-1$.

Mating	Tumorous-head abnormality		Male genital disc defect	
	<i>n</i>	percent	<i>n</i>	percent
Series 1 ($\text{♀ } \text{♀ } tu-1^+/tu-1$)				
1. $\text{♀ } \text{♀ } car/1 \times \text{♂ } \text{♂ } tu-h$				
Replication:				
1	..	..	288	33.0
2	..	..	163	29.4
3	..	..	248	24.2
4	..	..	91	8.8
5	..	..	409	35.5
6	..	..	392	7.4
7	..	..	322	8.4
8	..	..	247	54.7
9	..	..	311	31.8
10	..	..	207	31.9
Total			2678	26.6
2. $\text{♀ } \text{♀ } \gamma^2 v f car/1 \times \text{♂ } \text{♂ } tu-h$				
Replication:				
1	..	..	144	25.7
2	..	..	93	40.9
3	..	..	221	27.1
4	..	..	287	16.4
5	..	..	106	11.3
6	..	..	186	18.3
Total			1037	22.0
Series 2 ($\text{♀ } \text{♀ } tu-1/tu-1$)				
3. $\text{♀ } \text{♀ } \gamma^2/1 \times \text{♂ } \text{♂ } tu-h$	553	83.5	100	0.0
4. $\text{♀ } \text{♀ } \gamma^2 v/1 \times \text{♂ } \text{♂ } tu-h$	407	89.4	100	0.0
5. $\text{♀ } \text{♀ } \gamma^2 v f/1 \times \text{♂ } \text{♂ } tu-h$	326	76.1	100	0.0

ten progenies. The defect occurred in both carnation and non-carnation males. The average for all ten progenies was 26.6% ($n = 2678$). None of the ten matings showed evidence for maternal effect 1; hence no determinations were made of the frequency of offspring with the tumorous-head abnormality. In the absence of maternal effect 1, the tumorous-head abnormality still occurs at a small frequency (less than 5%), which has been described as resulting from independent action of $tu-3$ (GARDNER and WOOLF 1949; GARDNER, STOTT and DEARDEN 1952).

Six matings were made of the type $\text{♀ } \text{♀ } \gamma^2 v f car/1 \times \text{♂ } \text{♂ } tu-h$. These matings were also $tu-1^+/tu-1$. The frequency of the tumorous-head abnormality was clearly less than 5% in all progeny. The frequency of the genital disc defect ranged from 11.4% to 40.9% in the six progenies. The average was 22.0% ($n = 1037$).

Only one replication was made of the matings involving females with the chromosomes $\gamma^2/1$, $\gamma^2 v/1$ and $\gamma^2 v f/1$. These matings were *tu-1/tu-1*. As expected, maternal effect 1 was present (Table 2). The tumorous-head abnormality occurred at a high frequency in the progeny (76.1% to 89.4%). None of the male progeny dissected showed a manifestation of the genital disc defect.

The influence of one and two doses of tu-1⁺ on maternal effect 2 activity: Dosage studies with *tu-1⁺* were carried out by using X chromosomes known not to carry *tu-1* from four different laboratory stocks: *car*, *ma-l* (maroon-like), *M5* (Muller-5) and *Urbana* (wild type). These stocks were selected merely because of their availability in the laboratory.

Two types of third chromosomes are segregating in the tumorous-head strain; one with *tu-3* and the other with *In(3L)P st tu-3*. The latter is homozygous lethal (WOOLF 1965). Males of the type *M5; Cy/Pm; ru h D^s st ri In(3R)C e l(3)e/In(3L)P st tu-3* were mated with *tu-h* females. *F*₁ females of the type *M5/1; Cy/2; ru h D^s st ri In(3R)C e l(3)e/In(3L)P st tu-3* were backcrossed to *tu-h* males, and selection was made for females with the chromosomes *M5/1; 2; 3*. These females were mated with *tu-h* males. *M5/M5* females were obtained by mating *M5/1* females with their *M5* male siblings.

Urbana, *car* and *ma-l* males were mated with *M5; Cy/Pm; ru h D^s st ri In(3R)C e l(3)e/In(3L)P st tu-3* females. Two backcrosses were made to *tu-h* flies to yield females of the type $+^{Urb}/1$, *car/1* and *ma-l/1*, each with chromosomes 2 and 3 from the *tu-h* strain. These females were mated with *tu-h* males. Females homozygous for *car* and *ma-l* were obtained by mating *car/1* and *ma-l/1* females with their male siblings showing the mutant phenotype. *M5/ma-l* females were obtained by mating *M5/1* females with *ma-l* males. Females with one dose of *tu-1⁺* (*M5/1*, *car/1*, *ma-l/1* and $+^{Urb}/1$) and two doses of *tu-1⁺* (*M5/M5*, *car/car*, *ma-l/ma-l*) and *M5-ma-l* were then mated with *tu-h* males. Their male progeny were examined for the genital disc defect.

The results (Table 3) demonstrate that the frequency of the genital disc defect

TABLE 3

Frequency of the male genital disc defect when the parental females have one and two doses of tu-1⁺

Mating	Male genital disc defect	
	<i>n</i>	percent
Series 1 (♀ ♀ <i>tu-1⁺/tu-1</i>)		
1. ♀ ♀ <i>M5/1</i> × ♂ ♂ <i>tu-h</i>	1148	7.1
2. ♀ ♀ <i>ma-l/1</i> × ♂ ♂ <i>tu-h</i>	227	26.4
3. ♀ ♀ <i>car/1</i> × ♂ ♂ <i>tu-h</i>	473	31.1
4. ♀ ♀ $+^{Urb}/1$ × ♂ ♂ <i>tu-h</i>	462	35.9
Series 2 (♀ ♀ <i>tu-1⁺/tu-1⁺</i>)		
1. ♀ ♀ <i>M5/M5</i> × ♂ ♂ <i>tu-h</i>	581	52.8
2. ♀ ♀ <i>car/car</i> × ♂ ♂ <i>tu-h</i>	170	60.0
3. ♀ ♀ <i>ma-l/ma-l</i> × ♂ ♂ <i>tu-h</i>	77	68.8
4. ♀ ♀ <i>M5/ma-l</i> × ♂ ♂ <i>tu-h</i>	211	68.7

increases with the dosage of *tu-1*⁺ in the female parent. Females homozygous for *tu-1*⁺, i.e., *car/car*, *ma-l/ma-l* and *M5/ma-l*, produced about twice as many male progeny with the genital disc defect as heterozygous females of the type *car/1*, *ma-l/1* and *+^{urb}/1*. Although *M5/M5* females produced a relatively high frequency of sons with the defect, the frequency among the sons of the *M5/1* females was significantly lower (7.1%, *n* = 1148) than the frequency produced by the other three heterozygotes (26.4%–35.9%). The low value recorded among the sons of *M5/1* females was observed consistently in the subsamples. It is not known whether this reduced value is a function of the *tu-1*⁺ locus in the *M5* chromosome, modifiers in this chromosome, or a position effect resulting from one of the inversions in this chromosome.

Influence of modifiers on the frequency of males with the genital disc defect: Chromosome 2 in flies from the *tu-h* strain possesses modifiers that increase the frequency of males with the genital disc defect (Woolf 1966). Modifiers with a similar action are likely on other chromosomes as well. Males with the genital disc defect have a reproductive fitness value of zero; hence, in strains homozygous for *tu-1*⁺ and *tu-3*, there should be strong selection against any modifiers increasing the probability of abnormal development of the reproductive system. The pressure of backcrossing attached-X females with *tu-1*⁺ to *tu-h* males maintains the genital disc defect in the male progeny at a frequency over 60%. It necessarily follows that unless the modifiers become fixed, the frequency of the defect should decrease when the pressure of backcrossing is relaxed. This hypothesis was tested by using the newly synthesized *car*; 2; 3 and *ma-l*; 2; 3 females discussed in the previous section. Females of each type were mated with sibling males showing the mutant phenotype. This procedure established two strains, named the *car* and *ma-l* strains. Neither strain yielded many offspring in generation 1. For this reason only a small sample of males was dissected. However, it may be concluded that the frequency of males with the genital disc defect was relatively high in each case. 17 of 19 *ma-l* males (89.5%) had undeveloped testes; 31 of 55 *car* males (56.4%) were similarly affected. Although an attempt was made to set up 10 bottles of each strain, 8 of the bottles with *ma-l* flies and 5 of those with *car* flies had to be discarded because of low numbers of offspring and excessive growth of mold and bacteria. The remaining bottles were placed on the shelf and mass dumped each generation.

The frequency of the defect among *ma-l* males in generation 2 was 35.1% (*n* = 148); the frequency among *car* males was 36.7% (*n* = 270). In generation 5 the frequency among *ma-l* males was 0% (*n* = 155); the frequency among *car* males was 7.6% (*n* = 978).

A second set of experiments demonstrated that the decrease in frequency with time was not as pronounced in those strains where the pressure of backcrossing had likely brought about homozygosity for certain modifiers. When the experiments were initiated, females with *FMA3*, *γ*² attached-X chromosomes had been backcrossed to *tu-h* males for over four years. These females consistently produce 60%–70% male progeny with the defect. In one experimental line, the frequency dropped to 32% (*n* = 178) after 5 generations of mass

dumping. The frequency value remained approximately the same for 5 more generations suggesting that modifiers had been fixed in this line. *M5/1*; 2; 3 females were backcrossed to *tu-h* males for 15 generations. *M5/1* females were then mated with *M5* male siblings to initiate a *M5* strain. The frequency of males with the defect was 51.2% ($n = 80$) in generation 1. After 5 generations of mass dumping the frequency dropped to 28.0% ($n = 200$). Since this value is significantly higher than that observed in the *ma-l* and *car* strains after 5 generations of mass dumping, it is likely that the 15 generations backcrossing had brought about fixation of some of the modifiers.

Genital disc defect in transformed females: In the presence of maternal effect 2, modifiers and homozygosity for *tu-3*, the genital disc defect is limited to males. No morphological effect can be detected in females, indicating that female and male genital discs respond differently to the genetic mechanisms causing the abnormal development.

XX or *XXY* flies homozygous for the third chromosome transforming gene (*tra*) are male-like in appearance (STURTEVANT 1945). The gonads in these transformed females resemble testes (coiled and elongated), although they are reduced in size. Experiments were designed to determine whether the genital disc in transformed females would react as a male or female genital disc in the presence of the mechanisms causing the male genital disc defect. By using standard crossing over procedures, a chromosome was synthesized with the following markers: *ru h tra tu-3* (*ru* = roughoid, *h* = hairy). Males heterozygous for this chromosome and *In(3L)P st tu-3* were then mated with females of the type *FMA3*, γ^2/Y ; 2; *ru h D³ st ri In(3R)C e l(3)e/In(3L)P st tu-3*. Selection was made for non-Dichaete (*D³*) offspring, yielding a strain symbolized by *FMA3*, γ^2/Y ; *ru h tra tu-3/In(3L)P st tu-3*. Maternal effect 2 activity is produced by the females of this strain, *tu-3* is homozygous, and modifiers are present at a moderate frequency. One generation after this strain was synthesized, 116 males were dissected. Of this number, 24 (20.7%) showed the genital disc defect. The frequency of transformed females (roughoid and hairy in phenotype) in the offspring was low. An additional generation was required to isolate as many as 52 in four different bottles. Of this number, 15 (28.8%) showed small ellipsoidal gonads with no evidence of associated ducts. These results demonstrate that the genital disc in transformed females behaves as a male genital disc in the presence of the mechanisms giving the genital disc defect.

Effect of the age of the culture medium on the frequency of males with the genital disc defect: Even when the genetic background is reasonably controlled, a large variance is noted for the frequency of male offspring with the genital disc defect (see results in Table 2). Although it was not considered in the original design of the experiments, and, therefore, not controlled by the sampling procedure, it became evident during the course of the experiments that an important effect influencing the frequency of males with the genital disc defect was the age of the culture medium. Early eclosing males tend to have a lower frequency of the defect than late eclosing males. A preliminary test was made for this effect by mating *car*; 2; 3 males (possessing a moderate frequency of modifiers) with

tu-h females. The F_1 females ($tu-1^+/tu-1$) were then mated with tu-h males. A sample of males was taken on various days of eclosion and examined for the genital disc defect. The results are as follows: 1st and 2nd day, 15.9% ($n = 1026$); 3rd day, 12.3% ($n = 600$); 4th day, 12.6% ($n = 411$); 5th and 6th day, 18.6% ($n = 714$); 7th day, 26.7% ($n = 345$); 8th and 9th day, 27.4% ($n = 533$); 10th day, 33.3% ($n = 264$). The total percent based on 3890 males was 19.2% [18.9% ($n = 1880$) for carnation males and 19.5% ($n = 2010$) for non-carnation males]. An increase in the frequency of the genital disc defect with the day of eclosion was also noted for male offspring resulting from the mating ♀♀ $M5/1$; 2; 3 × ♂♂ tu-h: 1st day, 2.6% ($n = 196$); 2nd day, 4.5% ($n = 178$); 3rd day, 7.0% ($n = 143$); 4th day, 7.1% ($n = 84$); 5th day, 9.5% ($n = 74$); 6th day, 8.3% ($n = 60$); 7th day, 17.2% ($n = 64$); 8th day, 16.0% ($n = 25$); 9th day, 16.2% ($n = 59$). Muller-5 males have pigmentless testes because of the presence of w^u (apricot) in this chromosome, therefore in order to expedite scoring, only non- $M5$ males, with yellow testes, were dissected. The overall frequency with undeveloped testes was 7.4% ($n = 883$). Preliminary data have suggested that this effect is independent of parental age. Experiments are now in progress to determine the nature of this culture medium age effect.

DISCUSSION

The frequency of $tu-1$ has been studied in various natural populations in the western part of the United States (JOHNSON and GARDNER 1965). A linear relationship was observed between the frequency of $tu-1$ and degrees of northern latitude. The frequency of males hemizygous for $tu-1$ ranged from 13.6% ($n = 103$) in Phoenix, Arizona, to 31.9% ($n = 97$) in Yakima, Washington. Frequency studies carried out in the same geographical area (Utah) during different times of the year plus controlled laboratory experiments suggested that the primary environmental factor influencing the frequency of $tu-1$ is temperature: $tu-1$ is favored at lower temperatures.

The tumorous-head strain originated from a collection made at Acahuizotla, Mexico, in 1941. The maternal effect produced by $tu-1$ was first discovered in 1949 by GARDNER and WOOLF. This was made possible because of the presence of the mutant gene $tu-3$. The original mutation from the wild-type gene to $tu-3$ occurred in a strain with $tu-1$, and selection for flies with amorphous head growths resulted in a strain homozygous for $tu-1$ and $tu-3$. Over 17 years elapsed before it was realized that $tu-1^+$ is also responsible for a maternal effect (WOOLF 1966). The male genital disc defect was discovered accidentally when females with $FMA3$, γ^2 and $br\ ec$ attached-X chromosomes were backcrossed for many generations to tu-h males, and the male offspring were dissected for the purpose of determining the frequency of aspermia. A high frequency of aspermia is characteristic of certain tumorous-head strains (WOOLF, KNOWLES and JARVIS 1964; WOOLF 1965). The majority of the male progeny of the $FMA3$, γ^2 attached-X females showed undeveloped testes, while none of those produced by $br\ ec$ attached-X females exhibited the defect. The $br\ ec$ attached-X chromosomes contain $tu-1$. It is now evident that maternal effects resulting from wild type

isoalleles influence imaginal disc development in different regions of the larva, and the mutant gene (*tu-3*) used to detect these maternal effects acts as a semi-dominant gene in the presence of one maternal effect (produced by the recessive allele *tu-1*) and as a recessive gene in the presence of the other maternal effect (produced by the semi-dominant allele *tu-1*⁺). The reversibility of effects is further emphasized by the fact that the genetic disc defect is limited to males, while the tumorous-head abnormality occurs at a significantly increased frequency in females.

The development of the testes and associated organs in *Drosophila melanogaster* has been the object of extensive investigations (STERN 1940, 1941a, b; STERN and HADORN 1939; URSPRUNG 1963). BODENSTEIN (1950) has reviewed the literature on this subject. Any mechanism preventing the attachment of the developing seminal vesicles to the testes causes the latter to remain ellipsoidal. The presence of maternal effect 2, modifiers and homozygosity for *tu-3* is just one of these mechanisms. Non-attachment may also occur in gynandromorphs and the mutant sex-combless (STERN 1941a). Even though the usual expression of the genital disc defect consists of undeveloped testes and missing seminal vesicles and accessory glands, males frequently eclose suggesting there has been extreme abortive development of the genital disc. Such males exhibit undeveloped testes, absence of seminal vesicles, accessory glands and external genitalia (including the posterior end of the intestine), as well as the rudimentary development of the other structures derived from the genital disc.

The role of *tu-3* is decisive for the genital disc defect in the presence of maternal effect 2 and modifiers. However, even in the presence of maternal effect 2, *tu-3* may affect imaginal disc development in the head region causing the tumorous-head abnormality. Males have been observed with both the tumorous-head abnormality and the genital disc defect. In *tu-h* strains where maternal effect 1 is present, the influence of *tu-3* appears to be restricted to the development of the imaginal discs in the head region. Only an occasional male with an undeveloped testis was encountered when a large number of males from *tu-h* strains was dissected to determine the frequency of aspermia (WOOLF, KNOWLES and JARVIS 1964; WOOLF 1965). This observation is not unexpected since natural selection should eliminate very quickly any modifiers (if any exist) extending the influence of *tu-3* to the development of the genital disc in these strains. The necessary experiments have not been carried out to determine if a change of genetic background can extend the influence of *tu-3* to the development of the genital disc in the presence of maternal effect 1.

SUMMARY

A third chromosome gene (*tu-3*) in *Drosophila melanogaster* is primarily responsible for both the tumorous-head abnormality and the male genital disc defect. In the presence of a maternal effect produced by a sex-linked recessive gene (*tu-1*), and modifiers on chromosome 2, *tu-3* causes abnormal development of the imaginal discs in the head region resulting in the tumorous-head abnor-

malinity. In the presence of a different maternal effect produced by *tu-1*⁺, and modifiers also on chromosome 2, *tu-3* causes a defect of the genital disc in males. An obvious manifestation of this defect is undeveloped testes. The mutant gene *tu-3* acts as a semi-dominant gene in the presence of the maternal effect produced by the recessive gene *tu-1*, and reversely, it acts as a sex-limited recessive gene in the presence of the maternal effect produced by the semi-dominant gene *tu-1*⁺.—In strains homozygous for *tu-1*⁺ and *tu-3*, modifiers influence greatly the frequency of males with the genital disc defect. The frequency varies from 0% to over 60%. Since males with the genital disc defect have a reproductive fitness value of zero, the modifiers are quickly eliminated by natural selection in these strains, unless they have been fixed by backcrosses to flies from tumorous-head strains which contain these modifiers.—Although the genital disc defect is limited to males, it does occur in transformed females (XXY flies homozygous for *tra*), showing that the genital disc in these types acts as a male genital disc in the presence of the genetic mechanism (maternal effect, modifiers and homozygosity for *tu-3*) leading to the genital disc defect.

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