MATERNAL EFFECT INFLUENCING MALE GENITAL DISC DEVELOPMENT IN *DROSOPHILA MELANOGASTER*[1]

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THE development of the testes and associated organs in *Drosophila melanogaster* has been studied by many investigators (STERN 1940, 1941a, b; STERN and HADORN 1939; URSPRUNG 1963). A review of the literature has been given by BODENSTEIN (1950). During the larval and early pupal stages, the testes are ellipsoidal in form. Following attachment of the testes to the developing seminal vesicles, the testes elongate and become coiled (Figure 1). If attachment does not occur, the testes fail to develop in this manner. The seminal vesicles, accessory glands, vas deferens, ejaculatory duct, sperm pump and posterior end of the intestine develop from the genital disc (URSPRUNG 1963). A mechanism altering the development of the genital disc can inhibit attachment and therefore elonga-

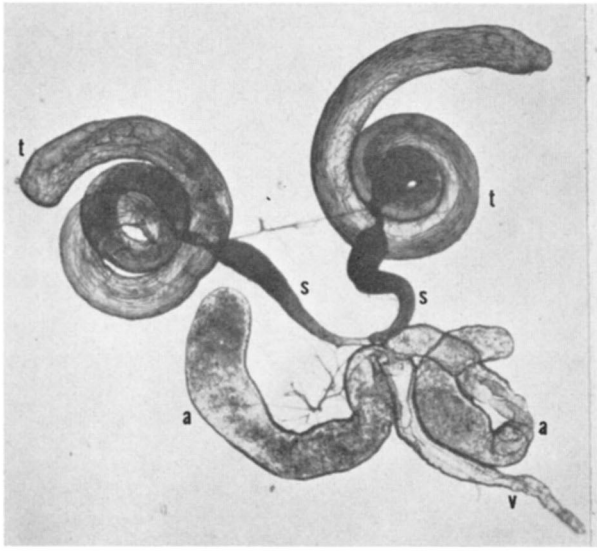

FIGURE 1.—Photograph of normal reproductive system of *Drosophila melanogaster* showing coiled testes (t) attached to seminal vesicles (s). Also shown are paired accessory glands (a) and vas deferens (v). 13.3×.

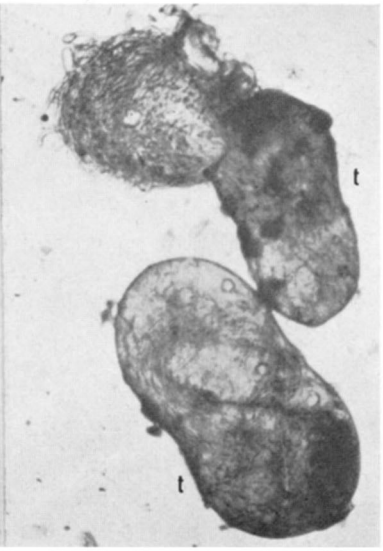

FIGURE 2.—Photograph of undeveloped testes (t). One testis was ruptured, yielding part of its contents. These uncoiled testes will produce motile sperm. Seminal vesicles and accessory glands were missing in this male. 13.3×.

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tion and coiling of the testes. Such a mechanism involving a maternal effect was encountered by chance when males from the tumorous-head (tu-h) strain of *Drosophila melanogaster* were backcrossed for several generations to FMA3, y^2 and *br ec* attached-X females. Over 60% of the male offspring showed undeveloped "bean-shaped" testes as well as other abnormalities of the reproductive system when the mothers used in the backcrosses were FMA3, y^2/Y (Figure 2). None showed this defect when the mothers were *br ec*/Y. (Where symbols are underlined, attached-X chromosomes are designated.)

The tumorous-head abnormality results from a third-chromosome gene (*tu-3*) showing reduced penetrance. The penetrance can be increased by a maternal effect attributed to a sex-linked recessive gene (*tu-1*) (GARDNER and WOOLF 1949), by polygenic modifiers largely on chromosome 2 (GARDNER, STOTT and DEARDEN 1952; WOOLF, KNOWLES and JARVIS 1964), and by high temperature during an early developmental period (GARDNER and WOOLF 1950). Females with *br ec* attached-X chromosomes are homozygous for *tu-1*. This wild-type isoallele is not present in females with FMA3, y^2 attached-X chromosomes. A detailed morphological study of the tumorous-head abnormality has been made by NEWBY (1949).

The objective of this paper is to present data which support the hypothesis that in the presence of one maternal effect, *tu-3* and modifiers may lead to the abnormal development of certain imaginal discs in the head region, resulting in the tumorous-head abnormality; while in the presence of another maternal effect, the same gene and modifiers may effect the development of the genital disc, resulting in abnormalities of the testes and associated organs. Both maternal effects are associated with the X chromosomes and both are present in attached-X stocks commonly found in the laboratory.

PROCEDURE

Backcross Series 1: Males from the tumorous-head strain maintained at Arizona State University were mated with females from nine different attached-X stocks. F_1 virgin females were then backcrossed to tu-h males. Backcrosses were continued for several generations. In each generation a sample of males was dissected in insect saline solution. Each male was classified as having either normal or unattached and therefore uncoiled testes. Because of ease of scoring, an undeveloped testis was used as the only criterion for a defective genital disc, even though a defective disc can manifest itself in another form. Progeny were also examined for the tumorous-head abnormality.

Backcross Series 2: WOOLF, KNOWLES and JARVIS (1964) synthesized strains with the following mixtures of tu-h and wild-type chromosomes: (a) +; +; 3 (b) 1; +; 3 (c) +; 2; +, where the first, second and third chromosomes from the tu-h strain are symbolized by 1, 2 and 3 and the corresponding wild-type chromosomes are symbolized by +. Chromosome 4 was not controlled. Males from these strains were mated with FMA3, y^2 attached-X females. Backcrosses dissections were made as outlined above. The purpose was to determine if the male genital disc defect is associated with the first, second or third chromosome from the tu-h strain.

Matings involving flies with dominant markers on the second and third chromosomes: FMA3, y^2/Y; 2; 3; 4 females were mated with 1: 2; *ru h* D^3 *st ri InRC e l3e*/3; 4 and 1; 2; 3; 4 males. Samples of the male offspring were dissected and examined for undeveloped testes. The objective was to determine the effect of homozygosity and heterogosity of chromosome 3 from

the tumorous-head strain on the testis defect. Chromosome 4 is controlled in these crosses; hence, they supply information on the role of this chromosome which was not controlled in backcross series 2.

Since modifiers increasing the frequency of the tumorous-head trait are largely on chromosome 2, matings were carried out in order to determine if heterozygosity for this chromosome from the tu-h strain influences the frequency of males with abnormal testes. Females of the constitution FMA3, y^2/Y; 2; 3; 4, and *br ec*/Y; 2; 3; 4, were mated with 1; *Cy*/2; 3; 4 males. Selection was made in the offspring for attached-X females with *Cy*/2; 3; 4 chromosomes. These females as well as the parental females were mated with 1; 2; 3; 4 and M5; *Cy*/2; 3; 4 males. A sample of each type of male progeny was then examined for abnormal testes.

RESULTS

Seven of the nine different attached-X females were effective in producing male offspring with abnormal testes (Table 1, Series 1). None of the F_1 males was abnormal, but the frequency of males with abnormal testes increased, in general, in each backcross generation as the genome of the tu-h strain became more established. The frequency of abnormal males in the third backcross generation varied from 57% when the mothers were *y*/Y to 13% when the mothers were *y w f*/Y. The observed variation in the frequency of abnormal males among the offspring of the seven types of females is not all due to chance. For example further experimentation has demonstrated that *y*/Y, *f B*/Y and FMA3, y^2/Y females consistently produce a relatively high frequency of male offspring with undeveloped testes within a few backcross generations, while females with *y w f* attached-X chromosomes produce a lower frequency of these males.

Two of the attached-X females (*br ec*/Y and y^2 *su-w^a w^a bb*/Y failed to produce males with undeveloped testes in any of the backcross generations (Table

TABLE 1

Frequency of males with abnormal testes in F_1 and backcross generations. Females resulting from each mating were backcrossed to males with the specified tumorous-head or wild-type chromosomes

Female (attached-X)	Male	Abnormal testes							
		F_1		BC_1		BC_2		BC_3	
		n	%	n	%	n	%	n	%
Series 1									
1) *y*	1; 2; 3; 4	100	0.0	83	13.3	96	25.0	93	57.0
2) *f B*	1; 2; 3; 4	61	0.0	85	23.5	90	40.0	100	53.0
3) *y v f car*	1; 2; 3; 4	100	0.0	100	42.0	109	43.1	100	48.0
4) *ac^3 w^a ct f*	1; 2; 3; 4	112	0.0	87	14.9	100	34.0	68	42.6
5) *y f*	1; 2; 3; 4	78	0.0	87	20.7	92	27.2	95	20.0
6) *RM, sc^8 B w^a sc^{S1}*	1; 2; 3; 4	100	0.0	102	7.8	100	10.0	100	16.0
7) *y w f*	1; 2; 3; 4	100	0.0	102	17.6	103	12.6	100	13.0
8) *br ec*	1; 2; 3; 4	100	0.0	100	0.0	100	0.0	100	0.0
9) *y^2 su-w^a w^a bb*	1; 2; 3; 4	100	0.0	100	0.0	100	0.0	100	0.0
Series 2									
10) FMA3, y^2	1; +; 3	82	0.0	100	9.0	93	15.0	56	41.1
11) FMA3, y^2	+; +; 3	88	0.0	100	7.0	98	20.4	103	24.3
12) FMA3, y^2	+; 2; +	100	0.0	93	0.0	87	0.0	81	0.0

1, Series 1). Both of these females are homozygous for tu-1 as revealed by the occurrence of the tumorous-head abnormality at a relatively high frequency in the progeny. Stocks have been maintained at Arizona State University for over a year by backcrossing each generation *br ec*/Y and *y² su-w^a w^a bb*/Y females to males from the tu-h strain. The frequency of offspring showing the tumorous-head abnormality (25°C) is about 25% (n = 241) when the mothers are *y² su-w^a w^a bb*/Y and about 76% (n = 319) when the mothers are *br ec*/Y. The latter frequency is similar to the one observed in the tu-h strain. Conversely, the tumorous-head abnormality occurs at a very low frequency among the offspring of the seven attached-X females capable of producing a high frequency of sons with undeveloped testes. For example, a stock has been maintained for over a year by backcrossing FMA3, *y²*/Y females to tu-h males. The frequency of male offspring with abnormal testes is over 60%, but the frequency of offspring with the tumorous-head abnormality is less than 1% (n = 219).

The second series of backcrosses (Table 1) suggest that chromosome 3 from the tu-h strain contains the principal genetic mechanism causing undeveloped testes in the presence of the proper maternal effect. Males with abnormal testes were not observed in any of the backcross generations when the backcross males were +; 2; +. However, they did occur in the backcross generations when the backcross males were +; +; 3 and 1; +; 3. Unfortunately, chromosome 4 was not controlled in the backcross males; hence these results by themselves do not give positive evidence that the genetic mechanism is located on chromosome 3. However, this premise is supported from the results shown in Table 2. Sixty-four percent of the male offspring showed the testis defect from the mating ♀ FMA3, *y²*/Y; 2; 3; 4 × ♂ 1; 2; 3; 4. Two types of male offspring result from the mating ♀ FMA3, *y²*/Y; 2; 3; 4 × ♂ 1; 2; *ru h D³ st ri InRC e l3e*/3; 4. None of the male offspring heterozygous for chromosome 3 showed abnormal testes, but 66.8% of the males homozygous for chromosome 3 in the same culture showed the testis abnormality. These observations plus the results of backcross series 2 indicate that the responsible mechanism is on chromosome 3, unless of course the *ru h D³ st ri InRC e l 3e* chromosome has suppressive action.

Evidence that chromosome 2 from the tumorous-head strain contains modifiers influencing the frequency of males with undeveloped testes is shown form the data in Table 3. The mating ♀ FMA3, *y²*/Y; *Cy*/2; 3; 4 × ♂1; 2; 3; 4 produces two kinds of males: 1; *Cy*/2; 3; 4 and 1; 2; 3; 4. The frequency of abnormal

TABLE 2

Frequency of males with abnormal testes. Samples of males were taken of each phenotype

Parents		Affected male offspring			
		1; 2; *ru h D³ st ri InRC e l 3e*/3; 4		1; 2; 3; 4	
Female	Male	n	%	n	%
FMA3, *y²*/Y; 2; 3; 4	1; 2; *ru h D³ st ri InRC e l3e*/3; 4	681	0.0	483	66.8
FMA3, *y²*/Y; 2; 3; 4	1; 2; 3; 4	..	..	206	64.0

TABLE 3

Frequency of males with abnormal testes. Samples of males were taken of each phenotype

Parents		Male offspring							
		1; Cy/2; 3; 4		1; 2; 3; 4		M5; Cy/2; 3; 4		M5; 2; 3; 4	
Female	Male	n	%	n	%	n	%	n	%
FMA3, y^2/Y; *Cy*/2; 3; 4	1; 2; 3; 4	123	43.0	142	57.7	..	..	..	..
br ec/Y; *Cy*/2; 3; 4	1; 2; 3; 4	75	0.0	81	0.0	..	..	..	..
FMA3, y^2/Y; 2; 3; 4	M5; *Cy*/2; 3; 4	..	..	..	..	176	46.0	106	71.7
br ec/Y; 2; 3; 4	M5; *Cy*/2; 3; 4	..	..	..	..	79	0.0	96	0.0

males was higher in the latter males (57%) than in the former (43.0%). The mating ♀ FMA3, y^2/Y; 2; 3; 4 × ♂ M5; *Cy*/2; 3; 4 also produces two kinds of males: M5; *Cy*/2; 3; 4 and M5; 2; 3; 4. Again, the frequency of abnormal males was higher among those males homozygous for tumorous-head second chromosomes (71.7%) than those heterozygous for the Curly chromosome (46.0%).

None of the male offspring had undeveloped testes when the female parent was *br ec*/Y (Table 3). The comparison of identical crosses involving FMA3, y^2/Y and *br ec*/Y females emphasizes the importance of a maternal effect in producing the testis abnormality.

DISCUSSION

The nine different attached-X females tested in this investigation produced either the undeveloped gonads in the male offspring, or the tumorous-head trait in female and male offspring, when backcrosses were made to tumorous-head males. There was no case of a tested attached-X female who produced neither trait in the offspring, and no female produced both traits at a high frequency in her offspring. Since nine constitutes a small sample, other attached-X females are being tested to determine if the maternal effects are independent of each other or are mutually exclusive. A multiple allele system does exist for the *tu-1* locus. In addition to *tu-1*, another allele (*tu-1*^{s}) is found in the Stephenville wild-type stock that results in a very high frequency of the tumorous-head trait in the offspring (GARDNER and SCOTT 1951). Another allele apparently exists in y^2 *su-w*^{a} *w*^{a} *bb* attached-X females because of the relatively reduced frequency of the tumorous-head trait in their offspring. Further investigation should reveal if the the maternal effect causing the abnormal testes is due to other alleles of *tu-1*, or if another locus is responsible.

Preliminary data have revealed that the maternal effect causing the testis defect is not restricted to attached-X chromosomes. Certain free X chromosomes also produce this maternal effect. For example, a stock was synthesized with the X chromosome containing the recessive gene for carnation (*car*) eyes and chromosomes 2, 3 and 4 from the tumorous-head strain. The frequency of males with abnormal testes in this strain was 14.5% (n = 533). Free X chromosomes from laboratory stocks and strains from nature are now being tested for this maternal effect activity.

Although definite evidence is lacking, it seems highly probable that the major genetic mechanism on chromosome 3, which interacts with the maternal effect giving the testis defect, is *tu-3*. Two types of third chromosomes occur in the tu-h strain used in these experiments (WOOLF and PHELPS 1960). Both contain *tu-3*, but one chromosome (symbolized 3B) contains the Payne inversion [*In(3L)P*] and the recessive gene for scarlet eyes (*st*). This chromosome is homozygous lethal. The other chromosome (symbolized 3A) lacks *In(3L)P* and *st*. The testis defect occurs in homokaryotypes (3A/3A) and heterokaryotypes (3A/3B), showing that the defect is not attributed to *In(3L)P* or *st*. Chromosome mapping should give the necessary proof that *tu-3* is the responsible mechanism.

The results shown in Table 2 demonstrate the presence of genetic modifiers on chromosome 2 which influence the frequency of males with abnormal testes. Likewise, chromosome 2 contains modifiers influencing the frequency of males with the tumorous-head phenotype. These may be the same modifiers. The frequency of the tumorous-head trait is increased if the flies are reared at a high temperature. The frequency of males showing abnormal testes is also influenced by temperature. Males resulting from the mating ♀ FMA3, y^2/Y; 2; 3; 4 × ♂ 1; 2; 3; 4 were raised in culture bottles maintained at 18° and 22°C. The frequency of males showing undeveloped testes at 18° was 21.4% (n = 205), while at 22° it was 43.3% (n = 208).

Male offspring resulting from the cross FMA3, y^2/Y; 2; 3; 4 × ♂ 1; 2; 3; 4 range from fertile males that are phenotypically normal to extreme cases of males exhibiting undeveloped testes, no external genitalia or posterior intestine (rectum) and only rudimentary structures derived from the genital disc. However, males with an obvious abnormality such as undeveloped testes and missing seminal vesicles and accessory glands usually have normal external genitalia. In rare cases, the abnormality is partially unilateral, with one testis being unattached and uncoiled while the other testis is coiled and associated with a seminal vesicle and accessory gland in various stages of development. WOOLF, KNOWLES and JARVIS (1964) demonstrated that about 20% of the males from the tumorous-head stock maintained at Arizona State University are aspermic. The same situation prevails for the undeveloped testes resulting from the cross ♂ FMA3, y^2/Y; 2; 3; 4 × ♂ 1; 2; 3; 4. About 80% (n = 642) of the males with uncoiled testes yield sperm, although the quantity is not large. The sperm are motile; however, since the testes are not attached, the males are obviously sterile.

The absence of seminal vesicles, accessory glands, and external genitalia (including the posterior end of the intestine), as well as abnormalities in the other organs of the reproductive tract, indicate that the primary defect lies in the genital disc (URSPRUNG 1963). Undeveloped testes are merely a symptom of this defect. Genetical, morphological and developmental studies of this genital disc abnormality are underway. It is a curiosity that no gross abnormality is present in the reproductive organs of the female siblings of the defective males. However, females resulting from the mating ♀ FMA3, y^2/Y; 2; 3; 4 × ♂ 1; 2; 3; 4 do produce few offspring.

Backcrossing tumorous-head males to *br ec* and FMA3, y^2 attached-X females for many generations results in males with identical genomes. Yet, these males may exhibit in one case the tumorous-head phenotype and in the other case an abnormal reproductive system. The only difference between these males is the genotype of their mothers. Since the tumorous-head phenotype results from an upset in the development of imaginal discs in the head region, principally the eye and antenna discs (NEWBY 1949), the experimental data demonstrate the unique situation of maternal effects upsetting the development of imaginal discs in opposite ends of the larvae. These results lead to the provocative question as to what role, if any, the maternal genotype plays in normal imaginal disc development.

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SUMMARY

In *Drosophila melanogaster*, the testes are ellipsoidal during early larval and pupal stages. Following attachment of the testes to the developing seminal vesicles, which are derived from the genital disc, the testes elongate and become coiled. A mechanism involving a maternal effect has been discovered which alters the development of the genital disc, and therefore inhibits attachment and development of the testes. The testes remain "bean shaped" in appearance.

The genetic mechanism causing this abnormality consists of a gene on chromosome 3 (probably *tu-3*) from the tumorous-head strain, and a maternal effect which is attributed to chromosome 1. Seven out of nine attached-X stocks tested possess this maternal effect. The other two attached-X stocks possess the maternal effect, produced by *tu-1*, which leads to a high frequency of flies with the tumorous-head trait. The hypothesis is presented that *tu-3*, in the presence of one maternal effect, affects development of the male genital disc, and in the presence of another maternal effect influences the development of the imaginal discs (principally eye and antenna discs) giving rise to head structures. Modifiers on chromosome 2 influence the frequency of flies with the tumorous-head or testis defect. The frequency of both traits is also influenced by temperature.

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