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THE INVESTIGATION OF DOSAGE PHENOMENA OF
tuh-1 AND tuh-1⁺ MATERNAL EFFECT WILD
TYPE ISOALLELES IN DROSOPHILA MELANOGASTER

by

Frank Charles Dukepoo

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tuh-1 AND tuh-1⁺ MATERNAL EFFECT WILD
TYPE ISOALLELES IN DROSOPHILA MELANOGASTER

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The development of certain imaginal discs in Drosophila melanogaster is influenced by two maternal effects (maternal effect 1 and maternal effect 2). In the presence of a mutant gene (tuh-3) in chromosome 3, and a maternal effect 1, produced by a wild type gene (tuh-1) in chromosome 1, abnormal development of the imaginal discs giving rise to the eyes and antennae may occur resulting in the tumorous-head abnormality. In the presence of tuh-3 and maternal effect 2 produced by the semidominant wild type allele tuh-1⁺, male progeny are afflicted with a defect of the genital disc. One consequence is the appearance of "bean-shaped" testes. The mutant gene tuh-3 acts as a semidominant gene in the presence of maternal effect 1 and as a recessive gene in the presence of maternal effect 2.

The objectives of this investigation were to study the effects of different doses of the wild type alleles controlling the two different maternal effects and to test the influence of the quantity of medium on the frequency of flies with the genital disc defect.

Four duplication fragments were produced by subjecting male flies to a source of ionizing radiation. Two fragments when tested indicated the presence of tuh-1. The frequency of flies with the tumorous-head abnormality was higher in any backcross generation when parental females possessed these duplications. It was also shown

that a lowering of productivity occurs when females possess the fragments containing tuh-1.

Three duplications believed to contain tuh-1⁺ were tested. All three indicated a dosage effect. The frequency of the genital disc defect was slightly higher in male progeny if mothers possessed one of these duplications. The frequency of the genital disc defect increased with successive backcrossing of duplication-carrying mothers. The duplications seem to have little effect on productivity. In addition to an increased penetrance of the genital disc defect, other anomalies were encountered in this dosage study. Among these were rectal tumors, genitalia anomalies and a podoptera phenotype. Of these, only the genitalia anomalies were restricted to male progeny. Rectal tumors are intestinal in origin and resemble the amorphous head growths characteristic of the tumorous-head abnormality.

The quantity of the medium was shown to influence the frequency of flies with the genital disc defect. Progeny cultured in half-pint milk bottles exhibited a higher frequency of the genital disc defect than progeny reared in population cages with a much greater quantity of medium.

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I. INTRODUCTION

The genital disc defect in Drosophila melanogaster results from an interaction between a mutant gene (tuh-3) in chromosome 3 and a maternal effect produced by the homozygosity or heterozygosity of a wild type gene (tuh-1⁺) in chromosome 1 (Woolf, 1966, 1968). During larval and early pupal stages, testes have an ellipsoidal appearance. Following attachment to the developing seminal vesicles, the testes elongate and coil. Failure of attachment results in uncoiled testes which are "bean-shaped" or "sac-like" in structure. The seminal vesicles are derived from the genital disc. Testes and the genital disc develop independently from one another (Dobzhansky, 1930). The action of tuh-3 and the maternal effect involves disruption of the development of the genital disc. One result is failure of the seminal vesicles to form; the consequence is nonattachment and therefore noncoiling of the testes. Since derivatives of the genital disc include the seminal vesicles, accessory glands, vas deferens, ejaculatory duct, sperm pump and the posterior end of the intestine (Ursprung, 1963), there are several other manifestations of the genital disc defect ranging from noncoiling of the testes to absence of most of these struc-

tures. A sac testis is an obvious manifestation which is easy to score.

Wolf, Knowles and Jarvis (1964) demonstrated that about 40% of males of the inbred tumorous-head stock maintained at Arizona State University are sterile. Approximately half of these sterile males are aspermic, as compared to less than 2% from tu-h stocks maintained at the California Institute of Technology. As a result of these sperm analyses, the genital disc defect was discovered (Wolf, 1966). When females from the FMA3,y2/Y strains were backcrossed for several generations to tu-h males, over 60% of the male offspring showed undeveloped testes as well as other abnormalities of the reproductive system. This was not observed when brec/Y females were backcrossed to tu-h males. Females with different types of attached-X chromosomes were then backcrossed to males of the tu-h strain. Seven of nine attached-X stocks tested produced males with the genital disc defect; two produced offspring which manifested amorphous head growths (tumorous-head). It was concluded that in the presence of tuh-3, the genital disc defect occurs if the attached-X chromosome (such as FMA3,y2/Y) contains tuh-1⁺, and the tumorous-head abnormality occurs if the attached-X chromosome (such as brec) contains tuh-1.

Thus, $tuh-1^+$ produces a maternal effect which interacts with $tuh-3$ to give the genital disc defect in males. The mutant gene $tuh-3$ is recessive in the presence of this maternal effect. Penetrance of the genetic mechanism is influenced by temperature and polygenic modifiers during an early developmental period (Woolf, 1968).

Woolf (1966, 1968) demonstrated that males homozygous for $tuh-3$ from FMA3,y2/Y mothers express varying degrees of the genital disc defect. One expression is sac testes. More extreme expressions included the absence of structures derived from the genital disc such as external genitalia and the posterior end of the intestine. Males which appear normal in regard to external genitalia may lack seminal vesicles and accessory glands. Sometimes the genital disc defect appears unilaterally. Females homozygous for $tuh-3$ from FMA3,y2/Y mothers are not physically deformed; however, they are subfertile.

The mutant gene $tuh-3$ is also responsible for another imaginal disc abnormality which has been named the tumorous-head trait. A review of the subject was given by Gardner in 1970. In 1941, research workers at the University of Texas made a collection of flies in a small Mexican village located near Acapulco. The stock was maintained at the University of Texas; flies were soon noted showing abnormal head growths. In 1946, the stock was sent to the

University of Utah where studies were initiated to determine the genetic mechanism responsible for the head growths. Two of the earliest references describing the phenotype, genetics of the growths and the influence of temperature on the expression of the phenotype are those of Gardner (1948) and Woolf (1948). In 1943, Newby gave an extensive description of the different expressions of the abnormal growths. The abnormal head growths became known as the tumorous-head abnormality and the strain possessing flies with these abnormal head growths was named the tumorous-head strain symbolized by tu-h (Newby, 1949; Gardner and Woolf, 1949).

The genetic mechanism for this abnormality consists of the mutant tuh-3 gene and a maternal effect produced by homozygosity of a wild type isoallele of tuh-1⁺, symbolized tuh-1 (Gardner and Woolf, 1949). In this case the interaction causes abnormal development of certain discs in the head region resulting mainly in amorphous growths in the regions of the eyes and antennae. The mutant gene tuh-3 is semidominant in the presence of this maternal effect. It was assumed that the maternal effect created a change in the egg cytoplasm, via accumulated substances, before fertilization. In the presence of these substances, tuh-3 causes the tumorous-head abnormality. The mechanism causing the tumorous-head abnor-

mality shows reduced penetrance. Flies of the tu-h strain are homozygous for tuh-1 and tuh-3; yet, only 70-80% manifest the tumorous-head abnormality. Females show a higher penetrance than males. Gardner and Woolf (1950) showed that penetrance can be influenced by temperature during an early developmental period, and also that a sex ratio favoring males is present in the tu-h strains. It has been well documented that penetrance is also increased by modifiers located mainly in the second chromosome (Gardner, Stott and Dearden, 1952; Woolf, Knowles and Jarvis, 1964). An aging culture medium has also been shown to decrease the penetrance and the individual size of the head tumors (Gardner and Ratty, 1952).

The gene (tuh-1) responsible for the maternal effect is located at position 64.5 in or near the heterochromatic region of the X chromosome (Gardner, 1959). The semi-dominant mutant gene tuh-3 is located in the right arm of the third chromosome at about position 58.9 (Gardner, 1959; Woolf, 1968).

It is apparent that the mutant tuh-3 gene in conjunction with two different maternal effects is responsible for the abnormal development of specific imaginal discs at opposite ends of the fly. Responsible maternal effects associated with the tumorous-head trait and genital disc defect are called maternal effects 1 and 2, respectively.

Since $tuh-1$ and $tuh-1^+$ regulate the level and type of maternal effect activity, the main objective of this investigation was to investigate dosage effects of these alleles by using diploid females carrying free duplication fragments carrying the appropriate allele. It was proposed that females carrying three doses of these alleles may manifest greater maternal effect activity than females with two doses of the same alleles. Another objective of this investigation was to study the effect of the quantity of medium on the expression of the genital disc defect.

PREVIEW

II. METHODS AND RESULTS

Unless specified otherwise, all flies were reared in half-pint sized milk bottles on the standard corn-meal-molassas-sucrose-yeast-propionic acid-agar medium at 24° C.

EXPERIMENT 1. Dosage Effect Study of tuh-1⁺.

The necessary matings were carried out to obtain FMA3,y2/Y females known to be homozygous for tuh-1⁺ and containing chromosomes 2 and 3 from flies of the tumorous-head stock. The synthesis of this stock (FMA3,y2/Y, 2, 3) is shown in Figure 1. The method involves retention of the attached-X chromosome and substitution of the second and third chromosomes with those of the tu-h strain. In the mating scheme shown in Figure 1, and in all other ensuing mating schemes in other figures, females are written first and Arabic numerals represent chromosomes from flies of the tumorous-head stock.

Free duplication stocks were received from the California Institute of Technology. The three used in this study were: Dp(1:f)3, Dp(1:f)18 and Dp(1:f)52. Each contains a free duplication fragment of the 19-20 region of the X chromosome and hence might contain the tuh-1⁺

gene. In Duplication 3, the free fragment carries wild type alleles of *y*, *ac*, *sc*, *su(f)* and *bb*; it is about 3.7-4 times as large as the fourth chromosome at metaphase. The fragment in Duplication 18 is about the same size and carries the wild type alleles of *y*, *ac*, *sc*, *su(w^a)* and *bb*. In Duplication 52, the fragment carries wild type alleles of *y*, *ac*, *sc*, *su(f)* and *bb*. It too, is approximately 3.7-4 times as large as the fourth chromosome (Lindsley and Grell, 1967).

The method of inserting the duplication into the females with attached-X chromosomes is shown in Figure 2. FMA3,y2/Y; 2; 3 females were mated with attached-XY males carrying a duplication. F₁ females of the type FMA3,y2/Y/Dp (nonyellow) each possessing a different duplication were then backcrossed to tu-h males for five generations to increase the probability of obtaining modifiers from the tumorous-head strain. The next generation was then arbitrarily chosen as the first generation in the duplication experiments.

In the dosage experiments, fifty virgin FMA3,y2/Y/Dp (nonyellow) females from each duplication strain were mated to an equal number of tu-h males. Males used in these experiments were from a tumorous-head strain expressing an average penetrance of about 70%. Parental flies were removed from milk bottles after nine days;

male progeny eclosing over a ten day period were then scored for the genital disc defect. All progeny eclosing over this period were scored for the tumorous-head abnormality.

Since penetrance of the genital disc defect can be increased by successive backcrossing to tu-h males with the incorporation of modifiers (Woolf, 1966, 1968), all dosage experiments were conducted over several backcross generations. In each generation three simultaneous replications were run of each duplication tested. Female progenitors of each generation were duplication (FMA3,y2/Y/Dp, yellow) and non-duplication (FMA3,y2/Y, nonyellow) members (siblings) of the same progeny. The latter were used as controls.

Table 1 summarizes the influence of Duplication 3 on the frequency of the male genital disc defect. When the females possess the duplication, the frequency of the genital disc defect increases successively in every generation from an initial value of 4.07% in generation one to 37.45% in generation seven. These values were consistently higher in each generation than those observed in the controls, however, in any given backcross generation the differences were not large (see Figure 3). After seven backcross generations the frequency of the genital disc defect in the controls was 32.98%.

The penetrance of the tumorous-head abnormality in both the experimental and control series was less than 1% in every generation. This trait occurs at a low frequency in the absence of the maternal effect produced by tuh-1 apparently because of the independent action of tuh-3 (Woolf, 1968).

Of interest is the influence of backcrossing on the total number of progeny eclosing (Figure 4). As the number of backcross generations increased the total number of progeny decreased. This occurred in both the experimental and control series and suggests that in the presence of the maternal effect produced by tuh-1⁺ and homozygosity for tuh-3, the accumulation of modifiers from the tu-h strain reduces fecundity (number of eggs produced), fertility (percentage of eggs that hatch) or viability of developing larvae or pupae. There is a suggestion in the data (Figure 4) that the duplication might have increased the productivity in some generations. Since productivity dropped in almost every generation, the experiment was terminated after seven generations of backcrossing. At this time there were so few surviving progeny in each bottle that the culture medium was nearly always overrun with mold and bacteria.

The influence of Duplication 18 on the frequency of the genital disc defect is summarized in Table 2. Al-

though fluctuation was present there was an increase in penetrance, in general, with each backcross generation in both the experimental and control series up to the eleventh generation. A decline then occurred in both series with a subsequent gain. The experiment was terminated at the fifteenth generation.

Although the differences were not large (Figure 5), a higher frequency of affected males occurred in the experimental series than in the controls in almost every generation. The penetrance of the tumorous-head abnormality was less than 1% in both series.

The productivity of both the experimental and control females decreased with successive backcross generations (Table 2, Figure 6). The presence of Duplication 18 seemed to have little effect on the productivity of females. A similar rate of decrease of productivity seemed to occur in both the experimental and control series. The experiment was concluded at the fifteenth generation because of the low productivity of the stock.

Results with Duplication 52 are summarized in Table 3. In this experiment, backcrosses were carried out for eleven generations. The frequency of male offspring with the genital disc defect was nearly similar in the experimental and control series until the ninth generation. At that time and in the succeeding tenth and eleventh