Phenocopies of Pigmentary and Behavioral Effects of the Yellow Mutant in Drosophila Induced by α-Dimethyltyrosine

Abstract. *α-Dimethyltyrosine induces a phenocopy of the body color mutant* yellow *when fed to larvae of* Drosophila melanogaster. *The compound also induces changes in male courtship behavior which are similar to the effects of the mutant gene.*

The courtship behavior of *Drosophila melanogaster* involves a complex interaction in which the male and female each transmit information to the other via a series of discrete behavioral elements (*1*). One important signal given by the male is the wing vibration display which serves to provide the female with patterned auditory stimuli that are picked up by way of her antennae (*2*). These auditory patterns form a species-specific code by means of which the female can identify a conspecific male (*3*); they are therefore important in the sexual isolation of certain species. Factors that give rise to changes in the wing vibration component of courtship may have consequences for the mating success of the male. An example of this is provided by males hemizygous for the sex-linked mutant gene *yellow* which show reduced success in fertilizing females. This reduction in mating success appears to be related to shortening of the mean bout length of vibration and to an increase in the mean interbout interval (*4*).

Adult flies of an inbred Amherst wild-type strain were cultured aseptically at 25°C on a modified version of Sang's (*5*) larval medium C containing the methyl ester of DL-α-dimethyltyrosine hydrochloride (α-DMT). The compound induced yellow body color in a proportion of flies related to the doses employed. α-DMT also reduced the rate of cephalopharyngeal retraction, and the duration of the larval period was lengthened. Above a concentration of 0.15 percent α-DMT in the larval medium no adults emerged. Many of the phenocopy flies showed difficulty in walking and died within 2 to 3 days after eclosion from the pupa. No yellow-bodied flies were obtained with a Pacific wild-type strain over the same dose range, an indication that the action of α-DMT is genotype-dependent.

Since other nonallelic light body color mutants, such as *straw*, do not show any obvious mating disadvantage, it was of interest to determine whether α-DMT induces changes in male courtship behavior comparable with the *yellow* mutant. Newly eclosed aseptic adult male flies cultured on control medium, and consequently having normal body color, were transferred to sterile tubes containing Sang and King's (*6*) adult medium with 1 percent α-DMT. These flies were used for behavioral tests when 3 days old. Control flies were kept on adult medium without α-DMT. For comparison, wild-type and *yellow* mutant males were generated from the F_1 of both reciprocal crosses of an inbred *yellow* strain to Amherst wild type, and also from the backcross of heterozygous F_1 females to parental wild-type males.

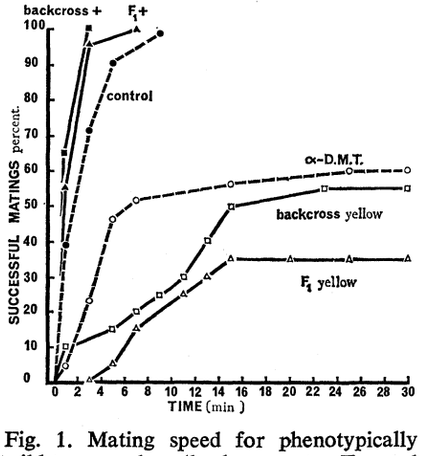

Fig. 1. Mating speed for phenotypically wild-type males (backcross +, F_1, and control), *yellow* males, and males fed on 1 percent α-dimethyltyrosine, shown as the cumulative percentage of successful matings with time.

Reciprocal 1:

♀ *y*/*y* × +/Y ♂

♀ *y*/+ *y*/Y ♂

yellow

Reciprocal 2:

♀ +/+ × *y*/Y ♂

♀ +/*y* +/Y ♂

wild type

Backcross:

F_1 ♀ +/*y* × *y*/Y ♂

+/*y* ♀ +/Y ♂ wild type

y/*y* ♀ *y*/Y ♂ *yellow*

Males were paired individually with 3-day-old virgin wild-type females in Perspex mating cells (*5*) and courtship elements were logged on a multichannel pen recorder (*7*).

Wild-type males achieved copulation within 2 to 4 minutes (Fig. 1), whereas *yellow* males were much less successful. Many of the mutant males failed to copulate within the 30-minute observation period. Wild-type males fed on α-DMT were less successful in mating than the control group, and many of the treated males also failed to copulate within 30 minutes. The male begins courtship by orientating his body axis toward the female, his head facing her. If she moves he follows. The wing vibration element is superimposed upon orientation. Measurements of orientation and vibration for the first half of the courtship period of wild-type males (approximately 2 minutes) were compared with the corresponding first 2 minutes of courtship by mutant and α-DMT treated males. The *yellow* mutant males show longer mean duration of breaks in orientation during courtship than wild-type males (Fig. 2), the difference in each case being significant ($P = .05$). The males treated with α-DMT similarly show significantly longer breaks in orientation ($P < .001$) compared with control males. The *yellow* males show a significantly longer duration of breaks in wing vibration compared with wild-type males (Fig. 3). The males treated with α-DMT are similar in having significantly longer breaks in wing vibration ($P < .001$) compared with the control males.

Failure to synthesize the black pigment normally present on certain areas of the body is associated with a behavioral abnormality in the *yellow* mutant. It is possible that a biochemical link between these phenes exists. Tyro-

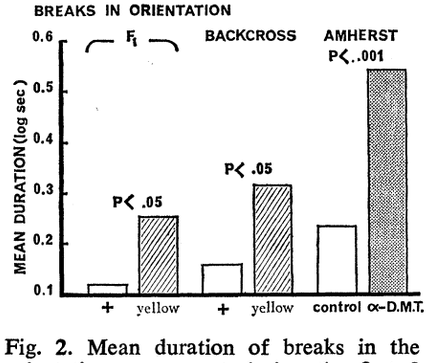

Fig. 2. Mean duration of breaks in the orientation component during the first 2 minutes of male courtship, with single pair matings with wild-type females. Differences between means were tested for significance with the use of an analysis of variance paradigm (*9*).

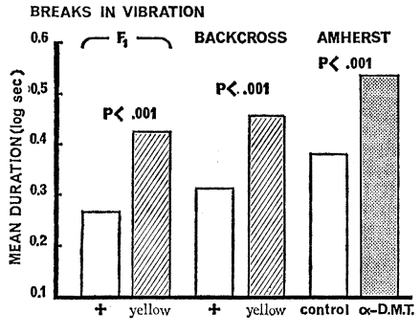

Fig. 3. Mean duration of breaks in the wing vibration component during the first 2 minutes of male courtship with single pair matings with wild-type females.

sine provides a common precursor for synthesis of melanin pigment and of catecholamines, such as dopamine and noradrenalin, which play an important role in the functioning of the nervous system (*8*). The action of α-DMT in mimicking the effects of the mutant may be an indication that the *yellow* gene is involved in some step common to both of these biosynthetic pathways.

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Cytomegalovirus: Conversion of Nonpermissive Cells to a Permissive State for Virus Replication

Abstract. *Human embryonic kidney cells are epithelioid cells which are normally nonpermissive for in vitro replication of human cytomegalovirus. These cells were converted to a permissive state for the virus by prior treatment with 5-iodo-2′-deoxyuridine. When this method was used, a nonpermissive cell was made permissive to an infecting virus.*

Human cytomegalovirus (CMV) is a member of the herpesvirus group (*1*). It is host specific and replication in vitro is limited to human fibroblasts (*2*). This specificity in vitro for fibroblastic cells is puzzling because the virus is often isolated from epithelioid cells taken from the host (*3*).

Certain thymidine analogs, such as 5-iodo-2′-deoxyuridine (IdU) and 5-bromodeoxyuridine, can induce cells to produce C type virus particles (*4*). They can also induce Epstein-Barr virus–negative (nonproducing) lymphoblastoid cells and somatic cell hybrids produced with these lymphoblastoid cells to produce Epstein-Barr virus, a member of the herpesvirus group (*5, 6*). These same analogs can also depress the synthesis of certain cell proteins such as interferon (*7*). We have reported that preliminary treatment of human fibroblast cells with IdU enhances the replication of CMV in these cells (*8*). We have now investigated the possibility that prior treatment with IdU might enable human CMV to replicate in nonpermissive epithelioid cells.

The epithelioid cells used in these studies were human embryonic kidney cells (HEM Laboratories, Rockville, Maryland). Second-passage confluent monolayers were grown in medium 199, supplemented with 10 percent tryptose phosphate broth, 10 percent fetal calf serum, 100 units of penicillin, 100 μg of streptomycin, and 0.075 percent sodium bicarbonate. The cells were dispersed with trypsin and centrifuged; the sedimented cells were resuspended to the desired concentration. The thymidine analog IdU (100 μg/ml) was added, and the cells were then placed in appropriate containers. The cells were incubated for 96 hours in the presence of IdU, washed two times with isotonic tris buffer, *p*H 7.4, and then exposed to either virus or growth medium containing the same concentration of fetal calf serum as the virus inoculum. Control cultures were treated in the same manner except that IdU was not added. The AD-169 strain of CMV was used throughout our studies. We detected virus DNA by labeling the DNA of infected and sham-infected cultures with tritiated thymidine ([^{3}H]dT) (17 c/mmole; Schwarz/Mann), separating cellular and virus DNA by isopycnic centrifugation in CsCl, and determining the amount of [^{3}H]dT incorporated into acid-insoluble material (*9*).

We first attempted to determine whether CMV DNA was produced in epithelioid cells. The HEK cells with IdU, both treated and untreated, were grown in 1-ounce (1 ounce about 30 ml) glass prescription bottles. The cultures were either infected with 1 plaque-forming unit (PFU) of CMV per cell or were sham-infected with medium. The cells were then grown in the presence of [^{3}H]dT (10 μc/ml) for 24-hour periods. The amount of [^{3}H]dT incorporated into acid-insoluble material was then determined (*9*). Figure 1 represents the cells exposed to [^{3}H]dT for 48 to 72 hours after inoculation with CMV. Figure 1A represents the cells not treated with IdU but infected with CMV. All DNA that contained [^{3}H]dT in these cultures banded at a density corresponding to that of reported values for cellular DNA (*9*). No evidence was found during the entire period of this experiment (0 to 120

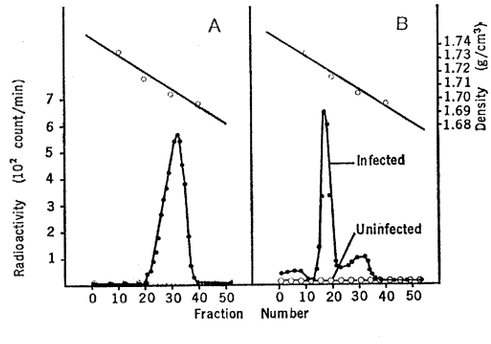

Fig. 1. DNA synthesis in cultures pretreated with 5-iodo-2′-deoxyuridine or untreated and inoculated with human cytomegalovirus strain AD-169 or growth medium containing the same serum concentration as the virus inoculum. The cells were treated with IdU (100 μg/ml) and then inoculated as described in Table 1. They were then treated for 24-hour periods with [H^3]thymidine (10 μc/ml; specific activity, 17 c/mmole). This figure represents the 48- to 72-hour treatment. The closed circles represent infected cultures, and the open circles represent the sham-infected cultures. The analysis of DNA has been reported (*9*). Cellular and virus DNA were separated by isopycnic banding in CsCl. DNA was precipitated in 5 percent trichloroacetic acid, and the incorporated [^{3}H]dT in the acid-precipitable material was determined (counts per minute) in a liquid scintillation counter.