by King between wobbly and albino (*c*). An F_2 population of 186 young consisted of the following classes:

	C Wo	*C wo*	*c Wo*	*c wo*
Observed	87	41	45	13
Expected	104.6	34.9	34.9	11.6

No indication of linkage is given by this population, since the critical recombination class *c wo* which could arise only from crossover gametes is even slightly greater than expected.

Summary—The mutant gene "wobbly" discovered by Daniels has been shown by linkage tests made by King and Castle to lie in the same chromosome (III) as hairless, with a crossover percentage of 40.3 ± 3.1.

[1] King, Helen Dean, and Castle, W. E., "Linkage Studies of the Rat," *Proc. Nat. Acad. Sci.*, **21**, 390–399 (1935).

[2] King, Helen Dean, and Castle, W. E., "Linkage Studies of the Rat, II," *Ibid.*, **23**, 56–60 (1937).

[3] Castle, W. E., and King, Helen Dean, "Linkage Studies of the Rat, III," *Ibid.*, **26**, 578–580 (1940).

[4] Castle, W. E., "On a Method for Testing for Linkage between Lethal Genes," *Ibid.*, **25**, 593–594 (1939).

[5] Grüneberg, H., "Linkage Relations of a New Lethal Gene in the Rat," *Genetics*, **24**, 732–746 (1939).

THE EFFECT OF ADULT BODY COLOR MUTATIONS UPON THE LARVA OF DROSOPHILA MELANOGASTER

BY KATHERINE S. BREHME

DEPARTMENT OF GENETICS, CARNEGIE INSTITUTION OF WASHINGTON, COLD SPRING HARBOR, N. Y.

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In an organism widely used, as is *Drosophila melanogaster*, for genetical, cytological and embryological experimentation, the effects brought about during development by those mutations which alter the adult phenotype are of importance from both the theoretical and the practical standpoints. From the theoretical point of view, the genetic factors whose effects are recognizable during embryogeny offer rich material for the study of the mechanism of gene action, since observations upon the early action of a gene may provide information which is important in understanding its effect upon the adult. From a utilitarian standpoint, mutants which can be identified during the larval period are useful in classifying the larvae required for salivary chromosome preparations, for transplantation experi-

ments and for studies of physiology and growth. The color of the larval Malpighian tubes of certain eye-color mutants and that of the mouth armature of larvae homozygous for the body color mutation, yellow, is available for use in this manner. Other genetic factors which affect the larva, such as chubby, giant, vestigial and the Minutes, cannot be used for classification because the expression of the mutant character overlaps the wild type or because penetrance is low.

It was found by Brehme[1] in 1937 that the cephalopharyngeal skeleton or "jaws" of the larva homozygous for yellow are lighter in color at all stages from hatching to pupation. The wild-type mouthparts are dark brown; those of the yellow larva are golden in the first instar, and golden brown at the basal region during the second and third instars. Classification of the living larva can readily be made at all stages by means of this difference. Kaliss,[2] in an embryological study of a deficiency, *Df*(1)260–2, in which the yellow and achaete loci are lacking, found that the larvae which are hemizygous for the deficiency and die in the late embryonic stage have yellow mouthparts. In view of these two cases, it has seemed desirable to examine the yellow alleles and other body and bristle color mutants for their effect upon the larval stage.

Methods.—Mass matings were made of each stock under consideration, and eggs were collected over a 24-hour period by a modification of the bottle-cap method of Schweitzer,[3] using a standard banana-agar. The medium on which the eggs had been laid was then placed in Petri dishes containing banana-agar seeded with live yeast suspension. As preliminary observations showed no difference in the color of the mouthparts of optimally fed and of small, inadequately fed larvae, no attempt was made subsequently to control the number of larvae in a culture. All experimental material was maintained at 25°C.

The larvae were examined under a binocular microscope, against a white background. Observations were facilitated when the larvae were immersed in water. Both living and dissected larvae were examined, and permanent mounts in balsam were subsequently made of the mouthparts.

At the moults which signalize the end of the first and second instars, the mouthparts are shed with the hypodermis. Previous to the moult, new mouthparts are formed by hypodermal tissue and underlie the older jaws until the latter are shed. The new mouthparts are colorless from the time of their appearance until the moult; they then darken within a few minutes. In observing the color of the larval jaws, it is therefore essential to examine larvae in which this process of darkening has been completed. For this reason larvae can be studied throughout the first instar but only during the late second and third instars.

Results.—All available mutants were examined which affect adult body or bristle color, and each was compared with larvae from the Florida or

Swedish-*b* wild-type stocks. The larval mouthparts of the wild type are heavily pigmented with what is generally conceded to be melanin. The mouth hooks, mentum and anterior part of the vertical plate (Fig. 1) are very dark brown, almost black; the posterior, basal prongs are less pigmented than the rest and appear golden brown at the tips.

The results are summarized in table 1. Of the mutations which darken adult body color (black, ebony, sable, dusky and speck) only the ebony alleles darken the color of the larval mouthparts. The difference between the ebony and wild-type larvae is not great and is not useful for classification. This result is to be expected, since the wild-type jaws are already very heavily pigmented. It was, however, observed that the sclerotization around the posterior spiracles, which is golden in the wild type, is dark in the ebony mutants at all stages of the larval period. The anterior spiracles, which are sclerotized only in the third instar, are similarly darkened at this time. This darkened color of the spiracle sheaths characterizes the alleles ebony, ebony[4] and ebony[11], and is visible, although the difference is less great, in ebony-sooty. This larval character can be used for classification as early as hatching from the egg. No difference from the wild type in color of the spiracle sheath has been observed in any other mutant.

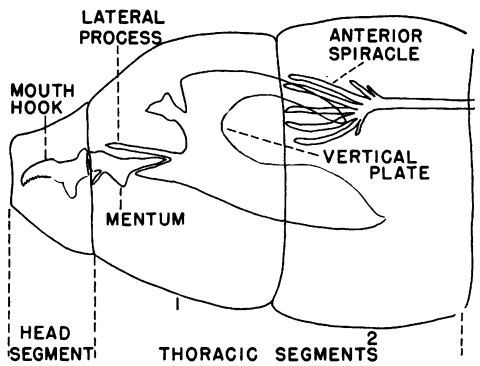

FIGURE 1

Mouthparts of the third instar larva of *D. melanogaster*, adapted from Sturtevant.[4] Lateral view; all parts shown in the diagram are paired.

Certain of the mutations which lighten the body or bristle color of the adult do not affect the larval mouthparts—silver, Blond translocation, straw, straw[4] and yellow^{34c}. In the majority of cases, however, lightened pigmentation of the adult is paralleled by a lighter color of the larval jaws; the tan alleles, straw[2] and straw,[3] and all the yellow alleles except yellow^{34c} produce this effect. The greatest difference from the wild type is seen in the cases of yellow^{35a} and yellow$^{260\text{-}28}$, although it cannot be said that the adults of these genotypes are markedly lighter in color than the other extreme yellow alleles, such as yellow or yellow.[4] One case of factor interaction was studied: the yellow-silver combination, in which the flies are lighter in body color than either yellow or silver, shows a corresponding difference in color of the larval mouthparts, which are lighter than those of yellow.

In general, it may be said that where the difference in color of mutant and wild-type larval jaws is great, classification is possible as early as hatching from the egg; where the difference is slight, only third instar jaws can be classified in the living larva, and in certain cases, classification is reliable only when the mouthparts are dissected from the larva. No sex difference has been observed in the color of the larval mouthparts of any genotype. Although a large number of eye color mutants and other stocks have been examined, no effect on color of the larval jaws has ever been found except in those cases listed in table 1.

Three of the stocks studied, yellow^{31c}, yellow ^{3P} and duplication 260-25*b*, show mottling for yellow in the adult. No indication of mottling was found in the larval mouthparts.

In examining the Malpighian tube color of the larvae of certain eye color mutants, Beadle[5] found one case of maternal or premeiotic effect; homozygous light larvae have colorless tubes if they are the offspring of homozygous light females, wild-type (yellow) tubes if the mothers are heterozygous for light. Each of the yellow alleles listed in table 1 were tested for such an effect by outcrossing to the Swedish-*b* wild-type stock and subsequent backcrossing to yellow; the larvae of the backcross generation segregated for yellow in the expected 1:1 ratio and the color of the larval jaws of the yellow segregants were in all cases indistinguishable from those of the parent yellow stock. The color of the larval mouthparts of the yellow alleles, therefore, does not show a maternal effect.

Discussion.—Melanin production is the end-result of a series of chemical reactions which involve tyrosinase, oxygen and a chromogen, and the amount of melanin produced may be affected through an effect upon any one of these substances, as well as through other factors which influence the reaction. The only quantitative study which has been made of the melanin-producing system in Drosophila is that of Graubard,[6] who measured the relative quantities of extractable tyrosinase in larvae and pupae of the wild-type, yellow, black and ebony genotypes. He found that yellow larvae and pupae do not contain less tyrosinase than the more heavily pigmented stocks; in fact, in the pupal period, the relative tyrosinase content may be indicated as: $y > + > b > e$. By different methods of extraction, he was led to conclude that inhibitors of tyrosinase activity are present in both larval and pupal stages, and that the production of melanin is not so much a question of the presence or absence of the enzyme or its quantity as of an internal environment which allows the enzyme to take part in the reaction. No study has been made of the oxygen consumption of the larvae and pupae of the body color mutants, nor of the nature of the chromogen involved in melanin production. The body color mutants may therefore have their effect on pigmentation through inhibitors of tyrosinase activity, through the rate of oxygen uptake, through some aspect of the

TABLE 1

THE EXPRESSION IN THE ADULT AND LARVAL STAGES OF MUTATIONS AFFECTING PIGMENTATION

LOCUS	MUTANT	ADULT BODY COLOR	ADULT BRISTLE COLOR	LARVAL SPIRACLE COLOR	LARVAL MOUTHPARTS	POSSIBILITY OF CLASSIFICATION IN LARVAL STAGE
black	*b*	Dark	+	+	+	Not classifiable
ebony	*e*	Very dark	+	Dark, sooty	Darker than +; almost no brown showing	Classifiable by spiracle sheath
	e^4	Very dark	+	Dark, sooty	Like *e*	Like *e*
	e^{11}	Very dark	+	Dark, sooty	Like *e*	Like *e*
	e^s	Dark	+	Slightly dark	Like *e*	Like *e*
sable	*s*	Dark	+	+	+	Not classifiable
	s^2	Dark	+	+	Probably +	Not classifiable
dusky	*dy*	+ but wings dark	+	+	+	Not classifiable
speck	*sp*	Dark	+	+	+	Not classifiable
	sp^2	Dark	+	+	+	Not classifiable
tan	*t*	Yellower than +, antennae light	+	+	Basal prongs light, difference slight	Difficult in dissected larva
	t^2	Slightly darker than *t*	+	+	Like *t*	Like *t*
	t^3	Lighter than +	+	+	Like *t*	Like *t*
suppressor of tan	*su-t t*	+	+	+	+	Not classifiable
straw	*stw*	Probably +	Light at tips, hairs yellowish	+	May be slightly lighter than + at prongs	Not classifiable
	stw^2	Yellowish	Brownish with light tips, hairs yellowish	+	Basal prongs strawish	Difficult in living larva
	stw^3	Yellowish	Hairs and bristles straw yellow	+	Basal prongs straw, vertical plate light	Classifiable in living larva
	stw^4	Very pale	Nearly black	+	+	Not classifiable
silver	*svr*	Pale, silvery	Dark	+	+	Not classifiable
Blond	*T*(1; 2)*Bld*	+	Yellow tips	+	+	Not classifiable
yellow	*y*	Yellow	Brown, yellow tips	+	Mouth hooks dark, rest brown, prongs golden	Classifiable in living larva
	y^2	Yellow	Black	+	Basal prongs lighter, rest dark	Not classifiable
	y^3	Tannish yellow	Black or nearly so	+	Basal prongs golden, lateral process and hooks light	Difficult in living larva
	y^4	Yellow	Brown, yellow tips	+	Mouth hooks dark, rest brown, prongs golden	Classifiable in living larva

	y^{6}	Yellow	Brown, yellow tips	+	Mouth hooks dark, rest brown, prongs golden	Classifiable in living larva
	y^{1S}	Yellow	Brown, yellow tips	+	Mouth hooks dark, rest golden brown	Classifiable in living larva
	y^{2S}	Tanner than *y*	Not quite black	+	Mouth hooks and mentum dark, rest golden brown	Classifiable in living larva
	y^{3P}	Tannish	Black	+	Basal prongs more golden, rest dark	Difficult in living larva
	y^{3d}	Tannish yellow	Black or nearly so	+	Golden brown, mouth hooks light	Classifiable in living larva
	y^{td}	Tan, antennae light	Brown	+	Golden brown, mouth hooks dark	Classifiable in living larva
	y^{r2}	Tan	Black	+	Basal prongs slightly lighter than +, rest dark	Not classifiable
	y^{34c}	Tannish	Black	+	+	Not classifiable
	y^{35a}	Yellow	Brown, gold tips	+	Golden, mouth hooks very light	Classifiable in living larva
	y^{31b}	Yellow	Brown, gold tips	+	Base golden, mentum light, mouth hooks slightly light	Classifiable in living larva
	y^{31c}	Yellow	Black, mutating to *y ac*	+	Basal prongs light, mouth hooks dark	Difficult in living larva
	y^{31d}	Yellow	Black	+	Basal prongs light, mouth hooks dark	Difficult in living larva
	y^{260-28}	Yellow	Golden	+	Basal prongs light, rest dark	Difficult in living larva
Deficiency affecting yellow*	*Df*(1)260-10	Yellow	Light	+	Base very light, vertical plate light, mouth hooks slightly light	Classifiable in living larva
yellow duplication†	*Dp*(1)260-25*b*	Yellow brown	Gold tips, mottling	+	Basal prongs light brown, rest dark	Difficult in living larva
yellow deficency	*Df*(1)260-2	Dies in embryonic stage			Yellow (Kaliss[2])	
yellow silver		Very light	Yellow	+	Lighter than *y*	Classifiable in living larva

* *Df*(1)260-10 lacks bands 1*A*1-2 of the salivary chromosome; the yellow locus, which is not included in the deficiency, is modified through a position effect to produce the yellow phenotype.

† The stock of *Dp*(1)260-25*b* is homozygous for the yellow mutation and also for a duplication carrying the wild-type alleles of *y* and *ac* inserted at the right of *bb* and causing mottling for yellow.

chromogen or through changes in the internal environment of the larva or pupa.

Graubard found by extraction of tyrosinase with chloroform that larvae contain the enzyme and a substrate, pupae only the enzyme, while no enzyme is present in the fly. It is thus probable that at least the early steps of formation of adult pigment take place in the larval and early pupal stages. It is not, therefore, surprising that genetically controlled differences in adult pigmentation are frequently paralleled by differences in larval pigmentation.

Certain of the mutations which lighten pigmentation affect three characters, body color, bristle color and color of the larval mouthparts. Others affect only two of the characters, while several mutants affect only one. The following relations may be derived from table 1:

Body color, bristles and larval mouthparts affected: *stw*^{2}, *stw*^{3}, *y*, *y*^{4}, *y*^{6}, *y*^{1S}, *y*^{2S}, *y*^{td}, *y*^{35a}, *y*^{31b}, *y*$^{260\text{-}28}$, *Dp*(1)260-25*b*, *Df*(1)260-10.
Body color and bristles affected, not larval mouthparts: none.
Body color and larval mouthparts affected: *t*, *t*^{2}, *t*^{3}, *y*^{2}, *y*^{3}, *y*^{3P}, *y*^{3d}, *y*^{v2}, *y*^{31c}, *y*^{31d}.
Body color only affected: *stw*^{4}, *svr*, *y*^{34c}.
Bristles and larval mouthparts affected: *stw* (slight effect).
Bristles only affected: Blond.
Larval mouthparts only affected: none.

These relations indicate that when the processes of melanin production are sufficiently upset to affect both adult body and bristle color, the effect begins early and influences larval melanin also; an effect on body color but not on bristle color may also be accompanied by an effect on larval melanin. In most cases, a considerable upset appears necessary to influence the deposition of the pigment as early as the larval stage. It would seem, however, that bristle color is less often affected by genetic agencies than either body color or color of the larval jaws. This may mean that less pigment-forming material is necessary for pigmentation of the bristles or, as is more likely, that bristle pigment is laid down at a time particularly favorable for melanin production.

Summary.—Pigmentation of the larval mouthparts and spiracle sheaths has been studied in the mutations of *D. melanogaster* which influence adult body or bristle color. Of the mutants characterized by darkened adult pigmentation, only the ebony alleles were found to increase pigmentation of the larval mouthparts. The ebony alleles considerably darken the spiracle sheaths at all stages of larval development; no other genetic factors were observed to have this effect. In general, the mutants whose adult body color is lighter than wild type are characterized by lighter pigmenta-

tion of the larval mouthparts. Bristle color appears to be affected less often than either body color or mouthpart pigmentation.

[1] Brehme, K. S., *Proc. Soc. Exp. Biol. Med.*, **37**, 578–580 (1937).
[2] Kaliss, N., *Genetics*, **24**, 244–270 (1939).
[3] Schweitzer, M. D., *Drosophila Information Service*, **6**, 19 (1936).
[4] Sturtevant, A. H., *Pub. Carnegie Inst.*, **301**, 150 pp. (1921).
[5] Beadle, G. W., *Amer. Nat.*, **71**, 277–279 (1937).
[6] Graubard, M., *Jour. Genet.*, **27**, 199–218 (1933).

THE SPERMATOPHORES OF TRITURUS TOROSUS AND TRITURUS RIVULARIS

By ROBERT EMRIE SMITH

DIVISION OF PHYSIOLOGY, MEDICAL SCHOOL, UNIVERSITY OF CALIFORNIA

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During the course of studies on the breeding activities of the western newts[1] the writer has had occasion to observe the spermatophores of *Triturus torosus* (Rathke) and of the closely related *Triturus rivularis* (Twitty). Concerning the latter, Twitty[2] gives incidental reference to the deposition of a spermatophore by a captive male. However, since there is in the literature no other allusion to the spermatophore either of this species or of *T. torosus*,* the following observations appear pertinent.

It is known that the glands in the cloaca produce the secretions which form the spermatophores. Moreover, these glandular products may be traced by their staining reactions and composition from the glands of origin directly to the spermatophore. In *Triturus torosus* the pelvic glands in the roof of the cloaca produce a coarse granular secretion which is deeply eosinophilic in prepared material but opaque white in the fresh condition. Its function has been described for other species (cf. Noble,[3] p. 287, and Noble and Brady[4]) as being that of agglomerating the sperm cells into a mass, or sperm capsule, and subsequently of attaching this mass to a gelatinous supporting structure, the spermatophore stalk. Studies of the cloaca and sperm mass in *T. torosus* disclose that in this species also the secretions of the pelvic gland operate in holding the sperm together and in attaching the oblong mass to the apical horn of the stalk. This is further shown in the stained spermatophore, where the pelvic secretion may be seen covering the tip of the apical horn.

The arrangement of the sperm cells within the mass is of some interest and may be contrasted with that observed by Noble and Brady (loc. cit., p. 104) in *Ambystoma opacum*. Here it is stated: "The spermatozoa are spread over the top of the spermatophore. A remarkable feature is that