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Developmental Study of the Tumorous-head Strain of Drosophila Melanogaster

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DEVELOPMENTAL STUDY OF THE TUMOROUS-HEAD STRAIN OF *DROSOPHILA MELANOGASTER*

BY

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THESIS

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INTRODUCTION

The original sample, from which all existing tumorous-head strains of Drosophila melanogaster were derived, was collected at Acahuizolta, Mexico in 1941 by field workers from the University of Texas. Amorphous head growths were first observed in 1945. In 1946 the strain was sent to the University of Utah where it was given the descriptive name "tumorous-head", symbolized tuh (Woolf 1965). Gardner and Woolf (1949) demonstrated that the tumorous-head trait in Drosophila melanogaster was caused by a third chromosome, semidominant gene symbolized tu-3, later mapped to position 58.5± (Gardner 1959). A chromosomal dimorphism exists in the tumorous-head strain (Woolf and Phelps 1960). Two types of third chromosome are involved, symbolized 3A and 3B. Both chromosomes 3A and 3B carry the mutant gene tu-3 on their right arms, the variation between them lies in the genetic composition of their left arms. Chromosome 3B, unlike chromosome 3A, carries a large paracentric inversion called the Payne inversion, symbolized (In(3L)P), and the recessive gene for scarlet eyes (st) on its left arm (Woolf and Phelps 1960). Since the 3B chromosome is a homozygous lethal, the tuh strain consists of homokaryotypes (3A/3A) and heterokaryotypes

(3A/3B). In order to distinguish homokaryotypes and heterokaryotypes phenotypically, the recessive gene h (hairy), position 26.5, was inserted into the left arm of chromosome 3A (Woolf, Knowles, and Jarvis 1964). Over 80% of the flies maintained in laboratory stock bottles and population cages are heterokaryotypes (Woolf and Church 1963; Kuhn 1970). Years of inbreeding and incorporation of modifying genes that increase the penetrance of the tumorous-head trait, have reduced the general fitness of the strain. Natural selection, under laboratory conditions, would favor any genetic system insuring at least minimal levels of heterozygocity. One such system involves a reduction in homokaryotype fitness, with the result being an increase in the proportion of flies heterozygous for In(3L)P (Kuhn 1970). Woolf and Church (1963) demonstrated that male and female homokaryotypes are less viable than heterokaryotypes. The left arm of chromosome 3A is responsible for the reduced longevity of homokaryotype females (Kuhn 1970). Knowles (1967) found that the increased productivity of female heterokaryotypes, over homokaryotypes, was attributable to their superior fecundity and fertility. The genes reducing homokaryotype fecundity are located near roughoid (0.0) in the left arm of chromosome 3A. Reduced homokaryotype fertility is produced by an interaction between a maternal effect associated with

chromosome 2 and genes in the left arm of chromosome 3A (Woolf and Knowles 1964; Knowles 1967; Kuhn 1970). Heterosis brought about by heterozygocity for the Payne inversion buffers development in the presence of the genetic mechanism producing the abnormal development in the head region (Woolf and Lott 1965). Also, the presence of the Payne inversion has led to the establishment of a natural heterosis mechanism by suppressing cross overs in the left arm of the third chromosome (Woolf and Phelps 1960). Another genetic system is responsible for a sex ratio in favor of males found in most tumorous-head strains. This system is controlled by an interaction of polygenes located on chromosome 2 with genes located in the left arm of chromosome 3B, probably In(3L)P, st (Kuhn 1971). The heterokaryotypic females show a maternal effect that reduces the number of each sex differentially, so that the number of adult females produced is always less than the number of adult males (Kuhn 1971).

Penetrance of the tu-3 gene is increased by a maternal effect controlled by a sex-linked recessive gene, symbolized tu-1 (Gardner and Woolf 1949) at 65.3 (Woolf personal communication) on the X chromosome. A maternal effect is the direct influence of a mother's genes in predetermining the expression of the trait in its progeny. In addition, Woolf (personal communication) has mapped a

gene that enhances tu-1 to the same region as tu-3. Other genetic modifiers, which further increase penetrance and expressivity of the tumorous-head trait, have been found in both laboratory and natural populations (Gardner, Stott and Deardon 1952). Environmental factors, such as quantity and quality of food, crowding conditions, and temperature have been shown to influence penetrance.

Gardner and Woolf (1950) demonstrated that an increase in temperature enhanced the maternal effect of tu-1 and thus increased tumor penetrance. Temperature was shown to have no influence upon the action of tu-3 in the absence of tu-1 (Gardner and Woolf 1950). Gardner and Ratty (1952) indicated that there was also a maternal effect associated with viability as well as penetrance. The gene tu-1 which controls the maternal effect was found to have no influence on the viability of flies when found alone at 21°C or at 30°C (Gardner and Ratty 1952). A marked differential sex viability was observed in the tumorous-head stock following a 30°C heat treatment during an early developmental stage (Gardner and Woolf 1949). In tuh, females express the greatest penetrance and expressivity at temperatures above normal (Gardner and Woolf 1949). A great proportion of abnormal flies, mainly females, can not survive the temperature treatment (Gardner and Ratty 1952).

Temperature effective periods for the expression of many mutant characters and phenocopies have been described (Gardner and Woolf 1950; Vogt 1946; Child, Blanc, and Plough 1940). Most of these temperature sensitivities have been identified as occurring during the later portion of the larval-developmental period. Gardner and Woolf (1950) demonstrated an early temperature effective period during the first 24 to 48 hours of development associated with the tumorous-head maternal effect. This early period seems to represent the stage of development when basic substances are being elaborated in the egg (Gardner and Woolf 1950).

Drosophila melanogaster is an insect with 4 major developmental stages. According to Strickberger (1962), at 25°C, embryonic development persists for about 22 hours after fertilization; followed by a larval stage of approximately 5 days, consisting of three instars; then a pupal stage which lasts for about 4 days; finally, an adult fly ecloses from its pupal case. Temperature can influence the length of time spent in each developmental stage. During the final metamorphosis many larval tissues break down and most portions of the adult's surface are formed from small structures in the larva called imaginal disks. Each disk is a small group of cells set aside early in embryogenesis which is determined to develop during meta-

morphosis into a particular adult structure: wing, leg, or antenna, for example. At the time of determination, each disk consists of about 10 to 40 cells (Nothiger 1972). During the ensuing three larval stages, the disks proliferate and by third instar each disk consists of several thousand cells. Each individual cell within the disk is determined (Fristrom 1970). Bryant and Schneiderman (1969), using genetic mosaics, found that the primorda for legs are present, in groups of approximately 20 cells, 3hours after fertilization. This observation supports the conclusion that the developmental fate of imaginal disks is determined 3-7 hours after fertilization (Fristrom 1970). Disk cells remain undifferentiated and never form a functional part of the larva. Despite their proliferation, each disk maintains its determined state and proliferates appropriately during metamorphosis. Hadorn (1966), testing the capacity of disk tissue to maintain its determined state for a very long period of time, found that whatever the molecular carriers of determination of the disk may be, the determined state is a persistently regenerated, self-maintained state. Hadorn discovered that tissue lines initially derived from one type of disk sometimes transdetermined and gave rise to adult tissues normally derived from another disk (Hadorn 1966). A transdetermination is an epigenetic change which causes cells

to alter their developmental program (Hadorn 1965). Hadorn and his co-workers (1966) found that each type of transdetermination occurs with a characteristic probability per generation. Evidence that transdetermination is not due to somatic mutation was provided by the discovery that transdetermination occurs simulataneously in groups of contiguous cells not clonally related (Gehring 1967) and is too frequent to be due to mutation. Kauffman (1973) proposed a transdetermination flow pathway, a systematic theory for major aspects of imaginal disk determination and transdetermination in Drosophila melanogaster. For example, genital disks transdetermine into antennal or leg structures; antennal or leg disks transdetermine into wing structures; wing disks transdetermine into mesothorax structures. Genital disks do not transdetermine directly into either wing or mesothorax structures. Kauffman's flow pathway uses a distance measure (a measure of the degree of difference) between the states of determination in various disks. Whatever the molecular circuits that maintain determination, a plausible control circuit would require the property of possessing sharply distinct alternate states. A normal characteristic of disk cells is an ability to modify continually their developmental fates (Fristrom 1970). In this context, transdetermination may be viewed as an

extension of the normal developmental capacity of disk cells so that cells can change their developmental assignments to different states (Fristrom 1970). The natural description of the determined state of each disk consists of specifying which alternate state of each circuit occurs in that disk. If more than one circuit is involved, then the state of determination in each disk is characterized by the combination of states specified by the different circuits. Kauffman (1973) predicted a symetric distance measure between the states of determination in various disks consisting of the number of circuits that must change state in order to change the determined state of one disk into the determined state of another disk. Kauffman (1973) maintained that determination is underlaid by a number of circuits, each with only two alternate states, one considerably more stable than the other, that there are at least four such circuits, and that one could make tentative state assignments for all the circuits in each disk.

The tumorous-head trait of Drosophila melanogaster is classified as a homoeotic mutant. According to Postlethwait et al. (1972), one of the most interesting and potentially informative classes of mutants in insects comprises those which result in homoeotic changes. In such a mutant, a particular organ is transformed partially

or completely into an organ normally found in a different area of the body. Gehring (1970) suggested that many homoeotic mutants are yet to be found because they result in lethal developmental defects. There is evidence indicating that one of the effects of a homoeotic mutant is to bring into action other genes - genes not normally expressed, in the affected imaginal disk (Waddington 1953). If the developmental alteration in a homoeotic mutant is essentially the same phenomenon as transdetermination, then the initial action of a homoeotic mutation could be to encourage transdetermination to occur at a high rate in a specific direction (Postlethwait et al. 1972). According to Kauffman's model, a homoeotic mutant would be involved with the initial setting of each circuit in the appropriate state for each disk (Postlethwait and Schneiderman 1973). Newby (1949) described external as well as internal alterations in the head region of tumorous-head flies. Some of the alterations reported appeared to be leg-like projections from the antenna. Postlethwait et al.(1972) later reexamined the tumorous-head stock and observed leg structures in place of antennal segments; structures that looked like abdominal tergites projecting from the eye; and genital structures projecting from the region between the eye and the proboscis, referred to as the rostralhaut. Kauffman, considering the findings of

Postlethwait et al. (1972), characterized the tumorous-head homoeotic mutant as demonstrating coordinated alterations from more than one disk to more than one target disk. His model predicted a coordinated increase in transdetermination from antenna to genitalia and also to leg. Furthermore, transition to leg should occur more frequently then to genital structures. Leg structures do occur, and are more common than genital structures, in place of antennal derivatives in adult tumorous-head flies (Postlethwait et al. 1972). Kauffman's model also speculates that the transdetermination frequency from wing to leg should occur in tuh flies where penetrance and expressivity are high enough.

Postlethwait and Schneiderman (1973), in a review of Kauffman's bistable memory circuits model, emphasized that Kauffman had developed a model with real predictive values. The most attractive feature of his model was its testability with regard to transdetermination frequency in homoeotic mutants and occurence of mutations that affect particular subsets of disks; a drawback was that his model provided no biochemical basis for transdetermination and homoeotic mutants. Postlethwait and Schneiderman (1973) regard homoeotic mutants and transdetermination as genetic and epigenetic alterations of the same regulatory system.

This study of the homoeotic effects of the tumorous-head phenotype was initiated in order to: (1) investigate the quantitative and qualitative effects of the In(3L)P, st upon specific head transformations; (2) determine the differential effects of the addition of a gene which enhances the tumorous-head maternal effect; (3) demonstrate types of transformations in the head region when penetrance and expressivity are high; (4) test the Kauffman (1973) prediction that transformations from wing to leg should be observed in flies displaying high penetrance and expressivity of the tumorous-head trait; (5) study the relative changes in viability, and penetrance of the tumorous-head trait when exposed to a cold shock during the temperature sensitive, early developmental stages; (6) study the daily development of the tumorous-head phenotype at 25°C. Due to the nature of the techniques involved, part I of the thesis deals with objectives (1)-(4); part II deals with objectives (5) and (6).

Part I

Homoeotic Effects of the Tumorous-Head Phenotype and the Differential Effect Due to the Addition of an Enhancer Gene in a Tumorous-Head Strain of *Drosophila Melanogaster*.

The first portion of the study of the tumorous-head phenotype was initiated in order to investigate the quantitative and qualitative differences in the expression of the tumorous-head trait between homokaryotypes (h 3A/h 3A) and heterokaryotypes (h 3A/3B), and then to determine the influence exerted on the tumorous-head phenotype due to the addition of a gene which enhances the tumorous-head maternal effect of tu-l. Next, using flies displaying high penetrance and expressivity of the tumorous-head trait, Kauffman's (1973) prediction that transformations from wing to leg should be observed was tested.

MATERIAL AND METHODS

The tumorous-head strain of Drosophila melanogaster originally maintained at Arizona State University, symbolized tuh(ASU), due to its known high tumor penetrance, was selected for study. The strain was propogated on standard Drosophila medium at 25°C by selecting approximately 75 males and 75 females demonstrating the tumorous-head trait. To increase penetrance, females carrying a 3rd chromosome gene, mapped to the same region as tu-3 and known to enhance the tumorous-head maternal effect (Woolf personal communication) was utilized. Females of this strain possess attached X chromosomes homozygous for tu-1, a free Y chromosome, 2nd and 4th chromosomes from a Stevensville laboratory strain, and Stevensville 3rd chromosomes homozygous for the enhancer of tu-1. The genetic composition of the females is: ♀♀ v tu-1/Y; $2^S/2^S$; ru E(tu-1) ca/ ru E(tu-1) ca; $4^S/4^S$. These females were mass mated to tuh(ASU) males of the following genetic composition: ♂♂ 1/Y; 2/2; h 3A/3B; 4/4. The resulting progeny of the cross were: ♀♀ v tu-1/Y; $2^S/2$; ru E(tu-1) ca/ h 3A or 3B; $4^S/4$, ♂♂ 1/Y; $2^S/2$; ru E(tu-1) ca/h 3A or 3B; $4^S/4$.

Heads and bodies of flies expressing the tumorous-

head trait, including homokaryotypes, heterokaryotypes, and flies carrying the enhancer gene, were collected and then digested in a 5% KOH solution leaving only exoskeleton. Each fly was placed on a glass slide and examined, using a dissecting scope, for abnormalities of the head and wing regions. The head was separated away from the body and carefully positioned so that the maximum number of alterations could be studied, then the specimen was mounted in lactophenol, covered with a cover slip and examined at X400 magnification. The total number of transformations per head was calculated from the sum of the distinct number of abnormalities observed on the head.

RESULTS

From a sample of 2509 flies, collected over 8 generations, the penetrance of the tumorous-head trait in the tuh(ASU) stock was calculated as 84.9%. From a sample of 9,587 flies, the progeny of a cross between the tu-1 enhancer gene and the tuh(ASU) males, the average penetrance was calculated as 93.9% (Table 4).

The bodies and heads of the flies expressing the tumorous-head trait were examined by the technique already described. Plate I demonstrates transformations found in the tuh(ASU) stock previously recognized and described by Postlethwait et al. (1972). However, in their study of the tumorous-head trait they used a tumorous-head stock of Drosophila melanogaster maintained at the California Institute of Technology, symbolized tuh(CT). The tuh(ASU) transformations represented in Plate I are: Figure 2 eye tissue transformed to abdominal tissue; Figure 5 rostral-haut transformed to genital tissue; Figure 8 antennal structures transformed to leg structures. Plate II demonstrates transformations found that previously were ur-reported as occurring in tumorous-head flies. The transformations represented are: Figure 2 eye tissue transformed to genital appearing tissue; Figure 3 rostralhaut tissue

PLATE I

Figures 1,2,3 (Figure 1) Wild-type eye X540. (Figure 2) Tumorous-head eye with a structure resembling an abdominal tergite X2240. (Figure 3) Wild-type lateral plate X2080. E, eye; AT, abdominal tergite.

Figures 4,5,6 (Figure 4) Wild-type rostralhaut X540. (Figure 5) Rostralhaut from which a structure appearing like a lateral plate extends X1920. (Figure 6) Wild-type lateral plate.X3120. R, rostralhaut; E, eye; LP, lateral plate.

Figures 7,8,9 (Figure 7) Wild-type antenna X740. (Figure 8) The 3rd antennal segment of a tumorous-head female fly from which a structure appearing like leg extends X528. (Figure 9) Wild-type female leg X333. R, rostralhaut; E, eye; L, leg; Ar, aristi; Al, All, Alll, first second, and third antennal segments.

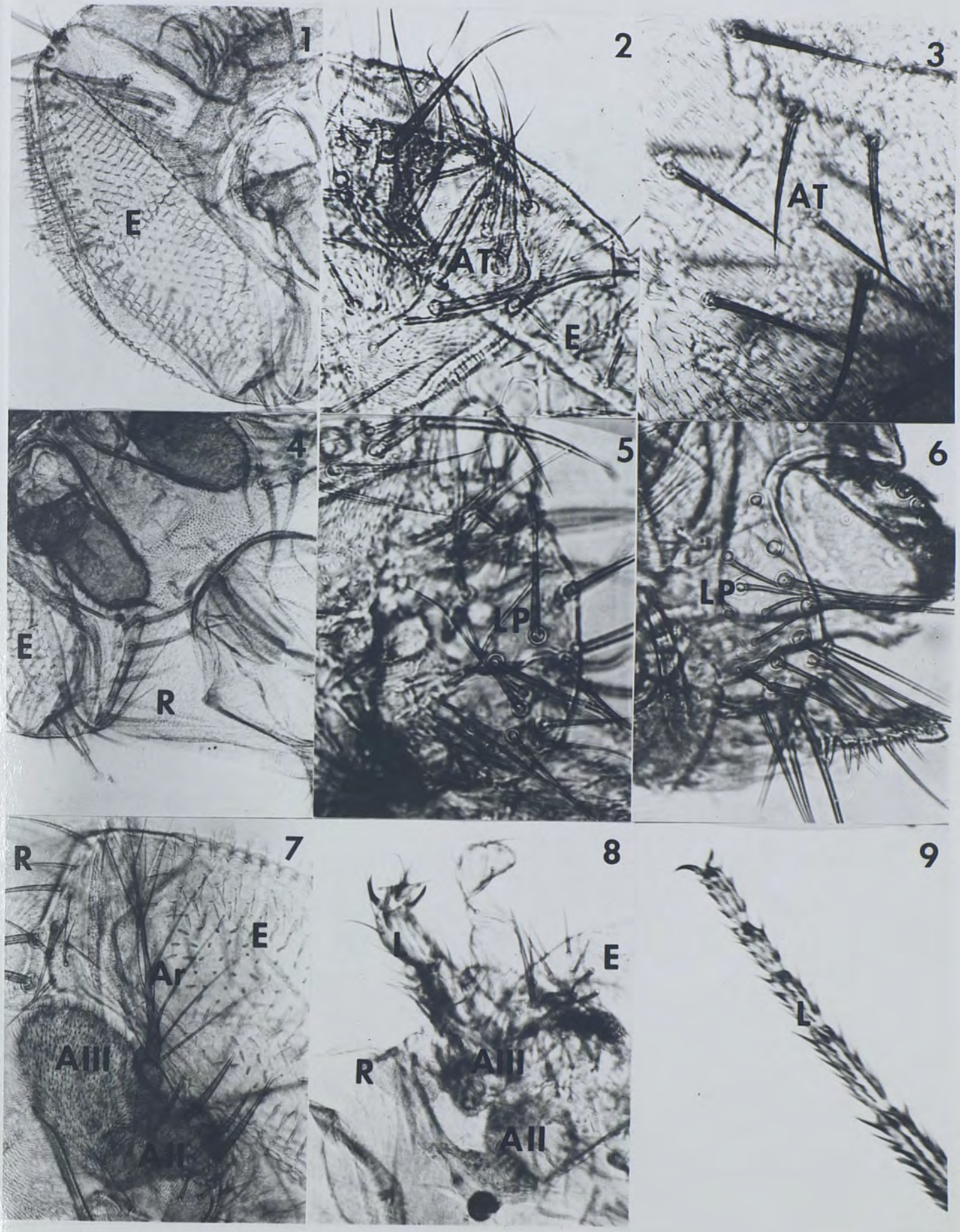

1
E
2
AT
E
3
AT
4
E
R
5
LP
6
LP
7
R
E
Ar
AIII
AII
8
L
E
R
AIII
AII
9
L

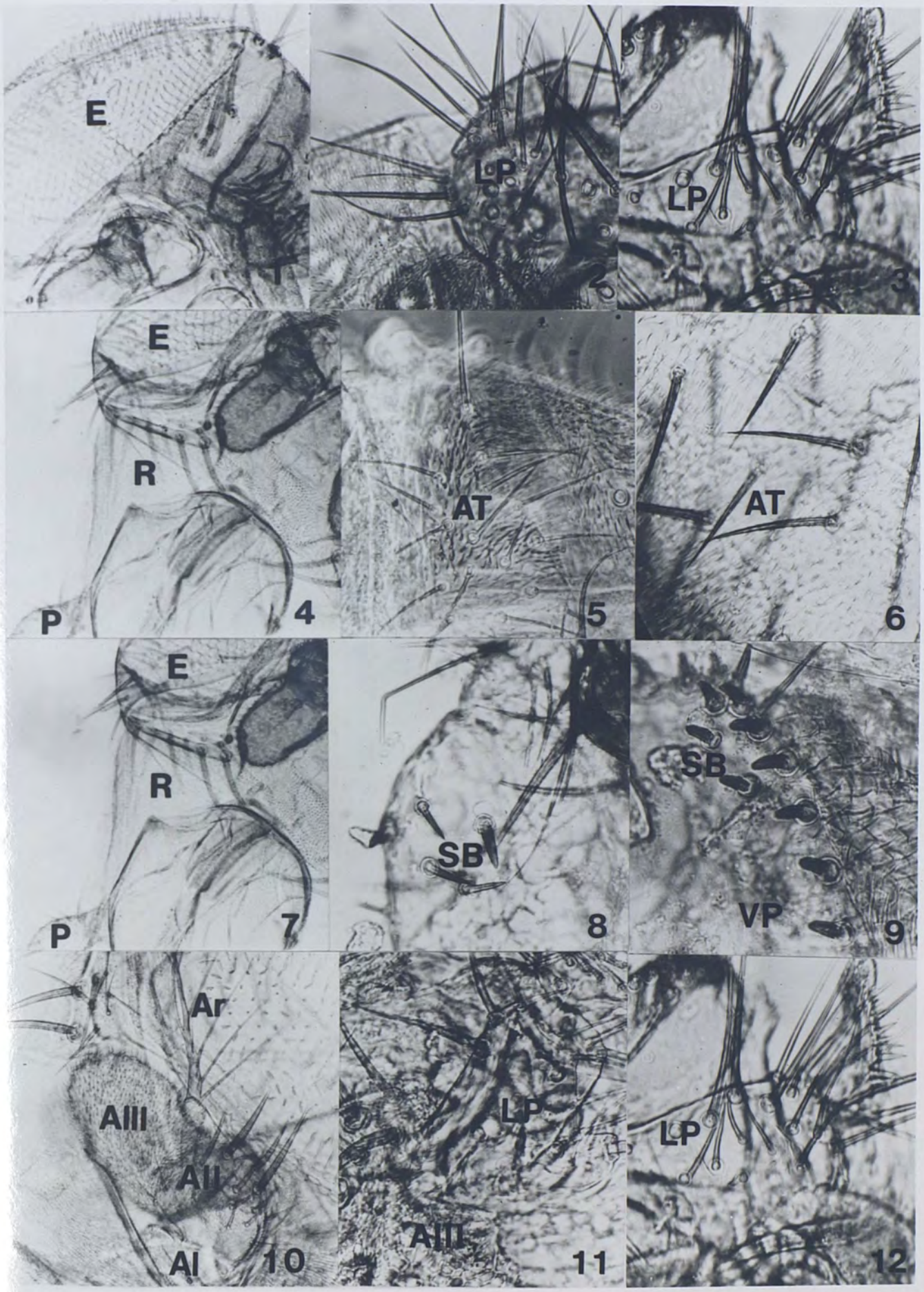
PLATE II

Figures 1,2, and 3. (Figure 1) Wild-type eye X360. (Figure 2) Tumorous-head eye with a structure resembling the lateral plate X1920. Since the eye of this male was almost entirely absent, no eye facets can be observed. (Figure 3) Wild-type lateral plate X1600. E, eye; Lp, lateral plate.

Figures 4,5, and 6. (Figure 4) Wild-type rostralhaut X360. (Figure 5) Rostralhaut of a female tumorous-head fly under phase contrast X1360, in which a structure resembling an abdominal tergite appears. (Figure 6) Wild-type abdominal tergite X1520. E, eye; R, rostralhaut; P, palpus; AT, abdominal tergite.

Figures 7,8, and 9. (Figure 7) Wild-type rostralhaut X360. (Figure 8) Rostralhaut of a tumorous-head female in which bristles resembling spiked bristles of the vaginal plate appear X1520. (Figure 9) Wild-type female vaginal plate with spiked bristles X2080. E, eye; R, roatralhaut; P, palpus; VP, vaginal plate; SB, spiked bristles.

Figures 10, 11, and 12. (Figure 10) Wild-type antenna X560. (Figure 11) The 3rd antennal segment of a tumorous-head fly from which a structure appearing like a lateral plate extends X1520. (Figure 12) Wild-type lateral plate X2080. Al, All, Alll, first, second, and third antennal segments; Ar, aristi; LP, lateral plate.


E
1
LP
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transformed to abdominal appearing tissue; Figure 8 rostralhaut tissue transformed to spiked bristles resembling those found on the female vaginal plate; Figure 11 antennal structures transformed to genital-like tissue.

The total number of transformations per head was calculated from the sum of the distinct number of abnormalities observed on the head. More than one abnormality could be located in one transformed area. The values presented in Tables 1,2, and 3 probably represent underestimates of alteration expressivity since it would be quite possible for a fly to have its entire eye region transformed into another tissue and yet have the alteration recorded as only one transformation. In other words, the recorded values for the number and types of tumors per head do not indicate the extensiveness of the transformation, they only reflect the numbers and types of observable and recognizable abnormalities.

Homoeotic effects of the tu-1; tu-3 combination among tuh(ASU) heterokaryotypes are presented in Table 1 (pg. 19). Tissue transformations were observed in the eye and head cuticle, in the antenna, in the rostralhaut, and in the proboscis. Transformations involving eye tissue represented an average of 63.8% of all transformations observed, 61.9% in the males and 65.6% in the females. Transformations from eye to abdominal tergites

TABLE 1

Homoeotic effects of the tumorous-head trait in tuh(ASU) heterokaryotypes.

Structure Transformed	ALTERATIONS					
	MALES		FEMALES		TOTAL	
	#of	%of total	#of	%of total	#of	%of total
EYE to						
Abdomen	156	34.1	171	37.4	327	35.8
Unidentified	114	25.0	119	26.0	233	25.5
Genital	9	02.0	9	02.0	18	02.0
Antenna	1	00.2	1	00.2	2	00.2
Palpus	3	00.7	0	00.0	3	00.3
TOTAL EYE ALTERATIONS	283	61.9	300	65.6	583	63.8
ANTENNA to						
Leg	7	01.5	9	02.0	16	01.8
Unidentified	16	03.5	22	04.8	38	04.2
Genital	3	00.7	1	00.2	4	00.4
Antenna Missing	2	00.4	4	00.9	6	00.7
Aristi Missing	8	01.8	11	02.4	19	02.1
Aristi Misshaped	9	02.0	21	04.6	30	03.3
Aristi Duplicated	1	00.2	1	00.2	2	00.2
TOTAL ANTENNAL ALTERA.	46	10.1	69	15.1	115	12.6
ROSTRALHAUT to						
Abdomen	7	01.5	7	01.5	14	01.5
Unidentified	55	12.0	40	08.8	95	10.4
Genital	17	03.7	8	01.8	25	02.7
Antenna	2	00.4	2	00.4	4	00.4
Palpus	1	00.2	2	00.4	3	00.3
TOTAL ROSTRAL. ALTERA.	82	17.9	59	12.9	141	15.4
PROBOSCIS to						
Palpus Missing	34	07.4	17	03.7	51	05.6
Palpus Modified	10	02.2	12	02.6	22	02.4
Palpus Doubled	2	00.4	0	00.0	2	00.2
TOTAL PROBOSCIS ALTERA.	46	10.1	29	06.3	75	08.2
TOTAL # OF ALTERATIONS	457		457		914	
# OF HEADS EXAMINED	214		218		432	
AVERAGE # OF ALTERATIONS PER HEAD	2.14		2.10		2.12	

and to unidentifiable tissue represented the majority of transformations with other eye tissue transformations such as eye to genital tissue (Plate II, Figure 2), to antennal structures, and to palpus occurring at lower frequencies. Alterations involving the various segments of the antenna represented 12.6% of all heterokaryotypic transformations, 10.1% in males and 15.1% in females. Listed by descending order of occurrence the antenna was transformed to: unidentifiable tissue; leg-like structures; antenna missing; genital tissue. Alterations involving only the aristi of the antenna were: misshaped aristi; aristi missing; aristi duplicated. Alterations involving the rostralhaut region occurred at an average frequency of 15.4%, 17.9% in males and 12.9% in females. The most frequent rostralhaut transformation was to unidentifiable tissue, with transformations to genital structures, abdominal tergites (Plate II, Figure 5), antennal segments, and palpus occurring less frequently. The remaining alterations occurred in the proboscis, 8.2% was the average frequency, 10.1% in the males and 6.3% in the females, and involved only changes of the palpus. Alterations of the palpus were: palpus missing; palpus modified; palpus doubled. A total of 916 alterations, 457 in males and 459 in females, were observed. An average of 2.14 alterations occurred in each of the 214 male heads ex-

amined and an average of 2.10 alterations per head in each of the 218 females examined, producing an overall average of 2.12 alterations per head. No significant difference in the average number of alterations exists between male and female heterokaryotypes ($\chi^2 = 0.025$; $0.90 > P > 0.80$).

The homoeotic effects of the tumorous-head trait observed among the homokaryotypic males and females are presented in Table 2 (pg.22). Transformations involving only the eye region represented 67.0% of all alterations, 64.6% in males and 69.5% in females. Within the eye, transformations to abdominal tissue and to unidentifiable tissue were the most frequent alterations observed, eye to genital tissue and to antennal segments occurred at lower frequencies. Alterations involving the antenna had an average frequency of 9.7%, 11.2% in males and 8.1% in females. The most common antenna alterations involved the aristi - aristi misshaped and aristi missing. At lower frequencies the following alterations were observed: aristi replaced by leg-like structures; unidentifiable alterations; antenna missing. Transformations of the rostralhaut region accounted for 16.9% of all observed transformations, 18.0% in males and 15.7% in females. The most frequent transformation within the rostral-haut region involved changes to unidentifiable tissue.

TABLE 2

Homoeotic effects of the tumorous-head trait in tuh(ASU) homokaryotypes.

Structure Transformed	ALTERATIONS MALES #of	MALES %of total	FEMALES #of	FEMALES %of total	TOTAL #of	TOTAL %of total
EYE to						
Abdomen	68	33.0	69	35.0	137	34.0
Unidentified	60	29.1	61	31.0	121	30.0
Genital	3	01.5	6	03.0	9	02.2
Antenna	2	01.1	1	00.5	3	00.7
TOTAL EYE ALTERATIONS	133	64.6	137	69.5	270	67.0
ANTENNA to						
Leg	3	01.5	4	02.0	7	01.7
Unidentified	5	02.4	2	01.0	7	01.7
Antenna Missing	2	01.0	0	00.0	2	00.5
Aristi Missing	5	02.4	5	02.5	10	02.5
Aristi Misshaped	8	03.9	5	02.5	13	03.2
TOTAL ANTENNAL ALTERA.	23	11.2	16	08.1	39	09.7
ROSTRALHAUT to						
Abdomen	6	02.9	3	01.5	9	02.2
Unidentified	21	10.2	22	11.2	43	10.7
Genital	9	04.4	6	03.0	15	03.7
Antenna	1	00.5	0	00.0	1	00.2
TOTAL ROSTRALHAUT ALTERA.	37	18.0	31	15.7	68	16.9
PROBOSCIS to						
Proboscis Modified	4	01.9	0	00.0	4	01.0
Proboscis to Leg	0	00.0	1	00.5	1	00.2
Palpus Missing	4	01.9	8	04.1	12	03.0
Palpus Doubled	1	00.5	1	00.5	2	00.5
Palpus Modified	4	01.9	3	01.5	7	01.7
TOTAL PROBOSCIS ALTERATIONS	13	06.3	13	06.6	26	06.5
TOTAL # OF ALTERATIONS	206		197		403	
# OF HEADS EXAMINED	110		103		213	
AVERAGE # OF ALTERATIONS PER HEAD	1.87		1.91		1.89	

Transformations to genital tissue, to abdominal tergites, and a single transformation of rostralhaut to antenna occurred at much lower frequencies. A small proportion of alterations involved the proboscis region, 6.5%, 6.3% in males and 6.6% in females. The proboscis itself was found modified in only a single female where leg-like structures were observed protruding from the proboscis. In descending order of frequency observed, alterations of the palpus were: palpus missing; palpus misshaped; palpus doubled. A total of 403 alterations were distributed between 206 males and 197 females. An average of 1.87 alterations was recorded for 110 male heads examined and an average of 1.91 alterations was recorded for the 103 female heads examined, producing a combined average of 1.89 alterations per head. No significant differences exist between homokaryotypic males and females in expressivity ($X^2 = 0.155$; $0.70 > P > 0.50$), nor were any significant differences noted between the results for homokaryotypes and heterokaryotypes ($X^2 = 0.118$; $0.80 > P > 0.70$).

Transformations in progeny that were v tu-1/Y; $2^s/2$; ru E(tu-1) ca/h 3A or 3B; $4^s/4$ are presented in Table 3 (pg. 24). Alterations involving only the eye region represented 45.6% of the total alterations, 50.3% in males and 42.2% in females. Eye transformations to abdominal tergites were the most prevelant of eye alterations, with

TABLE 3

Homoeotic effects of the tumorous-head trait among progeny resulting from a cross between females v tu-l/Y; $2^s/2^s$; ru E(tu-l) ca/ ru E(tu-l) ca; $4^s/4^s$ mated to males l/Y; 2/2; h 3A/3B; 4/4 from the tuh(ASU) strain.

Structure Transformed	ALTERATIONS					
	MALES		FEMALES		TOTAL	
	#of	%of total	#of	%of total	#of	%of total
EYE to						
Abdomen	138	35.2	143	26.7	280	30.3
Unidentified	47	12.0	77	14.4	124	13.4
Genital	11	02.8	5	00.9	16	01.7
Antenna	1	00.3	1	00.2	2	00.2
TOTAL EYE ALTERATIONS	197	50.3	226	42.2	423	45.6
ANTENNA to						
Leg	7	01.8	19	03.5	26	02.8
Abdomen	2	00.5	2	00.4	4	00.4
Unidentified	17	04.3	18	03.4	35	03.8
Genital	2	00.5	1	00.2	3	00.3
Antenna Missing	2	00.5	7	01.3	9	01.0
Aristi Missing	10	02.6	29	05.4	39	04.2
Aristi Misshaped	10	02.6	17	03.2	27	02.9
Aristi Duplicated	1	00.3	1	00.2	2	00.2
TOTAL ANTENNAL ALTERATIONS	51	13.0	94	17.5	145	15.6
ROSTRALHAUT to						
Abdomen	40	10.2	56	10.4	96	10.3
Unidentified	13	03.3	19	03.5	32	03.4
Genital	22	05.6	23	04.3	45	04.8
Clasper teeth/Spiked bris	1	00.3	1	00.2	2	00.2
Antenna	1	00.3	0	00.0	1	00.1
Leg	0	00.0	2	00.4	2	00.2
TOTAL ROSTRAL. ALTERA.	77	19.6	101	18.8	178	19.2
PROBOSCIS to						
Proboscis Modified	9	02.3	4	00.7	13	01.4
Leg	0	00.0	1	00.2	1	00.1
Proboscis Missing	0	00.0	1	00.2	1	00.1
Palpus Missing	38	09.7	76	14.2	114	12.3
Palpus Modified	16	04.1	26	04.9	42	04.5
Palpus Doubled	3	00.8	7	01.3	10	01.1
TOTAL PROBOSCIS ALTERA.	66	16.8	115	21.5	181	19.5
TOTAL # OF ALTERATIONS	392		536		920	
# OF HEADS EXAMINED	161		164		328	
AVERAGE # OF ALTERATIONS PER HEAD	2.43		3.27		2.83	

transformations to unidentifiable tissue, to genital tissue, and to antennal segments also observed. Antennal alterations comprised 15.6% of all transformations, 13.0% in males and 17.5% in females. In descending order of frequency, the antenna was transformed to: unidentifiable tissue; leg tissue; genital tissue (Plate II, Figure 11); abdominal tissue; antenna missing. At low frequencies the aristi was found misshaped or duplicated. Transformations involving the rostralhaut region represented 19.2% of total alterations, 19.6% in males and 18.8% in females. Of all alterations, 10.3% were from rostralhaut to abdominal tergites. Less frequently, transformations occurred from rostralhaut to genital tissue, to unidentifiable tissue, to antenna, to leg, to clasper teeth in one male, and in one female, to the spiked bristles found on the female vaginal plate (Plate II, Figure 8). Proboscis alterations comprised 19.5% of all alterations, 16.8% in males and 21.5% in females. By far the most frequent proboscis alteration was a missing palpus, with palpus modified, palpus doubled, and proboscis modified occurring at much lower frequencies. Of the 928 distinct alterations represented in Table 3, 392 were found in 161 males giving an average of 2.43 alterations per head, and 536 alterations were observed in 164 females giving an average of 3.27 alterations per head.

Expressivity in female enhancer heterozygotes is significantly higher than in male enhancer heterozygotes ($X^2 = 5.20$; $0.30 > P > 0.01$). Highly significant differences exist between the heterokaryotypes and flies heterozygous for the enhancer gene ($X^2 = 11.24$; $P < 0.01$). However, this difference results from a comparison of the females ($X^2 = 13.47$; $P < 0.01$) and not from a comparison of the males ($X^2 = 1.102$; $0.30 > P > 0.20$).

Table 4 presents data on 9,587 flies heterozygous for the E(tu-1) gene which showed 93.9% penetrance of the tumorous-head trait and an average of 2.83 distinct alterations per head (Table 3, p. 24). Females (4,730) showed 96.6% penetrance of the tumorous-head trait, while males (4,757) showed 91.2% penetrance of the trait. From a total of 18,974 wings examined, 9,514 from males and 9,460 from females, at X30 total magnification, no transformations from wing to leg were observed.

TABLE 14

A summary of penetrance of the tumorous-head trait and the transformations from wing to leg that occurred among the progeny of v tu-1/Y; ru E(tu-1) ca females crossed with tuh(ASU) males.

	MALES	FEMALES	TOTAL
With tumors	4,337	4,568	8,905
Without tumors	420	262	682
# Heads Examined	4,757	4,730	9,587
% Tumored	91.2	96.6	93.9
Wing to Leg Alterations			
# Wing to Leg Transformations	0	0	0
# Wings Examined	9,514	9,460	18,974

DISCUSSION

Transformations observed in the head region of tuh(ASU) homokaryotypic and heterokaryotypic flies were of the same type and, in most cases, found in similar frequencies (Tables 1 and 2). Penetrance of the tumorous-head trait is generally lower in homokaryotypes than in heterokaryotypes (Woolf and Lott 1965). However, no significant differences in expressivity exist between karyotypes or between the males and females of either karyotype. Expressivity is slightly higher in heterokaryotypes. Penetrance is normally higher in females than in males (Woolf and Lott 1965). From Tables 1 and 2, expressivity appears to be about the same between males and females in my study.

Many of the transformations observed in the tuh(ASU) strain were also observed in the tuh(CT) strain used by Postlethwait et al.(1972). However, some important differences were found between the transformations reported in my study and in the study by Postlethwait et al. (1972). A substantial number of transformations from eye tissue to genital tissue were observed (Tables 1,2, and 3; Plate II, Figure 2), as well as transformations of the rostral-haut region to abdominal tergites (Tables 1,2, and 3;

Plate II, Figure 5), which had not been reported previously by Postlethwait et al. (1972). Also, Postlethwait et al. (1972) had reported finding antennal transformations to leg structures (Tables 1,2, and 3; Plate I, Figure 8), but not to genital structures in tuh(CT). Kauffman (1973) predicted the transdetermination of antennal segments to leg structures and also to genital tissue. He also predicted that antenna transdetermined into leg should occur more frequently than antenna transdetermined into genital. Transition of antenna into leg structures and into genital tissue was found in tuh(ASU) (Table 1; Plate II, Figure 11), with the frequency of transition to genital tissue occurring less often than transition to leg structures. This supports Kauffman's proposed directional flow pathway of his bistabel memory circuits model (1973). The discrepancies between the two studies can be attributed to the tumorous-head strains used. Postlethwait et al. (1972) used a tumorous-head strain obtained from the California Institute of Technology stock center that has traditionally been known for low penetrance and expressivity (Woolf 1965). The tuh(ASU) strain,used in my study, is known to possess excellent penetrance and expressivity since the strain has been maintained for years by selecting parental flies, each generation that possess the tumorous-head trait.

Kauffman's model predicts that the tumorous-head genes should destabilize the eye disk and increase, within the eye area, transformations toward leg and genitalia, with transformations toward leg occurring at a higher frequency. Transformations to genitalia were observed within the eye region (Tables 1,2, and 3; Plate II, Figure 2). Although, no transformations from eye to leg were observed (Tables 1,2, and 3), transformations from rostralhaut to leg were observed in two females (Table 3). Therefore, Kauffman's predictions are true, but transformations of eye to genital are the more frequent. Kauffman's model also predicts that transformations from wing to leg should be observed in tumorous-head flies if penetrance and expressivity are sufficiently high enough. Females heterozygous for the enhancer gene (Tables 3 and 4) showed 96.6% penetrance of the trait and possessed an average of 3.27 alterations per head. If transformations from wing to leg occur, they should have been exhibited in this class of females. Wings from 9,487 flies were examined (Table 4) with no observable alterations from wing to leg found. It therefore appears that the tumorous-head trait involves no measurable destabilization of the wing disk. In tumorous-head the antennal-eye disk appears to be the only disk destabilized. The resulting transformations of the disk are nearly the same for the antenna,

rostralhaut, and eye (Tables 1,2, and 3) and vary from structure to structure only in frequency. Except for two examples of the proboscis being transformed to leg, the alterations of the proboscis are not homoeotic.

The addition of the enhancer gene of tu-l is responsible for increasing penetrance from 84.1%, as seen in tuh(ASU) flies, to 93.9%, as seen in flies heterozygous for the gene. There is a highly significant enhancement in expressivity when the average number of alterations per head for the tuh(ASU) heterokaryotypic flies 2.12 (Table 1), and for homokaryotypic flies 1.89 (Table 2), is compared to flies heterozygous for the enhancer, 2.83 (Table 3). It also appears that the enhancer differentially effects expression of the trait in structures of the eye-antennal disk other than the eye. According to Postlethwait and Schneiderman (1973), Kauffman's proposed model (1973) has the homoeotic mutant involved in the initial setting of each circuit in the appropriate state for each disk. There is evidence that a homoeotic mutant brings into action genes not normally expressed in the affected imaginal disk (Waddington 1953). If the developmental alteration in a homoeotic mutant is assumed to be essentially the same phenomenon as transdetermination, then the initial action of the homoeotic mutant is to encourage transdetermination to occur at a

high rate in a specific direction (Postlethwait et al. 1972). The addition of a gene which enhances the homoeotic effect in terms of penetrance has encouraged transdetermination to occur at higher rates in specific directions within the eye region, and more importantly, within other regions at even greater frequencies. Transformations involving the antenna, the rostralhaut region, and the proboscis were much more prevelant in flies heterozygous for the enhancer gene. While, transformations involving the eye were present at about the same frequency as observed among the tuh(ASU) flies. Additionally, the enhancer gene appears to increase transformations to identifiable structures. Flies heterozygous for the enhancer gene possessed 20.6% of the alterations classified as unidentifiable (Table3) whereas, heterokaryotypes possessed 40.1% (Table 1) and homokaryotypes 42.4% (Table 2). Only one transformation was unique to the flies resulting from the action of the enhancer gene (Plate II, Figure 8) when compared to the transformations of the tuh(ASU) flies. Therefore, enhancement seems to be quantitative rather than qualitative. The most obvious enhancement involved transformations of the rostralhau region to abdominal tergites. This transformation represented only 1.7% of all alterations in tuh(ASU) flies (Tables 1 and 2); it represented 10.3% of all alterations

in flies heterozygous for the enhancer gene (Table 3).

In my study, structures have been classified unidentifiable because they did not exhabit a "recognizable" trait characteristic of a tissue type or structure found on the fly. Often the alteration was classified unidentifiable due to the way the head was positioned on the slide. In future studies, a means should be developed, such as differential staining or labeling, to accurately determine the tissue types in alterations. Also, in this and past studies, identification of the tissue has been fairly subjective. Many alterations are straight forward as to their tissue type due to characteristic body bristle patterns. However, as the penetrance and expressivity of the alterations per head increases, the composition of the alterations are not as obvious, and eventually become a matter of subjective interpretation. Here also, a means of objectively identifying the tissue types of the alterations would prove invaluable. A differential staining or labeling technique could also be used to demonstrate whether large transformations, like antenna transformed to leg, actually are to "functional" body structures and tissue.

Perhaps the greatest area requiring work is the area dealing with the biochemical nature of homoeotic mutants and transdetermination. As Postlethwait and Schneider-

man (1973) pointed out in their review of Kauffman's model (1973), its main weakness was that it provided no biochemical basis for transdetermination and homoeotic mutants. The molecular mechanisms of early development, when the fate of the imaginal disks is thought to be determined, that are necessary for and unique to the expression of the tumorous-head phenotype in Drosophila melanogaster, must be found and studied before the mechanisms of mutants producing homoeotic changes and the nature of transdetermination can be understood.

Part II

Penetrance, Expressivity, Eclosion Rate and Sex Ratio of the Tumorous-Head Phenotype in *Drosophila melanogaster* Within Populations Exposed to Varying Lengths of a Cold Shock of 18°C, and Also Within Populations Reared at 25°C.

The present study was designed to determine the effects of environmental temperature, 25°C and 18°C, upon penetrance, eclosion rate, expressivity, and sex ratio in the tuh(ASU) strain, and upon the eclosion rate and sex ratio of a control, nontumorous strain, Canton-S. Secondly, this study was designed to analyze the penetrance, expressivity, sex ratio and eclosion rate of the tumorous-head phenotype in a strain of tuh(ASU), that was maintained at 25°C, in terms of the day of appearance in the third instar larval stage. This section utilized collection of emerging third instar larvae, a technique seldomly employed in previous developmental studies of tumorous-head populations. The technique provides a means for monitoring changes in the developing population that occur between the appearance of the third instar and eclosion as an adult.

MATERIALS AND METHODS

Tumorous-head flies are exposed to temperatures of 18°C and 25°C for varying lengths of time and during different developmental stages. Two methods are used for making temperature exposures. The fly strains studied are tuh(ASU) and Canton-S. Canton-S serves as the nontumorous control.

In the first method, tuh(ASU) and Canton-S flies, of varying ages, are placed into ½-pint milk bottles containing standard Drosophila medium. The flies are then subjected to one of the following temperature treatments: retention at 25°C; retention at 18°C; exposure to 18°C for either 48 hours, 4 days, or 10 days, followed by retention at 25°C. Parental flies are discarded after approximately 5 days (after 10 days for flies retained at 18°C for 10 days or longer). In Drosophila, at 25°C, embryonic development begins immediately after fertilization and continues for about twenty-two hours (Strickberger 1962). The developmental fate of the imaginal disks is determined 3-7 hours after fertilization (Fristrom 1970). Twenty-two hours after fertilization, at 25°C, the egg hatches and postembryonic development begins. A Drosophila larva is a white, segmented, worm-

shaped burrower with black mouth parts in a narrowed head region (Strickberger 1962). Since the larval cuticle does not stretch, the larva must periodically shed its skin in order to attain adult size. Such events are called "molts". The old cuticle is discarded and a new expanded integument is laid down, which the larva proceeds to fill out by rapid feeding. There are two such molts in larval development. During each period between molts, the larva is called an "instar". At 25°C, hatching from the egg (first instar) occurs approximately one day after fertilization; first molt (second instar) occurs approximately two days after fertilization; second molt (third instar) occurs approximately 3-6 days after fertilization. During the third instar, the larvae crawl out of the medium onto a relatively dry place, such as the walls of their containers, cease motion, and then enter a stationary, approximately 4 day, pupal stage. Third instar larval samples, in this procedure, are collected as soon as the third instar larvae appear and begin to crawl out of the medium, approximately 6 days after fertilization, at 25°C. For lower temperatures, the developmental rates are proportionally much longer. As many larvae as possible are collected from the bottles daily. The daily larval collection continues until no more third instar larvae are seen inside the

bottles or until the new generations of adult flies begin to emerge (at 25°C approximately 14 days after fertilization). Using a dissecting scope, the larvae are separated according to sex. Male larvae are distinguishable from female larvae by gonad size. The male larva has a visible, transparent, circular gonad area that is much larger and more distinct than the comparable gonad area of the female. Grouped by sex, the larvae are then transferred to shell vials containing standard Drosophila medium. No more than 100 larvae are placed into a vial in order to eliminate the effects of crowding. The number of larvae transferred to a vial is recorded. The remaining uncollected larvae of the bottle population are allowed to continue their development within the bottle. All adult flies which eclose in the bottles are examined to determine their sex so that an estimate can be made of the sex ratio within the bottle population. All adult tuh(ASU) flies which eclose in the vials are classified as to karyotype (homokaryotype or heterokaryotype), sex, and the absence or presence of head tumors. In other words, for tuh(ASU), the sex ratios, adult eclosion rates, and karyotype frequencies are recorded. For Canton-S, the eclosion rates and sex ratios are recorded. The sex ratios of the bottle populations can then be compared to the sex ratios of the vial popu-

lations.

In the second method of the temperature study population cages of tuh(ASU) and Canton-S are maintained at 25°C. Every 2 hours several of the small population cage bottles are cleared of their adults and removed from the population cages. These bottles contain standard Drosophila and all fertilized eggs that were laid in the bottle during the 2 hour sampling period. Since no egg within a bottle is more than two hours old, this method allows for greater control in exposing the developing eggs to a certain temperature at a specific stage in its development. The developmental fate of the imaginal disk is determined in the first 3-7 hours after fertilization (Fristrom 1970). Each imaginal disk is set aside during embryogenesis to develop into a particular adult structure during metamorphosis. In this study, eggs can be exposed to temperatures of 18°C and 25°C during this crucial developmental period. Immediately after the 2 hour collection period, the eggs are subjected to one of the following temperature exposure sequences: 48 hour exposure to 18°C then transference to 25°C; exposure to 18°C until the third instar larvae appear (approximately 12 days), then the larvae are transferred and retained at 25°C. As before, daily samples of the third instar larvae are collected for 2-4 days; separated according to their

sex; transferred to shell vials containing standard Drosophila medium; and allowed to develop. However, unlike the larvae collected in the first method, all larvae in the second method originated from eggs laid down within a single two hour period. A developmental rate discrepancy must exist between eggs laid within the same two hour period, that can cause them to disser by as much as four days in the length of time it takes for them to reach the third instar larval stage. The resulting tuh(ASU) adults which eclose in the vials are examined, and sex ratios, karyotype frequency, tumor penetrance and eclosion rates recorded. For Canton-S, the sex ratios and eclosion rates are recorded. Adult sex ratio measurements for the untransferred flies remaining in the population cage bottles were routinely made for both tuh(ASU) and Canton-S strains. A comparison can then be made of the results for the different temperature exposures on sex ratios, penetrance, expressivity, and eclosion rates in the tumorous-head phenotype.

The final procedure was designed to study the development of a tuh(ASU) bottle population maintained at 25°C in terms of tumorous-head phenotype and eclosion and its relation to the day of appearance in the third instar larval stage. Just as in the first tuh(ASU), Canton-S temperature exposure method, adult flies of varying

ages are placed into ½-pint milk bottles containing standard Drosophila medium and retained at 25°C. Parental flies are discarded after 5 days. Third instar larvae were collected daily as they crawled up onto the sides of the bottle to pupate. They were then classified as to sex; transferred to shell vials containing standard Drosophila medium, and placed at 25°C. When the flies eclosed , they were examined for head tumors. Heads and bodies of flies expressing the tumorous-head trait , grouped according to the day collected as third instar larvae, were then digested in a 5% KOH solution, leaving only exoskeleton. Each fly was placed on a glass slide and examined, using a dissecting scope, for abnormalities of the head and wing regions. The head was separated away from the body and carefully positioned so that the maximum number of alterations could be studied, then the specimen was mounted in lactophenol, covered with a cover slip and examined at X400 magnification. The average number of transformations per head was calculated just as in part I, however, now the head transformations of the adults are presented in terms of the day of their collection in the third instar larval stage.

RESULTS

Tables 5-10 present the results of 18°C and 25°C temperature exposures on breeding populations of tuh(ASU) and Canton-S. Within each temperature group, male eclosion is significantly higher than female eclosion (Tables 5,8, and 9) except for the groups exposed to 18°C for 48 hours, (% male eclosion is symbolized %E male), (%E male 73.6, %E female 66.1, Table 6) (X^2= 1.302; 0.30>P>0.20), and exposed for 4 days (%E male 78.5, %E female 73.5, Table 7) (X^2= 1.529; 0.30>P>0.20). For the 48 hour and 4 day 18°C temperature exposures the proposed maternal effect (Kuhn 1973) which produces a significant difference in male and female eclosion appears to be eliminated. However, the sample sizes of Tables 6, and 7 are relatively small, when compared to the sample sizes of Tables 5,8, and 9, and the validity of the X^2 may be questionable. At 25°C, between tuh(ASU) and Canton-S, there normally exists an extremely significant variation in the eclosion rates of their males (%E tuh(ASU) amle 76.2, %E Canton-S male 84.9, Table 5) (X^2= 47.84; P<0.01) and females (%E tuh(ASU) female 56.0, %E Canton-S female 82.9, Table 5) (X^2= 336.23; P<0.01). The difference in eclosion for Canton-S and tuh(ASU), for each sex, was

TABLE 5

The effects of a 25°C environment on cultures of tuh(ASU) and Canton-s, as obtained from the eclosion rates and phenotype frequencies of their adults. (The adults were from daily, third instar larvae collections.)

A. Tuh(ASU)

	MALES				FEMALES				TOTAL				
		Hetero	Homo			Hetero	Homo			%Larvae	%Adults		
DAY	%Eclosed	%Tum	%Tum	%Homo	%Eclosed	%Tum	%Tum	%Homo	%Eclosed	Transf. Male	Male	%Homo	%Tum
1	81.8	70.0	81.1	10.1	83.0	73.2	55.6	10.8	82.0	76.3	82.0	10.3	71.2
	526/643	334/477	43/53		166/200	109/149	10/18						496/697
2	79.5	78.5	64.3	15.7	70.5	76.4	70.8	11.3	75.9	59.8	62.6	14.0	76.2
	357/449	245/312	36/56		213/302	146/191	17/24						444/583
3	79.7	78.1	51.1	19.7	58.7	68.2	53.8	13.5	68.7	47.8	55.5	16.9	69.9
	239/300	150/192	24/47		192/327	116/170	14/26						304/435
4	66.0	85.4	76.6	31.3	49.4	83.5	73.7	10.4	55.8	38.0	45.0	19.8	82.6
	150/227	88/103	36/47		183/370	137/164	14/19						275/333
5	69.1	80.2	70.6	22.7	44.2	85.4	69.2	15.8	53.4	36.8	47.6	19.0	80.5
	150/217	88/106	24/34		165/373	117/137	18/26						244/303
6	61.0	76.2	96.2	29.2	36.8	85.2	68.8	21.3	46.9	41.7	54.3	25.6	81.9
	89/146	48/63	25/26		75/204	52/61	11/16						136/164

TABLE 5 A. Tuh(ASU) (continued)

DAY	%Eclosed	%Tum	%Tum	%Homo	%Eclosed	%Tum	%Tum	%Homo
Σ	76.2	75.8	71.5	17.4	56.0	78.5	65.1	13.0
	1516	950	188		991	677	84	
	1982	1253	263		1776	862	129	

%Larvae Misclassified 02.1 Males 06.0 Females

%Eclosed	%Larvae Transf. Male	%Adults Male	%Homo	%Tum
66.7	52.7	60.3	15.6	75.7
				1899
				2507

BOTTLE POPULATION %Adults Male: 67.1

B. Canton-S

DAY	MALES %Eclosed		FEMALES %Eclosed	
1	89.7	286/319	80.5	227/282
2	82.4	337/409	86.7	320/369
3	84.7	342/404	77.3	341/441
4	80.7	289/358	84.2	347/412
5	84.6	248/293	82.8	308/372
6	91.5	173/189	87.1	210/241
Σ	84.9	1675/72	82.9	1754/2117

%Larvae Misclassified: 04.0 Males 03.0 Females

TOTAL %Eclosed	%Larvae Transf. Male	%Adults Male
85.4	53.1	55.8
84.0	52.6	51.3
80.8	47.8	50.1
82.6	46.5	45.4
83.6	44.0	44.6
89.1	44.0	45.1
83.9	48.2	48.8

BOTTLE POPULATION %Adults Male: 47.7

TABLE 6

The effects of a 48 hour 18°C cold shock on cultures of tuh(ASU) and Canton-S as obtained from the eclosion rates and phenotype frequencies of their adults. (The adults were from daily, third instar larvae collections.)

A. Tuh(ASU)

	MALES				FEMALES				TOTAL				
DAY	%Eclosed	Hetero %Tum	Homo %Tum	%Homo	%Eclosed	Hetero %Tum	Homo %Tum	%Homo	%Eclosed	%Larvae Transf. Male	%Adults Male	%Homo	%Tum
1	75.0 30/40	45.8 11/24	66.7 4/6	20.0	50.0 1/2	100.0 1/1	00.0 -/-	00.0	73.8	95.2	96.8	19.4	51.6 16/31
2	83.5 86/103	32.4 23/71	40.0 6/15	17.4	80.0 21/26	42.1 8/19	100.0 2/2	09.5	82.9	79.8	80.4	15.9	36.4 39/107
3	54.3 25/46	50.0 7/14	18.2 2/11	44.0	65.0 13/20	53.8 7/13	00.0 -/-	00.0	57.6	69.7	65.8	28.9	42.1 16/38
4	50.0 4/8	100.0 2/2	00.0 -/2	50.0	42.9 6/14	40.0 2/5	00.0 -/1	16.7	45.5	36.4	40.0	30.0	40.0 4/10
Σ	73.6 145/197	38.7 43/111	35.3 12/34	23.4	66.1 41/62	47.4 18/38	66.7 2/3	07.3	71.8	76.1	78.0	19.9	40.3 75/186

%Larvae Misclassified: 00.0 Males 06.1 Females

BOTTLE POPULATION
%Adults Male: 87.0
40/46

47

TABLE 6 (continued)

B. Canton-S

DAY	MALES %Eclosed		FEMALES %Eclosed		TOTAL %Eclosed	%Larvae Transf. Male	%Adults Male
1	75.4	$\frac{43}{57}$	65.7	$\frac{46}{70}$	70.1	44.9	48.3
2	70.0	$\frac{14}{20}$	80.0	$\frac{12}{15}$	74.3	57.1	53.8
3	42.9	$\frac{3}{7}$	00.0	$\frac{-}{4}$	27.3	63.6	100.0
Σ	71.4	$\frac{60}{84}$	65.2	$\frac{58}{89}$	68.2	48.6	50.8

%Larvae Misclassified: 00.0 Males 02.2 Females

BOTTLE POPULATION %Adults Male: 52.4 $\frac{54}{103}$

TABLE 7

The effects of a 4 day 18°C cold shock on cultures of tuh(ASU) and Canton-S as obtained from the eclosion rates and phenotype frequencies of their adults. (The adults were from daily, third instar larvae collections.)

A. Tuh(ASU)

	MALES				FEMALES				TOTAL				
		Hetero	Homo			Hetero	Homo			%Larvae			
DAY	%Eclosed	%Tum	%Tum	%Homo	%Eclosed	%Tum	%Tum	%Homo	%Eclosed	Transf. Male	%Adults Male	%Homo	%Tum
1	83.7	50.0	22.2	10.3	86.3	59.0	60.0	11.4	86.5	67.1	67.2	10.4	51.1
	87/104	39/78	2/9		44/51	23/39	3/5						67/131
2	65.0	43.8	30.0	38.5	53.1	42.9	33.3	11.1	59.7	55.6	60.5	24.5	39.5
	26/40	7/16	3/10		17/32	6/14	1/3						17/43
Σ	78.5	48.9	26.3	16.8	73.5	54.7	50.0	13.1	76.7	70.2	64.9	15.5	48.3
	113/144	46/94	5/19		61/83	29/53	4/8						84/174

BOTTLE POPULATION
%Adults Male: No data available

%Larvae
Misclassified: 00.7 Males 05.7 Females

TABLE 7 (continued)

B. Canton-S

DAY	MALES %Eclosed		FEMALES %Eclosed		TOTAL %Eclosed	%Larvae Transf. Male	%Adults Male
1	80.0	$\frac{28}{35}$	76.3	$\frac{29}{38}$	78.1	47.9	49.1
2	80.0	$\frac{40}{50}$	78.7	$\frac{37}{47}$	79.4	51.5	51.9
3	100.0	$\frac{14}{14}$	76.5	$\frac{13}{17}$	87.1	45.2	51.9
Σ	82.8	$\frac{82}{99}$	77.5	$\frac{79}{102}$	80.1	49.3	50.9

%Larvae Misclassified: 00.0 Males 02.9 Females

BOTTLE POPULATION %Adults Male: 43.9 $\frac{54}{126}$

TABLE 8

The effects of a 10 day 18°C cold shock on cultures of tuh(ASU) and Canton-S as obtained from the eclosion rates and phenotype frequencies of their adults. (The adults were from daily, third instar larvae collections.)

A. Tuh(ASU)

	MALES				FEMALES				TOTAL				
		Hetero	Homo			Hetero	Homo			%Larvae			
DAY	%Eclosed	%Tum	%Tum	%Homo	%Eclosed	%Tum	%Tum	%Homo	%Eclosed	Transf. Male	%Adults Male	%Homo	%Tum
1	82.3 169/206	63.9 101/158	63.6 7/11	06.5	68.9 42/62	76.2 32/42	00.0 -/-	00.0	79.2	77.2	80.1	05.2	66.4 140/211
2	89.3 226/253	67.7 136/201	48.0 12/25	11.1	68.9 84/122	80.0 63/78	83.3 5/6	07.1	82.7	67.5	72.9	28.2	69.7 216/310
3	71.4 105/147	77.3 68/88	70.6 12/17	16.2	77.7 94/121	74.1 60/81	61.5 8/13	13.8	74.3	54.9	52.8	15.1	74.4 148/199
4	48.7 95/195	86.1 68/79	75.0 12/16	16.8	55.9 114/204	84.2 85/101	76.9 10/13	11.4	52.4	48.9	45.5	13.9	83.7 175/209
5	64.2 77/120	82.7 43/54	72.0 18/25	32.5	52.3 67/128	85.2 52/61	66.7 4/6	09.0	58.1	48.4	53.5	21.5	80.1 117/146

TABLE 8 A. Tuh(ASU) (continued)

DAY	Hetero %Eclosed	Homo %Tum	Hetero %Tum	Homo %Homo	Hetero %Eclosed	Homo %Tum	Hetero %Tum	Homo %Homo	%Eclosed	%Larvae Transf. Male	%Adults Male	%Homo	%Tum
6	69.4	95.8	100.0	04.0	25.8	93.8	00.0	05.9	41.2	35.3	59.5	04.8	95.1
	25/36	23/24	1/1		17/66	15/16	-/-						39/41
7	48.6	70.6	100.0	05.6	30.8	91.7	00.0	00.0	39.5	48.7	60.0	03.3	80.0
	18/37	12/17	1/1		12/51	11/12	-/-						24/30
8	62.5	100.0	100.0	60.0	22.7	80.0	00.0	00.0	33.3	26.7	50.0	10.0	90.0
	5/8	2/2	3/3		5/22	4/5	-/-						9/10
Σ	71.9	72.9	66.7	13.8	57.0	81.3	69.2	00.2	65.4	56.8	62.3	11.9	75.2
	720/1002	453/621	66/99		435/763	322/396	27/39						868/1155

%Larvae Misclassified: 00.3 Males 07.1 Females

BOTTLE POPULATION
%Adults Male: 65.9
209/317

B. Canton-S

DAY	MALES %Eclosed	FEMALES %Eclosed	TOTAL %Eclosed	%Larvae Transf. Male	%Adults Male
1	76.7 13/17	81.3 30/37	79.5	31.4	30.3
2	76.3 35/46	73.0 62/85	74.1	35.2	36.1

TABLE 8 B. Canton-S (continued)

DAY	MALES %Eclosed	FEMALES %Eclosed	TOTAL %Eclosed	%Larvae Transf. Male	%Adults Male
3	86.3 $\frac{104}{122}$	89.4 $\frac{101}{113}$	87.3	52.0	50.7
4	81.4 $\frac{70}{86}$	76.4 $\frac{97}{127}$	78.4	40.4	41.9
5	69.6 $\frac{32}{46}$	70.7 $\frac{41}{58}$	70.3	46.2	43.8
6	72.1 $\frac{49}{68}$	79.2 $\frac{76}{96}$	76.2	41.5	39.2
7	68.6 $\frac{35}{51}$	64.5 $\frac{40}{62}$	66.5	45.1	46.7
Σ	77.4 $\frac{322}{416}$	79.9 $\frac{428}{536}$	78.5	43.0	42.5

%Larvae Misclassified: 02.0 Males 05.0 Females

BOTTLE POPULATION
%Adults Male: 45.5 $\frac{455}{1000}$

TABLE 9

The effects of an 18°C environment on cultures of tuh(ASU) and Canton-S as obtained from the eclosion rates and phenotype frequencies of their adults. (The adults were from daily third instar larvae collections.)

A. Tuh(ASU)

	MALES				FEMALES				TOTAL				
		Hetero	Homo			Hetero	Homo			%Larvae			
DAY	%Eclosed	%Tum	%Tum	%Homo	%Eclosed	%Tum	%Tum	%Homo	%Eclosed	Transf. Male	%Adults Male	%Homo	%Tum
1	94.4	59.2	62.5	09.5	95.0	57.9	00.0	00.0	94.5	77.1	81.6	07.8	59.2
	84/89	45/76	5/8		19/20	11/19	-/-						61/103
2	92.2	63.6	33.3	06.4	82.1	81.8	100.0	04.3	88.6	64.6	67.1	05.7	68.6
	47/51	28/44	1/3		23/28	18/22	1/1						48/70
3	82.9	50.0	75.0	23.5	76.0	88.9	100.0	05.3	80.3	62.1	64.2	17.0	67.9
	34/41	13/26	6/8		19/25	16/18	1/1						36/53
4	83.0	71.4	63.6	28.2	78.6	60.0	100.0	09.1	79.2	62.7	63.9	21.3	67.2
	39/47	20/28	7/11		22/28	12/20	2/2						41/61
5	80.4	77.1	40.0	13.5	53.8	85.0	100.0	04.8	68.2	54.1	63.8	10.3	77.0
	37/46	27/35	2/5		21/39	17/20	1/1						47/61

TABLE 9 A. Tuh(ASU) (continued)

DAY	MALES %Eclosed	MALES Hetero %Tum	MALES Homo %Tum	MALES %Homo	FEMALES %Eclosed	FEMALES Hetero %Tum	FEMALES Homo %Tum	FEMALES %Homo	TOTAL %Eclosed	TOTAL %Larvae Transf. Male	TOTAL %Adults Male	TOTAL %Homo	TOTAL %Tum
6	85.1	70.4	66.7	18.2	38.2	81.8	100.0	15.4	56.8	58.0	71.7	17.4	73.9
	40/47	19/27	4/6		13/34	9/11	2/2						34/46
7	50.0	69.2	75.0	23.5	29.1	100.0	100.0	14.3	41.4	58.6	70.8	20.8	79.2
	17/34	9/13	3/4		7/24	6/6	1/1						19/24
Σ	82.8	64.7	62.2	15.5	62.6	76.7	100.0	06.5	75.6	70.3	70.1	12.8	75.9
	294/355	161/249	28/45		124/198	89/116	8/8						286/377

BOTTLE POPULATION
%Adults Male: 70.6

%Larvae
Misclassified: 01.7 Males 08.1 Females

B. Canton-S

DAY	MALES %Eclosed		FEMALES %Eclosed		TOTAL %Eclosed	TOTAL %Larvae Transf. Male	TOTAL %Adults Male
1	84.6	11/13	100.0	10/10	91.3	56.5	52.4
2	75.0	15/20	57.9	11/19	66.7	51.3	57.7
3	77.8	7/9	68.4	13/19	71.4	32.1	35.0

TABLE 9 B. Canton-S (continued)

DAY	MALES %Eclosed		FEMALES %Eclosed		TOTAL %Eclosed	%Larvae Transf. Male	%Adults Male
4	85.7	$\frac{6}{7}$	61.1	$\frac{11}{18}$	68.0	28.0	35.3
5	71.4	$\frac{10}{14}$	61.5	$\frac{16}{26}$	65.0	35.0	38.5
6	47.6	$\frac{10}{21}$	65.2	$\frac{15}{23}$	56.8	47.7	40.0
Σ.	70.2	$\frac{59}{84}$	66.1	$\frac{76}{115}$	67.8	42.2	43.7

%Larvae Misclassified: 00.0 Males 00.9 Females

BOTTLE POPULATION
%Adults Male: 36.4 $\frac{55}{151}$

TABLE 10

A summary of Tables 5-9's eclosion rates for Canton-S and tuh(ASU) and tumor penetrances for tuh(ASU) adults.

A. Eclosion Rates

	tuh(ASU)		Canton-S		Total	
SAMPLE	Male	Female	Male	Female	tuh(ASU)	Canton-S
Table 5						
25°C	76.2	56.0	84.9	82.9	66.7	83.9
	1516	991	1675	1754	2507	3429
	1982	1776	1972	2117	3758	4089
Table 6						
48 hr. 18°C	73.6	66.1	71.4	65.2	71.8	68.2
	145	41	60	58	186	118
	197	62	84	89	259	173
Table 7						
4 day 18°C	78.5	73.5	82.8	77.5	76.7	80.1
	113	61	82	79	174	161
	144	83	99	102	227	201
Table 8						
10 day 18°C	71.9	57.0	77.4	79.9	65.4	78.5
	720	435	322	428	1155	750
	1002	763	416	536	1765	952
Table 9						
18°C	82.8	62.6	70.2	66.1	75.6	67.8
	294	124	59	76	418	135
	355	198	84	115	553	199

Table 10 (continued)

B. Tumor Penetrance

	MALES			FEMALES			TOTAL
SAMPLE	Hetero	Homo	Total	Hetero	Homo	Total	%Tum
Table 5 25°C	75.8 950 1253	71.5 188 263	75.1	78.5 677 863	65.1 84 129	76.7	77.5 1899 2507
Table 6 48 hr. 18°C	38.7 43 111	35.3 12 34	37.9	47.4 18 38	66.7 2 3	48.8	40.3 75 186
Table 7 4 day 18°C	48.9 46 94	26.3 5 19	45.1	54.7 29 53	50.0 4 8	54.1	48.3 84 174
Table 8 10 day 18°C	72.9 453 621	66.7 66 99	72.1	81.3 322 396	69.2 27 39	80.2	75.2 868 1155
Table 9 25°C	64.7 161 249	62.2 28 45	64.3	76.7 89 116	100.0 8 8	78.2	68.4 286 418

eliminated in all the 18°C exposures (Tables 5,6,7,8, and 9) except in the females of the 10 day exposure (%E tuh(ASU) female 57.0, %E Canton-S female 79.9, Table 8) (X^2= 71.82; P<0.01)

At 25°C (Table 5) no significant difference between male and female tumor penetrance for both karyotypes was demonstrated, (X^2 for heterokaryotypes is symbolized X^2hetero), (X^2hetero= 0.948; 0.80>P>0.70), (X^2homo= 1.652; 0.20>P>0.10). This holds true for all 18°C exposure groups (Tables 6,7,8,9, and 10) except for the heterokaryotypes of the 10 day 18°C group. At 10 days, for heterokaryotypes, female tumor penetrance, (symbolized %Tum. female), was significantly higher than the male tumor penetrance (%Tum. male 72.9, %Tum. female 81.3; Tables 8,10) (X^2= 9.33; P<0.01). From Table 10, the greatest reduction in tumor penetrance appeared in the groups exposed to 18°C for 48 hours and 4 days. However, the sample sizes of the 4 day and 48 hour exposures are relatively small.

The 25°C sample produced a significantly higher proportion of male homokaryotypes than female homokaryotypes, (% homokaryotypes is symbolized %Homo.), (%Homo. male 17.4, %Homo. female 13.0, Table 5) (X^2= 9.13; P<0.01). For all the 18°C samples there was no significant difference produced in the proportion of

male and female homokaryotypes (Tables 6,7,8, and 9). Also, exposure to an 18°C cold shock produced no obvious enhancement of male and female homokaryotype production.

The sex ratios of the bottle populations do not vary significantly from the sex ratios of the sample populations. Under no temperature conditions used was a one-to-one, male-to-female sex ratio produced. The sample retained at 25°C had the lowest percentage of larvae transferred that were male, 52.7%, and also the smallest percentage of eclosed adults that were male, 60.3% (Table 5). Of the groups exposed to 18°C, the 10 day exposure group had the smallest ratio of transferred larvae that were male, 56.8%, and the smallest proportion of male adults, 62.3% (Table 8). The greatest percentage of transferred male larvae 76.1%, and percentage of male adults, 78.2%, were in the sample exposed to 18°C for only 48 hours (Table 6). In no case did the varied temperature exposures eliminate the maternal effect which is thought to be responsible for the sex ratio in favor of males.

Tables 11-13 are the results of 18°C and 25°C temperature exposures on egg samples of tuh(ASU) and Canton-s that were begun within the first two hours of development. For tuh(ASU) at both 18°C temperature

exposures, there is no significant difference in male versus female eclosion rates (X^2 48 hr. 18°C= 0.950; 0.50>P>0.30) ($X^2$18°C until the third instar stage= 0.531; 0.50>P>0.30) (Tables 11,12, and 13). The samples exposed to 18°C during the early developmental stages do demonstrate significant differences in Canton-S and tuh(ASU) eclosion. The sample exposed to 18°C until the third instar stage (12 days) has significantly different eclosion rates for tuh(ASU) and Canton-S adults (%E tuh(ASU) male 77.0, %E Canton-S male 86.6) (X^2= 7.72; P<0.01), (%E tuh(ASU) female 74.0, %E Canton-S female 85.0) (X^2= 9.13; P<0.01) (Table 11). Also, the group exposed to 18°C for the first 48 hours of the temperature shock shows a highly significant variation in the eclosion rates of adult tuh(ASU) and Canton-S flies (%E tuh(ASU) male 67.6, %E Canton-S male 75.4) (X^2= 7.79; P<0.01), (%E tuh(ASU) female 65.7, %E Canton-S female 78.6) (X^2= 18.34; P<0.01) (Table 12).

For both karyotypes, under the temperature conditions used, there was found no significant difference in tumor penetrance between the males and females of each sample (Table 13). The lowest tumor penetrance was in the sample exposed to 18°C for the first 48 hours of the temperature shock. The males always had the lower of the tumor penetrances for each sample (Table 13).

TABLE 11

The effects of maintaining egg samples, collected over two hour intervals from populations of tuh(ASU) and Canton-S, at 18°C until the third instar larval stage, (As the third instar appeared they were transferred to 25°C.) as obtained from the eclosion rates and phenotype frequencies of their adults. (The adults were from daily third instar larvae collections.)

A. Tuh(ASU)

	MALES				FEMALES				TOTAL				
		Hetero	Homo			Hetero	Homo			%Larvae			
DAY	%Eclosed	%Tum	%Tum	%Homo	%Eclosed	%Tum	%Tum	%Homo	%Eclosed	Transf. Male	%Adults Male	%Homo	%Tum
1	83.2	67.9	62.5	09.0	79.1	71.9	100.0	05.9	82.0	71.3	72.4	08.1	69.1
	89/107	55/81	5/8		34/43	23/32	2/2						85/123
2	79.5	60.8	37.5	07.6	81.4	60.7	00.0	01.8	80.2	65.3	64.8	05.6	51.9
	105/132	59/97	3/8		57/70	22/56	-/1						84/162
3	72.7	47.1	66.7	06.5	67.7	60.5	50.0	10.0	71.1	67.4	68.9	07.4	51.9
	93/128	41/87	4/6		42/62	23/38	2/4						70/135
4	82.7	56.9	68.8	23.9	81.7	77.6	44.4	15.5	82.2	53.3	53.6	20.0	65.6
	67/81	29/51	11/16		58/71	38/49	4/9						82/125
5	60.4	54.5	50.0	31.3	56.0	57.1	71.4	25.0	58.3	51.5	53.3	28.3	56.7
	32/53	12/22	5/10		28/50	12/21	5/7						34/60

TABLE 11 A. Tuh(ASU) (continued)

DAY	Hetero %Eclosed	Hetero %Tum	Homo %Tum	Homo %Homo	Hetero %Eclosed	Hetero %Tum	Homo %Tum	Homo %Homo	%Larvae %Eclosed	%Larvae Transf. Male	%Adults Male	%Homo	%Tum
	77.0	58.0	58.3	12.4	74.0	66.3	56.5	10.5	75.9	62.9	63.8	11.7	60.7
Σ	386/501	196/338	28/48		219/296	130/196	13/23						367/605

BOTTLE POPULATION
%Adults Male: 54.1
20
37

%Larvae Misclassified: 02.0 Males 07.1 Females

B. Canton-S

DAY	MALES %Eclosed		FEMALES %Eclosed		TOTAL %Eclosed	%Larvae Transf. Male	%Adults Male
1	77.3	17/22	82.9	34/41	81.0	34.9	33.3
2	87.2	41/47	78.0	39/50	82.5	48.5	51.3
3	93.3	28/30	87.2	34/39	89.9	43.5	45.2
4	86.8	33/38	86.3	44/51	86.5	42.7	42.9
5	86.0	43/50	92.3	36/39	88.8	56.2	54.4
Σ	86.6	162/187	85.0	187/220	85.7	45.9	46.4

BOTTLE POPULATION
%Adults Male: 42.9 21/49

%Larvae Misclassified: 00.0 Males 02.7 Females

TABLE 12

The effects of a 48 hour 18°C cold shock treatment on egg samples collected over two hour intervals from populations of tuh(ASU) and Canton-S, as obtained from the eclosion rates and phenotype frequencies of their adults. (The adults were from daily third instar larvae collections.)

A. Tuh(ASU)

	MALES				FEMALES				TOTAL				
DAY	%Eclosed	Hetero %Tum	Homo %Tum	%Homo	%Eclosed	Hetero %Tum	Homo %Tum	%Homo	%Eclosed	%Larvae Transf. Male	%Adults Male	%Homo	%Tum
1	76.1 401/527	55.7 192/345	46.4 26/56	14.0	74.1 100/135	59.3 51/86	35.7 5/14	14.0	75.7	79.6	80.0	14.0	54.7 274/501
2	67.4 215/319	52.7 79/150	57.8 37/64	29.8	71.6 169/236	54.5 84/154	66.7 10/15	08.9	69.2	57.5	56.0	20.6	54.8 210/383
3	37.9 47/124	44.4 12/27	30.0 6/20	42.6	56.6 69/122	57.7 30/52	29.4 5/17	24.6	47.2	50.4	40.5	31.9	45.7 53/116
4	17.6 3/17	00.0 -/2	100. 1/1	33.3	28.6 6/21	100.0 3/3	66.7 2/3	50.0	23.7	44.7	33.3	44.4	66.7 6/9
5	77.8 7/9	33.3 1/3	50.0 2/4	57.1	29.4 5/17	40.0 2/5	00.0 -/-	00.0	46.2	34.6	53.8	30.8	50.0 6/12

TABLE 12 A. Tuh(ASU) (continued)

DAY	%Eclosed	%Tum	%Tum	%Homo	%Eclosed	%Tum	%Tum	%Homo	%Eclosed	%Larvae Transf. Male	%Adults Male	%Homo	%Tum
	67.6	53.8	49.7	21.8	65.7	56.7	44.9	14.0	66.9	65.2	65.9	19.2	53.6
Σ	673/996	283/526	73/147		349/531	170/300	22/49						548/1022

%Larvae Misclassified: 00.5 Males 05.2 Females

BOTTLE POPULATION
%Adults Male: 49.7 (354/712)

B. Canton-S

DAY	MALES %Eclosed	FEMALES %Eclosed	TOTAL %Eclosed	%Larvae Transf. Male	%Adults Male
1	75.2 82/109	85.1 103/121	80.4	47.4	44.3
2	77.8 137/176	79.2 133/168	78.5	51.2	50.7
3	70.4 57/81	70.4 76/108	70.4	42.9	42.9
Σ	75.4 276/366	78.6 312/397	77.1	48.0	46.9

%Larvae Misclassified: 01.9 Males 01.2 Females

BOTTLE POPULATION
%Adults Male: 49.7

TABLE 13

A summary of Tables 11 and 12's eclosion rates for Canton-S and tuh(ASU) and tumor penetrance for tuh(ASU) adults.

A. Eclosion Rates

	tuh(ASU)		Canton-S		Total	
SAMPLE	Male	Female	Male	Female	tuh(ASU)	Canton-S
Table 11	77.0	74.0	86.6	85.0	75.9	85.7
18°C until 3rd	386	219	162	187	605	349
instar (12 days)	501	296	187	220	797	407
Table 12	67.6	65.7	75.4	78.6	66.9	77.1
48 hr. 18°C	673	349	276	312	1022	588
	996	531	366	397	1527	763

B. Tumor Penetrance

	MALES			FEMALES			TOTAL
SAMPLE	Hetero	Homo	Total	Hetero	Homo	Total	%Tum
Table 11	58.0	58.3	58.0	66.3	56.5	65.3	60.7
18°C until 3rd	196	28		130	13		367
instar(12 days)	338	48		196	23		605
Table 12	53.8	49.7	52.9	56.7	44.9	55.0	53.6
48 hr. 18°C	283	73		170	22		548
	526	147		300	49		1022

There was no significant variation in the number of male versus female homokaryotypes produced per sample group (Tables 11,12).

The sex ratios of the tuh(ASU) and Canton-S samples did not vary significantly from the sex ratios of the populations remaining uncollected within the bottles during the third instar larval stage. Under no conditions was a one-to-one sex ratio produced. The percent larvae transferred male and the percent adults that were male did not vary significantly between temperature exposure groups.

Table 14 is composed of the results received from a phenotype, eclosion rate, and sex ratio analysis of males and females within a developing population of tuh(ASU) maintained at 25°C. The data were derived from daily samples of third instar larvae that were collected as they appeared within the bottle population. The tuh(ASU) population was the same as the population presented in Table 5.

The males' (2.14) and females' (2.12) average number of alterations per head were virtually the same at the end of my study. However, the types and frequencies of transformations for males and females are not the same when grouped according to the day they were collected as third instar larvae. Male tumor expressivity was vari-

TABLE 14

Homoeotic effects of the tumorous-head trait in tuh(ASU) in daily samples of third instar larvae reared at 25°C.

A. MALES

Structure Transformed	Alterations (% of daily total) Day1	Day2	Day3	Day4	Day5	Day6	TOTAL
EYE to							
Abdomen	40.3	33.7	31.1	38.7	32.7	28.3	34.1
Unidentified	27.3	18.3	28.4	22.5	24.5	32.1	25.0
Genital	01.3	03.1	02.7	03.2	01.0	00.0	02.0
Antenna	00.0	01.0	00.0	00.0	00.0	00.0	00.2
Palpus	00.0	01.0	01.4	00.0	00.0	01.9	00.7
TOTAL EYE ALTERATIONS	50	56	47	40	57	33	283
ANTENNA to							
Leg	01.3	03.1	00.0	01.6	02.0	00.0	01.5
Unidentified	04.2	03.1	05.4	03.2	02.0	00.0	03.5
Genital	01.3	01.0	00.0	00.0	00.0	00.0	00.7
Antenna Missing	00.0	00.0	00.0	01.6	01.0	00.0	00.4
Aristi Missing	02.8	01.0	04.1	00.0	01.0	01.9	01.8
Aristi Misshaped	00.0	02.0	01.4	01.6	04.1	01.9	02.0
Aristi Duplicated	01.3	00.0	00.0	00.0	00.0	00.0	00.2
TOTAL ANTENNAL ALTERA.	8	10	8	5	10	5	46
ROSTRALHAUT to							
Abdomen	00.0	03.1	01.4	00.0	01.0	03.8	01.5
Unidentified	06.9	15.3	13.5	14.5	10.2	11.3	12.0
Genital	05.6	02.0	02.7	03.2	05.1	03.8	03.7
Antenna	00.0	00.0	00.0	00.0	02.0	00.0	00.4
Palpus	00.0	01.0	00.0	00.0	00.0	00.0	00.2
TOTAL ROSTRAL. ALTER.	9	21	13	11	18	10	82
PROBOSCIS to							
Palpis Missing	06.9	04.1	06.8	04.8	12.2	09.4	07.4
Palpus Modified	00.0	05.1	01.4	04.8	01.0	00.0	02.2
Palpus Doubled	00.0	02.0	00.0	00.0	00.0	00.0	00.4
TOTAL PROBOSCIS ALTERA.	5	11	6	6	13	5	46
TOTAL # OF ALTERATIONS	72	98	74	62	98	53	457
# OF HEADS EXAMINED	39	39	34	34	43	25	214
AVERAGE # OF ALTERATIONS PER HEAD	1.85	2.51	2.18	1.82	2.28	2.12	2.14
% ECLOSION	81.8	79.5	79.7	66.0	69.1	61.0	76.2
% OF TOTAL POPULATION MALE	82.0	62.6	55.5	45.0	47.6	54.3	60.3
% OF TOTAL LARVAE TRANSFER. MALE	76.3	59.8	47.8	38.0	36.8	41.7	52.7

TABLE 14 B. FEMALES

Structures Transformed	Alterations (% of daily total)						
	Day1	Day2	Day3	Day4	Day5	Day6	TOTAL
EYE to							
Abdomen	42.0	45.7	34.5	29.3	35.4	42.1	37.4
Unidentified	21.7	21.4	28.7	23.6	36.8	15.8	26.0
Genital	00.0	04.3	01.1	03.3	00.0	02.6	02.0
Antenna	00.0	00.0	00.0	00.8	00.0	00.0	00.2
Palpus	00.0	00.0	00.0	00.0	00.0	00.0	00.0
TOTAL EYE ALTERATIONS	44	50	56	70	57	23	300
ANTENNA to							
Leg	00.0	01.4	02.3	03.3	01.3	02.6	02.0
Unidentified	05.8	02.9	06.9	09.7	03.8	02.6	04.8
Genital	00.0	00.0	00.0	00.8	00.0	00.0	00.2
Antenna Missing	01.4	00.0	01.1	01.6	01.3	00.0	00.9
Aristi Missing	02.9	01.4	02.3	08.1	00.0	02.6	02.4
Aristi Misshaped	04.3	02.9	04.6	08.1	06.3	05.3	04.6
Aristi Duplicated	01.4	00.0	00.0	00.0	00.0	00.0	00.2
TOTAL ANTENNAL ALTERA.	11	6	15	23	10	5	70
ROSTRALHAUT to							
Abdomen	01.4	00.0	01.1	00.8	02.5	05.3	01.5
Unidentified	10.1	07.1	10.3	17.7	06.3	07.9	08.8
Genital	02.9	05.7	01.1	08.1	00.0	02.6	01.8
Antenna	00.0	00.0	00.0	00.8	01.3	00.0	00.4
Palpus	00.0	00.0	00.0	01.6	00.0	00.0	00.4
TOTAL ROSTRAL. ALTERA.	10	9	11	20	8	6	64
PROBOSCIS to							
Palpus Missing	02.9	04.3	03.4	08.1	03.8	02.6	03.7
Palpus Modified	02.9	02.9	02.3	08.1	01.3	00.0	02.6
Palpus Doubled	00.0	00.0	00.0	00.0	00.0	00.0	00.0
TOTAL PROBOSCIS ALTERA.	4	5	5	10	4	1	29
TOTAL # OF ALTERATIONS	69	70	87	123	79	35	463
# OF HEADS EXAMINED	38	41	39	42	37	21	218
AVERAGE # OF ALTERATIONS PER HEAD	1.82	1.71	2.23	2.93	2.14	1.81	2.12
% ECLOSION	83.0	70.5	58.7	49.4	44.2	36.8	56.0
% OF TOTAL POPULATION FEMALE	18.0	37.4	44.5	55.0	52.4	45.7	39.7
% OF TOTAL LARVAE TRANSFERRED FEMALE	23.7	40.2	52.2	62.0	63.2	58.3	47.3

able and random throughout the 6 day interval. Males showed a drop in eclosion rates from 81.8%, on day 1, to 61.0%, on day 6. The females showed the steepest drop in eclosion rates, 83.0%, day 1, to 36.8%, on day 6. The females of day 1 and day 2, with the highest female eclosion rates for the 6 day interval, (83.0%, dayl; 70.5%, day 2 (Table 14)), also had a high percentage of their tumors occurring in the eye region, especially as eye transformed to abdomen (42.0%, day 1; 45.7%, day 2 (Table 14)). By days 3 and 4, tumor expressivity had been increased in areas other than the eye; eye transformations to unidentified had increased; female eclosion rate had taken a sharp drop (58.7%, day 3; 49.4%, day 4 (Table 14)). In females, on day 4, within the antennal region, the rostralhaut region, and the proboscis region, all types of reported alterations were at their maximum values (Tables 14). The 4th day, in females, showed the highest average number of alterations per head, 2.93 (Table 14), and the highest percentage of females produced within the developing population, 55.0% (Table 14). On the 4th day, in males, they had their lowest percentage of alterations per head for any of the six days, 1.82 (Table 14), and the lowest average percentage of the population that was male, 45.0 (Table 14). On days 5 and 6, in females, the percentage

of tumors in areas other than eye and the number of tumors per head had again dropped; eclosion rate, as well as the female percentage of the population produced in the daily samples, continued to decline. Many females that appeared in the third instar larval stage on days 5 and 6 of larval collection, were not able to survive through the pupal stage and metamorphosis.

DISCUSSION

Throughout the results section, the data obtained by the first method of temperature exposure has been kept separate from the data obtained by the second method. The first method utilized a technique whereby, at times, even the parental flies were exposed to the temperature shock conditions; there was very little control over determining the age of the egg when it was exposed to a specific temperature. At least a day was usually required for adult flies to get acclimated to a sharp change in temperature, such as from 25°C to 18°C, and before the females would begin egg production. Also, eggs required different lengths of time to reach the third instar larval stage. It would have been quite possible, when larval collections were made, for third instar larvae from eggs that had been laid on the first day of the temperature exposure and eggs laid on a much later day to be collected in the same day's larvae sample. The larvae collected would have been exposed to different temperature conditions during different stages in their development. The results presented in Tables 5-10 are the products of such a general temperature exposure technique. Even though this exposure method might

not be considered precise, it is representative of what would normally occur within breeding populations of tuh(ASU) and Canton-S.

The developmental fate of the imaginal disk is determined in the first 3-7 hours after fertilization, at 25°C, in Drosophila melanogaster (Fristrom 1970). The imaginal disks are small groups of cells set aside early in embryogenesis which are destined to develop into a particular adult structure during metamorphosis. The disk cells remain undifferentiated and never form a functional part of a larva. Low environmental temperatures during the early critical period (3-7 hours after fertilization) are known to increase the length of the developmental period (Gardner and Ratty 1952). Also, low temperature, within this critical period is associated with a low expression of the tumorous-head trait (Gardner and Ratty 1952; Gardner and Woolf 1950). This suggests the possibility of a relationship between tumorous-head expression; rate of development; and temperature.

Normally at 25°C, due to a proposed maternal effect, tuh(ASU) male eclosion is much greater than female tuh(ASU) eclosion (Tables 5,10). When tuh(ASU) bottle populations were exposed to 18°C for either 48 hours or 4 days the female eclosion rate was comparable to the male eclosion rate. However, these samples were very

small when compared to the sample sizes of the other goups used in this study. Exposure to 18°C during the early developmental stages eliminated the differential viability in favor of males which occurs between the third instar and eclosion. Cultures exposed to an 18°C cold shock for only short periods of time during their early development, 48 hours or 4 days at 18°C, had shorter collection periods for appearing third instar larvae than those samples reared at 25°C. Eggs exposed to 18°C for 10 days or longer had longer third instar collection periods than those reared at 25°C. The duration of the cold shock played an important role in setting the appearance of the third instar larvae within the population.

For homokaryotypes, the sample exposed to 18°C for 4 days, which also had the shortest third instar larval collection stage (2 days, Table 7), had the lowest average tumor penetrance (%Tum. male 26.3, %Tum. female 50.0, Table 7, 10). Heterokaryotypes demonstrated their lowest tumor penetrances in the 48 hour 18°C exposure group (%Tum. male 38.7, %Tum. female 47.4, Table 6,10), which had a larval collection length of 4 days (Table 6). Exposure to 18°C during the early developmental period not only reduced the number of days required for the collection of the third instar

larvae, it also reduced tumor penetrance. However, extended exposure to low temperature, past the early developmentally critical period, reversed the effects seen for the short temperature shocks. Although Gardner and Woolf (1950), using 18°C temperature shocks of different lengths of time and during many different developmental stages, demonstrated this trend, they apparently did not recognize its importance. One possibility suggested by the reversal is the possibility of a second temperature sensitive stage, later in development, which influences the differentiation of the tumorous-head phenotype.

At 25°C, there are normally significantly more male than female homokaryotypes (Table 5). Under all 18°C conditions used there is no significant variation in the ratio of male to female homokaryotypes, probably due to their small numbers.

In no case did any of the temperature sequences employed eliminate the maternal effect which is responsible for a sex ratio in favor of males. The sex ratios of the sample populations did not vary significantly from the sex ratios of the uncollected bottle populations.

This method demonstrated that differences in male and female eclosion, tumor penetrance, and the time required for the eggs to develop into the third instar

larvae, could be reduced by an 18°C temperature shock during an early, developmentally critical period. It also showed that increased exposure to 18°C reversed the effects of the early temperature exposure. Accompanying the reversal was an increase in the number of days for the third instar larval collections, especially in females, and a reduction in eclosion rates, with the greatest drop in females.

The second method of temperature exposure, Tables 11-13 utilized a technique in which egg samples, collected over a period of 2 hours, could be exposed to specific temperatures during specific stages of their development, most importantly during the developmentally critical first 3-7 hours into embryogenesis. This method specifically tested the relationship between tumorous-head expression, temperature and developmental rate. One sample exposed the eggs to 18°C until they reached the third instar larval stage (about 12 days); the other sample exposed the eggs to an 18°C cold shock for the first 48 hours of the study. After both 18°C exposures, the samples were then retained at 25°C.

There was no significant difference in male versus female tuh(ASU) eclosion rates for both 18°C exposures. The maternal effect which normally favors male viability between third instar and eclosion, and found in popula-

tions at 25°C, had been eliminated. Both samples had an average larval collection length of 5 days, one day shorter than the average third instar collection period at 25°C. The lowest tumor penetrance was found in the group exposed to 18°C for 48 hours (Table 12). In this sample, male and female tumor penetrances were almost the same (Table 13). Due to different conditions in each sample group's medium, it would be questionable to directly compare the results of the two different exposure groups. Within both samples, there was no significant variation in male and female homokaryotype production. At no time in the temperature exposures was a one-to-one sex ratio produced. However, the ratio of males transferred as larvae to males eclosed remained constant within and between both exposure groups, due to the similar eclosion rates for males and females. The sex ratios of the bottle populations did not vary significantly from the sex ratios of the sample populations except for the sample exposed to 18°C until the third instar stage. In this case, the bottle sample was small.

The second method confirms that an early 18°C exposure eliminates the difference between male and female eclosion rates, and produces low tumor penetrance. By this exposure technique, the age of the egg when it is

first exposed to 18°C is known. Between males and females, exposure to 18°C for 48 hours produced the most similar eclosion rates and tumor penetrances. The 18°C exposure that lasted until the third instar stage, 12 days, caused a greater increase in female tumor penetrance in terms of male tumor penetrance; reduced female eclosion rates in terms of male eclosion rates. The difference between the male and female sex ratios remained constantly in favor of males, for both temperature groups throughout both the third instar and adult stages.

Results of method one and two temperature exposures suggests that there is a definite correlation between the number of days of appearance of third instar larvae within a population and its tumor expression. A cold shock was only effective at altering the visible effects of tumorous-head development if it was applied for a short period of time during an early critical developmental period. The changes were brought about by the application of the cold shock during the sensitive period of determination of the eye-antennal disk. This was also demonstrated by 18°C cold shocks employed in the study by Gardner and Woolf (1950). However, in both their study and my study, if the cold shock was applied for too long a period of time, the effects of the cold shock were completely reversed. Therefore, it was not

the cold temperature alone that changed the expression of the tumorous-head trait, but when in development the temperature was applied, that produced the changes.

Table 14 presented data for the analysis of the development of the tumorous-head trait at 25°C, for each sex, and in terms of the day of appearance and collection in the third instar larval stage. The results showed that even though the male and female average number of alterations per head were the same, after averaging together the results of the six larval collection days, the daily expressivity patterns differed. The majority of males reached the third instar larval stage within the first three days of collecting. For all six days, male eclosion was high and tumor expressivity was random and varied. In contrast, the female proportion of the population, as well as the tumor expressivity, started out low and continued to increase with each later day's third instar sample; they reached their maximum values on day 4. Meanwhile, female eclosion declined with each later day's larval sample. By day 4, over half the larvae collected and transferred to the shell vials were females. At 25°C, the majority of the female population appeared in the third instar larvae stage well after many males had pupated (days 4, 5, and 6, Tables 14). After day 4, tumor expressivity

decreased, and a large number of females that had been collected as third instar larvae did not survive through the pupal stage. This difference in eclosion pattern only helped increase the already present sex ratio in favor of males that is characteristic of the tumorous-head strain. Females collected on days 5 and 6 as third instar larvae apparently had not been able to survive the differentiation of their imaginal disks into the respective adult strucutres. The high tumor expressivity of these females may have been lethal, and only the less severely affected females were able to survive through eclosion. This could account for the apparent drop in tumor expressivity and number for females after day 4 (Table 14).

In tumorous-head, at 25°C, the females appeared to have had a different developmental rate than males, if appearance in the third instar larval stage can be used as an indication of rate. The longer the number of days required for reaching the third instar larval stage, the higher was the apparent tumorous-head expressivity. From this it appears that the greater the gap in the time required between males and females obtaining the third instar larval stage, the larger the gap between their average tumor expressivity and overall viability.

Due to early 18°C cold shocks, male and female

appearance in the third instar larval stage was apparently synchronized. Males and females then showed similar tumor penetrances and eclosion rates. The brief 18°C temperature, during the determination of the disks, was an affective shock factor for blocking the expression of the tumorous-head homoeotic mutant. The homoeotic mutant, in the first 3 to 7 hours of development, at 25°C, possibly brings into play within the cells of the disk, genes that had previously been unexpressed. When developing eggs were left at 18°C for longer periods of time, the number of larval collection days increased, with the majority of female larvae being collected on the later days. The shock advantage of the early and brief 18°C exposure was lost when the sample was retained at 18°C for one generation (Table 9), because tumor penetrance and differential viability returned to the normal rates. This also indicates that a second temperature sensitive period, during differentiation, might be involved in tumorous-head phenotype expression. Work by Gardner and Woolf (1950) also indicated the possibility of a later temperature sensitive stage which could influence expressivity.

Future work in studying homoeotic mutants will have to be done in areas which influence developmental

regulatory mechanisms such as the biochemical pathways involved, and the mechanisms which trigger the start and stop of homoeotic expression in the different developmental stages. It will be necessary to find out why females are taking longer to reach the third instar stage than males at 25°C; and why they apparently both reach the third instar stage at about the same time when exposed to a brief 18°C, early cold shock. It is necessary to find out how factors which change tumorous-head expression, such as enhancer genes, quantity and quality of food, alter the crucial regulatory mechanisms.

Larger samples will have to be made at 18°C exposures for 48 hours and 4 days. Samples should be made for days between the intervals already studied. The adults from these 18°C cold shocks then need to be analyzed in terms of their phenotype expressivity and day of appearance in a recognizable stage such as the third instar, as was done for samples reared at 25°C. Also temperature exposures will have to be made in order to identify the time when an 18°C cold shock's effect first begin to reverse. If there is a second temperature effective stage, as appears to be the case, it needs to be uncovered.

In my study, male and female survival rates have been reported between the third instar stage and eclosion.

In order to find out when the sex ratio in favor of males occurs, male and female survival rates and sex ratios must be recorded between earlier developmental stages. This could be done by adding some type of marker that will distinguish males and females apart earlier than the third instar stage.

SUMMARY

This study was initiated in order to analyze the development of a strain of Drosophila melanogaster which has been given the descriptive name "tumorous-head", symbolized tuh(ASU). The tumorous-head trait is classified as a homoeotic mutant. In such a mutant, a particular organ is transformed partially or comletely into an organ normally found in a different area of the body. The tumorous-head trait is caused by a third chromosome, semidominant mutant gene symbolized tu-3 (Gardner and Woolf 1949). Two types of third chromosomes are involved symbolized 3A and 3B (Woolf and Phelps 1960). Chromosome 3B carries a large paracentric inversion, called the Payne inversion, symbolized In(3L)P, on its left arm (Woolf and Phelps 1960). Heterosis brought about by heterozygocity for the Payne inversion buffers development in the presence of the genetic mechanism producing the abnormal development in the head region (Woolf and Lott 1965). Due to natural selection, the heterozygous condition (3A/3B) was found in 85% of the tumorous-head population used for my study. The penetrance of the tu-3 gene is increased by a maternal effect controlled by a sex-linked recessive gene, symbolized

tu-1 (Gardner and Woolf 1949). Other genetic modifiers and environmental conditions, which further increase penetrance and expressivity, have been found.

Through my study, it has been found that:

(1) Heterokaryotypes (3A/3B) have a slightly higher penetrance than homokaryotypes (3A/3A). However, no significant difference was found to exist in the expressivity of the two karyotypes, or in the males and females of either karyotype.

(2) The addition of an enhancer gene of tu-1 (Woolf personal communication) increased tumor penetrance. There was a highly significant enhancement in expressivity. Also, the addition of the enhancer gene differentially effected expression of the trait in areas other than eye, such as antenna and rostralhaut.

(3) When penetrance and expressivity of the tumorous-head trait were high, transformations of tissue to identifiable structures occurred at higher rates within the eye region, and more importantly, within other regions. Transformations were found of eye tissue to abdominal tissue; rostralhaut to genital; and antennal structures, as previously reported by Postlethwait et al. (1972). Transformations previously unreported that were found in my study were: eye transformed to genital tissue; rostralhaut transformed to abdominal tissue; antenna

transformed to genital tissue.

(4) Kauffman's transdetermination model (1973) for tumorous-head development was found to be correct in its prediction of finding transformations from antennal segments to genital tissue. Antenna to genital transformations had previously never been reported, and as predicted by Kauffman, the transformation occurred at lower frequencies than transformations of antenna to leg. However, his prediction of transformations of wing to leg occurring in tumorous-head flies showing high tumor penetrance and expressivity was not shown. There was no evidence of wing to leg transformations found in 4,730 female flies examined (The females had an average tumor penetrnace of 96.6% and an average of 3.27 alterations per head). In tumorous-head the eye-antennal disk appears to be the only disk destabilized.

(5) Using cold shocks of 18°C, applied for varying lengths of time during different developmental stages, it was shown that application of low temperature, during a developmentally early and critical stage, caused a decrease in tumor penetrance and eliminated the differential viability of males and females between the third instar and the adult stage. The higher the eclosion rates, the lower the corresponding tumor penetrance. However, extended exposure to low temperatures reversed

any effects in tumor penetrance and viability brought about by the short, early temperature shock. This reversal suggests the existance of a second temperature sensitive stage later in development.

(6) In tumorous-head populations, reared at 25°C, males developed much afaster than females. The majority of males were collected in the third instar stage approximately two days before the femlaes. The later the females appeared in the third instar stage, the lower their viability. The lower the female viability is, then the greater the possible tumor expressivity. Females demonstrated a higher mortality than males in the pupal stage, probably due to their increased tumor expressivity.

REFERENCES

Bryant, P.J., and H.A. Schneiderman. 1969. Cell lineage, growth and determination in the imaginal leg discs of Drosophila melanogaster. Dev. Biol.,16: 1-17.

Child, G.P., R. Blanc, and H.H. Plough. 1940. Somatic effects of temperature on development in Drosophila melanogaster. Physiol. Zool.,13: 56-64.

Fristrom, J.W. 1970. The developmental biology of Drosophila. Annual Review of Genetics,4: 325-346.

Gardner, E.J., and F.J. Ratty. 1952. Penetrance and expressivity of tumorous-head in Drosophila melanogaster and relative viability of flies carrying the tumorous-head genes. Genetics, 37: 49-61.

Gardner, E.J., and C.M. Woolf. 1949. Maternal effect involved in the inheritance of abnormal growths in the head region of Drosophila melanogaster. Genetics, 34: 573-585.

Gardner, E.J., and C.M. Woolf. 1950. The influence of high and low temperatures on the expression of tumorous-head in Drosophila melanogaster. Genetics, 35: 44-55.

Gardner, E.J., G.H. Stott, and D.M. Dearden. 1952. Modifiers of tumorous-head genes in natural populations and laboratory stocks of Drosophila melanogaster. Genetics, 37: 451-456.

Gehring, W. 1967. Clonal analysis of determination dynamics in cultures of imaginal discs in Drosophila melanogaster. Devel. Biol. 16: 438-456.

Gehring, W. 1970. A recessive lethal 1(4)29 with a homoeotic effect in Drosophila melanogaster. Drosophila Inform. Serv. 45: 103.

Hadorn, E. 1965. Problems of determination ant transdetermination in genetic control of differentiation. Brookhaven Symp. Biol.,18: 148-161.

Hadorn, E. 1966. Dynamics of determination. In Major Problems in Developmental Biology, M.Locke (ed.). Academic Press, New York, pp. 85-104.

Hadorn, E., and D. Buck. 1966. Uber entwicklungsleistungen transplantierter teilstucke von flugelimaginalscheiben von Drosophila melanogaster. Rev. Suisse Zool., 69: 302-310.

Kauffman, S.A. 1973. Control circuits for determination and transdetermination. Science, 181: 310-318.

Knowles, B.B. 1967. Fecundity and fertility in female homo- and heterokaryotypes of the tumorous-head strain of Drosophila melanogaster. Genetics, 57: 437-447.

Kuhn, D.T. 1970. The interaction between the Payne inversion and genetic background in the tumorous-head strain of Drosophila melanogaster. Evolution, 24: 181-187.

Kuhn, D.T. 1971. Coadaptation of the Payne inversion with a previously unrelated genetic background in Drosophila melanogaster. Genetics, 69:467-478.

Kuhn, D.T. 1973. A maternally effected sex ratio condition in the tumorous-head strains of Drosophila melanogaster. Genetics, 74: s146.

Newby, W.W. 1949. Abnormal head growths on the head of Drosophila melanogaster. J. Morphol., 5:177-185.

Nothiger, R. 1972. Introduction. In The Biology of Imaginal Disks, Results and Problems in Cell Differentiation, Vol. V, H. Ursprung and R. Nothiger (eds.). Springer-Verlag, Berlin, p.1.

Postlethwait, J.H., and H.A. Schneiderman. 1973. Developmental genetics of Drosophila imaginal discs. Annual Rev. of Genetics, 7: 381-433.

Postlethwait, J.H., P.J. Bryant, and G. Schubiger. 1972. The homoeotic effect of tumorous-head in Drosophila melanogaster. Devel. Biol. 26: 277-295.

Strickberger, M.W. 1962. Experiments in Genetics With Drosophila. John Wiley and Sons, Inc., New York.

Vogt, M. 1946. Zur labilen determination der imaginalscheiben von Drosophila 1. Verhalten verschiedenaltriger imaginalanlangen bei operativer defektsetzung. Biol. Zentr., 65: 223-238.

Waddington, C.H. 1953. Genetic assimulation of an acquired character. Evolution, 7: 118-126.

Woolf, C.M. 1965. Genetic divergence among the tumorous-head strains of Drosophila melanogaster. Genetics, 52: 809-817.

Woolf, C.M., and K. Church. 1963. Studies on the advantage of heterokaryotypes in the tumorous-head strain of Drosophila melanogaster. Evolution, 17: 486-492.

Woolf, C.M., and B.B. Knowles. 1964. Interchromosomal control of karyotype fitness traits in tumorous-head strain of Drosohila melanogaster. Genetics, 49: 165-173.

Woolf, C.M., and M.O. Lott. 1965. Relationship between penetrance and karyotype in the tumorous-head strain of Drosophila melanogaster. Am. Natur., 99: 511-513.

Woolf, C.M., and L.J. Phelps. 1960. Chromosomal polymorphism in the tumorous-head strains of Drosophila melanogaster. Science, 132: 1256-1257.

Woolf, C.M., B.B. Knowles, and M.A. Jarvis. 1964. Genetic analysis of fitness traits in tumorous-head strains of Drosophila melanogaster. Genetics, 50: 597-610.