TEMPERATURE-SENSITIVE CELL-LETHAL MUTANTS OF DROSOPHILA: ISOLATION AND CHARACTERIZATION

ROBERT ARKING

Center for Pathobiology, University of California, Irvine, California 92664

Manuscript received October 29, 1974

ABSTRACT

One hundred and twenty-one temperature-sensitive (ts) sex-linked lethals were screened by means of X-ray-induced somatic crossing over to determine if any were ts cell-lethal mutants. Cell-lethal mutations were identified by their ability to block the development of homozygous clones when raised under restrictive conditions (29°). Twenty-two ts cell-lethal mutants were isolated and categorized into three classes, depending upon the patterns of damage observed in larval and imaginal tissues. The phenotypes produced by these mutations ranged from those which affected only a limited set of structures (i.e., genital discs only) to those which affected diverse tissues at all stages of the life cycle. Each mutation has its own characteristic time-dependent pattern, frequency, and type of damage. All the mutations affect imaginal tissue, but only one-third of the mutations affect both larval and imaginal tissue. The fastest-acting lethals need 15 hours at the restrictive temperature to kill the cells and the slowest-acting lethals require at least 48 hours. By choosing the appropriate mutant and by manipulating the times of exposure to the restrictive temperature, it has proven possible to produce duplications and deficiencies in specific structures of the adult. A mechanism by which lethality might yield such structures is suggested. In addition, 15 of the mutants are ts female sterile mutants. Only one of these 15 mutants can recover its fertility when shifted back down to the permissive temperature (22°).

THE important role which cell death plays in the development of the imaginal structures of Drosophila has recently come to be appreciated. Stage-specific patterns of cell death have been observed during the normal development of Sarcophaga (WHITTEN 1969) and of Calliphora and Drosophila (SPREIJ 1971). Going a step further, it has been shown by FRISTROM (1968, 1969) that the production of specific morphological defects in structurally deficient mutants of Drosophila is due to the occurrence of cell death in specific regions of the affected imaginal discs. Cell death has also been cited as a cause of the duplicated adult structures observed after X-irradiation of the larvae (POSTLETHWAIT and SCHNEIDERMAN 1973).

A powerful tool for analyzing certain developmental processes such as pattern formation would consist of manipulating the regions of cell death in developing imaginal discs and analyzing the types and frequencies of the structural abnormalities produced in the adult. The mutants of choice for this technique would be temperature-sensitive (ts) autonomous cell-lethal mutations. Such mutations have recently been isolated by RUSSELL (1974) and by SIMPSON and SCHNEIDER-

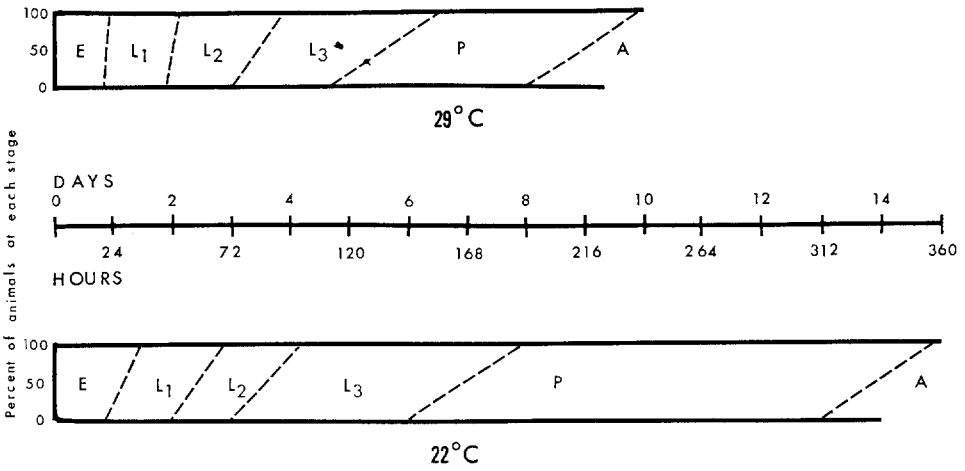
MAN (1975). This report describes the identification of twenty-two ts cell-lethal mutations of Drosophila by means of the twin spot test (STERN 1954) and a description of their general characteristics as a group, including their effect on pattern formation in the imaginal discs.

MATERIALS AND METHODS

Point mutations were chemically induced in the sperm of young adult males of *Drosophila melanogaster*, Oregon-R strain, by allowing them to feed for 12–14 hours on a sucrose solution containing 0.025 M ethylmethane sulfonate (LEWIS and BACHER 1968). These mutagenized males were then mass-mated to *yf C(1)RM/Y* (attached-*X*) virgin females at 22°. Each of the resulting sons received their (mutagenized) *X* chromosome from their fathers. Each of the F_1 male progeny was then mated with several attached-*X* virgin females at 22° to establish a stock culture at the permissive temperature. The F_1 parents were then transferred to new vials at 29° to establish a test stock at the restrictive temperature. The presence of F_2 males at 22° and their absence at 29° indicated a putative ts lethal. Each putative ts lethal was then retested. A mutant was classified as a ts lethal when the stock (balanced over *yf C(1)RM/Y*) exhibited a sex ratio of 0 ♂ : 1.00 ♀ at the restrictive temperature and a sex ratio of at least 0.5 ♂ : 1 ♀ at the permissive temperature in two successive tests. A total of 20,569 mutagenized *X* chromosomes were screened. Several additional mutants were obtained from a large number of putative ts lethals generously given to us by PROFESSOR D. L. LINDSLEY. These lethals were originally produced in a $XY^L:Y^S$, y^2 *su(*w^a*)* w^a chromosome.

Each mutation was simultaneously mapped using standard techniques. From the mapping crosses, chromosomes were recovered carrying a given lethal mutation and a marker mutation. The three markers employed were *y* (*yellow*), sn^3 (*singed*^{3}), and f^{36a} (*forked*), which affect bristle color and morphology respectively. (For a description of these and other mutants employed in this study, see LINDSLEY and GRELL 1968.) The basic procedure used to isolate the ts cell-lethal mutants was the twin spot test, utilizing X-ray-induced somatic crossing over (BECKER 1956). For this procedure *y* lethal males from each stock were mated with a large number of sn^3 virgin females. Fertilized eggs were collected over a two- or four-hour period, and at 22°. The larvae were floated off the food at 65–70 hours after oviposition, and X-irradiated with a dose of 1500 r (Picker X-ray machine, 110 kVp and 3 ma at about 500 rpm). Immediately after irradiation, the cultures were divided into two parts, transferred to new food bottles, and allowed to complete their development at either 22° or 29°. Within a few days after eclosion, the F_1 female progeny were mounted in Gurr's water mounting medium glass coverslips and the whole body except for the wings examined at 430 X magnification for the presence or absence of homozygous mutant tissue. A ts cell-lethal mutant would be expected to reduce the frequency of mutant clones recovered at 29°. At least ten flies were examined for each mutant in the initial screen. An additional ten animals were examined for each of those mutant stocks which appeared promising based on the initially depressed ratio of $y:sn^3$ clones. Calculations of statistical significance of the $y:sn^3$ ratio were made using chi-square analysis of 2×2 contingency tables.

For each mutant the duration of the temperature-sensitive period (TSP) and the timing of the effective lethal phase (LP) were determined from the analysis of reciprocal "shift-up" and "shift-down" experiments, as described by SUZUKI (1970). The beginning of the TSP is indicated by the earliest downward temperature shift ("shift-down") in which a significant number of animals die. The end of the TSP is indicated by the earliest upward temperature shift ("shift-up") in which a significant number of animals survive. Thus, the TSP is the developmental interval during which the animal is irrevocably committed to death by exposure to the restrictive temperature. The LP is the developmental stage at which this death occurs. The age of the animals, initially accurate to within ± one hour, was calculated from the time of oviposition. The shift interval was 24 hours unless otherwise specified. Figure 1 shows a comparison of the developmental chronology of the *Ore-R* wild-type strain used in this laboratory at 22° and 29°. Unless stated otherwise, all developmental ages refer to 22°.



DEVELOPMENTAL CHRONOLOGY OF THE ORE-R STRAIN AT PERMISSIVE AND RESTRICTIVE TEMPERATURES

FIGURE 1.—The progression of initially synchronous cultures of the Oregon-R wild-type strain through the different stages of the life cycle at 22° and 29° (E = embryo; L_1, L_2 and L_3 = first, second and third larval instars; P = prepupae and pupae; A = adults).

A detailed analysis of the timing and types of defects produced by each mutation in the imaginal disc derivatives was performed by subjecting larvae of known age to specific temperature pulses. Each stock was subjected to a minimum of 13 separate pulse treatments as follows: six 24-hr pulses (0–24, 24–48, 48–72, 72–96, 96–120, and 120–144 hrs); three 48-hr pulses (0–48, 48–96, and 96–144 hrs), and four 72-hr pulses (0–72, 24–96, 72–144, and 144–216 hrs). Each of these series of pulses covered the entire embryonic and larval stages (0–144 hrs after oviposition), with the exception of the 72-hr series which covered part of the pupal period as well (144–216 hrs after oviposition). In addition each stock was also subjected to two multiple pulse treatments. The 3 × 24-hr series involved three consecutive 24-hr heat pulses administered at 24–48 hrs, 72–96 hrs and 120–144 hrs after oviposition. The 2 × 24 series involved two consecutive 24-hr heat pulses administered at 48–72 hrs and 96–120 hrs after oviposition. The emerging adults were saved and examined for duplications and/or deficiencies of the imaginal disc derivatives. In many instances the animals died as pharate adults and had to be dissected out of the pupal cases. The number of animals per stock which were actually examined for the 48–96-hr pulse is indicated in Table 5. Comparable numbers were examined for the other pulses.

The structures affected by each mutation were determined by dissecting late third instar mutant larvae (about 140 hrs) which had been shifted up to 29° at 72 hrs after oviposition (mid-second instar) and by examining with the dissecting microscope the major imaginal discs, brain and ventral ganglion, fat body, salivary glands, Malpighian tubules, and gut.

Genetic mosaics, produced by the ring-*X* chromosome elimination method (HINTON 1955), were used to test the autonomy of the developmental effects of selected ts cell-lethal mutants. For the gynandromorph tests, virgin females heterozygous for the unstable ring-*X* chromosome (*R(1)w*^{vc}) and for the Binsinscy (*y sc*^{s1L} *B In(1)dl-49 sn*^{z2} *w sc*^{s8R}) female sterile balancer chromosome (BRYANT and ZORNETZER 1973) were mated at 22° with males hemizygous for a lethal and a marker mutation. The progeny from each stock tested were then allowed to develop at either 22° or 29°. Eclosed and pharate adult females containing the *R(1)w*^{vc} chromosome were fixed in 70% ethanol and examined at 25× in the dissecting microscope for any abnormal structures. Animals with abnormal patterns were more closely examined at 50× in the dissecting microscope, and then they were dehydrated, dissected, mounted between two coverslips in Euparol and set aside for a detailed examination with the compound microscope.

Two different vital staining techniques were used to visualize the dead and dying cells within the imaginal discs of heat-treated mutant larvae. These were the neutral red–trypan blue procedure of DE RENZIS and SCHECHTMAN (1973) and the acridine orange procedure of SPRELJ (1971). Mutant larvae were exposed to the restrictive temperature from 72–120 hrs or from 96–144 hrs after oviposition. At this time, the imaginal discs were dissected out, stained, examined, and photographed. In these procedures, the control larvae included both the mutant larvae raised only at 22° and the *Ore-R* larvae raised only at 29°.

RESULTS

I. *Efficiency of Mutant Recovery*

Out of 20,569 mutagenized *X* chromosomes, 2,581 putative ts lethals were obtained, of which 313 proved to be ts lethals when retested. Of these confirmed ts lethal stocks, 192 (62%) were unstable and gradually became non-lethal within 6 to 12 months of isolation while maintained against $y\ f\ C(1)RM/Yy^+$. In some of these cases the loss of the conditional lethality appeared to be due to the accumulation of modifiers at the permissive temperature, since the original penetrance could be restored by outcrossing. This high attrition rate, although it has not been reported before, seems characteristic of ts lethal mutations since it has also been observed in other laboratories which have done extensive work with such conditional mutants (SUZUKI, personal communication; WRIGHT, personal communication). Of the 121 stable ts lethals, 99 are not cell-lethal in their action, while 22 have proven to be autonomous ts cell-lethal mutations. Thus, 0.59% (121/20,569) of the mutagenized chromosomes carried a stable ts lethal. Of these lethals, one out of five eventually turned out to be a ts autonomous cell-lethal mutant. This report will concentrate on the developmental effects of the 22 ts cell-lethal mutants.

II. *Determination of Autonomy*

A. *Twin Spot test*

Strictly speaking, a ts cell-lethal mutation should be able to kill any cell at any developmental stage. Although one example of such a mutant has been recovered (see *l(1)ts-403*, Table 3), it was felt that too strict a definition would cause us to miss many other potentially interesting and useful mutants such as tissue- and/or time-specific cell lethals.

Throughout this study the term "cell lethal" is used in a manner identical to that of DEMEREC (1936), namely, it denotes a lethal which, when homozygous, prevents the appearance of a small patch of imaginal cuticular tissue for which the test is made. The designation does not necessarily imply that the mutation in question also has a lethal effect on the larval and/or internal tissues of the adult. This point is covered in more detail in the DISCUSSION.

The basic criterion used to separate the potential ts cell-lethal mutations from the other mutations was whether or not they allowed the survival and differentiation of small homozygous clones in X-ray-induced twin spots under restrictive temperatures. An analysis of the behavior of ts cell-lethal mutants in such X-ray-induced clones indicates that there should be approximately a 1:1 ratio of *y*

clones : sn^3 clones present at the permissive temperature. If the ts cell-lethal mutant is on a y chromosome, then the death of the homozygous mutant cells under restrictive conditions will be reflected in a decrease in the incidence of y, sn^3 twin spots and y single clones, with a corresponding increase in the number of sn^3 single clones. The number of y patches will only rarely decrease to zero since for each mutation there is a specific probability of the crossover events separating y from the *lethal* gene. Thus, a certain "noise level" of y non-lethal clones must be expected.

Table 1 records the observed behavior of the X-ray-induced clones under both restrictive and permissive conditions. At 22°, yellow and singed^3 clones were obtained in frequencies close to the expected ratio. At 29°, however, all but three of the mutants exhibit a significant excess of the singed^3 clones relative to yellow. Two of these apparent exceptions, *l(1)ts-1704* and *l(1)ts-1843*, both yield reasonably high levels of deficiencies and duplications (see Tables 3 and 6) and

TABLE 1

Twin spot analysis of ts cell-lethal mutations

Mutant no.	Number of clones observed 22° sn^3	22° y	29° sn^3	29° y	X^2	p*
Control: $y+/sn^3$	46	56(28)	41	48(25)	0.02	——
yl(1)ts-403	41	52(20)	48	17(3)	13.79	+++
yl(1)ts-1843	30	34(16)	43	28(7)	2.53	——
fl(1)ts-480	25	23(7)	61	4(0)	26.33	+++
yl(1)ts-1006	16	10(4)	18	2(1)	10.20	++
yl(1)ts-1251	25	30(14)	30	16(4)	3.87	+
yl(1)ts-3803	78	55(21)	41	14(8)	4.24	+
yl(1)ts-5697	57	82(27)	85	5(0)	20.85	+++
yl(1)ts-SD19	21	21(8)	45	8(0)	13.51	+++
yl(1)ts-UC32	17	19(9)	35	3(0)	17.82	+++
yl(1)ts-1704	31	29(12)	36	24(6)	0.82	——
fl(1)ts-5141	31	17(3)	45	8(0)	5.56	+
yl(1)ts-6225	24	25(7)	39	32(13)	0.38	——
yl(1)ts-UC13	15	22(4)	87	27(18)	16.32	+++
yl(1)ts-UC19	15	16(5)	53	16(3)	7.96	++
yl(1)ts-UC88	24	28(9)	52	7(0)	22.53	+++
yl(1)ts-UC259	29	36(12)	30	18(7)	3.86	+
yl(1)ts-2366	34	30(14)	42	9(3)	10.81	+++
yl(1)ts-2588	29	33(17)	28	10(3)	6.85	++
yl(1)ts-2864	37	40(19)	35	16(0)	5.24	+
yl(1)ts-3733	17	16(4)	17	4(2)	4.56	+
yl(1)ts-4975	18	19(10)	19	1(0)	12.16	+++
yl(1)ts-UC34	34	35(14)	76	29(7)	9.49	++

* Significance of deviation of the total number of yellow clones: total number of singed^3 clones observed at 22° as compared to the total number of yellow clones : total number of singed^3 clones observed at 29°. The figures in parentheses are the number of cases at that temperature in which singed^3 clones and yellow clones were found together in twin spots. + signifies $0.05 > p > 0.01$; ++ $0.01 > p > 0.001$; +++ $p > 0.001$. *l(1)ts-1843*, *-1704* and *-6225* were retained as ts cell-lethal mutations for the reasons presented in the text.

have been retained as putative ts cell-lethal mutants. The remaining mutant (*l(1)ts-6225*) exhibited *y* patches on some areas and none on other areas and has been shown to be a disc-specific ts cell-lethal mutation (VENTA and ARKING, unpublished observations).

B. *Gynandromorph test*

These mutants have also been analyzed by means of the gynandromorph test. These tests have identified three different ways in which hemizygous ts cell-lethal tissue can behave in gynandromorphs raised at 29°. The first class consists of those 21 mutations (i.e., all except *l(1)ts-6225*) in which no lethal-bearing gynandromorphs were observed when the animals were raised continuously at 29° although ring-*X* elimination should have occurred at about the same frequency as in their sibs in the corresponding 22° control crosses.

Mutant *l(1)ts-6225* is a disc-specific mutant and is the only mutation to show the second class of gynandromorph behavior. Lethal-bearing gynandromorphs did survive at the restrictive temperature, but with a much lower probability than their sibs raised at the permissive temperature (11% gynandromorphs at 29° compared to 56% at 22°. In these gynandromorphs hemizygous lethal tissue was not observed in the head or wing but has been seen in the thorax, legs, halteres, and abdominal structures. Focal-point mapping has confirmed that this mutation is disc-specific in its effects (VENTA and ARKING, unpublished observations).

The third class consists of those mutations which will yield both structurally abnormal gynandromorphs and non-gynandromorph females following a temperature pulse. A summary of the data obtained from the fourteen mutations tested to date is shown in Table 2. It has been assumed that the structually abnormal non-gynandormorph females were originally gynandromorphs in which the hemizygous lethal tissue has been killed as a result of the temperature pulse. Such animals have been designated as "cryptic gynandromorphs". The following points support the validity of this assumption: (a) In all but the last pulse the percentage of ring loss in the heat-pulsed groups is very close to that of the 22° control only when the number of cryptic gynandromorphs are included in the numerator (see Table 2). (b) It has been possible to find gynandromorphs which have structural abiormalities in the non-mutant tissue (Figures 2 and 3).

TABLE 2

Effect of temperature pulses on the recovery and the morphology of gynandromorph offspring arising from R(1)w^{ve}/*Binsinscy* + × y l o

Timing of temperature pulse (hrs)	Gynandromorphs: Visible + Cryptics	Percent ring loss	Percent cryptic gynandromorphs	Percent cryptic gynandromorphs with: Deficiencies	Duplications
22° Control	351 + 7	14.3	1.9	100	0
48–96	86 + 103	11.6	54.5	81	41
48–120	16 + 42	11.6	72.4	78	56
72–120	122 + 29	10.5	19.2	86	21
120–192	68 + 31	5.6	31.3	100	0

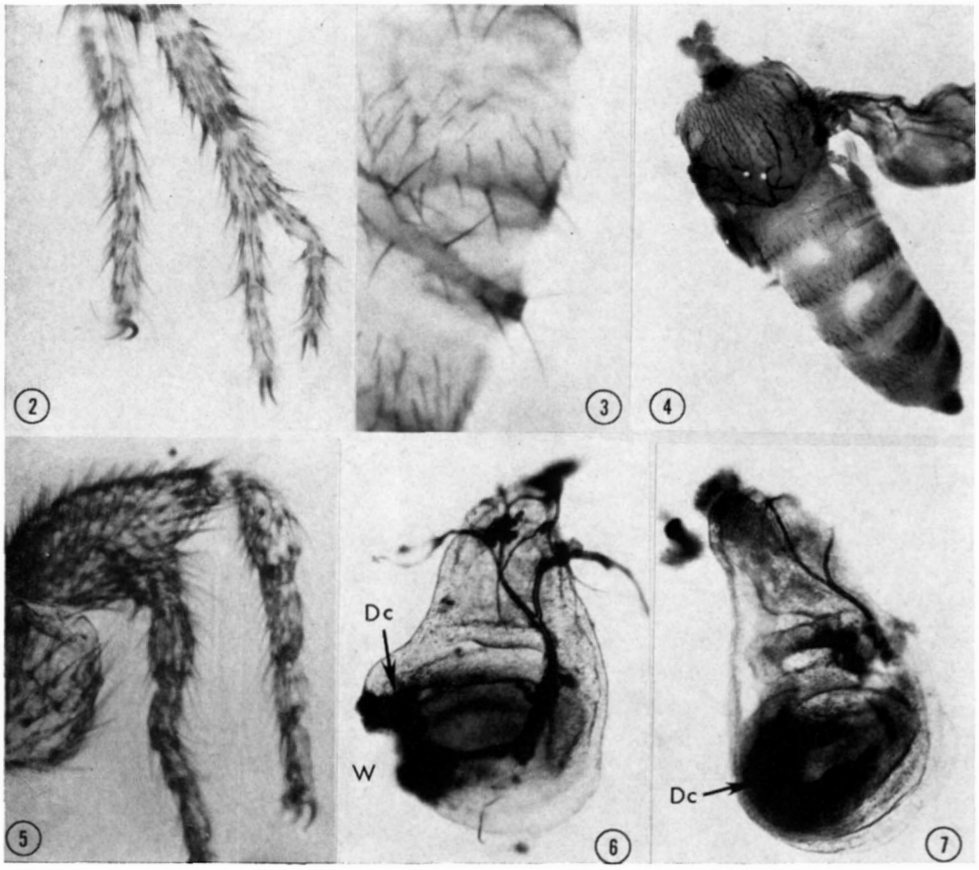


FIGURE 2.—A triplication of the second leg induced in a *R(1)w^{vc}/y l(1)ts-UC13* cryptic gynandromorph by a 48–96-hr temperature pulse. The affected structures are composed entirely of nonmutant tissue.

FIGURE 3.—Polarity reversal as seen in a duplicated tergite of a *R(1)w^{vc}/y l(1)ts-UC13* gynandromorph subjected to a 48–96 hr temperature pulse. The affected structures are composed entirely of nonmutant tissue.

FIGURE 4.—An example of tissue-specific cell death. A homozygous *l(1)ts-480* pharate adult subjected to a heat pulse from 72 to 96 hours after oviposition. Note that the eye and antennal disc derivatives are completely absent but that all other adult structures are normal.

FIGURE 5.—A leg duplication induced in a homozygous *l(1)ts-2366* animal by a heat pulse administered 48–96 hours after oviposition.

FIGURE 6.—A wing disc obtained from a homozygous *l(1)ts-5697* mature third instar larva raised at 22°. After dissection from the host, the disc was deliberately wounded with a hot needle and then immediately stained with a solution of 0.25% trypan blue-neutral red in Ringer's solution. The dark-staining areas represent areas of cell death caused by the wounding procedure. (Dc = dead cells; W = wound area).

FIGURE 7.—A wing disc obtained from a homozygous *l(1)ts-5697* third in star larvae which had been exposed to the restrictive temperature from 72–120 hours after oviposition. After dissection from the host, the disc was immediately stained with a solution of 0.25% trypan blue-neutral red in Ringer's solution. The dark-staining areas represent a localized area of heat-induced cell death. (Abbreviations as in Figure 6).

This last point is quite important since it clearly demonstrates that it is not the mere presence of mutant tissue that is responsible for these structural abnormalities but the decisive point is rather its sudden removal from a developing imaginal disc. This point is more fully developed in the DISCUSSION. (c) The type of structural abnormality produced (i.e., deficiencies and/or duplications) is dependent primarily on the timing of the temperature pulse and only secondarily on the particular mutation involved. Although deficiencies occur at a high rate in all pulses, it is clear that duplications are more frequently found in those animals subjected to early temperature pulses (compare Table 2 and Table 5).

III. *Classification of the Mutants*

All of the ts cell-lethal mutants have been characterized in terms of the patterns of damage exhibited by the larval and/or imaginal tissues, and each mutant can be put into one of three different classes (Table 3). The data in Table 3 are derived from the dissection and examination of heat-treated (72–144 hrs after oviposition) third instar larvae, as well as from the analysis of heat-pulse-induced duplications and deficiencies of imaginal structures. A structure was considered to be abnormal when either the imaginal discs or larval organs were of abnormal appearance (i.e., size, shape, color, presence and location of folds, etc.) upon dissection, and/or the adult structures possessed duplications and/or deficiencies following a heat pulse treatment.

The presence of abnormal discs in the larvae is often, but not always, correlated with the presence of abnormal disc derivatives in the adult (Table 3). This correlation is very strong in those structures most susceptible to the effects of cell death (i.e., leg discs) and much weaker in those structures not so susceptible (i.e., haltere discs). The morphological abnormalities observed in adults which had been heat pulsed during some part of their larval development consisted of deficiencies and/or duplications of various structures. Figure 4 shows an example of eye-antennal disc deficiencies caused by a 72–96-hr heat pulse of *l(1)ts-480*. The proboscis is attached directly to the thorax and there is no trace of the eyes, antennae or head capsule itself. Figure 5 shows an example of a leg duplication induced by a 48–96-hr heat pulse of *l(1)ts-2366*.

A. *Class 1: Mutants affecting both larval and imaginal tissue*

Approximately one-third (7) of the ts cell-lethal mutants affect both larval and imaginal tissues (Table 3). The brain is the larval structure most often affected, being much reduced in size relative to the size of the mature larvae in six different mutants. Only three mutants (*l(1)ts-403*, *l(1)ts-1251*, and *l(1)ts-5697*) also affected the larval fat body and/or salivary glands. No mutant was observed to affect any other of the larval structures examined. Although these conclusions are based on the dissection, vital staining and observation of heat-treated larvae, it is highly probable that defects at the cellular level may exist in many of these supposedly unaffected tissues. To answer this point will require a detailed histological investigation.

TABLE 3

Classification of ts cell-lethal mutations according to patterns of damage

Tissues affected	Mutant no.	Locus*	TSP	LP	Specific structures affected†											
					Lab‡	Ant	Eye	Wing	Ha	Lg1	Lg2	Lg3	Hist	Gen	Br	FB/SG
(Class 1)	*l(1)ts-403*	42	0–340	E-A	d	d	d	d	d	d	d	d	p	d	d	d
Larval and	*l(1)ts-1843*	46	24–192	L1-PhA		d	d	d		d	dp	dp		p	d	
imaginal	*l(1)ts-480*	54	24–168	L2-P		dp	dp	dp		dp	dp	dp	p	p	d	
tissue	*l(1)ts-1006*	—	24–168	L1-P		dp	dp	dp		dp	dp	dp	p	p	d	
	l(1)ts-1251	43	24–168	L2-P		p	p	p		p	p	p	p	p		d
	l(1)ts-3803	53	24–168	L2-PhA		d	dp	dp		dp	dp	dp	p	p	d	
	l(1)ts-5697	55	24–168	L1-PhA		d	dp	dp	d	dp	dp	dp	p		d	d
(Class 2)	*l(1)ts-SD19*	—	24–144	L1-Pp		p	p	p		p	p	p		p		
Imaginal	*l(1)ts-UC32*	45	0–216	L1-PhA										p		
tissue	*l(1)ts-1704*	46	24–168	L2-P		dp	dp	dp	d	dp	dp	dp		p		
only	*l(1)ts-5141*	34	24–96	L1-L3		p		p				p				
	l(1)ts-6225	20	0–288	E-PhA		d	d	d					p	p		
	l(1)ts-UC13	29	24–216	L1-PhA		dp	dp	dp	d	dp	dp	dp	p	p		
	l(1)ts-UC19	55	24–216	L2-P		p	p	dp	d	dp	dp	dp	p	p		
	l(1)ts-UC88	45	24–216	L1-P		p	dp	p	p	p	p	p	p	p		
	l(1)ts-UC259	53	24–216	L1-PhA		p	p	dp	d	dp	dp	dp	p	p		
	l(1)ts-2366	51	24–168	L2-P		dp	dp	dp	d	dp	dp	dp	p	p		
	l(1)ts-2588	—	24–168	L2-P		p	dp	dp		d	dp	dp	p	p		
	l(1)ts-2864	35	24–216	L2-PhA		d	dp	dp		d	dp	dp	p	p		
	l(1)ts-3733	38	24–168	L2-P		dp	dp	p		dp	dp	dp	p	p		
	l(1)ts-4975	49	24–192	L1-PhA									p			
(Class 3) Neither larval nor imaginal	*l(1)ts-UC34*	18	24–264	L3-P												
Summary: A. Larval dissections only					1	12	14	14	7	14	14	14	—	1	6	3
B. Pulse data only					0	12	15	16	1	14	16	17	16	17	—	—

* The map positions presented above are approximate since all classes of recombinant progeny were reduced for many mutants.

† The letter "d" means that the particular structure was determined to be abnormal upon dissection of the heat-treated larvae while the letter "p" means that the particular structure was abnormal in adults which had been heat-pulsed at some time during development.

‡ Abbreviations: Lab: labial disc or derivatives; Ant: antennal disc or derivatives; Ha: haltere disc or derivatives; Lg1, Lg2, Lg3: first, second and third leg disc or derivatives; Hist: histoblasts or derivatives; Gen: genitalia; Br: larval brain and ventral ganglion; FB/SG: larval fat body and salivary gland; E: embryo; L1, L2, L3: first, second and third larval instars; Pp: prepupae; P: pupa; PhA: pharate adult.

TABLE 4

*Correlation between occurrence of different abnormalities within the same imaginal disc derivatives**

Types of abnormalities	*N*	Percent
Deficiency + duplication	72	(61)
Deficiency only	39	(33)
Duplication only	7	(6)
	118	

* These data were obtained from all the mutations which caused structural abnormalities in the first, second or third legs following a 48–96-hr temperature pulse.

B. *Class 2: Mutants affecting imaginal tissue only*

There are 14 mutants in this class, none of which affect the labial discs or any larval structures. The extent of the duplications observed in the various stocks has ranged from the complete duplication of one structure to the duplication of only one portion of a structure. In general, however, it has been observed (Table 4) that deficiencies are much more commonly induced by the ts cell-lethal mutations than are duplications. It appears that, within any given structure, deficiencies can occur without duplications but that duplications usually appear to be associated with deficiencies.

C. *Class 3: Mutants affecting neither larval nor imaginal tissues*

l(1)ts-UC34 is the only member of this class. Although heat treatment does, in actuality, affect imaginal tissue since a significant reduction of *y* lethal clones was observed in the twin spot analysis (Table 1), the imaginal discs appear small but healthy following a 72–144-hr heat pulse. Neither deficiencies nor duplications were produced from any of the 15 heat pulses tested. Heat treatment appears to delay development of this mutant.

TABLE 5

Correlation of defects in any one adult structure with timing of the heat pulses

No. of stocks in which pulse yields	Timing of heat pulse 0–48	48–96	96–144	144–216
No effect	12	4	5	5
Lethality only	7	2	10	2
Deficiencies only	2	1 *	0	13
Duplications only	0	1 *	0	0
Deficiencies and duplications	1	15	7	0
Total	22	22	22	20

* Both of these numbers refer to the same stock, *l(1)ts-5141*, which produced deficiencies only in the wing and third leg but produced duplications only in the antennae (see Table 6).

IV. *Determination of TSP for Duplications and Deficiencies*

Of the 13 separate heat-pulse treatments administered to each stock, only the data from the 48–96-hr pulse will be presented in detail. In general, significant levels of structural abnormalities were obtained with pulses of 48 hrs duration (Table 5). A brief summary of the results of temperature pulses longer or shorter than 48 hrs follows. There were only three stocks (*l(1)ts-UC32*, *l(1)ts-UC88*, and *l(1)ts-480*) in which a 24-hr pulse could induce structural deficiencies and no stocks in which a 24-hr pulse induced structural duplications. A 72-hr heat pulse applied anytime during larval development caused most of the 22 ts cell-lethal mutants to die as larvae or as pharate adults. Such pharate adults possessed very severe and very extensive morphological abnormalities. The multiple heat pulses (i.e., either the 3 × 24-hr series or the 2 × 24-hr series) had absolutely no effect on 19 of the mutant stocks. In only one stock (*l(1)ts-UC259*) did a multiple heat pulse (2 × 24) induce structural deficiencies which were not induced by any single 24-hr heat pulse.

Thus, the single time period during which almost all of the mutant stocks are most sensitive to the restrictive temperature is the interval from 48 to 96 hrs after oviposition (see Table 5). It is clear that during this developmental period the highest level of heat-induced duplications involves the legs (Table 6). There are nine different mutations which cause at least 20% of the imaginal leg discs

TABLE 6

*Frequency of tissue-specific abnormalities observed in adults following exposure of the larvae to restrictive temperature from 48–96 hours**

Mutant	*N*	Lab		Ant		Eye		Wing		Ha		Lg1		Lg2		Lg3		Hist	Gen
		Df	Dp	Df	Dp	Df	Dp	Df	Dp	Df	Dp	Df	Dp	Df	Dp	Df	Dp	Df	Df
l(1)ts-480	255	0	0	52	0	54	0	43	0	0	0	6	1	1	1	1	1	25	27
l(1)ts-1006	21	0	0	14	0	21	0	21	0	0	0	17	21	12	26	5	21	33	33
l(1)ts-1251	25	0	0	6	0	16	4	58	0	0	0	62	30	30	38	46	26	0	72
l(1)ts-3803	12	0	0	0	0	8	0	25	0	0	0	42	8	0	63	4	17	0	33
l(1)ts-5697	27	0	0	0	0	41	0	11	0	0	0	7	26	0	26	0	35	0	4
l(1)ts-SD19	47	0	0	13	0	23	0	32	3	0	0	1	27	2	24	2	5	0	34
l(1)ts-1704	35	0	0	29	0	29	0	37	0	0	0	40	1	20	16	10	13	3	20
l(1)ts-5141	31	0	0	0	2	0	0	2	0	0	0	0	0	0	0	2	0	0	0
l(1)ts-UC13	19	0	0	21	0	24	0	24	5	0	0	37	24	16	18	16	5	0	68
l(1)ts-UC19	52	0	0	2	4	28	1	8	0	0	0	7	12	2	15	2	4	0	4
l(1)ts-UC88	135	0	0	0	1	70	4	0	0	0	0	2	2	2	2	2	1	0	4
l(1)ts-UC259	40	0	0	4	4	31	0	15	3	0	0	3	11	8	24	0	5	0	30
l(1)ts-2366	121	0	0	2	0	8	0	6	1	0	0	26	8	19	42	12	20	0	45
l(1)ts-2588	97	0	0	2	1	3	0	3	1	0	0	22	10	8	30	5	7	0	38
l(1)ts-2864	22	0	0	0	0	11	2	14	5	0	0	0	0	2	0	2	0	0	41
l(1)ts-3733	52	0	0	25	3	40	0	20	0	0	0	17	18	12	18	15	8	0	35

* *N* = number of abnormal animals examined.

All frequencies refer to number of abnormal imaginal disc derivatives/number of imaginal discs involved × 100, except for histoblasts where frequency refers to number of abnormalities/number of abdomens involved × 100.

Abbreviations used: Df-deficiencies; Dp-duplications. Other abbreviations as in Table 3.

to undergo duplication. Significant numbers of duplications (i.e., more than 20% of the particular imaginal discs involved) are not induced in any other structures by the 48–96-hr heat pulse. Significant levels of deficiencies (greater than 20% of the imaginal discs involved), however, are produced in the antennal, eye, wing, and genital disc by many of the ts cell-lethal mutations.

The imaginal discs all behave similarly in that they are most sensitive to the heat pulse during the 48–96-hr interval. The histoblasts, on the other hand, are most sensitive to heat treatment during the 144–216-hr interval. Heat treatment during this latter period predominantly results in the "naked abdomen" syndrome (ARKING 1973). This phenotype is found in pharate adults with abnormal abdomens. These abdomens possess genitalia which appear to have rotated in a normal manner but do not possess any histoblast derivatives. The rest of the abdomen is covered with what appears to be pupal cuticle. Apparently the histoblasts are affected but the genital discs and other imaginal discs are unaffected by heat pulses during this period of time.

V. *Other Studies*

A. *Vital staining*

The absence of X-ray-induced *y*, sn^3 twin spots in a *y lethal*/sn^3 heterozygous female raised at the restrictive temperature could be due to cell death, cell division arrest, or an inability to metamorphose. The imaginal discs of at least some of the mutants which were known to cause deficiencies and/or duplications were examined for the presence of cell death. The trypan blue-neutral red prosedure of DE RENZIS and SCHECHTMAN (1973) was utilized in order to detect cell death in the imaginal discs. The effectiveness of this procedure was tested in several ways. The simplest method involved the deliberate wounding with a hot needle of an imaginal disc obtained from a late third instar homozygous *l(1)ts-5697* larva raised at 22° and its subsequent staining with a solution of 0.25% trypan blue-neutral red in Ringer's solution. It can be seen in Figure 6 that the area immediately surrounding the wound has stained very deeply with trypan blue, thereby indicating the presence of dead and dying cells. Imaginal discs obtained from late third instar homozygous *l(1)ts-5697* larvae which had been exposed to the restrictive temperature from 72–120 hours after oviposition also stain deeply with trypan blue (Figure 7). In many of these cases the staining pattern indicates that the area of cell death was localized. At 22°, the mutant discs cannot be distinguished from the wild-type controls. Similar results have been obtained with other mutants as well as with the acridine orange staining procedures (SPREIJ 1971). However, a more detailed histological analysis at least certain selected mutants will have to be performed before more definitive conclusions can be reached.

B. *Mapping studies*

The 22 ts cell-lethal mutants which we have isolated and mapped (Table 3) involve 16 complementation groups. There are 13 groups containing one mutant

in each, one group containing two alleles (*l(1)ts-3803* and *l(1)ts-UC259*), one group containing three alleles (*l(1)ts-2366*, *l(1)ts-2588*, and *l(1)ts-5697*) and one group containing four alleles (*l(1)ts-1006*, *l(1)ts-1251*, *l(1)ts-1704*, and *l(1)ts-1843*).

C. *Female sterility*

Twenty of the ts cell-lethal mutants were tested to determine whether any of them display a decreased fecundity when the homozygous adult females were allowed to lay eggs at the restrictive temperature for extended periods of time. Fifteen of the mutants tested are ts female steriles when exposed to 29° as adults for periods of time ranging from two days to one week. In one mutant (*l(1)ts-1006*), a heat pulse of three days or longer results in the sterility of the homozygous females. However, if these same animals are subsequently kept at the permissive temperature for about one week, then the homozygous females once more become fecund.

DISCUSSION

I. *Frequency of Cell-Lethal Mutations*

DEMEREC (1936), using the twin spot test, determined that 40% (10 out of 24) of his sex-linked, X-ray-induced lethals (free of any detectable chromosome abnormalities) were cell lethals. However, since at least some of the X-ray-induced cell lethals he isolated were probably small deficiencies and not just point mutations, this percentage is most likely an over-estimate of the actual percentage of loci in the genome that can mutate to cell lethality. BRYANT and ZORNETZER (1973), in their analysis of tissue autonomy in gynandromorphs, found that about 40% of EMS-induced, sex-linked lethals were gynandromorph inviable. This value is most likely an over-estimate of the actual percentage of cell-lethal loci, since, as they point out, cell lethals represent only one of several mutant classes which would be expected to be gynandromorph inviable. RIPOLL and GARCIA-BELLIDO (1973), using both a gynandromorph test and a twin spot test, determined that 16.3% of their EMS- induced non-ts lethals were cell lethals. RUSSELL (1974) has also used the twin spot method and found that 17.8% of his stable ts lethals were ts cell lethal mutants. These values are in excellent agreement with the results of the present study, which has also used the twin spot test and has found that 18.2% of these EMS-induced, sex-linked ts lethal mutants are actually ts autonomous cell-lethal mutants. SIMPSON and SCHNEIDERMAN (1975) used the twin spot technique as a method of directly screening for ts cell-lethal mutations and have found that they occur at a frequency of 0.092% of the mutagenized chromosomes screened. This value is very close to my independently derived estimate of 0.11% (22/20, 569).

Thus it appears that approximately one out of every five ts lethal mutations prevents the appearance of small patches of homozygous imaginal cuticular tissue and meets the criteria for a ts cell-lethal mutation. These cell lethals would include not only those mutations which affect functions necessary for the survival of the cell, but would also include mutations which affect the cell cycle in some

manner and block cell division, as well as mutations which block the ability of cells to metamorphose into cuticular structures. None of the mutants obtained in this study appear to be cell cycle mutants, although SIMPSON and SCHNEIDERMAN (1975) have recently isolated and analyzed one such mutant. Mutant *l(1)ts-UC34*, so far as it has been tested, fulfills the criteria set up by MURPHY (1974) as a means of uniquely identifying hormone receptor mutations. More work will be needed to determine whether *l(1)ts-UC34* is, in fact, a hormone receptor mutation. In principle, all of these types of mutations could be detected by the twin spot technique, although only the cell lethal mutations would be capable of inducing deficiencies and duplications as a result of heat pulses. The procedure of examining the head, thorax, and abdomen—and not just one area—of the irradiated heterozygotes for *y* and sn^3 clones has allowed the detection of at least one disc-specific ts cell lethal mutation. The twin spot technique, however, does not detect those cell-lethal mutations which affect only larval tissues nor those which affect only cells of the internal organs of the adult.

On the basis of the Poisson distribution, it has been calculated that the *X* chromosome of Drosophila should contain 20 complementation groups which are capable of mutating to ts cell lethality. Thus this study has achieved an 80% saturation of the *X* chromosome. Since the *X* chromosome represents approximately one-fifth of the genome, this implies that there should be a total of about 100 ts cell-lethal complementation groups. SUZUKI (1970) has shown that 10% of his EMS-induced mutations are ts lethals. However, WRIGHT (1970) has shown that one locus may have both ts and non-ts alleles. Thus, on the basis of the present evidence, it is not possible reliably to estimate the number of loci throughout the genome capable of mutating to imaginal cell lethality, except to indicate that the number is probably not less than 100 nor more than 1,000. SHEARN and GAREN (1974), working with non-ts imaginal disc mutants on the third chromosome, have estimated that there exist about 1,000 complementation groups controlling gene functions necessary for formation of all imaginal discs.

II. *Distribution of ts cell lethal mutations*

The map positions presented in Table 3 indicate that the ts mutants which have been isolated are non-randomly distributed along the *X* chromosome, i.e., most of them occur in the proximal half of the *X* chromosome. STEWART, MURPHY and FRISTROM (1972) also obtained a non-random distribution of non-ts lethals affecting imaginal disc development, except that they observed a preponderance of mutants mapping in the distal half of the *X* chromosome. In particular, four of their "disc-degenerate" mutants map near *cv* (13.7). Such mutants are probably non-ts cell-lethal mutants (MURPHY 1974).

III. *Cell death and the formation of abnormal adult structures*

The question now arises as to the nature of the mechanism which translates heat-induced cell death in imaginal discs of a larva into the appearance of deficient, duplicated and/or normal structures in the adult.

Firstly, the fact that 19 of the 22 ts cell-lethal mutations can induce some deficiencies and/or duplications in adult structures suggests that cell death is the only precondition required for the formation of these morphological abnormalities. In other words, it is not necessary to postulate the involvement of any "pattern" mutations in order to explain the mechanism which probably causes deficiencies and duplications of the imaginal discs. In fact, it has recently been shown that the lethal effects of one "prepattern" mutant, eyeless-Dominant (ey^D), are probably due to cell death (ARKING, PUTNAM and SCHUBIGER 1975). A similar mechanism may cause the duplications of the ocelli and antennae, as well as the gaps in the intersegmental membranes between the first and second tarsal segments which are believed to be responsible for the duplications of sex combs in the ey^D mutant (POODRY and SCHNEIDERMAN 1975).

Secondly, the production of structural abnormalities by the non-mutant tissues of temperature-pulsed $R(1)w^{vc}/y$ lethal cryptic gynandromorphs (see Table 2 and Figures 2 and 3) strongly supports the view that the heat-induced cell death disrupts the developmental organization of the imaginal disc and results in abnormal growth.

Thirdly, there exist a large number of other mutations which also cause morphological abnormalities. RUSSELL (1974) and SIMPSON and SCHNEIDERMAN (1975) have each analyzed in detail the pattern-disrupting effects of two different ts cell-lethal mutants. As in the present study, their experiments have shown that the same temperature conditions which produce cell death in the imaginal discs (as determined by vital staining) are also effective in producing deficiencies and/or duplications in the resulting adults. There are also a number of non-ts mutations which are known to cause mirror-image duplications. These mutants include reduplicated (HOGE 1915), crippled (KOMAI 1926), extra organs (SCHALET 1972), eyeless (SANG and BURNET 1963), vestigial (WADDINGTON 1939), scalloped (VYSE and JAMES 1972), and erosion (INOUYE and TAKAYA 1964). Cell death has been shown to occur in the presumptive facet region of eyeless (FRISTROM 1969) and in the presumptive wing blade region of vestigial (FRISTROM 1968) and of scalloped (JAMES, personal communication). Finally, the production of deficiencies and duplications as a result of treating developing larvae with agents such as X-rays (POSTLETHWAIT and SCHNEIDERMAN 1973; POSTLETHWAIT 1975) strongly supports the belief that there is a causal connection between cell death in a developing imaginal disc and the consequent production of duplications and deficiencies.

BRYANT (1974) has advanced a model of pattern formation which postulates that a gradient of developmental capacity exists within the disc such that, after removal of a section of the gradient, the cells at the cut surface can produce only structures lower on the gradient. This hypothesis and its logical implications are diagrammatically summarized in Figure 8. This hypothesis provides at least formal explanations for certain of the observations. Firstly, the hypothesis implies that heat pulses administered late in development should yield a much higher frequency of deficiencies than would be found in heat pulses administered early in development. This situation would arise because a disc treated early in

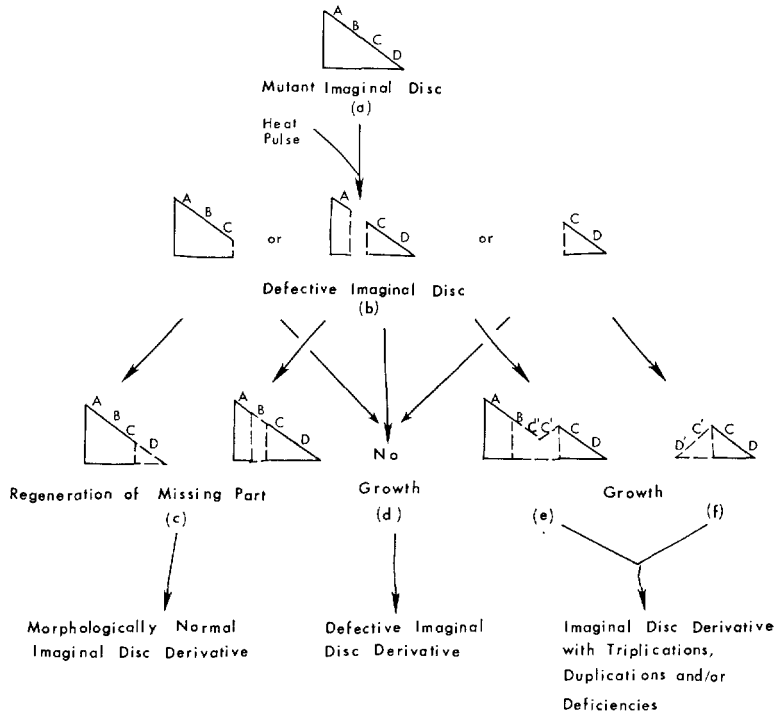


FIGURE 8.—The postulated gradient of developmental capacity showing the three possible types of defects caused by heat-induced cell death and the three different developmental processes the disc may undergo in order to yield normal, defective or duplicated imaginal disc derivatives. The letters A, B, C and D represent different adult structures which all arise from the same disc. The value of the ordinate at each point represents different levels of developmental capacity. It is assumed that any part of the imaginal disc can regenerate areas below it on the gradient but not those above it. (Adapted from BRYANT 1974).

development would have more time to undergo regeneration than would a disc treated late in development. Conversely, the optimum timing of heat pulses designed to yield duplicated disc derivatives must occur at some earlier developmental stage than those of heat pulses primarily intended to yield deficiencies. The data summarized in Table 5 clearly support these implications of this working hypothesis. A second prediction implicit in this hypothesis is that genetically mosaic imaginal discs should exhibit duplications of the non-mutant tissue as a result of heat treatment during the larval stages. The widespread occurrence of what we have termed "cryptic gynandromorphs" (Table 2) supports this last prediction. It is implicit in the hypothesis that duplications of an imaginal disc derivative should always be found associated with a deficiency for other derivatives of the same imaginal disc. The data shown in Table 4 suggest that this association occurs in 91% of the cases.

However, the production of morphologically abnormal structures depends not only on the location of cell death in the disc but is also dependent upon the extent of the affected areas and the rapidity with which this cell death occurs. For

example, *l(1)ts-480* kills the cells of the eye and antennal discs so rapidly (15 hrs at 29°) that the entire disc dies. No duplications are produced, presumably because there are no surviving eye-antennal imaginal disc cells. On the other hand, a mutation which takes a long time to kill (such as *l(1)ts-5141*) produces only low levels of deficiencies and of duplications, since most of the dying cells are presumably being replaced at a rate high enough to insure the developmental continuity of the imaginal disc. In addition to these locus-specific effects on cell deaths, an analysis of the optimum TSP for the production of structural abnormalities suggests that the extent of cell death taking place in a given tissue may be related to the mitotic activity of that tissue. Thus the imaginal discs are most susceptible to heat-induced cell death during mid-larval life, while the histoblasts are most susceptible only after puparium formation. In both cases these TSP's correlate very well with the periods of intense mitotic activity (GARCIA-BELLIDO and MERRIAM 1971; GUERRA, POSTLETHWAIT and SCHNEIDERMAN 1973). Thus, those mutations which do yield significant levels of deficiencies and/or duplications probably kill a large proportion—but not all—of the cells of the homozygous mutant imaginal discs. In these mutants, furthermore, the cells that do die are probably spatially localized in one portion of the disc. It is thus possible that such an intra-disc localization of cell death may reflect the cellular specificity of gene action.

Judging from the ease with which deficiencies and duplications can be induced in them, it appears that the leg discs are the most sensitive of all the imaginal discs to the pattern-disrupting effects of cell death, while the labial and haltere discs are the least sensitive. The other imaginal disc derivatives appear to be preferentially affected by certain of the mutations. As seen in Table 6, the eye antennal disc is preferentially affected by *l(1)ts-480* and *-UC88*, the wing disc by *l(1)ts-480* and *-1251* and the genital disc by *l(1)ts-1251* and *-UC13*. The remaining mutants appear to be much more similar to one another than they are dissimilar. It is reasonable to assume that this tissue specificity is the direct result of tissue-specific gene activity.

It is a pleasure to acknowledge the many helpful suggestions offered by my colleagues Ms. PATRICIA SIMPSON and DR. HOWARD A. SCHNEIDERMAN and I would like to especially thank DRS. PETER J. BRYANT, DARREL FALK, TOM CLINE, and MR. RANDALL SMITH for their critical reading of the manuscript. I would also like to thank Ms. ANNA GREENHOUSE for technical assistance. This research was supported by a Grant-in-Aid from the Zoecon Foundation and by GB 26278 from the National Science Foundation.

LITERATURE CITED

ARKING, R., 1973 Developmental effects of a temperature-sensitive cell lethal. Genetics **74:** s9 (Abst.).

ARKING, R., R. L. PUTNAM and M. SCHUBIGER, 1975 Phenogenetics of the eyeless-Dominant mutant of Drosophila. II. The involvement of the nervous system. J. Exptl. Zool. In press.

BECKER, H. J., 1956 On X-ray induced somatic crossing over. Drosophila Inform. Serv. **30:** 101.

BRYANT, P. J., 1974 Determination and pattern formation in the imaginal discs of *Drosophila*. Current Topics in Developmental Biology (A. A. Moscona and A. Monroy, eds.) **8:** 41–80.

BRYANT, P. and M. ZORNETZER, 1973 Mosaic analysis of lethal mutations in Drosophila. Genetics **75**: 623–637.

DEMEREC, M., 1936 Frequency of "cell-lethals" among lethals obtained at random in the X-chromosome of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. **22**: 350–354.

DE RENZIS, F. A. and A. SCHECHTMAN, 1973 Staining by neutral red and trypan blue in sequence for assaying vital and non-vital cultured cells. Stain Technol **48**: 135–136.

FRISTROM, D., 1968 Cellular degeneration in wing development of the mutant *vestigial* of *Drosophila melanogaster*. J. Cell Biol. **39**: 488–491. ——, 1969 Cellular degeneration in the production of some mutant phenotypes in *Drosophila melanogaster*. Mol. Gen. Genet. **103**: 363–379.

GARCIA-BELLIDO, A. and J. MERRIAM, 1971 Clonal parameters of tergite development in *Drosophila*. Devel. Biol. **6**: 264–276.

GUERRA, M., J. H. POSTLETHWAIT and H. A. SCHNEIDERMAN, 1973 The development of the imaginal abdomen of *Drosophila melanogaster*. Devel. Biol. **32**: 361–372.

HINTON, C. W, 1955 The behavior of an unstable ring chromosome of *Drosophila melanogaster*. Genetics **40**: 951–961.

HOGE, M., 1915 The influence of temperature on the development of a mendelian character. J. Exptl. Zool. **18**: 241–297.

INOUYE, S. and H. TAKAYA, 1964 On the occurrence of hereditary irregularities of antennae in *Drosophila melanogaster*. Japan J. Genet. **38**: 28–36.

KOMAI, T., 1926 Crippled, a new mutant character of *Drosophila melanogaster* and its inheritance. Genetics **11**: 280–293.

LEWIS, E. B. and F. BACHER, 1968 Method of feeding ethyl methane sulfonate (EMS) to Drosophila. Drosophila Inform. Serv. **43**: 193.

LINDSLEY, D. L. and E. H. GRELL, 1968 Genetic variations of *Drosophila melanogaster*. Carnegie Institute Wash. Publ. No. **627**.

MURPHY, C., 1974 Cell death and autonomous gene action in lethals affecting imaginal discs in *Drosophila melanogaster*. Devel. Biol. **39**: 23–36.

POODRY, C. A. and H. A. SCHNEIDERMAN, 1975 Pattern formation in *Drosophila melanogaster*: The effects of mutations on polarity and "prepattern" in the developing leg. (Manuscript in preparation.)

POSTLETHWAIT, J. H., 1975 Pattern formation in the wing and haltere imaginal discs after irradiation of *Drosophila melanogaster* first instar larvae. Roux' Archiv (In press).

POSTLETHWAIT, J. H. and H. A. SCHNEIDERMAN, 1973 Developmental genetics of Drosophila imaginal discs. Ann. Rev. Genet. **7**: 381–433.

RIPOLL, P. and A. GARCIA-BELLIDO, 1973 Cell autonomous lethals in *Drosophila melanogaster*. Nature New Biol. **241**: 15–16.

RUSSELL, M. A., 1974 Pattern formation in the imaginal discs of a temperature sensitive cell-lethal mutant of *Drosophila melanogaster*. Devel. Biol. **40**: 24–39.

SANG, J. H. and B. BURNET, 1963 Environmental modification of the eyeless phenotype in *Drosophila melanogaster*. Genetics **48**: 1683–1699.

SCHALET, A., 1972 New mutant. Drosophila Inform. Serv. **49**: 36.

SHEARN, A. and A. GAREN, 1974 Genetic control of imaginal disc development in *Drosophila*. Proc. Natl. Acad. Sci. U.S. **71**: 1393–1397.

SIMPSON, P. and H. A. SCHNEIDERMAN, 1975 Temperature-sensitive cell autonomous mutations in Drosophila. I. Isolation by use of somatic recombination. (Submitted for publication.)

SPREIJ, T. H., 1971 Cell death during the development of the imaginal discs of *Calliphora erythrocephala*. Neth. J. Zool. **21**: 221–264.

STERN, C., 1954 Genes and developmental patterns. Caryologia (Suppl.) **6**: 355–369.

STEWART, M., C. MURPHY and J. W. FRISTROM, 1972 The recovery and preliminary characterization of X-chromosome mutants affecting imaginal discs of *Drosophila melanogaster*. Devel. Biol. **27**: 71–83.

SUZUKI, D. T., 1970 Temperature-sensitive mutations in *Drosophila melanogaster*. Science **170**: 695–706.

VYSE, E. R. and A. JAMES, 1972 New Mutant. Drosophila Inform. Serv. **49**: 39.

WADDINGTON, C. H., 1939 Preliminary notes on the development of the wings in normal and mutant strains of Drosophila. Proc. Natl. Acad. Sci. U.S. **25**: 299–307.

WHITTEN, J. M., 1969 Cell death during early morphogenesis: parallels between insect lines and vertebrate limb development. Science **163**: 1456–1457.

WRIGHT, T. R. F., 1970 The genetics of embryogenesis in *Drosophila*. Advan. Genet. **15**: 262–395.

Corresponding editor: D. T. SUZUKI