A STUDY OF THE AUTONOMOUS BEHAVIOUR OF SEX-LINKED TEMPERATURE-SENSITIVE LETHAL MUTANTS IN *DROSOPHILA MELANOGASTER*

by

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

in the Department
of
Genetics in Zoology

We accept this thesis as conforming to the
required standard

THE UNIVERSITY OF BRITISH COLUMBIA
July, 1969

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ABSTRACT

The autonomous behaviour of sex-linked recessive temperature-sensitive lethal mutants in Drosophila melanogaster could be demonstrated by the presence of mosaic patches of tissue hemizygous for the mutant created by loss of a ring X chromosome in cells at the permissive temperature (21.5°C) and the absence of such patches at the restrictive temperature (29°C). The presence of patches at both temperatures indicated that the mutant was non-autonomous. Such non-autonomous behaviour might be attributed to the existence of a substance capable of diffusing from the wild type tissue to supplement the mutant tissue.

The experiments carried out showed that the presence or absence of mosaic patches could not be directly interpreted as demonstration of autonomous or non-autonomous properties of the mutant. Other factors such as the time of activity of the ts mutant and the type of tissue undergoing ring X loss affected mosaic tissue production. Therefore, the mere presence of mosaic tissue at 29°C could not be used as a criterion for the non-autonomous behaviour of the ts mutants. However, these mutants can be graded according to the degree of autonomy of ts lethality after alterations due to XO survival frequencies, lethal periods, and temperature-sensitive periods have been placed on

mosaic frequencies at 29°C. Of the thirteen ts mutants studied, six can be classed as autonomous lethals. The others are equally autonomous as lethals but only in specific tissues, while others do not appear to be as autonomous. In fact, one of these may be considered non-autonomous.

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ACKNOWLEDGEMENT

I would like to thank Dr. D. T. Suzuki for his valuable assistance and advice in preparing this paper.

I am also deeply appreciative of the encouragement and patience of Dr. Leonie Piternick during the course of this work.

INTRODUCTION

The function of a gene is defined as autonomous if the phenotype developed in a given tissue reflects the genetic constitution of that tissue and is not influenced by the genotypes of neighbouring cells. Non-autonomous genetic behaviour, on the other hand, is indicated when a cellular phenotype does not correspond to the genetic constitution of the cells which produce it owing to an influence of genetically different cells (Hadorn 1951). These aspects of gene function are generally studied in higher organisms by surrounding cells or tissue of the genotype under investigation with cells of a different genotype.

The definition of autonomy implies that phenotypes can be influenced by the action of substances produced by genes of a different genotype. The existence of such a substance has been demonstrated by Sussman and Lu (1955) although the mechanism of its action remains speculative. They showed that a diffusible substance described earlier by Bonner (1947) as acrasin, produced by the wild type myxamoeba of the slime mould Dictyostelium discoideum, could pass through an agar membrane and cause aggregationless mutants to cluster directly opposite wild type centres of aggregation. However, they found that different pairwise combinations of morphogenetically deficient variants, each of which cannot com-

plete the normal developmental sequence in spore formation alone but which can do so when mixed in pair combinations, cannot complete development when placed on opposite sides of a membrane. They concluded, therefore, that the exchange of low weight diffusible intermediates was not the only requirement for complete non-autonomous development of those deficient stocks.

Early studies on the autonomy of various non-lethal and lethal mutants in Drosophila melanogaster have given ambiguous results. Morgan and Bridges (1919) showed that, in general, the sex and phenotype of thirty different sex-linked characters in gynandromorphs produced by the occasional elimination of one of the X chromosomes from a female cleavage nucleus, were completely autonomous in development. Later, Sturtevant (1932), studying mosaics produced by chromosome elimination caused by $M(1)^2$-n (see Lindsley and Grell 1968) found the following exceptions to complete autonomy: 1) the Bar phenotype at certain stages of eye development, 2) scute and yellow phenotypes when in small patches on the cuticle, and 3) the vermilion-eye phenotype. At that time it was also noted that specific genes affected certain parts of the body at specific times in development. Demerec (1934) and Ephrussi (1934) studied the autonomy of cells hemizygous for chromosomal deletions of various sizes. Using aberrant somatic segregation and chromosome elimination to produce spots of tissue

hemizygous for the deletions surrounded by cells carrying the complete segments, they arrived at different conclusions. Demerec claimed that the lethal expression of deletions was autonomous in development, whereas, Ephrussi recovered small spots of tissue hemizygous for the deletions and concluded that the lethal phenotype of the deletion was suppressible by surrounding wild type tissue. Stern(1934) corroborated Ephrussi's conclusions when he showed that a deficiency lethal was non-autonomous. These interpretations may be questioned since Ephrussi and Stern did not consider the possibility of the genes associated with the deleted segments not being functional in the tissues studied. Poulson (1945) and Oster and Sobels (1956) studied the autonomous properties of different sex-linked lethals in females carrying the $\underline{X^{c2}}$ chromosome which is somatically unstable and frequently lost. They concluded that autonomous behaviour varied depending on the tissue affected and on the time of elimination of the wild type allele. Hannah (1953), using a similar experimental procedure, showed that the mutant yellow was slightly non-autonomous. The synthesis of cuticular pigmentation was found to be completely autonomous in the first four abdominal segments and the thorax and the head, but non-autonomous in tergites genetically dimorphic for genes affecting pigmentation at the junction of wild type female and yellow male tissue. Bristles in the

border zone showed complete gradation in colour from yellow (mutant phenotype) through brown to black (wild phenotype) while in other sections large yellow sectors were usually autonomous while small patches may not have been so. From extensive studies on the behaviour of cells carrying different lethals transplanted into wild type hosts, Hadorn (1951) has concluded that the phenotypic behaviour of the mutants is influenced by the following: 1) phase specificity of the mutant, referring to the different times in the development of the organism during which the effect of the mutant can be observed, since different tissues and organs react differently to the lethal constitution; 2) damage due to primary and secondary (because surroundings are abnormal) effects of the lethal; 3) penetrance or expressivity of lethal effects as determined by genotypic milieu, sex, temperature, nutritional conditions, and other environmental influences. All these studies point to the complex nature of cell to cell interactions.

Temperature-sensitive (ts) mutants are conditional mutations that survive at permissive temperatures but are lethal at restrictive temperatures (Epstein et al. 1963). Analyses of temperature-sensitivity in microorganisms have shown thermolability to be a property of the protein product of the mutant gene rather than due to an effect on the actual process of transcription or trans-

lation (Jockusch 1966; Naono and Gros 1967; Sundaram and Fincham 1967). In Drosophila melanogaster, ts mutants with properties of lethality similar to those of microorganisms have been recovered (Suzuki et al. 1967; Baillie, Suzuki and Tarasoff 1968; Suzuki 1969). Several of these mutants have been shown to have delineable temperature-sensitive periods (TSP) in development during which exposure of the organism to restrictive temperatures irrevocably commits the organism to death (Suzuki and Duck 1967; Tarasoff 1968; Suzuki and Procunier 1969) and an effective lethal phase (LP) when the exposure to restrictive temperatures during the TSP is phenotypically manifest in death. The coincidence of the LP and TSP of each mutant varied from concomitance to separation by several days (Suzuki and Procunier 1969).

The present study was undertaken in order to detect a non-autonomous temperature-sensitive lethal and to determine whether the non-autonomy might result from the diffusion of a substance produced by the wild type tissue which would supplement the deficiency of the mutant tissue at restrictive temperatures. Recognition of such "supplementable" mutants could result in a bio-assay for the isolation of such a substance with a view to determining the nature of the genetic activity of a locus. Furthermore, once autonomy has been established, survival of cells carrying a ts lethal in certain spe-

cific regions of a wild type host at restrictive temperatures could separate tissues in which the locus is genetically functional from those in which it is not required.

In order to determine the autonomous behaviour of ts mutants, a scheme similar to that designed by Hannah (1951) was used. The mitotic instability of the ring chromosome, $\underline{X^{c2}}$, in somatic cells (Hinton 1955) is used to generate patches of tissue in which recessive mutants carried on the homologous X chromosome are expressed due to the loss of the corresponding wild type alleles when the ring X is eliminated. With the sex-linked ts lethal mutants at permissive temperatures, mosaic patches of tissue should occur. However, under restrictive temperatures, mosaic patches should be produced only if the ts mutant studied is non-autonomous or is not functional in the tissues observed.

It was found that the presence or absence of mosaic patches of tissue was not governed solely by the autonomous or non-autonomous behaviour respectively of the ts gene, under restrictive conditions. Other factors such as the time of activity of the ts gene and the time of loss of the wild type allele influenced the recovery of mosaic tissue. Thus, the detection of mosaic patches, per se, was not found to be a reliable criterion of the non-autonomy of sex-linked ts lethal mutants.

METHODS AND MATERIALS

I. Establishment of y..ts.. stocks

Sex-linked ts mutants which have been induced in adult Oregon R males (Suzuki et al. 1967), localized genetically (Suzuki 1969), and shown to give no survivors at 29°C, were used in this experiment. They wil be referred to as "non-leaky ts mutants" (leaky mutants being defined as those giving a few survivors at 29°C). Some of the chromosomes bearing the ts lethals were marked with different recessive mutations (see Lindsley and Grell 1968, for complete description): y (0.0, yellow body colour), sc (0.0, scute bristle mutant), cv (13.7, crossveins missing), v(33.0, vermilion eye colour), f (56.7, forked bristles), car (62.3, carnation eye colour) in the course of the genetic localizations (Suzuki 1969). In the cases in which the ts-bearing X chromosome did not carry other recessive markers, females heterozygous for the lethal and an inversion marked with the dominant mutant, Bar, (ts/FM-6 or ts/M-5) were mated to males with chromosomes marked with y, sc, cv, v, f and car. F_1 females heterozygous for the ts lethal and the multipli-marked X chromosome were test-crossed at 21.5°C and male progeny carrying the ts lethal, y, and in some instances the other recessive markers, were isolated. Henceforth, these marked ts-bearing chromosomes will be referred to as y..ts..

Table Ib

Description of stocks tested for temperature-sensitivity

mutant	markers inserted into ts chromosome	stock maintenance
E5	y sc cv	homozygous
E7	y sc	homozygous
E9	y sc cv car	♂
E25	y	homozygous
E27	y sc f car	homozygous
E34	y sc	homozygous
E45	y sc cv	♂
E46	y sc cv v	♂
E76	y sc	♂
E82	y v	♂
E88	y sc cv v	♂
E94	y sc	homozygous
X8	y	homozygous
M10	y	♂
6IV ES	y cv	homozygous
EIII E52	y cv	♂

Table Ia

General genetic properties of stocks tested for temperature-sensitivity

mutant	mutagen	viability index[1]		regional localization[2]	viability at 29°C		
		21.5°C	29°C		very	slight	not
E5	EMS	homo=30.6	homo=0.0	v-sn		✓	
E7	EMS	1.25	0.0	cv-sn			✓
E9	EMS	homo=73.7	homo=0.0	near cv	✓		
E25	EMS	homo=86.8	homo-0.0	cv-sn			✓
E27	EMS	homo=35.6	homo=0.0	cv-sn			✓
E34	EMS	0.98	0.08	at sn			✓
E45	EMS	homo=60.3	homo=0.0	v-f		✓	
E46	EMS	homo=35.6	homo=0.0	car-l			✓
E76[3]	EMS	0.17	0.0	car-l		✓	
E82	EMS	0.77	0.0	at cn			✓
E88	EMS	0.54	0.0	at wy			✓
E94	EMS	0.92	0.0	cv-sn	not determined		
X8	X-rays	homo=47.0	homo=0.0	right of v			✓
M10[4]	Mitomycin C	0.42	0.0	f-car			✓
6IV BS EIII	EMS	0.13	0.0	g-f		not ts	
E52	EMS	0.75	0.0	v-wy			✓

1 Viability index = $\frac{\text{frequency of ts males}}{\text{frequency of heterozygous (ts/FM-6 or M-5) females}}$
In the homozygous ts stocks (homo) the figures represent the average number of offspring hatching in cultures set up in an identical manner except at different temperatures.

2 Example: v-sn means that the mutant is located betwen v and sn

3 Males not fertile at 29°C

4 Homozygous females not fertile at 29°C

chromosomes. The y..ts.. chromosomes were made homozygous by mating males to a balancer stock, FM-6/y, and backcrossing the y..ts../FM-6 females to y..ts.. males. In cases where homozygous females were sterile or lethal, the y..ts.. chromosomes were maintained in males by mating to females carrying the compound X chromosome, C(1) RA, marked with y and f. Before the determination of autonomy was started, each stock was tested to ensure the presence of non-leaky ts genes. The ts lethals tested and their genetic properties are shown in Tables Ia and Ib (mode of origin, viability indices, genetic positions, markers on the ts chromosomes).

II. Determination of autonomy

The unstable ring X chromosome, In (1) X^{c2}, w^{vC}, (referred to as w^{vC}), was generated by irradiation, with X-rays, of the ring chromosome, R(1)2, containing the inversion, In (1) w^{vC} (Hinton 1955). The resulting chromosome, w^{vC}, is characterized by variable degrees of instability which produces gynandromorphs, XO males, and dominant lethals in progeny of w^{vC}-bearing females. w^{vC}-bearing males are sterile and the ring is maintained in females balanced over the In (1) dl-49, y w lz^{s} chromosome (referred to as dl-49) contributed by fertile males in the stock. The males also carry the sc^{8}·Y

chromosome which includes the entire Y chromosome and the tip of the X including $\underline{1(1)J1}^{+}$, $\underline{y}^{+}$, and $\underline{sc}^{+}$.

Virgin $\underline{w}^{vC}/\underline{dl\text{-}49}$ females raised at room temperature and less than 3 days old were mated to $\underline{y..ts..}$ males also raised at room temperature. 20 pairs were allowed to mate and lay eggs for 5 days at 29°C in quarter pint bottles containing standard Drosophila medium. The same procedure was carried out at 21.5°C. The P_1 flies were then discarded and the F_1 flies allowed to develop at these temperatures. All viable progeny including those that adhered to the medium or remained in the pupal cases were counted and classified. Dead unhatched pupae were classified in the 29°C cultures by dissecting them to determine the phenotype of the developing imago. Any morphological abnormalities in the $\underline{w}^{vC}/\underline{y..ts..}$ females, whether they were stuck to the medium, dead, or unhatched were noted.

In crosses of $\underline{w}^{vC}/\underline{dl\text{-}49}$ ♀ x $\underline{y..ts..}/\underline{Y}$ ♂, primary non-disjunction in females generated $\underline{w}^{vC}/\underline{dl\text{-}49}/\underline{Y}$ females which are phenotypically similar to $\underline{w}^{vC}/\underline{y..ts..}$ females. In order to minimize the number of such females erroneously classified as $\underline{w}^{vC}/\underline{y..ts..}$, several precautions were taken. Putative $\underline{w}^{vC}/\underline{y..ts..}$ females were separated into two classes, those displaying external mosaicism and those phenotypically wild type. Mosaic females from the 29°C

cultures were tested intensively in the following manner. Those mosaic for y and other recessive markers were classified unequivocally as y..ts.. mosaics. Those displaying only y patches of tissue but having normal female genitalia were tested to determine whether the dl-49 chromosome was in fact carried. If this chromosome was detected, the females were classified as primary exceptional females. However, most of these females were sterile. They were included in the y..ts.. mosaic class if dissection did not reveal the presence of colourless Malpighian tubules or testes due to the presence of the w gene. The majority of y mosaic females were gynandromorphs showing mosaicism of the external genitalia and therefore were sterile. These were dissected likewise and classed as products of primary non-disjunction if they had unpigmented Malpighian tubules or testes. Otherwise, these were considered to be y..ts.. mosaics. At 21.5°C mosaic females were classed as y..ts..-bearing females unless patches of w lz^s tissue occurred. All other non-y females which did not show mosaic patches under a dissection microscope at 25X magnification were, at both temperatures, classed as w^{vC}-bearing non-mosaics as regards external characters. Henceforth, females manifesting external mosaicism will be referred to as mosaics while those females bearing the w^{vC} chromosome which are not mosaic for external phenotypes will be called non-

mosaics.

Any y..ts.. males that survived at 29°C were mated to C(1) RA/Y females at room temperature to check their fertility. These adults were transferred to fresh vials and kept at 29°C to determine by absence of male F_1 progeny whether the ts gene had still been present.

In control experiments, w^{vC}/dl-49 females were mated to y sc cv v f car/Y males and to y cv v f/B^S Y y^+ males in the same manner as described above to measure the survival of non-lethal mosaic and non-mosaic females. They were also mated to w^e bb^1/B^S Y males to measure these frequencies with a non-ts lethal. The latter test should give results comparable to those obtained with the y..ts.. chromosomes at 29°C. In all cases, the classification procedure described for the y..ts.. tests was followed. However, since primary non-disjunctional females could be detected in female progeny by the presence of the Bar marker in the last two crosses, the precautions in scoring mosaic and non-mosaic females did not have to be taken. (For a description of all the mutants and special chromosomes used above, see Lindsley and Grell 1968).

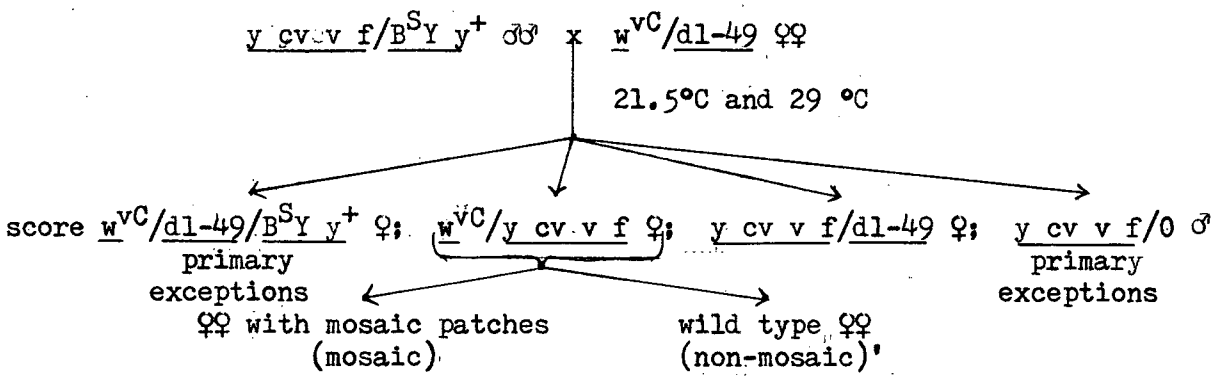
III. Determination of the frequency of non-disjunction in the w^{vC} stock

Since the w^{vC}/dl-49/Y females resulting from primary non-disjunction

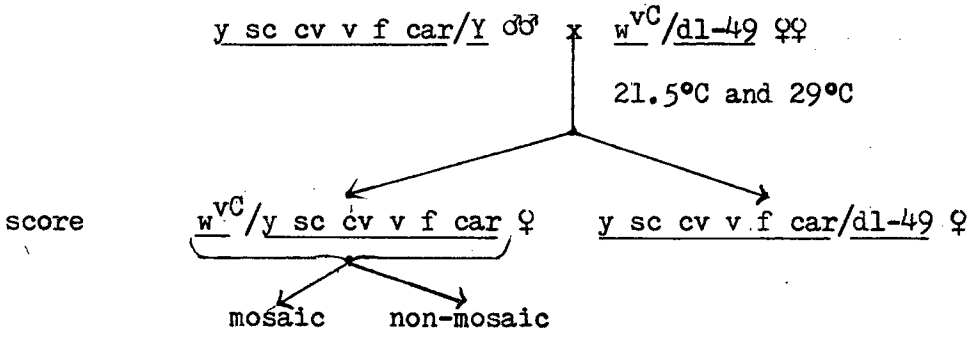
Figure 3

Other crosses made in the investigation of autonomy

A. Control test of primary non-disjunction in $\underline{w^{vC}}$-bearing ♀♀



B. Controls to determine regular mosaic and non-mosaic frequencies



C. Test of a non-temperature-sensitive autonomous lethal

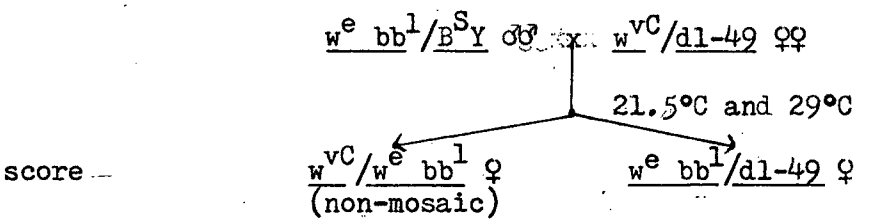


Figure 2

Cross carried out for a determination of the autonomy of each temperature-sensitive mutant

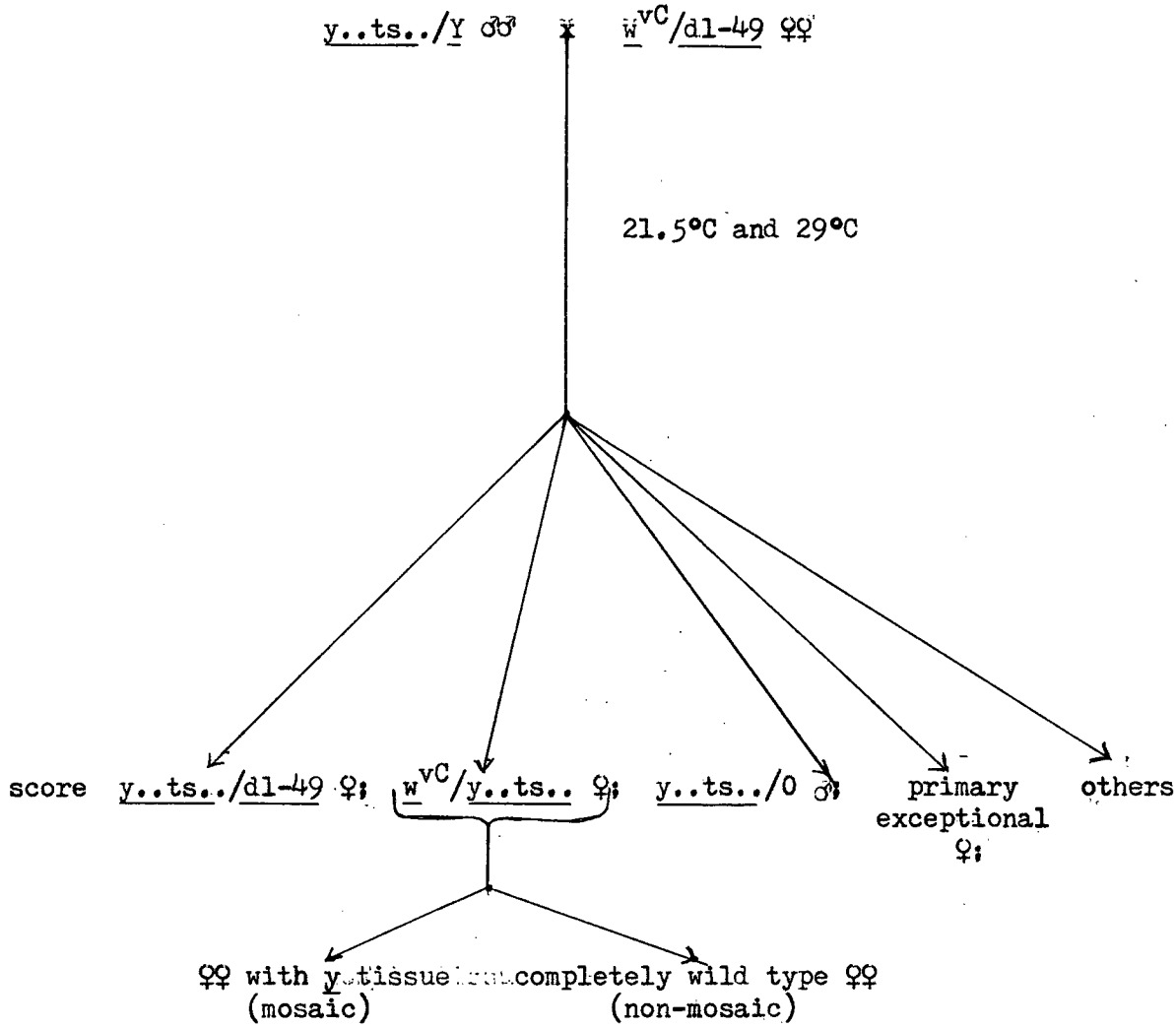
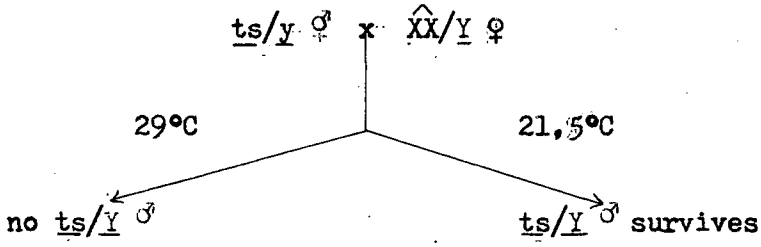


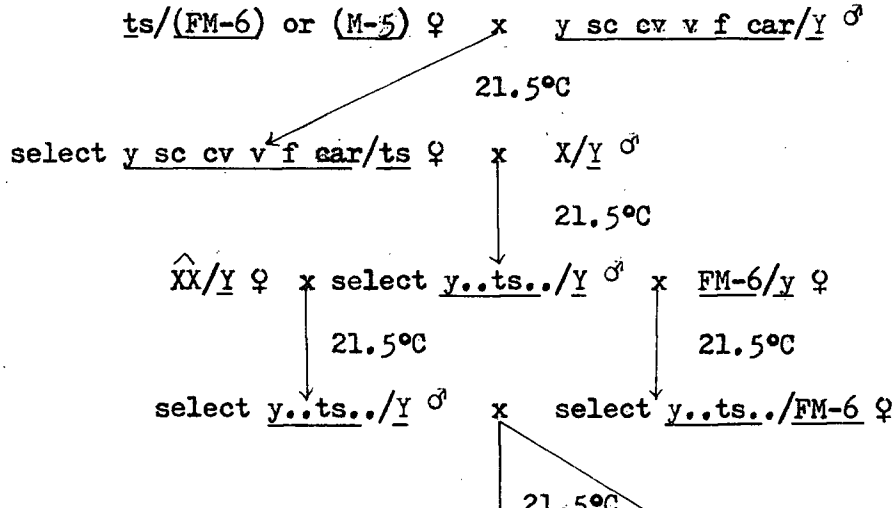
Figure 1

Crosses made for different aspects of the study

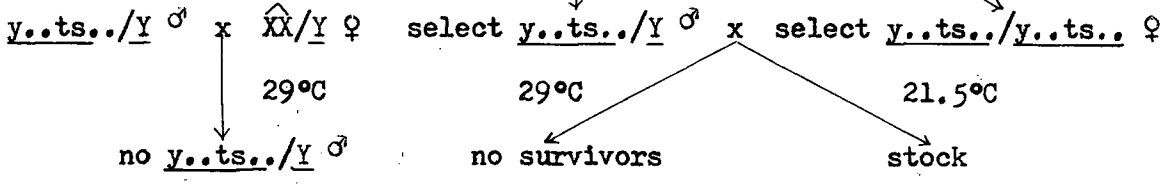
A. Determination of temperature-sensitive lethals



B. Insertion of markers on the chromosomes carrying the ts lethal



c. Retest of marked stock



mimic $\underline{w^{vC}}/\underline{y..ts..}$ females, their frequencies of occurrence had to be estimated. In those cases where mosaicism involved tissues affected by $\underline{w}$ and $\underline{lz^{s}}$, these females could be recognized. However, this criterion could only be applied in eye tissue, not in the cuticular areas where only $\underline{y}$ could be detected.

The frequencies of $\underline{y..ts..}/0$ exceptional males does not give an indication of the rate of primary non-disjunctional female production at permissive temperatures since X0 males are produced at higher frequencies than XXY females in crosses involving $\underline{w^{vC}}$ (Hinton 1955). Under restrictive temperatures these males should not survive.

In order to estimate the rate of primary non-disjunction in females the results of one of the control crosses for the autonomy tests was used. In the cross involving $\underline{y\ cv\ v\ f}/\underline{B^{S}\ Y\ y^{+}}$, the Y-linked Bar marker indicated non-disjunction. The exceptional females carrying Bar were then tested to estimate the number resulting from paternal non-disjunction.

Summaries of all the crosses made are shown in Figures 1, 2, and 3.

IV. Determination of temperature-sensitive period and effective lethal phase

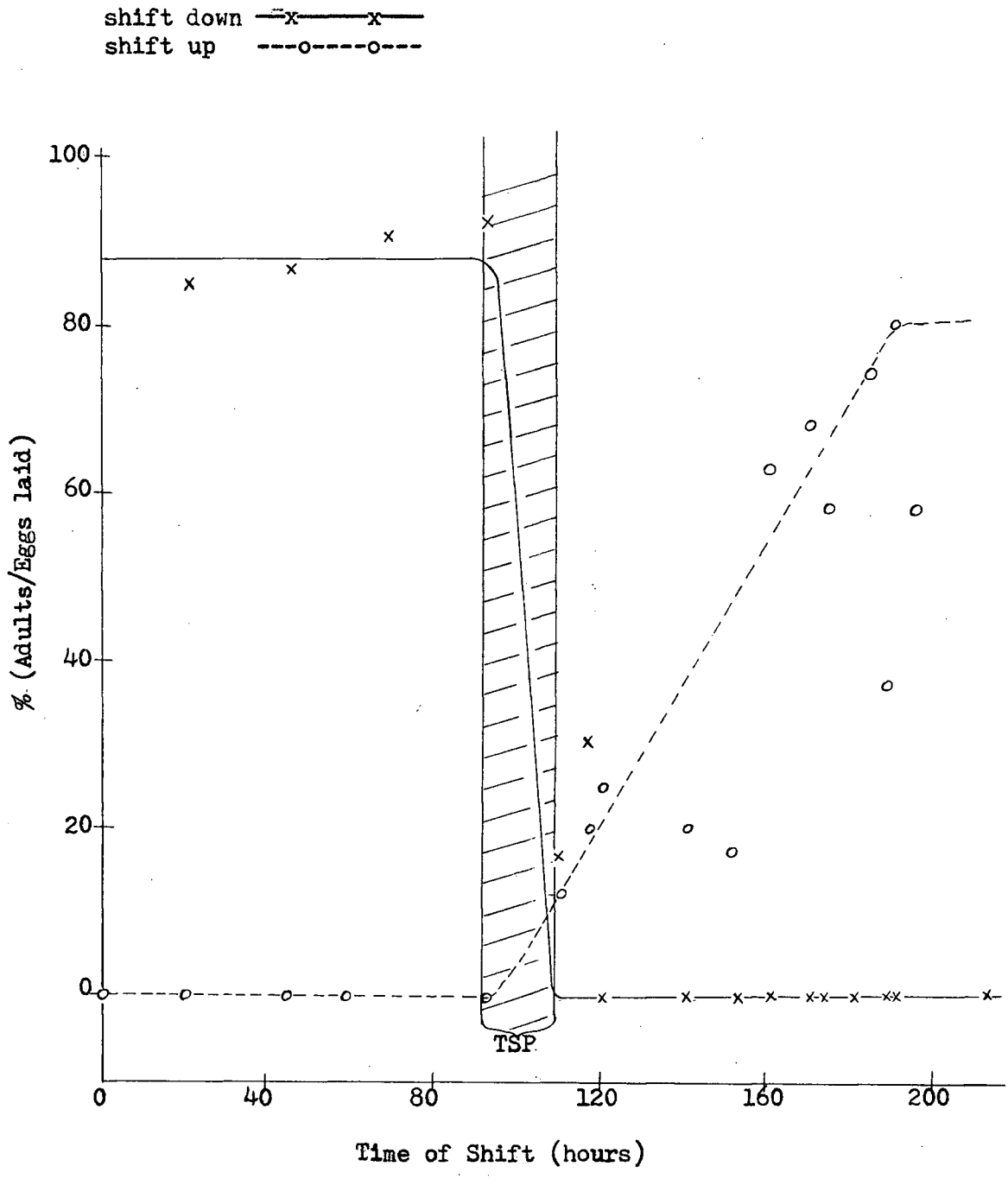
Since the assessment of the autonomous or non-autonomous behaviour of a

given ts lethal requires knowledge of the time at which temperature affects the viability of the individual carrying the ts mutant (the temperature sensitive period or TSP) and also of the time at which exposure to the restrictive temperature during the TSP manifests itself phenotypically by death (the lethal period or LP), these periods were determined by the following procedure. Groups of 50 - 100 pairs of flies from each ts stock were placed in empty half pint bottles inverted over petri plates containing standard Drosophila medium and allowed to lay eggs at 21.5°C and 29°C for 1 - 2 hour periods. Usually, 50 - 100 eggs could be collected within such an interval. These were maintained at the respective laying temperatures and at successive twelve hour intervals, a 29°C culture was shifted to 21.5°C (shift down) and vice versa (shift up) for total periods as long as 240 hours.

After sufficient time to allow emergence of adults had elapsed, the cultures of homozygous ts lethals were examined for the number of adults, for the presence of pupa cases containing dead fully pigmented imago, for dead early pupae, and for dead larva at various developmental stages as determined by examination of mouth parts and tracheal development (Bodenstein 1950). The onset of the TSP was indicated by the first culture of a shift down which showed any evidence of death due to exposure to the restrictive temperature.

Figure 4

A model of reciprocal shift experiments to determine the TSP based on results obtained with y E25



and the end of the TSP was indicated by the first culture in a shift up which began to yield viable adults (Figure 4). Since loss of the $\underline{w^{vC}}$ chromosome can occur at any time in development, all possible LP's resulting from the exposure of a culture to restrictive temperatures at different times were determined. This was done by noting all the stages at which death occurred in the shift cultures.

In those stocks in which the ts mutant was maintained in males only, cultures were examined in a manner similar to that outlined above except that certain precautions were taken to differentiate between male and female progeny. Fully pigmented pupa were sexed by the presence of sex comb and genital apparatus, whereas the presence of testis or ovary upon dissection was used as the criterion in early unpigmented pupae and third instar larvae. Extensive death in the first and second instar larval stages with the emergence of the expected number of female progeny was assumed to indicate effective lethal phase in the early instars.

Controls for these shift experiments were carried out using a y sc cv v f car stock maintained in the males. The procedure described for the y.. ts.. stocks was followed in this case also.

Table II

Number in each class of offspring of the cross: y..ts../Y ♂♂ x w^{vC}/dl-49 ♀♀

chromosome tested		dl-49 y..ts.. ♀	non-mosaic w^{vC} ♀ y..ts..	mosaic w^{vC} ♀ y..ts..	XO ♂	1°exceptional ♀	others	total
	a	835	79	65stuck	94stuck	5	206	1284
y sc cv E5	b	8	3	9	10	-	7	
	c	377	171	10	67	3	99	816
	a	1760	127	103	22	3	386	2402
y sc E7	b	17	14	22	9	3	38	
	c	726	371	211	86	3	267	1674
	a	431	47	30	29	1	62	510
y sc cv E9 v car	b	11	2	12	21	-	6	
	c	336	146	134	44	5	41	706
	a	956	92	52	4	4	109	1221
y E25	b	12	10	63	152	-	16	
	c	546	270	166	287	4	78	1355
	a	893	80	18	1	6	163	1161
y sc E27 f car	b	6	6	14	-	-	7	
	c	895	364	253	149	9	84	1754
	a	886	51	29	6	9	285	1276
y sc E34	b	9	9	48	104	-	31	
	c	750	372	293	149	7	110	1680
	a	2524	222	71	19	13	383	3233
y sc cv E45	b	2	6	8	11	-	6	
	c	494	222	150	70	5	73	1014
	a	1241	110	8	3	5	302	1671
y sc cv v E46	b	11	20	10	10	-	16	
	c	852	384	238	148	15	132	1709
	a	874	61	44	14	1	126	1120
y sc E76	b	10	8	9	6	-	9	
	c	656	307	187	70	5	49	1276
	a	1153	76	37	1 dead	3	195	1465
y E82 v	b	2	14	25	49	-	25	
	c	620	290	117	71	12	33	1203
	a	1542	125	15	1	5	234	1922
y sc cv v E88	b	15	17	14	-	-	18	
	c	494	222	179	117	6	116	1134
	a	1423	154	100	3	2	229	1913
y sc E94	b	17	7	13	3	-	6	
	c	731	254	216	158	-	40	1399
	a	2209	194	11	-	15	433	2865
y X8	b	23	28	30	-	19	87	
	c	1110	487	303	212	4	326	2444
	a	187	9	-	-	2	219	
w^e bb^1/B^SY	b	4	6	-	-	-	3	
	c	732	219	-	-	24	98	1073
	a	556	65	86	65	3	136	921
y sc cv v f car	b	6	9	18	13	6	-	
	c	562	216	210	75	8	147	1214
	a	1045	131	96	106	8	897	2284
y cv v f/B^SY y^+	b	18	13	12	6	1	77	
	c	623	206	188	98	31	560	1708

a at 29°C
b unhatched pupa at 29°C
c at 21.5°C

RESULTS

After a number of y..ts.. mutants were tested for complete lethality at 29°C and survival at 21.5°C, 16 stocks listed in Table I were selected for tests of autonomy. Of these, 3 were subsequently found to be unsuitable for further intensive study: y cv v E52 was found to be very leaky (i.e. gave large number of survivors at 29°C) when outcrossed to the unstable ring stock; M10 was completely sterile when mated to w^{vC}/dl-49 females at both 21.5°C and 29°C (at both temperatures, a number of white opaque eggs which usually represent unfertilized eggs or very early embryonic death were recovered; this may be related to the fused wing phenotype of the y M10-bearing males); the third y..ts.. mutant, 6IV BS EIII, was too poorly fertile to merit investigation.

The number of individuals in each of the expected phenotypic classes in tests of each lethal at 21.5°C and 29°C, as described in Figure 2, are found in Table II. From these numbers, the relative viability of females in which the ts lethal might be unmasked by loss of the w^{vC} chromosome was indicated by the ratio of the number of w^{vC}/y..ts.. mosaic and non-mosaic females to the number of y..ts../dl-49 females at 21.5°C and 29°C, respectively. The degree of leakiness of the ts mutants when outcrossed was estimated by

Table III

Ratio of the number of $\underline{w^{vC}}$-bearing females and exceptional males to the number of y..ts../dl-49 females (value referred to as relative viability ratio or RVR)

chromosome tested		mosaic ♀*	non-mosaic ♀*	XO ♂	matro-clinous ♀	mosaic ♀**	non-mosaic ♀**	total ♀**
y sc cv E5	a	.078	.095	.113	.006	.078	.091	.169
	b	.276	.454	.178	.008	.259	.429	.688
y sc E7	a	.058	.072	.013	.002	.056	.068	.124
	b	.291	.511	.118	.004	.270	.486	.756
y sc cv E9 v car	a	.087	.138	.085	.003	.086	.134	.209
	b	.399	.435	.131	.015	.389	.410	.829
y E25	a	.054	.099	.004	.004	.054	.095	.149
	b	.304	.495	.526	.007	.286	.470	.756
y sc E27 f car	a	.020	.090	.001	.007	.020	.086	.106
	b	.283	.407	.166	.010	.268	.382	.650
y sc E34	a	.033	.056	.007	.010	.033	.052	.085
	b	.391	.496	.199	.009	.375	.471	.846
y sc cv E45	a	.028	.088	.008	.005	.028	.084	.112
	b	.304	.449	.142	.010	.289	.424	.713
y sc cv v E46	a	.006	.089	.002	.004	.006	.085	.091
	b	.279	.451	.174	.018	.272	.426	.698
y sc E76	a	.050	.070	.016	.001	.047	.066	.113
	b	.285	.468	.107	.008	.268	.443	.711
y E82 v	a	.032	.066	.001	.003	.031	.062	.093
	b	.264	.468	.115	.019	.258	.443	.701
y sc cv v E88	a	.010	.081	.001	.003	.009	.077	.086
	b	.362	.449	.237	.001	.338	.424	.762
y sc E94	a	.070	.108	.002	.001	.067	.104	.171
	b	.295	.347	.216	-	.270	.422	.692
y X8	a	.005	.088	-	.007	.005	.084	.089
	b	.273	.439	.191	.004	.252	.415	.666
$\underline{w^e\ bb^1}/\underline{B^SY}$	a			-	-	-	.048	.048
	b			-	-	-	.299	.299
y sc cv v f car	a			.117	.005	.155	.117	.272
	b			.134	.014	.374	.384	.758
$\underline{y\ cv\ v\ f}/\underline{B^SY\ y^+}$	a			.101	.008	.092	.125	.217
	b			.152	.050	.302	.331	.633

* not corrected for primary non-disjunction in females
** corrected for primary non-disjunction in females
a at 29°C
b at 21.5°C

the ratio of y..ts../0 males to y..ts../dl-49 females at the two temperatures. These ratios will be referred to as relative viability ratios or RVR.

RVR were adjusted to eliminate distortion caused by misclassification of non-disjunctional females as w^{vC}/y..ts... Although extensive precautions were followed to eliminate such misclassification, it could not be eliminated completely. Control tests showed that the ratio of primary exceptional to regular females was 0.008 at 29°C and 0.050 at 21.5°C (Table III). Furthermore, at both 21.5°C and 29°C, half of the exceptional B^S females showed mosaicism, thus contributing 0.004 at 29°C and 0.025 at 21.5°C to each of the mosaic and non-mosaic class ratios. These values were used to correct the ratios of the y..ts.. results.

In all autonomy experiments, females could be unambiguously classified as primary non-disjunctional offspring only if they were mosaic for external tissue since only those displaying y patches were tested or dissected to determine the presence of the dl-49 chromosome. Therefore, the ratios of matroclinous females (Table III) represent only non-disjunctional mosaic females. The corrections were made in the following manner; if the frequency of verified non-disjunctional females in the crosses was greater than 0.004

Table IV

Ratios of the Relative Viability Ratios of $\underline{w^{vC}}$-bearing females

(21.5°C/29°C)

chromosome tested	mosaic ♀	non-mosaic ♀	total ♀
y sc cv E5	3.3	4.7	4.1
y sc E7	4.8	7.2	6.1
y sc cv E9 v car	4.5	3.1	4.0
y E25	5.2	5.0	5.1
y sc E27 f car	13.4	4.4	6.1
y sc E34	11.4	9.1	10.0
y sc cv E45	10.3	5.1	6.4
y sc cv v E46	45.3	5.0	7.7
y sc E76	5.7	6.7	6.3
y E82 v	8.3	7.2	7.5
y sc cv v E88	37.6	5.5	8.9
y sc E94	4.0	4.1	4.1
y X8	50.4	4.9	7.5
average	15.9	5.6	6.4
$\underline{w^e\ bb^1}/\underline{B^SY}$	-	6.2	6.2
y sc cv v f car	2.4	3.3	2.8
$\underline{y\ cv\ v\ f}/\underline{B^SY\ y^+}$	3.3	2.7	2.9

or 0.025 at 29°C and 21.5°C respectively, no change was made. However, if it was less than this figure, the difference was subtracted from the y..ts.. mosaic RVR. For non-mosaic females, the established control value was subtracted from the RVR of non-mosaics at both temperatures. An analysis of variance on the corrected and uncorrected ratios showed that the statistical distortion of mosaic and non-mosaic values by misclassification of non-disjunctional offspring was negligible.

These corrected mosaic and non-mosaic values at 21.5°C (Table III) were taken as a ratio of the respective values at 29°C (Table III). Thus, a value greater than 1.0 was an indication of decreased viability at 29°C (Table IV).

Similar ratios were calculated from the results of non-lethal control experiments. It can be seen that both classes of $\underline{w^{vC}}$-bearing females of the controls decreased in frequency at 29°C, and that this decrease was of the same order to magnitude in both phenotypic classes for both controls (Table IV, average ratio = 2.9). This decrease never exceeded the values observed in tests of y..ts.. chromosomes, where it varied from 3 - 9 in non-mosaic females and 3 - 51 in mosaic females. These control values may simply reflect a decreased viability of females which carry the unstable ring at 29°C.

Results using the $\underline{bb^1}$ mutant to obtain mosaic and non-mosaic frequencies for an autonomous non-temperature-sensitive lethal gave a ratio of $\underline{w^{vC}/w^e\ bb^1}$ to $\underline{w^e\ bb^1/dl\text{-}49}$ of 0.299 at 21.5°C. This was decreased by 6.3 times to 0.048 at 29°C. These frequencies of surviving $\underline{w^{vC}/w^e\ bb^1}$ females may indicate the proportion of zygotes in which no ring loss had occurred, loss occurred in tissue in which activity of the locus was not required, or loss occurred internally very late. Decreases in viabilities of $\underline{w^{vC}/y..ts..}$ females of similar magnitude were found for some ts mutants at 29°C (Table IV).

Before a closer analysis of the ratios is made, results indicating the penetrance of the ts genes will be investigated. Penetrance of a gene, that is, the actual phenotypic manifestation of an allele, has been shown to vary with differing environmental and genetic factors so that "Durchbrenner" or lethal-bearing "escapees" may either survive to the adult stage or die only at a later effective lethal phase (Hadorn 1951). Upon outcrossing, some of the ts mutants survived as hemizygous males at 29°C, indicating that the lethal phenotype had been affected by genetic modifiers. Therefore, although all 13 y..ts.. mutant stocks were initially confirmed as complete lethals at 29°C, viability could be affected by altering the "environmental milieu"

upon outcrossing to the ring-X stock. Indeed, only two of the thirteen mutants tested (y sc E27 f car and y X8) remained completely lethal upon outcrossing (Table II). Both y E82 v and y sc cv v E88 gave only one y..ts.. male (In the case of y E82 v, the male was dead.) in all crosses at 29°C and so can be classed with those that maintained their temperature-sensitivity. Another group of mutants showed a very slight leakiness; y E25, y sc E34, y sc cv E45, y sc cv v E46, and y sc E94 with ratios of XO males to y..ts../ dl-49 females varying from .002 to 0.008 at 29°C, and RVR of 0.142 to 0.526 at 21.5°C (Table III). A third class appeared to be more strongly affected by the change in genetic background for the autonomy tests showed that XO male survival had increased to give RVR of 0.013 for y sc E7, 0.085 for y sc cv E9 v car, and 0.016 for y sc E76 at 29°C. However, the higher RVR for these mutants at 21.5°C indicated that their relative viabilities at the high temperature were still very low. In all these tests, the XO males that did hatch at 29°C either died at eclosion or were stuck to the medium and therefore were adult lethals. Even in the case of y sc cv E5 where emergence of XO males was high, all adults died soon after eclosion. Since all surviving males lacked a Y chromosome, the presence of the ts gene could not be verified genetically.

In order to determine whether the frequency of surviving XO males at 29°C, which could be a measure of the penetrance of the ts gene in a new genotype, was correlated with the frequency of females with mosaic patches at 29°C, a correlation test was carried out. The correlation coefficient, r, measures whether mutually dependent variables x (the number of XO males) and y (the number of mosaic females) are related and ranges in value from +1 (which shows perfect positive correlation) to -1 (which shows perfect negative correlation); a value of 0 indicates no correlation. The correlation coefficient, r, may also be an index of the closeness of fit of the observed points (n) to the estimated line of regression. The larger the absolute value of r, the closer the points will fit the line; if $r = \pm 1$, every point will be exactly on the line. Also,

$$r = \frac{\sum(x-\bar{x})\,(y-\bar{y})}{\sqrt{\sum(x-\bar{x})^2\sum(y-\bar{y})^2}}$$

where $\bar{x}$ and $\bar{y}$ are the means of the x's and y's

To test a hypothesis of correlation, t-tables can be used since

$$t = \frac{r}{\sqrt{1-r/n-2}}$$

The correlation coefficient was calculated on the raw data (that is, with the number of mosaics as one variable and the number of XO males as the other),

Table VI

Data collected in the scoring of "shift" cultures of y E25

time of shift down(hr.)	# of eggs laid	results
0	50	40 live adults
20	50	42 live adults
44	50	43 live adults
68	50	45 live adults
92	50	46 live adults
111	45	7 live adults, few dead third instar larvae
116	50	30 live adults, few dead third instar larvae
120	30	all dead third instar larvae
140	86	few live adults, dead third instar larvae,
152	59	dead late pupae, partly eclosed dead adults,
160	34	and dead adults
170	30	1 live adult, dead third instar larvae
175	54	dead third instar larvae, dead early and late
185	50	pupae
188	50	mostly dead third instar larvae, some dead
190	75	pupae, dead partly eclosed adults, dead adults
212	100	mostly dead late pupae

Death occurred in the culture that had been shifted down after 111 hours at 29°C, therefore, TSP must begin somtime between 92 and 111 hours. LP extended from third larval instar to adult stage.

time of shift up(hr.)	# of eggs laid	results
0	50	dead late pupae
20	40	dead late pupae
44	40	dead late pupae
68	40	dead adults, dead partly eclosed adults, dead
92	32	late pupa
111	40	6 live adults, dead adults and pupae
116	30	6 live adults, dead adults and pupae
120	76	19 live adults, dead adults and pupae
140	35	7 live adults, dead adults and pupae
152	38	6 live adults, dead adults and pupae
160	38	24 live adults, dead adults and pupae
170	32	22 live adults, dead adults and pupae
175	43	25 live adults, dead adults and pupae
185	39	29 live adults, dead adults and pupae
188	30	11 live adults, dead adults
190	39	31 live adults, dead adults
212	30	16 live adults, dead adults

Live adults emerged in the culture that had been shifted up after 111 hours at 21.5°C, therefore, the TSP must have ended sometime between 92 and 111 hours.

Table V

TSP and LP of the different mutants tested

mutant tested	TSP	LP
y sc cv E5	♀♀: indispensible ♂♂: 150 hours (pupa) to adult	late third instar to adult
y sc E7	25 to 70 hours (first instar larva to third instar larva)	first instar larva to adult
y sc cv E9 v car	70 to 180 hour (third instar larva to late pupa)	prepupa to adult
y E25	92 to111 hours (end of third instar larva stage)	late third instar to adult
y sc E27 f car	60 to 165 hours (third instar larva to mid-pupa)	third instar larva to late pupa
y sc E34	50 to 80 hours (second instar larva to third instar larva)	third instar to adult
y sc cv E45	0 to 140 hours (egg to mid-pupa)	second-third larval intermolt to adult
y sc cv v E46	0 to 120 hours (egg to early pupa)	first instar to eclosion
y sc E76	0 to 240 hours (indispensible)	first instar to adult
y E82 v	60 to 140 hours (third instar larva to mid-pupa)	second-third larval intermolt to eclosion
y sc cv v E88	80 to 150 hours (third instar larva to mid-pupa)	third instar to late pupa
y sc E94	28 to 125 hours (first instar larva to early pupa)	first and second instar larva, pupa, adult
y X8	82 to 97 hours (during late third instar larva)	pupa to adult

The value of r was 0.330 and of t (11 degrees of freedom) was 1.158. With 11 degrees of freedom, the value of t at the 5% level of significance is 1.796; thus, no statistically significant correlation was indicated in this analysis. In comparing the RVR of XO males and mosaic females, r was found to be 0.593. The corresponding value of t (11 degrees of freedom) was 2.444, while t at the 2.5% level of significance is 2.201. Therefore, at the 2.5% level of significance, the null hypothesis that there is no correlation can be rejected, meaning that XO survival is related to viability of mosaics.

Besides the penetrance of the ts gene, its TSP and LP might be expected to affect the frequency of mosaicism at 29°C. The temperature-sensitive periods and lethal phases of all the mutants studied are shown in Table V. A sample of the method of scoring for one y..ts.. mutant is given in Table VI. It must be pointed out that the determinations of the TSP were very crude owing to considerable asynchrony in larval development. Because of this, organisms in shift cultures were of different ages and could, therefore, react to the temperature change in a number of ways.

Although control shift experiments using the non-ts, y sc cv v f car/Y stock were set up in an attempt to standardize developmental time and rates

at the two temperatures, these could not be used as stringent controls for the ts experiments since the rates of development may vary with each genotype. However, these controls did provide a measure of the amount of lethality in a culture at different developmental stages that might have resulted from the temperature change alone. This level of lethality was taken into account when the y..ts.. shift experiments were being recorded.

A statistical test was used to determine whether there was any correlation between the length of the TSP and the magnitude of the decrease encountered by the mosaic class due to the temperature difference as measured by $RVR^{21.5°C}/RVR^{29°C}$. The tests showed that there was no correlation at the 5% level of significance.

In addition to the above results, information on three ts mutants was obtained from the autonomy and TSP experiments. Red pigment granules were found in the Malpighian tubules of larvae, pupae, and adults, both alive and dead, of the mutant y sc E27 f car at 29°C. y sc cv E45 males developing up to the late pupal stages at 29°C had dark pigment deposits on their dorsal abdominal surfaces. These abnormalities were definitely associated with the ts gene since they did not occur at 21.5°C. Also, they did not occur at 29°C in non-ts individuals which had the other recessive markers. The sexual

dimorphic nature of y sc cv E5 (Tarasoff 1968) was confirmed (Table V).

DISCUSSION

The study of cellular autonomy is of biological interest as a method of gaining an insight into the nature of cell to cell interactions. The influence of one functioning cell on the activity of another is an integral part of the differentiation and regulation of a multicellular organism. From the definition of autonomy used in this study, genetic cell to cell interactions can be studied by determining whether cells of one genotype can alter the phenotype of a genotypically different cell. Although such a condition does not normally arise in a developing individual, such genetically contrived mosaics may, in fact, mimic states of differential genetic activity in differentiated cells. Thus, studies of autonomy could parallel the process of differentiation, and whatever information about genetic interactions between cells that is gained from these studies might reveal interactions taking place during development.

These studies of autonomy involved the analysis of mosaics in which cells or tissues of one genotype are juxtaposed to genetically different cells. Thus, if a mutant genotype being studied is autonomous and viable, mosaic patches of mutant tissue will be detectable phenotypically, adjacent to wild type tissue. The size and location of mosaic tissue may suggest the devel-

opmental time at which genetic activity is initiated and repressed and its tissue specificity. In these studies, however, the phenotype of each mutant was lethality and therefore chromosomes carrying the lethal gene were marked with autonomous visible mutants in order to detect the presence of non-autonomous mutant tissue. In such a case the size and location of mosaic tissue may suggest the time after which autonomous genetic activity is repressed and/or tissue in which the lethal gene does not function.

A demonstration of non-autonomous genetic behaviour of a mutant suggests the presence of a diffusible substance in the wild type tissue which can modify the phenotype of mutant cells. Thus, the recognition of non-autonomous mutants may provide a bio-assay which would permit the isolation of diffusible factor(s) produced by the wild type tissues. Characterization of the requirements of such "supplementable" mutants could ultimately permit cell culture selection techniques comparable to those used in microorganisms.

These studies of autonomy were facilitated by the use of temperature-sensitive lethal mutations. Previous studies of autonomy in Drosophila melanogaster involved the use of non-conditional lethal mutations (Demerec 1934; Ephrussi 1934; Stern 1934; Poulson 1948; and Oster and Sobels 1956). where the frequencies of mosaic patches in flies heterozygous for the lethal

mutant were compared with the frequencies of mosaics in non-lethal-bearing individuals. The ts mutant provides a more rigid control since mosaic frequencies in flies of the same genotype can be compared at 29°C and 21.5°C. Genotypic control and the ease of imposition of the selective condition greatly enhance the study of lethal autonomy.

In addition, in studies of non-ts lethals, it cannot be said definitely that the mosaic patch of mutant tissue was produced during lethal activity of the mutant. The mutant patch could have been produced after lethal activity had ceased. However, the TSP of a ts mutant is the time during which genetic activity can be altered to result in lethality of the mutation. Thus, mosaic patch production after exposure of the developing fly to lethal temperatures during its TSP may be the result of non-autonomy. Yet, it must be remembered that mutant activity will be expressed only in tissue requiring genetic functioning of that particular locus. So, although by using a ts mutant, it can be said that lethal genetic activity was being expressed during a specific time interval, this activity results in lethality of certain tissue causing death of the whole individual. It does not necessarily mean a small patch of other tissue will be affected by the ts lethal mutant. In order to say specifically that the mosaic patch was expressing mutant activity,

knowledge of both TSP and tissue specificity is required. In this respect, the use of a ts mutant is no more advantageous. However, once autonomy has been confirmed, the advantages of the ts mutant in further investigations are numerous.

Turning now to the experiments performed, the technical difficulties in evaluating the results will be outlined initially. A major problem arises because the mere presence of mosaic patches at 29°C does not necessarily indicate non-autonomy of a mutant since other factors may contribute to the production of mosaic tissue. In the following discussion a number of these will be considered and it will be determined whether they affect the frequency of mosaics scored in these tests.

Mosaic patches can be produced by abnormal genetic events not involving actual loss of the ts^+ gene; for example, loss of fragments of the ring-X chromosome unmasking only certain recessive markers while maintaining the wild type allele of the locus has been suggested (Singer 1969, personal communication). In the present experiments, it was found that in most cases where y tissue was detected, other recessive visible markers linked to y (sc, cv, v, f, and car) were also expressed. Thus, if loss of only small fragments of the X-chromosome occurs, it is infrequent and the loss generally involved the

entire ring-X (including the locus of the $\underline{ts}^+$ gene). Moreover, since the final analysis of the data was made by comparing the relative frequencies of mosaics at the restrictive and permissive temperatures, this factor should cancel out if the rate of loss of y^+ was independent of temperature. Thus, generation of mosaic patches through loss of small regions of the ring-X, I feel, is probably unimportant.

The production of mutant tissue through somatic exchange also should not distort the estimate of autonomy based on the frequency of recovery of mosaics. Somatic crossing over between the mutant marker and the ts lethal results in the formation of twin spots, tissue homozygous for the lethal and the mutant marker. If death of cells homozygous for the ts lethal does not kill the fly, non-autonomy of the lethal could be suggested by surviving mosaic tissue. Also, if somatic recombination is independent of temperature, the contribution to mosaic frequencies by somatic crossing over should be similar at both temperatures. Moreover, since most mitotic crossing over occurs in proximal heterochromatin (Stern 1936) the marker and the ts lethal should remain linked.

The possible distorting effects of a number of other factors have also been ruled out. The presence of females resulting from primary non-disjunction and therefore having mosaic patches which do not carry the ts lethal

was found to be too small to affect the estimated frequencies of lethal mosaics. The markers (y, sc, cv, f, and car) used to identify the mosaic patches of ts tissue were shown to be autonomous (Sturtevant 1932) at both temperatures. The number of recessive markers linked to the ts gene would not affect the relative frequencies at the two temperatures unless there was a drastic and unexpected temperature sensitivity of the markers.

In spite of the elimination of these possibilities, the presence of mosaics at 29°C does not confirm the non-autonomy of a ts lethal. Mosaics could survive if hemizygosis of the y..ts.. chromosome at 29°C took place after the time that ts gene lethal activity had taken place. Although the tissue specificity of the mutants was not investigated, the TSP was determined. It was expected that the longer the TSP, the greater would be the chances of w^{vC} loss during that interval. Thus, it was anticipated that there would be a slightly decreased frequency of mosaic survival with non-autonomous mutants and a markedly decreased frequency of mosaic survival with autonomous mutants of long TSP's at 29°C. Analysis of the results showed that the length of the TSP and the magnitude of the decrease in the mosaic class at 29°C were not statistically related. This could mean that both non-autonomous and autonomous mutants were present in the sample. On the other

Table VII

Description of y..ts.. mosaics surviving at 29°C

y sc cv v E46

1. half of sixth abdominal tergite yellow, half of fifth abdominal tergite missing
2. eyes vermilion, half of fifth and sixth abdominal tergites yellow, genitalia mosaic
3. parts of fifth and sixth abdominal tergites yellow, half of fourth abdominal tergite missing
4. half of fifth and sixth abdominal tergites yellow, genitalia mosaic eyes partly vermilion
5. fifth and sixth abdominal tergites yellow, genitalia mosaic, fly stuck to medium
6. half of fifth and sixth abdominal tergite yellow, genitalia mosaic
7. scutellar bristles missing, genitalia mosaic
8. left half of abdominal tergites yellow, missing scutellar bristles

y sc cv v E88

1. left legs yellow, genitalia mosaic*
2. left half of abdominal tergites yellow, genitalia mosaic
3. half of sixth abdominal tergite yellow
4. genitalia mosaic
5. all abdomen yellow except for half of the first, second and third abdominal tergites
6. left half of head yellow, left eye vermilion, fifth and sixth abdominal tergites yellow, genitalia mosaic
7. left half of abdomen yellow
8. right eye vermilion, right antenna yellow
9. right half of abdominal tergites yellow
10. half of fifth abdominal tergite yellow
11. half of fifth and sixth abdominal tergites yellow
12. half of fifth and sixth abdominal tergites yellow
13. half of first to fifth abdominal tergites yellow
14. bristles on head yellow
15. not described

*only mosaic patch detected on legs; could be due to primary exceptional female

y X8

1. half of fourth abdominal tergite yellow
2. half of fourth and fifth abdominal tergites yellow, genitalia yellow, dead
3. parts of abdominal tergites yellow, other parts missing, genitalia abnormal
4. male genital arch, stuck to medium
5. one antenna yellow
6. one wing and half thorax yellow, individual dead
7. parts of head and thorax yellow, individual dead
8. small patches of tissue all over the body yellow, dead

9-11. mosaics stuck and dead, yellow patches all over the body

hand, it could be argued that all of the mutants are autonomous but are required only in certain tissues during the TSP; mutant tissue not requiring activity of the ts gene at this time will survive and yield mosaics. Therefore, only when it is known in which tissue the ts mutant is lethal during the TSP will the TSP be of great significance in understanding autonomy. Thus, in these studies, only when total absence of mosaics or complete absence of one type of tissue displaying mosaicism is demonstrated, can a mutant definitely be called autonomous. Mutants E46 and E88, which yielded no mosaic patches on the thorax, wings, and legs (Table VII) at 29°C, are examples of the latter.

The survival of y..ts.. males at 29°C in some of the tests probably resulted from altered gene expression under different genetic and environmental conditions. It must be asked whether such survival might result from a different mechanism such as the loss of the ts mutant by somatic crossing over. Hinton (1955) established that XO males result primarily from early somatic loss of the w^{vC} chromosome from X/w^{vC} zygotes. Therefore, the occurrence of a somatic double exchange in the earliest stages of cleavage before or at the time of w^{vC} elimination, could result in replacement of the ts lethal by its wild type allele, permitting survival of the XO male. A test for

such an elimination could not be performed since these males were XO and therefore sterile. Somatic exchange, if it occurs, can be used to account for the survival of these XO males at 29°C, but the possibility of the early occurrence of such an event spanning only the ts locus, followed by w^{vC} loss, is remote since the frequency of mitotic double crossovers is itself rare (Stern 1936). Thus, y..ts../O male survival is undoubtedly primarily the consequence of "escapee" activity.

Many of the y..ts../O males that did hatch at 29°C died immediately or stuck to the medium and died shortly thereafter. The weakness of these males probably resulted from a prolongation of the LP into late pupal and early adult stages. Five y..ts.. mutants (E46, E88. E27, E82, and X8) that gave virtually no XO males did not have LP's in the adult stage, whereas all of the others which yielded some XO male "breakthroughs" had LP's extending into the adult stage. The higher frequency of XO adult male of y E5 was expected since its lethal period is exclusively from pupal to adult stage. So, the survival of XO males to the adult stage due to "escapee" activity at 29°C appears to be enhanced by the presence of a lethal period in adults.

Analysis of results showed that higher mosaic frequencies at 29°C were correlated with XO "breakthrough" frequencies at 29°C. XO male survival in

turn has been found to be greatest in those ts mutants with adult lethal periods. Therefore, mosaic frequencies at 29°C are higher among those mutants having an adult lethal period. Such results may mean that in some cases the ts gene in the hemizygous mosaic tissue, as in XO males, is susceptible to similar genotypic and environmental modifications. Such altered activity must not be misinterpreted as non-autonomous behaviour.

Besides mosaic tissue production at 29°C, which does not reflect the autonomous property of the ts gene, the manipulation of the data could affect estimates of mosaic frequencies. Since the evaluation of the results is based on the ratios of the frequency of a particular class to the frequency of sibling In(1) dl-49/(X-chromosome from the male) females, and since these relative viability ratios (RVR) were used in further calculations, it is important to establish the validity of using the frequency of these heterozygotes as a common denominator. Are discrepancies caused by differential viability of the In(1) dl-49/(X-chromosome from the male) female at 29°C and 21.5°C which might then distort the relative viabilities of the mosaic class? If temperature did affect the development of this class, decreased viability at higher temperatures is expected since it has generally been shown that the frequency of emergence of adults from cultures kept at higher temperatures

is much lower than the frequency at optimal temperatures. Parsons (1959) demonstrated such decreased viability by testing the effect of 31°C as compared with 24°C on various wild type stocks and their F_1 hybrids. Therefore, in the present experiment, the ratios at 29°C would yield an overestimate of mosaic viability and tend towards classification of a mutant as non-autonomous. In fact, the ratios at 29°C of both mosaic and non-mosaic classes were greatly reduced over that at 21.5°C. Thus, even if the ratio were overestimated at 29°C, decreased viability was indicated. If temperature had no effect on the denominator, then this ratio would have been much smaller. Since it is of interest to look at the amount of decrease encountered at the higher temperature, it should be kept in mind that the magnitude of the decrease measured would be minimal by these criteria. Therefore, even if temperature did affect the viability of the In(1) dl-49/(X-chromosome from the male) female, the inferences drawn from the ratios would be conservative but reasonable.

Comparison between RVR of different ts's are a different matter. In these cases, differences in heterozygote viabilities in each cross would affect the ultimate ratios compared. Although adequate tests of differential heterozygote viability in different crosses were not carried out, there are no compelling reasons for expecting severe differences between the crosses.

Thus, in the discussion which follows, it will be assumed that viability of the heterozygote remains relatively constant from experiment to experiment.

Although all y..ts.. mosaic and non-mosaic frequencies were decreased at the restrictive temperatures, the total reduction cannot be wholly attributed to the lethal effects of the y..ts.. mosaic patches. It was found that non-ts control w^{vC}-bearing mosaics and non-mosaics also underwent an approximate three fold reduction at 29°C. The decrease may result from a reduced viability of flies mosaic for male and female tissue at higher temperatures. This basic level of decrease must be considered when discussing ts mutant activity.

In this experiment we are comparing survival of mosaic tissue at 21.5°C and 29°C. This includes external as well as internal mosaic tissue. A difficulty arises in not knowing how representative external mosaicism is of internal mosaicism. The degree of internal mosaicism which is not detectable externally can be estimated by looking at the total decrease of all w^{vC}-bearing females at 29°C. The decreased frequency of the mosaic class at 29°C represents the lethality incurred by flies mosaic both externally and internally at 29°C, whereas the decreased numbers in the non-mosaic class reflect additional lethality of zygotes which are completely mosaic internally.

The results of the $\underline{bb^1}$ experiments can also be used as a base level to measure the total mosaic frequency of the ts mutants. The frequency of $\underline{w^e}$ $\underline{bb^1}/\underline{w^{vC}}$ females recovered is a measure of females which are non-mosaic in both internal and external tissue if it is assumed that loss of $\underline{bb^+}$ activity results in lethality at any time in any cell. Since Ritossa et. al. (1966) have shown that the bb locus directs the synthesis of ribosomal RNA, it is highly probable that this assumption is indeed valid. The ratio between the two classes is about 0.30 at 21.5°C, so it can be said that about three times out of ten, $\underline{w^{vC}}/\underline{bb^1}$ females have few if any cells in which the ring is lost in tissue vital for viability. In other words, seven out of ten developing $\underline{w^{vC}}/\underline{bb^1}$ females suffered ring loss in vital tissue and therefore were lethal mosaics. The ratio of external mosaic to non-$\underline{w^{vC}}$-bearing females as shown by control experiments, is also about 0.30 (ie., three out of ten ring-bearing females suffered loss of $\underline{w^{vC}}$ in external somatic cells). If these mosaics in the controls can be considered representative of the mosaic females resulting from loss of $\underline{w^{vC}}$ in any external tissue (although they are based on the numbers of females mosaic for the specific markers, y, sc, cv, v, f, and car) and if the numbers seven out of ten are taken to be an estimate of the frequency of females that undergo any loss of $\underline{w^{vC}}$ (although it is specifically

the frequency pertaining to any loss in tissue affected by bb activity), then it can be said that external mosaics represent less than half of all individuals in which the ring-X is lost at some stage.

The results obtained from tests of the autonomous mutant, bb^1, also provide values of the viability ratios against which the ts values may be compared. The decreases of the RVR of the $w^{vC}/w^e\ bb^1$ females at 29°C compared to the ratio at 21.5°C (6.3 times) gives a measure of the lethality incurred by individuals carrying an autonomous lethal and the w^{vC} chromosome at 29°C. This decrease would result from reduced viability of the w^{vC}-bearing females at 29°C plus death due to mosaicism for an autonomous non-ts lethal. An average decrease of 6.4 times was found for all y..ts../w^{vC} females at 29°C, a value very similar to the bb^1 decrease. If bb^1 is truly autonomous, it sets an upper limit on viability ratios of autonomous lethal mutants. The ts mutants that have decreases greater then 6.3 times therefore must be autonomous; the greater values may result from an interaction between the ts and w^{vC} chromosomes at 29°C or from a greater lethal effect of the ts mutant in mosaic tissues. This figure will be used as a basis for the classification of the viability ratios of the ts mutants.

The following mutants yielded relative ratios of w^{vC}-bearing females

(21.5°C/29°C) greater than the ratio observed in $\underline{w^{vC}}/\underline{bb^{1}}$ females (6.3) and therefore were considered to be autonomous: E34 (10.0), E45 (6.4), E46 (7.7), E82 (7.5), E88 (8.9), and X8 (7.5).

The greatest decrease of the $\underline{w^{vC}}$-bearing female class was shown by E34. The reduced viability in the mosaic and non-mosaic classes are 11.4 and 9.1 times respectively; so both classes are reduced equally. It should be pointed out that the decrease suffered by the non-mosaic class in E34 is the greatest of all the ts mutants tested (average decrease = 5.6) while the decrease in the mosaic class is below average (15.9). Since decreases in the non-mosaic class reflect lethality of internal mosaics, it can be said that E34 is more effective as a lethal in internal tissue than in external tissue. The very low frequency of mosaic tissue is correlated with a very low frequency of escapees. XO survival frequency is 0.007 at 29°C as compared with 0.199 at 21.5°C.

E82, which can also be classed as autonomous, is like E34 in that it shows the same amount of decrease in both classes (mosaic reduction = 8.3 times, non-mosaic reduction = 7.2 times). But unlike E34, escapee activity cannot account for the survival of mosaics since XO flies are inviable at 29°C and no adult lethal period was observed. Since mosaic patches are found

on all external parts of the body, it could be speculated that this mutant is lethal exclusively in internal tissue. Such speculation seems reasonable when it is noted that the lethality of the non-mosaic class is greater than average. If this is true, then ts lethality of E82 is specific for internal tissue.

E46 and E88, which have reductions of the w^{vC}-bearing female class of 7.7 and 8.9 times, respectively, have very similar properties. Besides the overall reduction of the w^{vC}-bearing female class, the mosaic and non-mosaic classes show a similar pattern of decrease: in E46 the mosaic class was reduced by a factor of 45.3 times and the non-mosaic class by 5.0 times and in E88 the reduction factor for the mosaic class was 37.6 and for the non-mosaic class, 5.5. This means that both are more effective as lethals in external tissue. In both, XO male survival is negligible (0.001 at 29°C). While the TSP for E46 is prolonged from egg to early pupal stage, the TSP for E88 was confined to the third instar to mid-pupal interval. Another striking property common to both is the complete absence of external mosaic patches on the thorax, wings and legs, tissues which develop from the wing and leg imaginal discs. Also, morphological abnormalities in the same tissues and the absence of parts of these tissues were frequent in non-mosaic and mosaic females.

The occurrence of mosaic patches on the scutellum, as indicated by the absence of scutellar bristles in E46, may be a result of mutant tissue lethality at 29°C and not necessarily of scute phenotype manifestation due to the survival of mutant tissue. Therefore, these genes appear to function autonomously in cells of the wing and leg discs that will eventually be located on the external surface. Whether the similarity between these mutants is fortuitous or whether they are genetically related cannot be said at this time. However, they are genetically distinct with respect to map position, E46 being located to the right of car and E88 mapping at wy.

X8 is another autonomous mutant which is more active in external tissue (the mosaic class was reduced by 50.4 as compared with the non-mosaic class reduction of 4.9). Of the 11 mosaic adults recovered out of a total of 2865 progeny scored at 29°C, only 3 appeared to be fully viable, the rest were poorly viable (stuck to the medium) or dead, and all had mosaic patches all over the body. Also, many abnormalities in external morphology were noted in the w^{vC}-bearing females. Since it cannot be disputed that this gene is autonomous and functional in external tissue, how can the survival of the few mosaics be explained when there was complete absence of XO males at 29°C? w^{vC} loss after the very specific TSP at the end of the third larval instar

is a reasonable explanation. This assumption is strengthened when it is noticed that most mosaic patches involved very small areas (Table V), the largest covering half a thorax.

By the standards set with the $\underline{bb^1}$ experiments, E45 is also autonomous. The decrease in mosaic and non-mosaic classes at 29°C are 10.3 and 5.1 respectively, so this lethal appears to be more active in external tissue. The very low XO survival (0.008 at 29°C) and the presence of an adult lethal period can account for the viability of the mosaics which involve all body parts. Nothing more can be said about the genetic activity of this ts except that the dead y..ts.. males that developed up to the late pupal stage at 29°C had dark pigment deposits on their dorsal abdominal surfaces. This phenotype is a temperature-sensitive phenomenon, but how it is related to the ts lethal is not known.

Reduction of $\underline{w^{vC}}$-bearing females at 29°C similar in magnitude to that found with $\underline{bb^1}$ occur in E76 (6.3 times) and E7 (6.1 times). Nothing exceptional was noted from the results of E76. Mosaic and non-mosaic classes were equally susceptible to 29°C, being reduced by 5.7 and 6.7 times respectively. The observed escapee activity and the presence of an adult lethal period explains the survival of mosaics. This mutant is temperature-sensitive at all

times so the XO males that do pupate are all dead at hatching time.

The mutant, E7, is exceptional. It has an unexpected pattern of decrease: the non-mosaic class undergoes a much greater reduction (7.2 times) than does the mosaic class (4.8 times). Such results can only mean that E7 is most active in internal tissue. The data are made more interesting in light of the fact that the TSP occurs early in development, between the first and third instar. More can be said about the genetic activity of E7 but it would all be conjecture. However, it can definitely be stated that E7 functions autonomously in internal tissue.

Although the survival of the w^{vC}-bearing female with the rest of the mutants is reduced to a lesser degree than with the bb^{1}, it cannot be said that these mutants are non-autonomous. $E27/w^{vC}$ females are 5.9 times less viable at 29°C but its mosaic class is reduced by 15.5 while its non-mosaic class is reduced by only 3.7 (N.B., control decrease is about 3.0). Such data suggest that E27 is autonomous in external tissue but non-functional or non-autonomous in internal tissue. Although some individuals recovered had mosaic patches all over the body at 29°C, these mosaics were either dead or nearly dead (stuck to the medium) at the time of eclosion. It is interesting to note that like E45, this mutant has a temperature-sensitive phenotype -

red pigment granules in the Malpighian tubules in individuals developing at 29°C.

The mosaic and non-mosaic classes with E25 are reduced to a similar extent at 29°C (5.3 and 5.0 respectively) to give a total reduction of the w^{vC}-bearing female of 5.1. This pattern of decrease is reminiscent of those shown by E34 and E82 except the degree of viability is greater at 29°C in this case. Mosaic survival does not reflect XO breakthrough activity which is negligible in this case (0.004 at 29°C as compared with 0.526 at 21.5°C). The very limited TSP of about twenty hours at the end of the third larval instar may account for survival of females with mosaic patches if w^{vC} loss occurs after this period. If this were so, relatively small patches of tissue should be mosaic. However, viable mosaics involving more than half the whole organism are recovered. Therefore, it can be argued that like E34, E25 is autonomous and more active in internal tissue, but unlike E34 and E82, its period of activity is short-lived so that its lethal-inflicting ability is curtailed.

The sexually dimorphic ts mutant, E5, has a TSP from 150 hours to the adult stage in males and continuously in females. Decreases in viability of E5 were not much greater than those found in the controls, as might be expected since the male TSP occurs very late in development with a LP exclu-

sively in the late pupal and adult stage. It is interesting to note that the pattern of genetic activity of E5 in hemizygous tissues reflected the male pattern even when it was surrounded by female tissue. Therefore, with respect to sexually dimorphic temperature-sensitive activity, E5 is autonomous.

Of all mutants studied, E94 appears to be the most non-autonomous. This ts mutant has equal decreases of 4.0 in the mosaic class and 4.1 in the non-mosaic class. External mosaicism was detected throughout the body. The decreased magnitude of lethality cannot be explained by a short TSP as in E25 since its TSP lasts from first instar to early pupal stage. Nor can it be accounted for by "Durchbrenner" activity since XO male survival frequency at 29°C is only 0.002 compared with 0.216 at 21.5°C.

The temperature-sensitive lethality was least noticeable in the case of E9, which had decreases in the mosaic class of 4.2 and in the non-mosaic class of 3.8 at 29°C. Survival of both external and internal mosaics can be wholly explained by the XO survival frequency, which is only slightly changed by temperature (0.088 at 29°C and 0.131 at 21.5°C), an indication of the excessive leakiness of the mutant upon outcrossing.

The above discussion demonstrates that a study of somatic mosaicism resulting from unstable ring loss can indicate the relative autonomy of the ts

mutants but is not precise enough to identify a non-autonomous mutant unequivocally. In order to prove non-autonomy within this scheme, methods must be devised to exclude the production of mosaics in tissues where mutant activity is not lethal at 29°C. A more difficult task is the elimination of survival of mosaics made possible by "Durchbrenner"effects. By choosing ts lethals that are not influenced by changes in genetic background and whose tissue specificity is known, non-autonomous behaviour can be detected if mosaic patches are found in tissue requiring functioning of that locus.

However, this study has proven fruitful in other respects. It has shed light into the tissue specificity of some ts mutants. With a more detailed investigation of the occurrence of mosaic patches in mutants such as E46 and E88, the tissue affected by a given lethal may be pin-pointed more exactly.

SUMMARY

The survival of mosaic patches of tissue at 29°C cannot be used as a valid criterion for the non-autonomous behaviour of sex-linked recessive temperature-sensitive lethal mutants.

However, the relative degrees of autonomy of the mutants were determined after considering the relative viability ratios of mosaics and non-mosaic females, the XO survival frequencies, the lethal periods, and the temperature-sensitive periods. Those thought to be autonomous are E34, E45, E46, E82, E88, and X8. E46 and X8 are definitely autonomous in cells of the thorax, wings and legs, since no mosaic patches appear in these tissues at 29°C. E76 is not as strict an autonomous lethal as the preceding ones while E7 is thought to function autonomously in internal tissue only. E27, on the other hand, is thought to act autonomously in external tissue. E25 is another lethal acting only in internal tissues but not as effectively as E7. The sexual dimorphic ts mutant, E5, functions autonomously according to its sexual dimorphic nature. Of all the ts mutants studied, E94 appears to be the least autonomous. The apparent non-autonomous character of E9 can be explained by a high XO survival frequency. Therefore, although it cannot be said definitely that any of the ts mutants act non-autonomously, their potency as lethals in mosaic patches varies.

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