GENETIC AND DEVELOPMENTAL STUDIES OF PROXIMAL SEGMENTS OF CHROMOSOME 3 OF *DROSOPHILA MELANOGASTER*

by

DONALD A.R. SINCLAIR
B.Sc.(Hons), University of Manitoba, 1969
M.Sc., University of Manitoba, 1972

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Department of Zoology (Genetics)

The University of British Columbia
2075 Wesbrook Place
Vancouver, Canada
V6T 1W5

Date 6/14

ABSTRACT

The present work deals with several approaches to the study of regions near the centromere of chromosome 3 of Drosophila melanogaster. The goals of this research were: (i) to examine spontaneous crossing over near the centromere in detail; (ii) to locate the Deformed locus genetically and to determine whether this lesion is a recessive lethal; (iii) to test the efficacy of radiation-induced crossing over as a method of producing proximal aberrations; and (iv) to genetically and developmentally characterize a temperature-sensitive (ts) allele of a Minute locus, located near the centromere.

CHAPTER 2 describes a study of recombination, which deals with short genetic regions near the centromere of chromosome 3, using the intervals, st-in-ri-eg^2-Ki-p^p and Gl-p^p-Sb-H. The following generalizations have emerged: (i) an excess of multiple crossover chromosomes was recovered, and the intervals which immediately span the centromere showed the highest negative interference; (ii) a positive correlation of simultaneous exchange within closely-linked intervals, was noted for many of the multiple crossovers; and (iii) several classes of reciprocal crossover products were not recovered equally. Three possible explanations for these results are: pre-meiotic exchange, chromatid interference and gene conversion. The results of one experiment also indicated that the interchromosomal effects of C(1)M3 are most pronounced within the st-in and Ki-p^p intervals.

CHAPTER 3 describes a genetic study of the Deformed locus. The

mapping results confirmed that Dfd is closely linked to Ki. Genetic analysis of the crossover chromosomes suggested that Dfd is homozygous viable and this was confirmed by the synthesis of homozygous Dfd stocks. This indicates that the Dfd locus is not located within section 84F in proximal 3R.

CHAPTER 4 deals with experiments involving the use of radiation to produce crossovers near the centromere of chromosome 3, in males. Crossovers originating from exchange nearest the centromere, were associated with clusters more frequently than those originating from exchange within other proximally-adjacent segments. Induced exchange was frequently accompanied by mutation and/or chromosome damage, at or near the site of exchange. This was particularly true for crossovers resulting from exchange in wholly euchromatic segments. It is suggested that many of the radiation-induced crossovers arise through asymmetrical exchange, and that this approach will permit the isolation of proximal aberrations.

CHAPTER 5 describes the genetic and developmental analysis of a ts Minute. As a ts allele of a proximally-located Minute locus, Q-III exhibits the classical dominant M traits, recessive lethality, and a highly pleiotropic phenotype, at 29°C. This phenotype was analysed in detail through the use of various temperature shift experiments.

Q-III possesses a polyphasic temperature-sensitive period (TSP) for lethality extending from the first larval instar to late pupation. Shorter heat pulses defined discrete larval, larval/pupal, and pupal TSPs for lethality. In addition, homozygous Q-III females exhibit ts sterility and maternal effects, indicating that the Q-III gene product is essential throughout development.

Heat-pulse experiments revealed a number of adult developmental abnormalities, involving derivatives of eye-antennal, leg, wing and genital imaginal discs. Many defects, for example, those involving the eye or antenna (eye-antennal disc), male genitalia (genital disc), and scutellum (wing disc), have larval TSPs; whereas others, such as bristle or sex comb traits, have pupal TSPs. It is suggested that the former defects may be related to cell death in the larval anlagen; while the latter are more likely due to blockages in differentiation during pupation.

Q-III also interacts in ts fashion with several non-allelic mutations. Thus, at 29°C, Q-III is lethal when combined with Dl, Ly and Dfd; suppresses the sex comb phenes of Msc and Pc; and produces wing nicking effects when combined with vg or Scx. TSPs were defined for the vg, Dl and Scx interactions. It is suggested that many of these interactions are metabolic rather than specific.

The fact that Q-III phenotypically resembles bobbed and suppressor of forked^{ts}, strengthens the notion that Minute gene products are active in translation. It is concluded that translational defects can fully account for the pleiotropy of Q-III.

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Parts of CHAPTER 2 are published in Genetical Research.

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CHAPTER 1

GENERAL INTRODUCTION

I. Background

For many years, geneticists have studied segments that lie near the centromeres of chromosomes. Attempts to characterize these proximal segments have been marked by considerable speculation (see Cooper, 1959), as well as by definitive experimentation.

1. Heterochromatin

Long ago, Heitz (1933) reported that specific chromosome segments possess special cytological properties, in that they remain permanently condensed. Such segments are particularly prominent in regions near the centromeres of chromosomes of eukaryotes, and more recently they have been called constitutive heterochromatin (Brown, 1966).

When cytological length is considered, constitutive heterochromatin includes 20 to 25 percent of the two autosomes, 35 to 50 percent of the X chromosome and the entire Y chromosome of Drosophila melanogaster.

The previous discovery of highly redundant satellite DNA sequences in Drosophila and their preliminary localization within heterochromatin through in situ hybridization (Gall et al., 1971) has recently led to the important study of Peacock et al. (1974). These workers detected 7 species of satellite DNA and determined that they were highly repetitive. In situ analysis of labelled RNA complementary to these DNA sequences revealed that the latter are located mainly in the chromocentre in salivary gland chromosome preparations. Nucleotide analysis of 3 of the satellites showed that the basic repeating units

are relatively short and simple, thereby supporting the idea that most of this repetitive DNA is incapable of coding for a gene product of average complexity.

2. The Genetic Importance of Proximal Regions

Directly or indirectly, several workers have given valuable information concerning the nature of proximally-located genes. This information provides ample justification for continued study of this region of the chromosome.

The search for heterochromatic loci

Few genes capable of being mutated have been localized within proximal heterochromatin in any of the chromosomes of *Drosophila*. This observation led to the hypothesis that these chromosome regions are genetically inert (Muller and Painter, 1932). To test this, several approaches have been adopted to attempt to genetically dissect the various heterochromatic regions in the genome of this organism.

Since the only phenotype which accompanies the lack of the entire Y chromosome is male sterility (Bridges, 1916), it seemed that no vital genes resided on this chromosome. Such a sterile phenotype was successfully exploited by Brosseau (1960), who provided a minimum estimate of 7, Y-linked male fertility factors.

Muller *et al*. (1937) used the method of selecting for reciprocal products of meiotic exchange within overlapping inversions to recover heterochromatic deletions and duplications on the X-chromosome. They found no inviability associated with large duplications or deficiencies and concluded that aside from *bobbed* (*bb*), no essential genes are

present in the major blocks of heterochromatin near the centromere. This has recently been corroborated by Schalet and Lefevre (1973). However, the latter workers mentioned that the suppressor of forked locus may be located within heterochromatin.

The bobbed (bb) phenotype (short, fine bristles and delayed eclosion) has been mapped to a proximal location. Quantitative correlations between the dosage of bb and bristle length (Stern, 1929), as well as the existence of a lethal allele, pointed to a probable hypomorphic basis for bb alleles. High frequency mutation and reversion of bb had been noted in several studies. The cytological position of the Nucleolus Organizing (NO) region was located close to the bb locus (Cooper, 1959). Consequently, a combined genetic-biochemical approach was initiated to see if the bb locus and the NO region were identical.

First, Ritossa and Spiegelman (1965) used a nucleic acid hybridization technique to measure the proportion of total DNA which codes for the 2 species of ribosomal (r)RNA (18S and 28S) and they found that each X chromosome carried about 130 copies of both types. Furthermore, they showed that flies bearing different numbers of NO regions possessed different numbers of rRNA genes, thereby indicating that the NO region and the site(s) of these genes are either identical or closely linked. Finally, Ritossa et al. (1966a) compared phenotypically different stocks of bb and found that the numbers of rDNA copies varied inversely with the phenotypic intensity. The instability of bb is attributed to the propensity of such a highly redundant locus to cause asymmetrical pairing of homologues, because via crossing over this would generate deficiency and duplication products.

The apparent lack of loci within proximal heterochromatin of the

autosomes has stimulated a great deal of interest. Since highly repetitive satellite DNA had been localized to constitutive heterochromatin and because of the existing evidence which equated bb with the tandemly redundant rRNA genes, Suzuki (1970; 1974a) suggested that genes controlling vital functions such as cell division might be highly redundant and reside in heterochromatin. Thus, the loss of one or even a few copies of such genes through recessive mutation could be normalized by the presence of many wild-type duplicates.

Two alternative methods of testing the hypothesis were formulated by Suzuki and his co-workers. First they used Ethyl methanesulphonate (EMS) to screen for Dominant temperature-sensitive (DTS) lethal mutations, following the rationale that any unconditional dominant mutation in such essential genes would of necessity be lethal or sterile. Subsequent screens yielded several such mutants, both on the second (Suzuki and Procunier, 1969) and the third (Holden and Suzuki, 1973) chromosomes. However, none was definitely localized to proximal heterochromatin, although DTS-6 mapped to a proximal position in chromosome 3.

The second approach involved attempts to synthesize extensive deletions within heterochromatin through the method of attaching and detaching compound third chromosomes (Baldwin and Suzuki, 1971). The rationale was based on the idea that formation of compounds is frequently accompanied by asymmetrical exchange within the proximal segments of the chromosome. This method enabled these workers to isolate a large number of recessive lethals whose complementation pattern suggested that many of these lesions were deficiencies. Many also displayed the dominant Minute phenotype (Minutes are recessive lethal

mutations having the dominant visible traits, thin bristles and delayed development). However, as with the DTS lethals, none of the above mutations could be unequivocally assigned to heterochromatin (although in this instance many were mapped to proximal positions).

The lack of known heterochromatic mutations, as well as the relative dearth of lesions in proximally-adjacent euchromatic segments, has made work with this region of chromosome 3 extremely difficult. In contrast, rolled (rl) and light (lt) have been genetically localized near or within the proximal heterochromatin of chromosome 2 and Df(2R)M(2)S210 lacks all of the heterochromatin in 2R (see Lindsley and Grell, 1968). Hilliker and Holm (1975) used a method involving detachment of compound second chromosomes to isolate a large number of recessive lethals in both 2L and 2R heterochromatin. Several of these were shown to be deletions through pseudodominance tests with rl and lt and complementation tests with Df(2R)M(2)S210. Hilliker (1976) next isolated EMS-induced alleles of some of these deficiencies and he was able to resolve a total of 13 heterochromatic loci (including lt and rl) in this chromosome. He argues that the relatively high mutability of these genes is inconsistent with the idea that they are redundant.

The above genetic evidence, coupled with previously mentioned biochemical evidence indicating that the basic repetitive units of DNA in heterochromatin are simple, would suggest that heterochromatic genes are likely structurally and functionally distinct from the surrounding simple sequence DNA. However, it would be interesting to genetically probe the constitutive heterochromatin of chromosome 3 in a similar fashion and to extensively study such uniquely-placed loci.

The genetic study of proximally-adjacent segments of chromosome 3

Several approaches have proved successful in helping to genetically characterize regions near the constitutive heterochromatin of chromosome 3. Three studies involved the direct selection for chromosome aberrations near the centromere, while an additional study provided relevant information even though it was not initially designed to investigate proximal loci.

Lindsley et al. (1972) exploited the segregation properties of a large number of Y-autosome translocations to survey the viability and phenotypic effects of aneuploidy for specific segments of the autosomes. They verified the existence of two previously identified, proximal Minutes (M) on chromosome 3, M(3)S34 in 3L and M(3)S39 in 3R; and they discovered a new M site, M(3)LS4 in 3L and an additional haploinsufficient locus, Splayed (Spl) which is located between the 3R heterochromatin and M(3)S39. The existence of a Triplo-lethal (Tpl) locus in a proximal part of 3R did not allow the characterization of an appreciable portion of this segment, since this locus is inviable when present in either haploid or triploid doses. A major drawback of this method is the fact that the duplications and deficiencies synthesized do not exist as stable stocks, but must be re-synthesized each time they are to be used. However, this characterization of the dosage-sensitivity of the genome is extremely valuable. In particular, the findings concerning the proximal Minutes in chromosome 3 are important since many workers are presently attempting to study these loci with a view to determining their primary functions.

A group of so-called homeotic loci has been localized within proximal segments of the right arm of chromosome 3. Since mutations

in these loci cause switches in developmental fates of imaginal discs resulting in the production of structures normally derived from other discs, such loci have generated considerable interest (for a review see Postlethwait and Schneiderman, 1973).

One of the homeotic genes, Nasobemia (Ns) was suspected to be neomorphic (Muller, 1932). It had been previously shown that the neomorph, Killer of prune (K-pn) could be phenotypically reverted through the use of radiation and the genetic evidence suggested that such revertants were often deficient for the K-pn locus (Lifschytz and Falk, 1969). Denell (1972, 1973) extended this method to the study of Ns, and recovered several revertants including a putative deletion. In a subsequent study, Duncan and Kaufman (1975) used this approach to isolate a number of deletions in proximal 3R, 2 involving Ns revertants and 3 involving doublesex (dsx). They corroborated Denell's findings which indicated that Antennapedia (Antp), Ns and Extra sex comb (Scx) are mutations in the same locus (possibly including Multiple sex comb). Their experiments have also provided a number of stable deficiencies in proximal 3R which have been very useful for mapping other mutants, especially since the restrictive nature of proximal crossing over makes the map positions of proximal loci less meaningful.

In a study that was designed to select for temperature-sensitive lethals along the entirety of chromosome 3, Tasaka and Suzuki (1973) obtained interesting results. Ninety percent of the ts mutations were mapped to the proximal region between scarlet (st) and Stubble (Sb). Genetically the st to Sb interval is small, although cytologically it includes nearly 40 percent of the chromosome. One of the mutants was lethal at 29°C but viable at 25°C and at the latter temperature,

heterozygotes displayed a phenotype similar to that of Spl, which is located near the heterochromatin in the right arm. Some of the aforementioned Ns and dsx deficiencies have been used to map at least five of these ts lethals (T. C. Kaufman, personal communication), thereby supporting the results of the recombination mapping.

The basis for the apparent preferential selection of ts mutations in proximal loci of chromosome 3 is not known. However, the eventual cytological mapping of all of the ts lethals may provide information concerning the genetic organization of these loci. The well-documented utility of ts mutations for investigating developmental properties of a given gene (see Suzuki, 1970; Hartwell, 1974), should provide the impetus to determine how these proximal loci function during development.

3. Other Genetic Properties of Proximal Regions

For decades, geneticists have observed numerous properties of chromosome segments residing near the centromere, particularly in Drosophila. The following category is the most relevant to my work.

Crossing over

The area of investigation involving the centromeric regions which has produced the most striking results, is that of crossing over. Several approaches to the study of crossing over near the centromere have been adopted, and in most cases, the results are descriptive in nature.

Spontaneous meiotic crossing over in females is the type of exchange most frequently studied. The work of several people has provided a number of interesting observations. For example, it was found that while the most proximal segment makes up about 20 to 25 percent of the

mitotic length of chromosome 3 cytologically, it constitutes only 1 percent of the total genetic length (Dobzhansky, 1930; Painter, 1935). Thus, the obvious suggestion was that for meiotic crossing over in females, exchange occurs only within euchromatin. The idea that no crossing over occurs within heterochromatin has been supported by the findings of Baker (1958) and Roberts (1965), and of particular importance in this regard is the work of Hilliker (1975). In addition to this, however, it has long been recognized that crossing over between markers spanning proximal regions is severely restricted relative to comparable regions in more distal locations. Beadle (1932) used appropriate chromosome aberrations to displace markers from distal to more proximal positions and was able to show that crossing over between them was reduced, thereby suggesting that some sort of inhibitory effect of the centromere on crossing over exists. More recently, Thompson (1963a,b) proposed that if exchange pairing of centromeric intervals was rapidly followed by localized centromeric repulsion just prior to exchange, this may explain the observed decrease in crossing over between proximal loci.

Another interesting observation concerning spontaneous crossing over is that while positive interference usually governs double exchange in adjacent intervals of the same chromosome arm (Morgan et al., 1925), studies of Drosophila have shown that simultaneous exchange within closely linked intervals which span the centromere is independent (Graubard, 1934; Stevens, 1936). In fact, Morgan et al. (1925) found coincidence values of 1.3 for crossing over near the centromere of chromosome 3.

The above information poses some interesting questions with respect to spontaneous crossing over near the centromere. Does negative interference definitely appear in multiple exchange between proximal loci? If so, is it restricted to one arm of the chromosome? Besides causing mapping difficulties in work with proximally-located mutant loci, could centromeric inhibition obscure the nature of a given gene by maintaining close linkage of accumulated lethals to that gene?

Induced crossing over has been demonstrated in both females (reviewed by Schultz and Redfield, 1951; and see Whittinghill, 1955) and males (Friesen, 1933; 1937a,b; Patterson and Suche, 1934; Whittinghill, 1937). It has long been known that in females, recombinagenic agents increase crossing over preferentially near the centromere of the chromosome (see Schultz and Redfield, 1951; and Lucchesi and Suzuki, 1968). Similarly, it has been reported by several people that induced exchange in males occurs mainly within proximal regions (Friesen, 1937b; Whittinghill, 1937; Puro, 1966; Hannah-Alava, 1968). However, no published study has conclusively established that induced crossing over in proximal regions occurs principally within heterochromatin.

Friesen (1933) was the first to report that radiation could induce crossing over in males of Drosophila. From his data he concluded that radiation-induced exchange in males closely resembles spontaneous meiotic exchange in females. Contemporaries in this field (Patterson and Suche, 1934; Whittinghill, 1937) agreed with this conclusion and felt that induced exchange was relatively precise, since crossover chromosomes were not usually associated with lethality. However, Muller (1954, 1958) has argued that induced crossing over in both

sexes occurs by a mechanism similar to that which produces translocations, and thus, crossover chromosomes formed in such an asymmetrical manner could be associated with aberrations. Recently, studies have shown that radiation-induced crossover chromosomes are frequently associated with lethality or sterility and in some cases chromosome aberrations (Hannah-Alava, 1968; Mglinets, 1972).

If induced crossing over does involve asymmetrical exchange, and if such crossing over occurs preferentially within or near heterochromatin, the selection of induced crossovers might prove to be an important way of enriching for stable proximal deficiencies.

II. The Present Work

These background studies have established an important foundation for subsequent investigations of the proximal regions of the chromosome. Since many of the features described above are in some ways related, I decided to adopt a multifaceted approach in my work with these regions. This thesis represents a report of the results of such an approach.

CHAPTER 2 describes a series of experiments designed to explore proximal recombination in chromosome 3 of females with a view to examining: (a) the degree and nature of negative interference in different genetic intervals near the centromere and (b) the extent of interchromosomal effects within these intervals.

CHAPTER 3 represents a genetic study of the Deformed locus, including both an experiment designed to map this gene relative to other proximal markers in 3R and the subsequent analysis of resulting lethal crossover chromosomes. Since evidence exists which suggests that the lethality formerly ascribed to this locus is due to a closely-linked but separate mutation, the main aim of this study was to determine if the Dfd lesion is itself a recessive lethal. The potential use of Dfd in future crossover studies, as well as the developmental interest of this mutant stemming from its temperature-sensitivity and its effects on the derivatives of the eye-antennal imaginal disc, provided the impetus for this study.

CHAPTER 4 describes the use of radiation-induced, male crossing over to recover proximally-located aberrations and lethals. The rationale for this method arises from recent evidence which argues that

many induced crossovers may be the result of asymmetrical exchange events.

Finally, CHAPTER 5 is a report concerned with the genetic and developmental investigation of a ts allele of a Minute locus, located proximally in chromosome 3. By studying the mutant I hoped to provide more information about (a) the basis of the Minute phenotype and (b) the effects of such a lesion on the development of the organism.

CHAPTER 2

CROSSING OVER BETWEEN CLOSELY LINKED MARKERS SPANNING THE CENTROMERE OF CHROMOSOME 3

I. Introduction

Recently, the correct location of the centromere of chromosome 3, relative to the position of loci known to be tightly linked, has been ascertained. Thus, radius incompletus and inturned have been assigned to the left arm (Arajärvi and Hannah-Alava, 1969) along with Polycomb (Puro and Nygren, 1975) and eagle (Holm, et al., 1969), while Kinked (Merriam and Garcia-Bellido, 1969) and Deformed (Holm et al., 1969) have been positioned in the right arm.

The unequivocal left and right localization of these genes makes it possible to more accurately interpret crossover data from this region and therefore an intensive study of crossing over in the intervals adjacent to the centromere of chromosome 3 was initiated.

II. Materials and Methods

Tables 1 and 2 represent summaries of all third chromosome mutations and special chromosomes used in these experiments. All third chromosome balancers that have been used are described fully in Lindsley and Grell (1968). The balancer referred to as TM3 should henceforth be considered as equivalent to TM3, Stubble Serrate unless otherwise indicated. All experiments were performed at 22 $\pm$ 0.5°C unless otherwise specified.

Recombination was measured in the proximal region of chromosome 3 using the following markers (for a complete description, see Table 1 and consult Lindsley and Grell, 1968): st - scarlet (44.0), in - inturned (47), ri - radius incompletus (47.0), eg^2 - eagle-2 (47.3), Ki - Kinked (47.6), and p^p - pink peach (48.0). Figure 1 is a schematic representation of the map positions of the markers along the chromosome (Lindsley and Grell, 1968). The centric blocks of heterochromatin are believed to be immediately flanked by eagle (Holm et al., 1969) and Kinked (Merriam and Garcia-Bellido, 1969) on the left and right respectively. The st - in interval was designated as 1, in - ri as 2, ri - eg^2 as 3, eg^2 - Ki as 4 and Ki - p^p as 5. Note that the centromere lies in interval 4.

Three different major studies of crossing over were performed: Experiment I. 100 st in ri eg^2 Ki p^p/ + + + + + + females were test-crossed for 5 consecutive 3-day broods and their progeny were scored. Experiment II. Crossing over was similarly measured in 213 st in ri eg^2/ + + + + Ki p^p females; 108 of these were studied for five 3-day broods (Expt. IIa) and 105 for one 3-day brood (Expt. IIb). Experiment III.

Table 1

Summary of All Third Chromosome Mutations Used

Mutant	Symbol	Genetic Position	Phenotype
Lyra	Ly	40.5	Excised wing margins (rec. lethal)
Glued	Gl	41.4	Rough small eyes
scarlet	st	44.0	Bright red eyes
transformer	tra	45	Transformation of females into sterile males
inturned	in	47	Thoracic hairs and bristles directed towards midline
radius-incompletus	ri	47.0	Interruptions in L2
Polycomb	Pc	47.7	All legs of male possess sex combs (rec. lethal)
eagle^2	eg^2	47.3	Wings spread
Deformed	Dfd	47.5	Reduced eyes (rec. lethal)
Deformed-recessive	Dfd^r	47.5	Recessive allele of Dfd
Kinked	Ki	47.6	Short and twisted bristles and hairs
roughened-eye	roe	47.6	Eyes rough
proboscipedia	pb	47.7	Transformation of oral lobes into tarsus or arista
Extra sex-comb	Scx	47	All legs of male possess sex combs (rec. lethal)

Table 1 (continued)

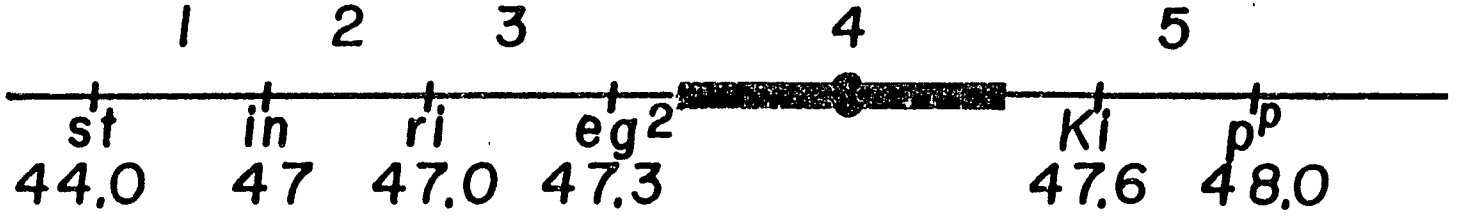
Mutant	Symbol	Genetic Position	Phenotype
Antenna-pedia	Antp	48	Transformation of antenna into leg structures (rec. lethal)
Multiple-sex comb	Msc	48.0	All legs of male possess sex combs, associated with In(3R)84B;85F (rec. lethal)
Nasobemia	$Antp^{Ns}$	48.0	Transformation of antenna into leg structures
double sex	dsx	48	Males and females intersexual
pink peach	p^{p}	48.0	Dull ruby eyes
Stubble	Sb	58.2	Short and thin bristles (rec. lethal)
Delta	Dl	66.2	Termini of wing veins thick and broad (rec. lethal)
Hairless	H	69.5	Missing postvertical and abdominal bristles (rec. lethal)
ebony-sooty	e^{s}	70.7	Black body

Table 2

Summary of Special Mutant Chromosomes Used

Chromosome	Abbreviation	Cytology	Reference
Df(3R)Antp^{+R2}	Antp^{+R2}	Df(3R)84B3;84D1-2	Duncan and Kaufman, 1975
Df(3R)Antp^{Ns+R21}	Ns^{+R21}	Df(3R)84A-B;84D-E	"
Df(3R)dsx^{D+R2}	dsx^{D+R2}	Df(3R)84D9-12;84F16	"
Df(3R)dsx^{D+R5}	dsx^{D+R5}	Df(3R)84F2-3;84F16	"
T(3;Y)P92	Dp-P92	Insertion of 3R(84D10-11;85A1-3) into Y	"
Dfd kar^{26}ry^{2}	Dfd-rk	—	Chovnick et al., 1971

FIGURE 1 Schematic representation of proximal regions of the third chromosome showing the genetic markers used. Published map positions are given below the symbols with numerically designated crossover intervals indicated above the line.


1
2
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47
47.0
47.3
47.6
48.0

Crossing over was measured in 25 C(1)M3/Y; st in ri eg^2 + + / + + + + Ki p^p females for five 3-day broods. Expt. III females were tested in order to determine any interchromosomal effects of the inversions contained in each arm of the compound X (Lucchesi and Suzuki, 1968).

All females tested were mated individually (within 40 hours of eclosion at 22 $\pm$ 0.5°C) with 2 or 3 males homozygous for st in ri eg^2 Ki p^p e^s. The third chromosomes of all females were isogenized prior to use in order to minimize the presence of lethals in the stocks. However, the other chromosomes were not made coisogenic and the Ki p^p chromosomes of Expt. IIb females were of different origins than those of Expt. IIa females. Progeny in each vial were scored daily until the eighteenth day after the parents had been introduced.

III. Results

A summary of the numbers of progeny examined and the crossover values for the region studied (including published map distances, Lindsley and Grell, 1968) are given in Table 3. Data for females carrying normal X chromosomes (columns 2 and 3, Table 3) reveal that recombination was consistently higher in Expt. I than in Expt. II (X^2 = 108.9, P = 0.05). These differences probably reflect random differences in genetic backgrounds in the two series. The insertion of C(1)M3 into test females (columns 4 and 5, Table 3) noticeably augmented recombination, thereby reconfirming its interchromosomal effects on crossing over near proximal heterochromatin. These effects were more prominent for the distalmost intervals; for example, recombination in regions 1 and 5 increased 3- and 4-fold, respectively.

Table 4 summarizes the number of different crossover chromosomes recovered. A total of 3,603 single, 85 double and 20 triple crossover chromosomes was scored. The most frequent class of doubles occurring in Expts. I and II involved regions 1 and 5 (nearly a third of the total) and 3 and 4 (more than a third of the total). Other doubles frequently recovered were 3, 5 and 1, 4.

Double crossovers involving exchange in intervals known to be on the same side of the centromere were never recovered. However, 1, 2, 4 and 1, 3, 4 triple crossovers, which included exchange in two of these intervals, were recovered.

Since double crossover chromosomes involving exchange in the two distalmost intervals (1 and 5) were frequently encountered, these data

Table 3

Crossover Frequencies in The st to p^p Interval in Chromosome 3

Genetic Region	Reference Values	Experiment Number I	IIa rod X	III C(1)M3	Ratio III/IIa
1	3.0	3.99	1.77	5.39	3.05
2	0.06	0.25	0.20	0.30	1.50
3	0.30	0.21	0.08	0.17	2.13
4	0.30	0.43	0.22	0.64	2.90
5	0.40	0.65	0.25	1.11	4.44
Number of Fertile Females	-	92	108	25	-
Number of Progeny	-	36,948	33,139	4,063	

Table 4

Types and Numbers of Recombinant Chromosomes Recovered

	Experiment Number			
Region	I	IIa	IIb	III
SINGLES				
1	1393	574	217	547
2	71	63	12	13
3	27	17	4	30
4	101	50	24	58
5	183	64	41	114
Totals	1775	768	298	762
DOUBLES				
1,2	0	0	0	0
1,3	0	0	0	0
1,4	3	2	0	1
1,5	14	8	1	2
2,3	0	0	0	0
2,4	2	0	0	1
2,5	4	0	0	1
3,4	22	6	1	4
3,5	5	2	2	1
4,5	0	3	0	0
Totals	50	21	4	10
TRIPLES				
1,2,3	0	0	0	0
1,2,4	3	0	0	0
1,2,5	0	0	0	0
1,3,4	2	2	0	0
1,3,5	0	0	0	0
1,4,5	4	4	2	2
2,3,4	0	0	0	0
2,3,5	0	0	0	0
2,4,5	0	0	0	0
3,4,5	1	0	0	0
Totals	10	6	2	2

Table 5

Coefficients of Coincidence Computed From All Multiples Recovered

	Experiment Number		
Intervals	I	IIa	III
1,2	0.81	-	-
1,3	0.64	4.25	-
1,4	1.90	6.21	1.43
1,5	1.90	8.17	1.23
2,3	-	-	-
2,4	12.50	-	-
2,5	6.75	-	-
3,4	75.20	137.16	22.36
3,5	11.90	30.00	49.20
4,5	4.83	38.40	6.93

support the contention of other work in Drosophila (Graubard, 1934; Stevens, 1936), in Neurospora (Bole-Gowda et al., 1962) and yeast (Hawthorne and Mortimer, 1960), that positive interference does not extend to regions in different arms of the chromosome. This is further emphasized by the fact that most of the triple crossovers (12 of 20) involved exchanges in intervals 1, 4 and 5. However, it should also be noted that all of the triple crossovers involved the most proximal interval (eg^2 to Ki).

Coefficients of coincidence were calculated for doubles in all three experiments (excluding Expt. IIb). In all cases but 1, 2 and 1, 3 doubles of Expt. I, these values exceeded unity (Table 5). Extremely high values for 3, 4 and 4, 5 exchanges (and 3, 5 exchanges for Expt. III) indicate a very high negative interference in these intervals. Therefore, in spite of very tight linkage between st and p^p, the recovery of multiple crossover chromosomes greatly exceeds conventional expectations. It is noteworthy that while single exchanges were increased in all intervals by C(1)M3, a concomitant increase in the occurrence of multiple exchanges (except for 3, 5 doubles) did not occur, as shown by coincidence values.

In order to further test different proximal intervals for interference, two other mapping experiments were carried out with the markers: Glued (Gl), Stubble (Sb), Hairless (H) and p^p (see Table 1 and consult Lindsley and Grell, 1968 for descriptions of these loci). Heterozygous Gl + Sb H/+ p^p + + females were crossed to p^p/p^p males (20 males and 20 females per quarter pint bottle) and crossing over was measured for the three intervals: (a) Gl to p^p; (b) p^p to Sb; and (c) Sb to H. Note that the (a) interval spans the centromere.

Table 6

Results of Mapping Experiments Using The Markers G1 Sb H/p^p

Experiment Number	Number of Progeny	Single Crossovers			Multiple Crossovers			
		a	b	c	a,b	a,c	b,c	a,b,c
IV	1148	68	98	123	10	9	5	1
Map Distances (percent)		7.67	9.93	12.02				
Coefficients of Coincidence					1.26	0.95	0.44	
V	909	57	73	84	9	9	4	1
Map Distances		8.36	9.57	10.78				
Coefficients of Coincidence					1.37	1.20	0.53	

Table 6 summarizes the data from Experiments IV and V. The map distances for the three intervals show good agreement with book values (see Lindsley and Grell, 1968) although (a) is slightly larger in both experiments. Coincidence values were calculated for the different interval combinations: a, b (1.3 to 1.4); a, c (0.95 to 1.2); and b, c (0.44 to 0.53). It is apparent that more multiple crossovers than expected occurred for the combination involving the centromeric and the immediately adjacent intervals (a, b), while that involving the most distal and centromeric intervals (a, c) showed about the expected number of multiple crossovers. However, interference was positive when the most distal intervals are considered. The level of negative interference for this series of experiments was also much less marked than that of the major series which had involved more proximal markers. Thus, these data further support the idea that multiple exchanges are more common in adjacent intervals more closely associated with the centromere, and that although positive interference does not extend to both sides of the centromere, negative interference does.

Previous workers have suggested that some rare multiple exchange chromosomes could, in fact, result from successive single crossover events. Thus, a mitotic crossover in a gonial cell could be followed by a meiotic exchange to produce an apparent double crossover chromosome (Whittinghill, 1955; Suzuki et al., 1966). Such a Two-Step model predicts that the gonial exchange could be amplified through mitotic divisions, thereby generating doubles amidst a cluster of single crossovers (Suzuki et al., 1966). The progeny of individual females yielding

Table 7

Interval-Specific Examination of Data of Females Producing Double Crossovers (Experiment I)

Female	Type of Double	Number of Doubles	Singles Occurring in Either Interval	Total Number of Progeny
J	1,5	1	23 (16.8)*	391 (360)
K	3,4	2	2 (1.3)	388 (360)
L	2,4	1	3 (1.6)	465 (360)
M	3,5	2	2 (2.6)	466 (360)
N	1,4	1	13 (15.6)	429 (360)

*Numbers in parentheses represent mean values of comparable data for 52 non-multiple females

double exchanges (but not triples) were examined for evidence of clustering of single crossovers in the regions where the doubles had occurred. A sample of 5 such females (Expt. I) is given in Table 7 (along with mean values for females yielding no multiple exchange progeny). No noticeable clusters of singles appeared to accompany doubles for the regions in question.

If multiple recombinant chromosomes are generated by a Two-Step mechanism, then crossover values for multiple-producing females would be expected to be higher than the values from females producing no multiples. These subsets of data were significantly different (Table 8) and in both experiments, crossover values for regions 3 and 4 were higher in those females producing multiple crossovers. However, when the crossover data of the 9 females of Expt. I that had produced triple recombinant progeny (within the st to p^P interval) were examined, in each case the distribution of crossover types followed a Poisson distribution (Table 9).

A tetrad analysis as inferred from single strand recovery (Weinstein, 1936), was initiated with a view to distinguishing between a meiotic and a gonial origin of the triple exchanges (Table 10). The Two-Step production of rare multiple exchange chromosomes was inferred from an insufficient number of double exchange chromosomes predicted from a tetrad analysis of the multiple exchange chromosomes (Suzuki et al., 1966). In every case examined in present tests (Expts. I, IIa and IIb), the number of triple exchange tetrads was equivalent to or exceeded that of the double exchange tetrads. This supports a

Table 8

Crossover Values in Progeny of Females Producing Multiple Crossovers Compared to Those of Females Producing None

		Female Type			
		Multiples		No multiples	
		Expt I	Expt IIa	Expt I	Expt IIa
Number of Females		42	24	52	85
Number of Progeny		19,284	7,111	15,378	26,029
Crossover Values					
Intervals	1	4.09	1.65	3.71	1.76
	2	0.28	0.22	0.17	0.18
	3	0.26	0.21	0.07	0.05
	4	0.53	0.36	0.24	0.15
	5	0.56	0.40	0.59	0.20

Significant difference for X^2 was indicated for a subset comparison of both experiments at P = 0.01

Table 9

Analysis of Crossing Over in Those Females Which Produced Triple Crossover Chromosomes (Experiment I)

Female	Types of exchange (st to p^p)				*Chi-Square Values
	0	1	2	3	
A	431	28	0	1	0.002
B	438	35	1	1	0.329
C	460	27	0	1	0.060
D	482	25	0	1	0.081
E	423	15	1	1	0.046
F	163	10	0	2	2.200
G	467	23	0	1	0.427
H	401	29	2	1	2.280
I	295	21	1	1	1.333

0 = no exchange

1 = 1 exchange

2 = 2 exchanges

3 = 3 exchanges

*In each case recombination was found to approximate a Poisson distribution at P = 0.05

Table 10

Tetrad Analysis of The Crossover Data (Inferred From Recovery of Single Strands)

	Experiment Number		
Tetrad Distribution	I	IIa	IIb
Triple Exchange	80	48	16
Double Exchange	80	12	16
Single Exchange	3,410	1,536	734
No Exchanges	33,378	31,543	10,088
Total Tetrad Sample	36,948	33,139	10,854

Table 11

Comparison of Observed With Expected (From Meiotic Triple Exchange Tetrads) Numbers of Double Exchanges

Classes of Doubles	Experiment Number					
	I		IIa		IIb	
	Expected	Observed	Expected	Observed	Expected	Observed
1,2	3	0	0	0	0	0
1,3	2	0	2	0	0	0
1,4	9	3	6	2	2	0
1,5	4	14	4	8	2	1
2,3	0	0	0	0	0	0
2,4	3	2	0	0	0	0
2,5	0	4	0	0	0	0
3,4	3	22	2	6	0	1
3,5	1	5	0	2	0	2
4,5	5	0	4	3	2	0

Table 12

Types and Numbers of Recombinant Chromosomes Recovered

Region	Experiment I Genotype	Number Recovered	*R	Experiment IIa Genotype	Number Recovered	R
SINGLES						
1	st	726	1:1	st Ki p^p	302	1:1
	in ri eg^2 Ki p^p	667		in ri eg^2	272	
2	st in	56	3.5:1	st in Ki p^p	39	1.5:1
	ri eg^2 Ki p^p	15		ri eg^2	24	
3	st in ri	16	1.5:1	st in ri Ki p^p	5	2.5:1
	eg^2 Ki p^p	11		eg^2	12	
4	st in ri eg^2	58	1.5:1	st in ri eg^2 Ki p^p	31	1.5:1
	Ki p^p	44		+ + + + + + +	19	
5	st in ri eg^2 Ki	75	1.5:1	st in ri eg^2 p^p	19	2.5:1
	p^p	108		Ki	45	
DOUBLES						
1,4	st Ki p^p	3	3:0	st	2	2:0
	in ri eg^2	0		in ri eg^2 Ki p^p	0	
1,5	st p^p	7	1:1	st Ki	0	8:0
	in ri eg^2 Ki	7		in ri eg^2 p^p	8	
2,4	st in Ki p^p	1	1:1	st in	0	-
	ri eg^2	1		ri eg^2 Ki p^p	0	
2,5	st in p^p	2	1:1	st in Ki	0	-
	ri eg^2 Ki	2		ri eg^2 p^p	0	

Table 12 (continued)

Region	Experiment I Genotype	Number Recovered	R	Experiment IIa Genotype	Number Recovered	R
DOUBLES						
3,4	st in ri Ki p^p	19	6:1	st in ri	6	6:0
	eg^2	3		eg^2 Ki p^p	0	
3,5	st in ri p^p	1	4:1	st in ri Ki	2	2:0
	eg^2 Ki	4		eg^2 p^p	0	
4,5	st in ri eg^2 p^p	0	-	st in ri eg^2 Ki	3	3:0
	Ki	0		p^p	0	

* R = Ratio of reciprocal classes

Two-Step explanation for the origin of the multiples. Comparison of the minimum expected numbers of doubles (i.e., doubles generated by triple exchange tetrads) with the actual numbers recovered (generated by both double and triple exchange tetrads) reveals (Table 11) that in a few cases (1, 2; 1, 3 and 4, 5) the observed numbers were appreciably less than expected, while in most of the remaining classes the observed numbers exceeded or approximated the expected.

Table 12 shows a summary of the reciprocal crossover classes recovered from Expts. I and IIa females, along with the numbers of each class obtained. In several cases (particularly for the more proximal intervals), these classes do not appear to be equally represented, despite the apparent lack of any obvious selective advantage for nonmutant alleles.

In order to rule out high revertability of the markers studied as a contributive factor to some of the multiple exchange chromosomes, homozygous stocks were screened for revertants and none was found among 1.1×10^4 st in ri eg^2 and 2.0×10^4 st in ri eg^2 Ki p^p e^s chromosomes.

IV. Discussion

Although genetically small, the region studied in these experiments represents a large portion of the physical length of chromosome 3. Unexpectedly, these experiments have revealed evidence for the existence of non-classical, non-reciprocal recombination events in this region of the chromosome. Thus, interference (expressed as coefficients of coincidence) for these intervals was high and negative.

The present work is concerned solely with intergenic recombination near the centromere of one of the autosomes of Drosophila. Previously, it had appeared that exchange within genetically short regions in this organism was generally accompanied by high positive interference (Morgan et al., 1925). Exceptions to this in Drosophila usually involved intragenic crossing over (e.g. Hexter, 1958; Green, 1959, 1960), although Sturtevant (1951) reported that negative interference could be detected in the study of intergenic crossing over in the fourth chromosome of triploid females. Similarly, negative interference was seen in intergenic crossing over in Aspergillus (Calef, 1957; Pritchard, 1960) and barley (Søgaard, 1974). One explanation offered to account for such negative interference is equivalent to the idea of effective pairing in bacteriophage (see Chase and Doermann, 1958), which essentially assumes that short localized regions of pairing exist, and within these regions recombination is highly probable. Both Calef (1957) and Pritchard (1960) used this hypothesis to explain coincidence values exceeding 100 for exchange between tightly linked loci. However, Søgaard (1974) discounted any explanation invoking

localized pairing for his work with the eciferum loci of barley, since in this case he was dealing with relatively large interlocus intervals.

Recently, workers have promoted another possibility to account for the recovery of multiple crossovers at unexpected frequencies. Thus, the occurrence of successive gonial and meiotic exchange to produce rare multiple crossovers (i.e. the Two-Step model), has been postulated (Whittinghill, 1955; Suzuki et al., 1966). In the present study some evidence supports this idea, viz. the relative lack of double compared to triple exchange tetrads and the higher levels of recombination in those females producing multiple exchange progeny. However, examination of the data for individual females failed to show the clustering phenomenon that would be predicted for the females generating multiples. Furthermore, the types of double crossover chromosomes encountered were not dissimilar to those that would be predicted to arise from the different types of meiotic triple exchange tetrads. In most cases, the numbers of the recovered double crossovers exceeded those of the expected (see Table 11). However, the Two-Step model should be considered as a possible contributive factor to the results of these experiments, particularly since C. Sharpe (personal communication) finds high coincidence values for proximal recombination near the centromere of chromosome 2 and claims that gonial exchange is involved.

As previously mentioned, work in several organisms has indicated that exchange across the centromere is marked by a lack of positive chromosome interference and, in the case of chromosome 3 of Drosophila, negative interference has been observed (Morgan et al., 1925). In the

present study, the occurrence of negative chromatid interference for crossing over near the centromere could provide an explanation for the high negative chromosome interference that was detected, since an excess of two-strand doubles would generate more double relative to single crossover chromatids thereby inflating coincidence values. In this regard, it is noteworthy that Strickland (1961) and Bole-Gowda et al. (1962) found evidence of chromatid interference in Neurospora, particularly with respect to centromeric crossing over. Hawthorne and Mortimer (1960) have mentioned a similar situation in yeast. Howe (1956) and Stadler (1956) had repudiated earlier claims that this phenomenon occurs in Neurospora.

Welshons (1955) reported that negative chromatid interference could be seen in the study of crossing over in short genetic intervals in attached-X chromosomes of Drosophila. However, Baldwin and Chovnick (1967) found no chromatid interference in exchange in compound third chromosomes. Davis (1974) also failed to detect chromatid interference when using the meiotic mutant mei-s332 for the recovery of half-tetrads. It should be mentioned that neither of the latter 2 studies examined crossing over in proximal regions and therefore chromatid interference cannot be eliminated as a characteristic of exchange in these segments of the chromosomes.

The demonstration of conversion in Drosophila has previously been invoked as an explanation for the occurrence of exceptional events in intragenic exchange. For example, in his study of the white locus, Green (1959, 1960) could account for some exceptional chromosomes by assuming that rare true double exchange was involved. However, in

crossover studies of different white-apricot pseudoalleles, four exceptions appeared which could be explained by gene conversion, while no single crossovers were recovered. Recently, it has been argued that recombination and conversion may be manifestations of the same homologous exchange event, particularly in light of the findings in work with maroon-like (Smith et al., 1970) and in yeast (Hurst et al., 1972), which revealed that half of the convertants were associated with exchange of flanking markers.

In this study, conversion must be considered as a possible mechanism for the frequent production of multiple recombinant chromosomes. For example, simple conversion of eg^2 to its wild-type allele and vice versa, would result in apparent 3, 4 double crossovers. Triples involving 3,4 exchange might be explained by the conversion of eagle accompanied by exchange of either of the most distal markers. Extending this logic, 1, 4, 5 triples could result from conversions of Ki or Ki^+ with a crossover in region 1, while 1, 2, 4 triples could be generated by conversion of ri or ri^+ and an exchange in region 4. It must be emphasized that previous failure to detect intergenic exchange events which resemble conversion in Drosophila is likely related to the effects of high positive interference. The absence of interference across the centromere might permit the appearance of such a phenomenon. Indeed, evidence from other organisms suggests that when tightly-linked markers are studied, crossing over produces multiple exchange chromosomes at inordinately high frequencies (see Calef, 1957; and Søgaard, 1974). Conversion has been mentioned as a possible contributor. Crossover frequencies in this present work indicate that the region from in to Ki is particularly small genetically.

The use of the inverted attached-X chromosome (C(1)M3) actually resulted in lower coincidence values (in most cases), suggesting that fewer multiple relative to single crossovers occurred. Previous demonstration of intrinsically (Schultz and Redfield, 1951) and extrinsically (Suzuki and Parry, 1964) mediated recombinagenesis in Drosophila were marked by decreased positive interference. Therefore, the present data are consistent with the suggestion that conversion may be contributing to the appearance of multiple crossover chromosomes, since one would expect recombinagenic agents to effect similar increases in the occurrence of true multiple crossovers as well as of singles.

Green (1975) has reported similar results from his work with this region of the chromosome. Puro and Nygren (1975) also observed a double crossover involving radius incompletus when they were mapping Polycomb. These workers raise the possibility that conversion is involved.

Other support for the conversion-based explanation for the multiple crossovers is provided by the inequalities of reciprocal crossover classes (Table 12); even though spontaneous reversion of these loci was not observed.

The different possibilities discussed above might be distinguished by using females carrying third chromosome pericentric inversions which include all of the loci used in this study. Recombinants recovered from such heterozygotes would almost certainly arise from even-numbered crossovers, since odd-numbered crossovers would produce inviable progeny bearing extensive duplications or deficiencies. Comparisons of the control frequencies of multiple crossovers with the number of progeny produced by these females should provide information about the origin of multiple crossover chromosomes.

It might also be possible to use meiotic mutants which effect increased levels of non-disjunction but do not alter recombination and in this way capture half-tetrads to test for reciprocality and chromatid interference for crossing over in proximal regions of this chromosome. However, given the low rates of recombination in these regions, this would be a formidable project.

Finally, it is noteworthy that the interchromosomal effects of C(1)M3 were more marked for the distal crossover intervals that were examined. This raises the possibility that most of the proximal increases noted previously (see Lucchesi and Suzuki, 1968) may have occurred near, but not in heterochromatin. In fact, since several lines of evidence suggest that no crossing over occurs within heterochromatin (Baker, 1958; Roberts, 1965; Hilliker, 1975), it is possible that this sort of recombinagenesis is wholly euchromatic.

It would be interesting to study this further. For example, one could compare these effects to the artificial induction of crossing over, which also occurs preferentially near the centromere in both males and females (see Schultz and Redfield, 1951). Since it appears that both lt and rl lie within heterochromatin (Hilliker and Holm, 1975), chromosome 2 would probably be more useful for this purpose. This type of approach will tell us a great deal about the properties of heterochromatin, particularly with respect to crossing over.

CHAPTER 3

A GENETIC STUDY OF THE *DEFORMED* LOCUS

I. Introduction

Deformed (*Dfd*) is a mutation mapping in the proximal part of chromosome 3 at 47.5. *Dfd* mutants express a dominant phenotype resulting in ventral and lateral reduction of ommatidial tissue in the eyes of the adult. Concomitantly, tufted vibrissae are often observed. The penetrance of the eye phenotype is variable and sometimes *Dfd*/+ flies are indistinguishable from wild-type. Recessive alleles (e.g. Dfd^{r}) have been described, with allelism based on the observation that *Dfd*/Dfd^{r} flies are phenotypically more extreme than Dfd^{r}/Dfd^{r}. Previously, it had been thought that the *Dfd* lesion was also lethal when homozygous, although the recovery of aberrant homozygotes at a very low frequency has been reported (Lindsley and Grell, 1968).

This locus is of particular interest from a developmental standpoint. A temperature-sensitive allele (Dfd^{rL}) has permitted the delineation of a TSP for the *Dfd* gene in the first to second larval instars (Vogt, 1947). The eye phenotype could be due to the promotion of localized cell death within the eye-antennal disc (Fristrom, 1969). This may explain the frequent occurrence of mirror-image duplications of antennae of *Dfd* flies. This is supported by the observation that at 29°C, *Dfd* interacts synergistically when heterozygous with a temperature-sensitive *Minute*, causing lethality by preventing the formation of eye-antennal structures (see CHAPTER 5). The occurrence of extensive proliferation of vibrissae, which is also diagnostic of the

Dfd phenotype, may be due to a repatterning of surviving cells within the disc following cell death (see Postlethwait and Schneiderman, 1973). Furthermore, Dfd interacts with ophthalmoptera to produce homeotic wing tissue in the eye (Ouweneel, 1969). Thus, further study of this locus with respect to patterns of cell death in the eye disc during development, should prove interesting.

The cytological location of Dfd has not been determined. Its genetic location does not define the chromosome arm in which this locus resides. However, Holm et al. (1969) found that the synthesis of compound chromosomes heterozygous for Dfd (Dfd/+) from normal homologues, resulted in the high frequency association of this gene with the 3R elements, while no 3L element containing Dfd was ever recovered, thereby proving that Dfd is in the right arm of this chromosome.

Duncan and Kaufman (1975) synthesized an array of proximal chromosomal aberrations in 3R by selecting for radiation-induced revertants of the homeotic mutant Nasobemia (Ns) as well as of the dominant allele of double sex (dsx^{D}). Three revertants of the latter are cytologically-observable deficiencies, with the smallest (dsx^{D+R5}) lacking the 3R material in the 84B-F interval. All three are lethal in combination with either of two different chromosomes bearing Dfd. However, none of these deficiencies exposes the phenotype of Dfd^{r}, that is, Dfd^{r}/ deficiencies are wild-type. Moreover, they were able to show that the lethality associated with both original Dfd stocks is covered by Dp-P92, which includes the region of 3R between 84D and 85A (see Tables 1 and 2). They offered two possible explanations for these results: (i) The Dfd locus is actually located in 84B-F, but Dfd^{r} is not an allele of

Dfd (i.e. the recessive lethality is associated with the Dfd locus). (ii) The lethal mutation is distinct from the Dfd locus, and therefore Dfd is located elsewhere in proximal 3R and is viable when homozygous.

A minimal genetic characterization of a locus is a prerequisite to any analysis of its developmental properties. Therefore, the present study was initiated to accomplish two things: (a) to map Dfd with respect to Kinked in the hope that their correct relative positions might aid future genetic studies in this region of the chromosome; and (b) to genetically analyse any recombinant chromosomes derived from this mapping study, in order to determine which of the aforementioned possibilities concerning the nature of the locus is correct.

II. Materials and Methods

One hundred Dfd/Ki p^p females were collected within 40 hours of eclosion and mass mated to homozygous st in ri eg^2 Ki p^p males (henceforth I will refer only to the markers scored, Dfd, Ki and p^p) in six quarter-pint milk bottles (20 females and 20 males per bottle). Every three days, the parents were transferred to fresh bottles and after nine days these flies were discarded. Progeny were scored until the fifteenth day after the introduction of the parents. An early experiment using Dfd/Ki p^p females crossed to Dfd/TM3 males was abandoned because of poor penetrance of Deformed in Dfd/TM3 flies. However, several Dfd p^p and Ki as well as one p^p recombinant chromosomes from the latter were saved for genetic analysis. All recombinant and one parental chromosomes were balanced over either TM3 or CxD and examined for recessive lethality.

All of the lethal-bearing recombinant chromosomes were tested for complementation inter se as well as with the following stocks: the Dfd parental stock; Dfd-rk (kindly contributed by Dr. D. G. Holm); and four deficiency stocks, Ns^{+R21} $Antp^{+R2}$ dsx^{D+R2} dsx^{D+R5} (all kindly contributed by Dr. T. C. Kaufman, see Table 2 for cytological descriptions).

III. Results

The results of the Deformed mapping experiment are presented in Table 13. A total of 5822 progeny was scored and 28 confirmed crossovers between Dfd and p^p were recovered (17 Dfd p^p and 11 Ki types). Initially, 34 progeny were scored as wild-type (i.e. $Dfd^+/Ki\ p^p$) recombinants but upon subsequent testing, 32 of these proved to be carrying Dfd (i.e. were parentals) and the remaining 2 (males) were sterile. If the latter 2 were genuine crossovers, this would argue that Dfd is proximal to Ki in 3R (Figure 2a). The computed map distance (excluding the two unconfirmed wildtypes) from Ki (or Dfd) to p^p is 0.48 percent, a value reasonably close to that predicted from standard book values (Lindsley and Grell, 1968).

Fourteen Dfd p^p and seven Ki recombinant chromosomes were balanced. Recessive lethals were present on all of the former while 6 of 7 of the latter carried recessive lethals. In addition, 8 recessive lethal stocks including six Dfd p^p and one Ki recombinants along with a single p^p type were found in the early crossover experiment. The existence of the p^p recombinant argues that Ki is proximal to Dfd in 3R (Figure 2b). For complementation purposes, the lethal-bearing recombinant stocks were designated as follows: Ki, lethals 1 to 7; Dfd p^p, lethals 8 to 27; and p^p, lethal 28.

The results of the complementation tests are summarized in Table 14 and Figure 3. Since all of the lethal crossover chromosomes were inviable in combination with the original Dfd parental chromosome, all must share a lethal site(s) in common with the parental stock. All

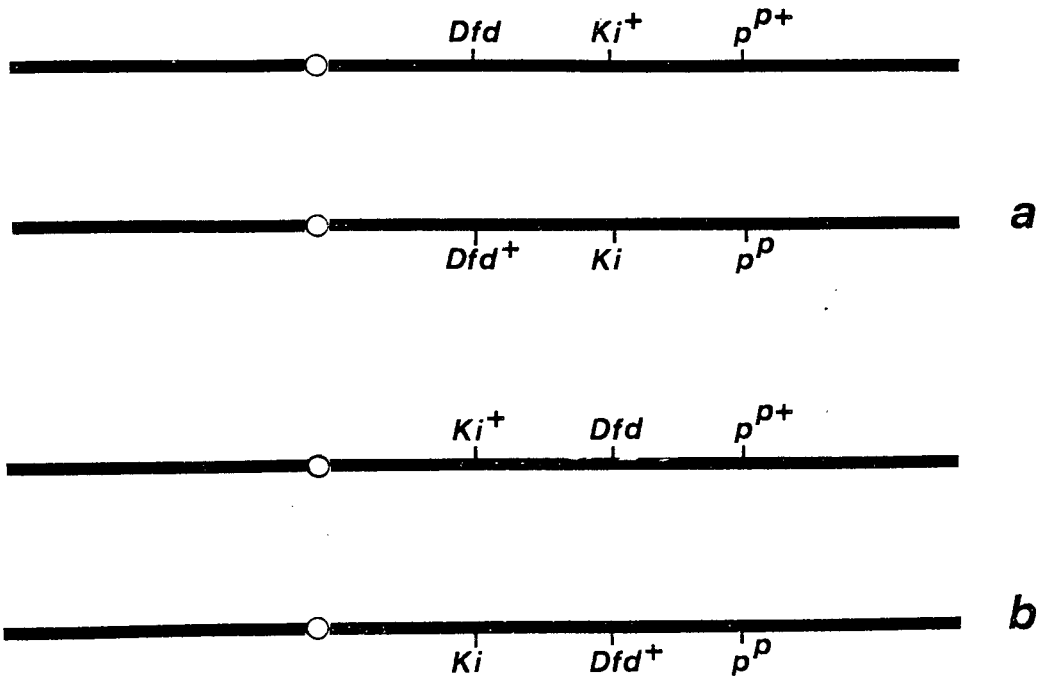
Table 13

Results of The Cross of Dfd/Ki p^p Females to Ki p^p/Ki p^p Males

Genotype	Progeny			
	Number of Parentals	Number of Recombinants	Unknowns	Totals
Dfd/Ki p^p	2789			2789
Ki p^p/Ki p^p	3003			3003
Dfd p^p/Ki p^p		17		17
Ki/Ki p^p		11		11
Dfd^+/Ki p^p			2 (sterile)	2
				5822

Map distance Dfd to p^p = 0.48 percent

FIGURE 2 Schematic representation of possible relative arrangements of Ki and Dfd in the proximal portion of the right arm of chromosome 3. In a, Dfd is proximal, Ki distal; in b, Ki is proximal and Dfd distal.


Dfd
Ki+
p p+
a
Dfd+
Ki
p p
Ki+
Dfd
p p+
b
Ki
Dfd+
p p

inter se combinations of the Dfd p^P(p^P) stocks as well as those of the Ki stocks were inviable, indicating that at least one common lethal is present on all of the chromosomes of a given recombinant class. However, when members of the different recombinant classes were tested together in turn, and also each with the Dfd-rk and deficiency stocks, a differential pattern of complementation emerged (Figure 3). Although the results are not unequivocal, i.e. the deficiency or Dfd-rk stocks may contain more lethal sites, the simplest explanation is that the original Dfd parental chromosome contained a minimum of 3 lethal sites designated as m^a, m^b, m^c, in addition to the Dfd locus.

One possible arrangement of these lethals on the original Dfd chromosome is shown in Figure 4. A single crossover in region 2 would generate a Dfd p^P recombinant carrying m^a (Group IV also including lethal 28) and reciprocally a Ki recombinant carrying both m^b and m^c (Group I). Similarly, a single exchange in region 3 would provide Dfd p^P recombinants carrying both m^a and m^b (Group III) and the Ki reciprocals bearing m^c (Group II). A double exchange involving regions 2 and 4 could produce Dfd p^P chromosomes carrying m^a and m^c, thereby accounting for Group V types. Finally, if Ki were proximal to Dfd, a crossover between them could generate a p^P recombinant bearing m^a (lethal 28, Group IV). Thus, it is proposed that m^b is the lethal site which is carried by the Dfd-rk chromosome and that this site is exposed by both dsx^{D+R} deficiencies.

The above proposal satisfies the requirement that all Ki recombinants carry a common lethal (m^c in Groups I and II) as do Dfd p^P types (m^a in both Group III and IV). Furthermore, the members of Groups I and III are non-complementing by virtue of the m^b site.

Table 14

Results of *Inter Se* and Deficiency Complementation Tests With Recombinant Lethals

lethals	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	Dfd-rk*
1	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+
2		-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+
3			-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+
4				-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+
5					-	-	-	+	+	-	-	-	-	-	-	+	-	+	+	-	+	+	-	-	+	+	-	+	-
6						-	-	+	+	-	-	-	-	-	-	+	-	+	+	-	+	+	-	-	+	+	-	+	-
7							+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+
8								-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9									-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
10										-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
11											-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12												-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13													-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14														-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15															-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16																-	-	-	-	-	-	-	-	-	-	-	-	-	+
17																	-	-	-	-	-	-	-	-	-	-	-	-	-
18																		-	-	-	-	-	-	-	-	-	-	-	+
19																			-	-	-	-	-	-	-	-	-	-	+
20																				-	-	-	-	-	-	-	-	-	+
21																					-	-	-	-	-	-	-	-	+
22																						-	-	-	-	-	-	-	+
23																							-	-	-	-	-	-	-
24																								-	-	-	-	-	+
25																									-	-	-	-	+
26																										-	-	-	+
27																											-	-	-
28																													+
dsx^{D+R2}	+	+	+	+	-	-	+	-	+	+	-	-	-	-	-	-	+	-	+	+	+	+	+	-	+	+	+	-	+	-
dsx^{D+R5}	+	+	+	+	-	-	+	-	+	+	-	-	-	-	-	-	+	-	+	+	+	+	+	-	+	+	+	-	+	-
$Antp^{+R2}$	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ns^{+R21}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Dfd-par	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

*Dfd-rk = Dfd ry stock (see Table 2)

Dfd par. = original Dfd parental chromosome

FIGURE 3 Complementation pattern emerging from inter se and deficiency complementation crosses. The numbers 1 to 7 represent Ki recombinant lethal stocks while 8 to 27 are the Dfd p^P lethals and 28 is the p^P lethal. m^a, m^b and m^c represent proposed lethal sites originally present in Dfd parental stock.

Def^{dsx} = $\frac{dsx^{D+R2}}{}$ and $\frac{dsx^{D+R5}}{}$

Def-rk = Dfd ry stock

I, II, III, IV and V are distinct complementation groups.

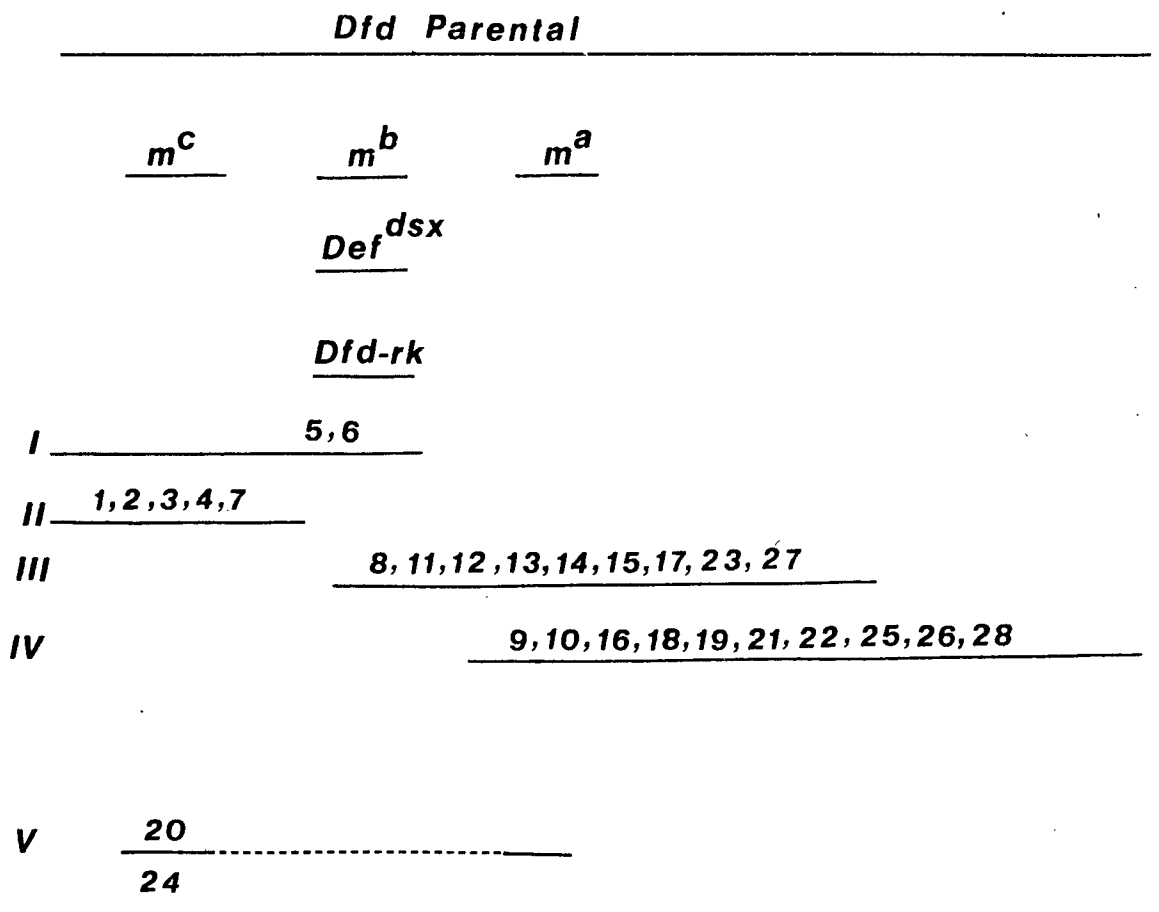
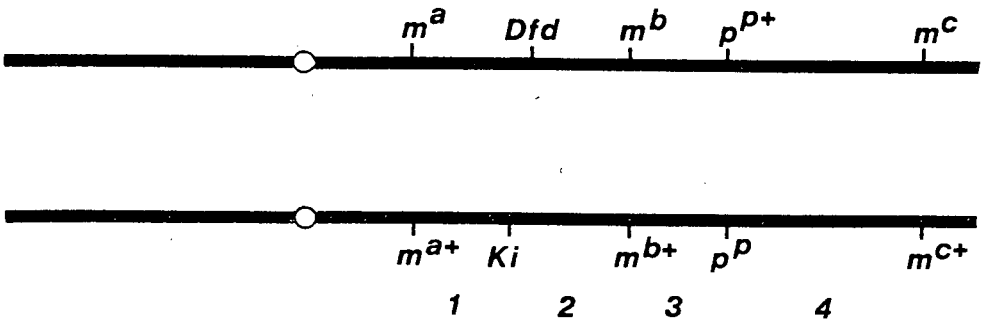

Dfd Parental
m^c
m^b
m^a
Def^dsx
Dfd-rk
I
5,6
II
1,2,3,4,7
III
8, 11, 12, 13, 14, 15, 17, 23, 27
IV
9, 10, 16, 18, 19, 21, 22, 25, 26, 28
V
20
24

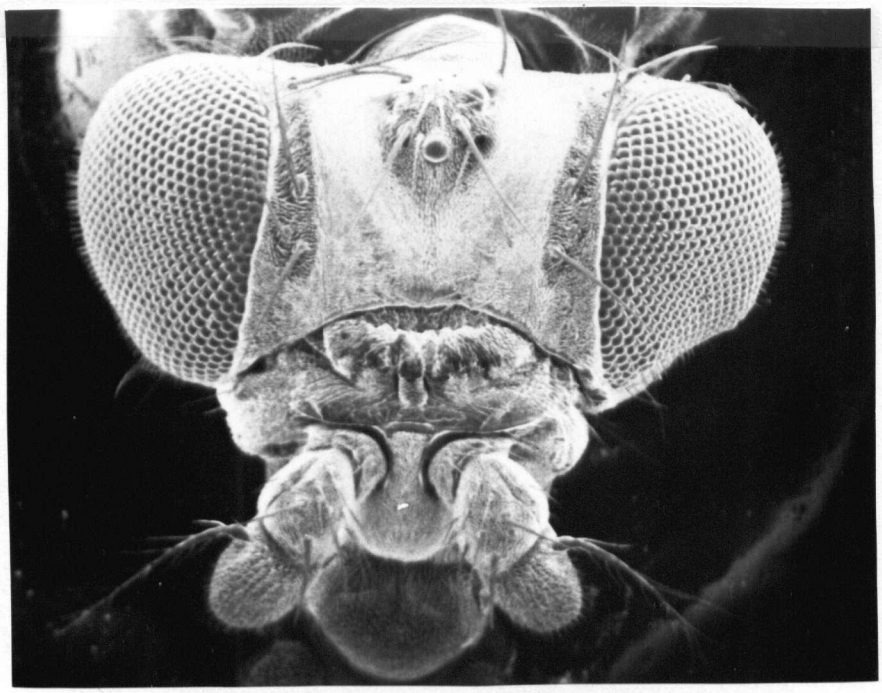
FIGURE 4 Proposed arrangement of lethal sites in proximal 3R in the original Dfd/Ki p^p female in which recombination was studied. m^a, m^b and m^c represent the lethal sites. Regions 1, 2, 3 and 4 are possible crossover intervals.


m^a
Dfd
m^b
p^{p+}
m^c
m^{a+}
Ki
m^{b+}
p^p
m^{c+}
1
2
3
4

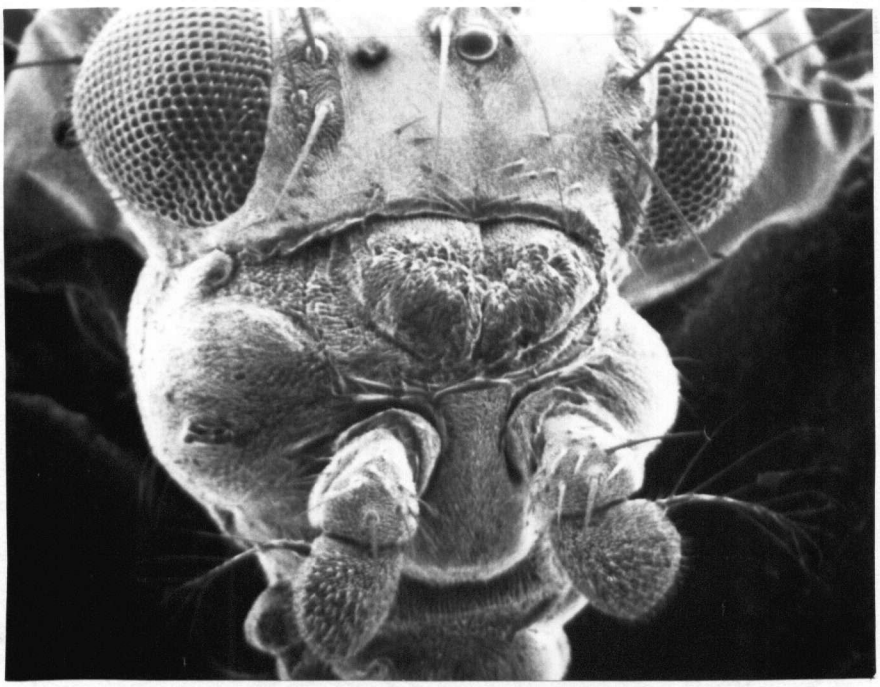
As represented in Figure 3, all Group IV Dfd p^p recombinants are viable in combination with Dfd-rk. However, all of the heterozygotes (except lethal 28/Dfd-rk) displayed a very extreme form of the Deformed phenotype, which suggested that Dfd is viable when homozygous. The verification of the explanation for lethality of Dfd chromosomes would be the synthesis of Dfd homozygotes and such a stock was isolated in the following manner: Dfd p^p/+ + (the lethal-26 stock was used) females were crossed to Dfd p^p/TM3 males (from the lethal-26 stock, presumably carrying only m^a) in bottles and the progeny scored. Surviving Dfd p^p/Dfd p^p progeny should arise only from the fertilization of a m^{a+} Dfd p^p recombinant oocyte by a m^a Dfd p^p sperm. Three such recombinant flies (one male and two females) and a single p^p (non-Dfd) individual were scored in a total of 2772 progeny. Stocks of the three Dfd p^p recombinant chromosomes were produced and individual lines were established for each. In all three cases fertile Dfd p^p/Dfd p^p homozygotes were produced. Figure 5 is a scanning electron microscope picture of Dfd p^p/+ + and Dfd p^p/Dfd p^p adults. The latter characteristically possess extremely reduced eyes (sometimes antennal structures are also missing) with extensively tufted vibrissae. The eye phenotype appears to be even more extreme than that of Dfd/Dfd^r. In addition, mirror image duplications of the antennae (or in some cases aristae only) are frequently observed.

That the Dfd locus does not appear to be located within the cytological interval 84A,B to 85A (which is delineated by the dsx^{D+R} deficiencies) is suggested by the observation that combining the newly isolated Dfd p^p chromosome with the deficiency chromosomes, does not expose the more extreme phenotype which is characteristic of the homozygote.

FIGURE 5 Scanning electron micrographs showing the eye development of Dfd heterozygotes (a, Dfd p^P/+) and homozygotes (b, Dfd p^P/Dfd p^P), (magnification about x400).



a



b

IV. Discussion

This study has shown that the Dfd locus is genetically separable from a lethal site present in the 84F interval in the proximal part of 3R of Dfd stocks (Duncan and Kaufman, 1975). Kinked and Deformed are very tightly linked and their relative positions are not definite and await further clarification through crossover studies. This should be facilitated through the use of Dfd in the homozygous condition, thereby eliminating the problem of misclassification of heterozygotes because of incomplete penetrance. Thus recombination experiments, for example, using females of the constitution st in ri eg^2 Dfd p^p/Ki, should allow unequivocal ordering of the two genes in question and shed further light on the nature of proximal recombination in general.

The question of the exact functional defect of the Dfd mutation remains unresolved, as does that of the cytological location of the locus. Deformed may be an amorph (Muller, 1932) in that the initial mutation may produce the Dfd phenotype and the homozygote would simply exhibit an extreme version of the phenotype. Alternatively, Dfd could be an hypomorph, that is, haplo-abnormal or dosage-sensitive. This is clearly possible since in their study of segmental aneuploidy in Drosophila, Lindsley et al. (1972) were unable to synthesize flies heterozygous for a deficiency spanning the region 82CD to 83EF, an interval which includes the Triplo-lethal segment. This unique locus is lethal in either a haploid or triploid condition. In a hypomorphic situation, +/deficiency $\frac{Dfd}{\quad}$ would presumably be equivalent to Dfd/+. The possibility that Dfd is an hypermorph is more remote, since no Dfd phenotype was produced in flies bearing interstitial duplications for

most of the proximal regions of 3R and the only region resistant to trisomy was the 83DE (Tpl) interval (Lindsley et al., 1972). Dfd could be interpreted as an antimorph, particularly since there is evidence that it is dominant in triploids (Lindsley and Grell, 1968). This could be tested directly by determining if Dfd/+/+ flies are phenotypically less mutant than Dfd/+ individuals.

Finally, Dfd could be a neomorph. If the latter is the case, it should be possible to induce deficiencies of the locus as has been done previously (Lifschytz and Falk, 1969; Denell, 1973; Duncan and Kaufman, 1975). However, it should be noted that such attempts to revert Ki have not been successful (Duncan and Kaufman, 1975). If radiation-induced revertants of either of these mutations are phenotypically indistinguishable from their respective heterozygotes, it may not be possible to use such an approach to acquire proximal chromosomal aberrations.

This present study emphasizes the need to be cautious when one is assessing the viability of a given mutation. Dfd has been known and studied for more than fifty years and all reports have assumed that lethality is a property of the Dfd mutation itself. This cautionary note is particularly important in the case of mutants isolated through the use of chemical or radiation mutagenesis, since their potent mutagenicity enhances the probability that double lesions will be induced in single chromosomes. The lack of completely correct knowledge about the viability of an allele is apt to mislead workers' attempts to genetically dissect a locus. Furthermore, the use of lethal flanking markers in recombinant systems demands precise knowledge of whether or

not an allele is lethal. Finally, studies of the developmental effects of specific genes would be difficult unless their viability characteristics are well-defined.

CHAPTER 4

A STUDY OF INDUCED CROSSING OVER NEAR THE CENTROMERE OF CHROMOSOME 3

I. Introduction

The lack of duplications and deficiencies for specific intervals has been one of the principal factors limiting the complete genetic dissection of Drosophila. The dosage dependent expression of many segments of the genome (Lindsley et al., 1972), particularly those where the structural genes of enzymes have been localized (Grell, 1962; Stewart and Merriam, 1974; Hodgetts, 1975), has permitted the cytological mapping of several functions where aberrations which include them exist. However, segmental aneuploidy, produced by combinations of different translocations involving the Y chromosome and the autosomes (Lindsley et al., 1972), suffers from limitations stemming from the variety of disjunctional possibilities. Therefore, workers have attempted to isolate heritably stable aberrations. The use of small, stable aberrations has permitted important genetic analyses. For example, a study of the interval between zeste and white on the X chromosome led to the conclusion that one functional unit exists in each polytene chromosome band (Judd et al., 1972).

A recently reported method for the production and recovery of localized aberrations involved selection of crossover chromosomes, where the crossovers were induced by radiation (Mglinets, 1972, 1973). Since normal meiotic crossing over occurs in females, it follows that crossovers arising from irradiated females would include both induced and

meiotic types. However, since no meiotic crossing over occurs in Drosophila melanogaster males, all exchange chromosomes recovered must be of the induced variety.

The question of the origin of induced crossovers has previously centered on two distinct proposals: (a) in both sexes, induced exchanges result from intimate pairing of homologues coupled with precise crossing over (breakage and rejoining), a situation analogous to the production of meiotic crossovers in females; or (b) in both sexes, induced exchange occurs in a manner similar to that of the production of translocations and therefore may involve mispairing and different breakpoints on each homologue and the potential for asymmetrical exchange.

According to (a), induced crossover chromosomes should not be preferentially associated with lethals and/or chromosome aberrations at or near the sites of exchange. Evidence for this has been provided by several studies. Patterson and Suche (1934) found that of a total of 59 third chromosome crossover progeny of X-irradiated males, only 9 carried recessive lethals. Moreover, most of these lethals were mapped to sites other than where exchange had occurred. They suggested that radiation might promote crossing over in males by releasing the normal constraints on meiotic crossing over. Friesen (1937a) found a similar situation for crossovers involving both chromosomes 2 and 3 as did Whittinghill (1937) for heat-induced crossovers from males. Ives and Fink (1962) found that while only a low frequency of crossover progeny produced by gamma-irradiated males carried recessive lethals, translocations involving non-crossover chromosomes were relatively frequent. Finally, Raytnayake (1970) found that the majority of crossover chromosomes of progeny produced by formaldehyde-treated males could be homozygosed.

Muller (1954, 1958) favoured the second alternative (b) and argued that the production of radiation-induced crossover chromosomes resembles the formation of translocations. In support of this idea, Herskowitz and Abrahamson (1957) found that radiation-induced exchange in centromeric regions in X chromosomes of females, conformed to a two-hit kinetic situation. Olivieri and Olivieri (1964) confirmed this in males and also showed that dose fractionation or administration in a nitrogen atmosphere decreased crossing over, while delivery of radiation in oxygen enhanced exchange. Recently, Williamson et al. (1970) found that induced exchange involving the fourth chromosomes of females, followed two-hit kinetics.

After studying her own data and those of other workers, Hannah-Alava (1968) concluded that there is a correlation between radiation-induced crossing over and the occurrence of recessive lethals and dominant sterility, particularly for meiotic broods sampled from male parents. She also found that crossovers involving intervals outside the centromeric region (the centromeric region is loosely defined as that region spanned by the most proximal markers used), were preferentially recovered in intermediate broods, while proximal crossovers (i.e. those occurring within the centromeric interval) were detected in later broods and were primarily associated with large clusters. Apparently, this brood pattern is a reflection of the meiotic and pre-meiotic origins of so-called non-proximal and proximal crossovers, respectively. She claims that earlier studies (e.g. Patterson and Suche, 1934; Whittinghill, 1937) failed to compensate for the occurrence of crossovers in clusters when estimating lethal frequencies amongst crossover stocks. She concluded that induced crossovers frequently arise in a translocation-like fashion or less likely, that exchange chromosomes must somehow be predisposed towards the possession of lethal sites.

Mglinets (1972) provided definitive support for the asymmetrical-exchange proposal through his finding that about 20 percent of recombinant third chromosomes from gamma-irradiated males, possessed chromosome aberrations (especially duplications and deficiencies). Furthermore, nearly all of the aberrations had at least one breakpoint at or near the point of exchange. The majority of the crossover rearrangements were present in 'meiotic' broods and few occurred in 'gonial' broods, a finding which parallels the results of Hannah-Alava (1968). He also found a significant correlation between sites of chromosome damage and sites of exchange in recombinant third chromosomes derived from irradiated females. Thus, his data have raised the possibility that aberrations involving particular regions of the chromosome can be recovered through the selection of appropriate crossover progeny.

The aim of this present study was to use radiation to induce crossovers within proximal intervals in chromosome 3 and to analyse the resulting crossover chromosomes genetically. I hoped that it would be possible to determine if such a method could provide a source of useful proximal deficiencies.

II. Materials and Methods

Three separate experiments were performed. Heterozygous st in ri eg^2 Ki p^p e^s/+ + + + + + males, collected within 30 hours of eclosion, were irradiated in gelatin capsules (the source of radiation was a cobalt-60 Gammacell in the U.B.C. Chemistry Department) and then mass mated to homozygous st in ri eg^2 Ki p^p e^s virgin females (these virgins were maintained for at least six days prior to mating) in quarter-pint milk bottles (using 8-10 males with 10 to 15 females per bottle). At the end of various intervals (depending upon the experimental protocol), new virgin females along with the irradiated males were added to fresh bottles. Wicks of filter paper were added to each bottle in order to maximize freedom of movement and mating ability. The mass mating technique, coupled with observed differences in viability of female parents, precluded the use of a standardized definitive brooding procedure for any of the experiments.

Two-hundred forty and 80 males were irradiated with 1000 R (Expt. I) and 2000 R (Expt. II) respectively and test crossed to females for six successive intervals of 3, 4, 4, 5, 5 and 5 days, for a total of 26 days.

Two-hundred fifty males were irradiated with a dose of 3000 R and mated to females for five successive intervals of 3, 3, 6, 5 and 5 days, for a total of 22 days. Because the female parents frequently became mired in the wet food at the beginning of the third brood interval of this experiment, a new interval was started on the seventh day (the 55 non-crossover progeny recovered in the short interval were not included in the total).

As a control, 90 males of the heterozygous genotype were test crossed for five 3-day intervals.

The crossover (between st and p^p) and non-crossover progeny were scored until the twenty-fourth day after the parents had been introduced. Although crossing over was not strictly monitored between p^p and e^s, several crossovers for this region were noted.

All crossovers and 30 st in ri eg^2 Ki p^p e^s/+ + + + + + + non-crossover males (the latter were selected at random from cultures at days 12-22 in Expt. III) were balanced with one of TM1, TM3 or CxD and tested for recessive lethality and each of the crossovers was coded according to its genotype.

Sixteen of the lethal- and semi-lethal-bearing chromosomes (henceforth the mutants will all be referred to as lethals unless otherwise specified), collected from these experiments, were tested for complementation inter se and with 4 cytologically-identifiable deletions lacking specific proximal segments in the right arm of chromosome 3 (see Table 2). In addition, some of the mutants were tested for complementation with the following dominant mutations: Pc, Ki, Msc, Scx and $Antp^G$; and for pseudodominant expression of the recessive visible mutations: tra, pb, Dfd^r, roe and dsx. All of these loci are known to be genetically located in the proximal regions of chromosome 3 (see Table 1).

Salivary gland chromosomes of 11 of the 16 mutant stocks were inspected by Dr. T. C. Kaufman using a standard technique.

III. Results

1. Radiation-Induced Crossing Over in Males

The crossover intervals will hereafter be referred to as follows: st to in - region 1; in to ri - region 2; ri to eg^2 - region 3, eg^2 to Ki - region 4; Ki to p^p - region 5; and for reference, p^p to e^s - region 6. Note that the centromere and proximal heterochromatin lie in region 4 (see Figure 1). Hereafter, region 4 will be known as the Proximal interval while regions 1, 2, 3 and 5 will be collectively known as the Non-Proximal interval. Since this study was designed to recover crossovers near the centromere and because some p^p to e^s crossovers may not have been scored, data involving region 6 will be considered only briefly. Although phenotypes such as eg^2, st or e^s could arise from mutation at a low frequency, all were scored as crossovers.

Numbers of progeny and frequency of crossing over

The control and experimental crossover data for the progeny of treated and untreated males are presented in APPENDIX 1. For all three experiments it is evident (APPENDIX 1a) that the single most frequent type of crossover occurred in the Proximal interval as has been previously noted (see Hannah-Alava, 1968). In Expt. I, the total number of Proximal crossovers was slightly less than that of Non-Proximal crossovers (19 versus 22), while for both Expts. II and III the numbers of Proximal types greatly exceeded those of the Non-Proximal types (Expt. II, 13 versus 3; Expt. III, 63 versus 12). Double crossovers were detected and these have been reported in an earlier study (Mglinets, 1972).

Table 15

Distribution of Crossovers in Proximal and Non-Proximal* Intervals for st to p^p Exchanges

Treatment of Male Parent	Crossovers (Crossover Events)**					
	Intervals					
	1	3	4	5	3,4	5,6
Expt. I 1000 R	11(7)	2(2)	20(8)	9(6)	-	-
Expt. II 2000 R	3(3)	-	13(5)	-	-	-
Expt. III 3000 R	4(4)	-	61(19)	5(4)	2(2)	1(1)

* Proximal = Exchange in region 4
Non-Proximal = Exchange in region 1, 3 or 5
** Crossover Event = Each cluster counted as one event

APPENDIX 1b is a summary of the total crossover progeny for the entire st to p^p and p^p to e^s regions as well as an estimate of crossing over between st and p^p in Expt. III. Since it is thought that gametic samples of earlier broods are mainly post-meiotic (i.e. with respect to irradiation, see Hannah-Alava, 1968), the crossover frequency includes only progeny recovered from day 7 to 22 inclusive. Furthermore, only the progeny of fourteen randomly selected cultures which had produced a minimum of 25 progeny per brood interval, were used for this estimate. Thus, the estimated frequency of induced crossing over in males treated with 3000 R is 0.59 percent, while the comparable level for untreated males is 0.078 percent. A similar analysis of Puro's (1966) data gives a crossover frequency of 0.65 percent. The fact that he used a single male technique probably accounts for the frequency difference.

Numbers of crossover events

Presumably, radiation-induced crossovers can arise either in germ cells which have stopped dividing (i.e., those that have reached late gonial or early meiotic stages), or in germ cells which are still dividing. In the former case, one would expect to recover single crossover progeny of a given type (or of each reciprocal type); while in the latter case, one could recover clusters of identical crossovers (and/or reciprocals) that arose from a single exchange event. The size of the cluster would depend upon the number of gonial divisions occurring after irradiation.

In the present work, the crossover frequency is low (about 1 in 150 to 200 progeny). Therefore, the probability that 2 (or more) phenotypically identical crossover progeny in the same culture had arisen from independent crossover events, is even lower. Consequently, when

2 or more of the same (e.g., st in ri eg^2) or reciprocal (e.g., st in ri eg^2 and Ki p^p e^s) types were seen in the same culture, they could be collectively scored as a single crossover event. Many clusters were observed, with the largest including 26 Ki p^p e^s progeny in two consecutive brood intervals (Expt. III).

Table 15 gives the total numbers of crossover progeny, along with the numbers of crossover events (in parentheses) for the three experiments. These data underscore the fact that in Expt. I, Non-Proximal and Proximal crossovers occurred at the same frequency and in Expts. II and III, the Proximal types were scored much more frequently. However, when crossover events are considered, the Non-Proximals are about twice as frequent as the Proximals in Expt. I, while the differential between the two types is markedly reduced in both Expts. II and III (Expt. II, 13 Proximal:3 Non-Proximal crossovers versus 5 Proximal:3 Non-Proximal crossover events; Expt. III, 63 proximal:12 Non-Proximal crossovers versus 19 Proximal:11 Non-Proximal crossover events). Thus, it seems that clustering is more characteristic of crossovers occurring within the region which includes heterochromatin than of those occurring outside of this region. This may indicate that Proximal crossovers are primarily gonial in origin, while Non-Proximal crossovers are not and this would support the findings of Hannah-Alava (1968).

Recovery of mutants amongst crossovers

Table 16 summarizes the distribution of recessive lethals (including 3 semi-lethal visibles) and putative dominant steriles amongst the various types of crossover chromosomes. The appropriate numbers of lethal or sterile events (after correcting for clusters) are included in parentheses. A total of 16 independently-induced lethals (or semi-lethals) was recovered amongst 52 tested crossover chromosomes which

Table 16

Regional Summary of Lethals* and Steriles Present on Crossover Chromosomes

Experiment Number		1	3	4	5	6	3,4	Total Proximal	Total Non-Proximal**
I	Lethals	1(1)***	-	1(1)	5(3)	1(1)	-	1(1)	6(4)
	Steriles	2(2)	1(1)	1(1)	-	-	-	1(1)	3(3)
II	Lethals	1(1)	-	1(1)	-	-	-	1(1)	1(1)
	Steriles	1(1)	-	-	-	-	-	-	1(1)
III	Lethals	3(3)	-	3(3)	2(2)	2(1)	1(1)	3(3)	6(6)
	Steriles	-	-	2(2)	1(1)	-	1(1)	2(2)	2(2)

* Includes semi-lethals
** Not including interval 6
*** Number in parentheses = Number of lethal or sterile events (each cluster counted as one event)

arose from independent exchanges between st and p^p; 5 in Expt. I, 2 in Expt. II and 9 in Expt. III. The totals show that overall, most of the lethals occurred in Non-Proximal crossover chromosomes (11 of 16). A total of 9 of the crossover progeny were sterile (3 Proximals and 6 Non-Proximals). Since the mass mating technique makes it difficult to define these as genuine steriles, they will not be mentioned further.

Regional comparison of crossovers and mutants

Table 17 shows the relative occurrence (as percent of total crossover events) of Proximal versus Non-Proximal crossover events for the three experiments as well as the frequencies (in percent) of lethals amongst the crossover events. The latter frequencies were based upon the percentage of independent crossover stocks which carried lethals (since steriles could not be tested, they were omitted). These data (Table 17) reveal that in Expt. I most of the independent crossovers occurred outside the Proximal interval, while this trend was reversed at the higher doses of radiation (although in Expt. II only 2 Non-Proximals were tested since 1 was sterile). While 14 percent of the tested independent Proximal crossovers were associated with lethals in Expt. I, these frequencies were 20 percent in both Expts. II and III. In contrast, the lethal frequencies for Non-Proximal crossover events were 33.3, 50 and 66.7 percent in Expts. I, II and III, respectively (in Expt. II only two stocks could be tested). For reference, it was determined that 16.7 percent (5 of 30) of wild-type parental chromosomes from day 7 to 22 in Expt. III, carried recessive lethals.

The above results support the idea that there is a preferential association of lethality with proximal crossovers that are induced in

Table 17

Relative Occurrence of Crossovers and Lethal Events for Proximal and Non-Proximal Intervals

Experiment Number	Crossover Events (Percent of Total)		Number of Lethal-Associated Crossovers (Percent of Region-Specific Total)*	
	Proximal	Non-Proximal	Proximal	Non-Proximal
I	8(35.0)	15(65.0)	1(14.3)	4(33.3)
II	5(62.5)	3(37.5)	1(20.0)	1(50.0)
III	19(63.3)	11(36.7)	3(20.0)	6(66.7)

*Percent Lethality = ((number of lethal events)/(number of crossovers - number of steriles)) x 100

the euchromatic portions of the chromosome in the region between st and p^p. It would also appear from these results that more induced crossing over occurs (or at least is detected) outside of the Proximal interval at 1000 R, while at higher doses of radiation (i.e. 2000 and 3000 R), this trend is reversed and Proximal crossover events are more frequently observed. The question of whether the latter difference (between Expts. I, and II and III) is real or is due to artifacts of the technique, must await more analyses involving precise brood and single male studies.

2. Analysis of Lethal Stocks

Inter se complementation, pseudodominance, and additional tests

The results of the inter se and deficiency complementation involving the 16 crossover mutants are shown in Table 18. The mutants have been coded according to their genotypes and where more than 1 member of a given class occurred as a lethal, each was assigned a different number. The mutant stocks st-3, st-4 and st in ri eg^2-4 actually possessed recessive semi-lethal sites and in each case less than 10 percent of the expected number of homozygotes survived to adulthood and these were extremely small (about 1/3 to 1/2 of normal size).

It is clear from the inter se complementation results that most mutant combinations were viable. The non-complementing exceptions are: (a) st-1, st-2; (b) st in ri eg^2-2, st in ri eg^2-3; (c) in ri eg^2 Ki p^p e^s, st in ri eg^2-2; and a group of five (d) st-3, st-4, st in ri eg^2-4, st in ri eg^2 Ki-1 and st in ri eg^2 Ki-2. Note that in groups a and b, non-complementation occurred between different lethals associated with crossovers within the same region, whereas in group c, crossing over

Table 18

Results of Inter Se and Deficiency Complementation Crosses Involving Crossover Lethals

	(A) st-1	(B) st-2	(C) st-3	(D) st-4	(E) in ri eg^2 Ki p^p e^s	(F) st in ri eg^2-1	(G) st in ri eg^2-2	(H) st in ri eg^2-3	(I) st in ri eg^2-4	(J) Ki p^p e^s	(K) st in ri eg^2 Ki-1	(L) st in ri eg^2 Ki-2	(M) p^p e^s-1	(N) p^p e^s-2	(O) p^p e^s-3	(P) eg^2
(A)		-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
(B)			+	+	+	+	+	+	+	+	+	+	+	+	+	+
(C)				-	+	+	+	+	-	+	-	-	+	+	+	+
(D)					+	+	+	+	-	+	-	-	+	+	+	+
(E)						+	-	+	+	+	+	+	+	+	+	+
(F)							+	+	+	+	+	+	+	+	+	+
(G)								-	+	+	+	+	+	+	+	+
(H)									+	+	+	+	+	+	+	+
(I)										+	-	-	+	+	+	+
(J)											+	+	+	+	+	+
(K)												-	+	+	+	+
(L)													+	+	+	+
(M)														+	+	+
(N)															+	+
(O)																+
(P)																
Ns^{+R21}	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+
Antp^{+R2}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
dsx^{D+R2}	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+
dsx^{D+R5}	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+

occurred in different regions for the 2 lethals (it is possible that st in ri eg^2-2 carries two lethal sites). Group d presents a heterogeneous situation i.e. non-complementation occurred between similar as well as different crossovers. It should be mentioned that in the latter group, both st in ri eg^2 Ki mutant stocks were lethal as homozygotes and in combination with each other, but in combination with any of the other 3 members of this group, produced the characteristic small flies at low frequencies.

Most of the combinations between the crossover mutants and the proximal deficiencies were viable (Table 18). Four exceptions to this were Ki p^p e^s/dsx^{D+R2}, Ki p^p e^s/dsx^{D+R5}, p^p e^s-3/Antp^{+R2} and p^p e^s-3/Ns^{+R21}. Since all of these deficiencies lack chromosome material between Ki and p^p, these data reveal that both the Ki p^p e^s and p^p e^s chromosomes possess lethals near or at the sites of exchange. The failure of the Ns^{+R21} and dsx^{D+R2} deficiencies to complement with st in ri eg^2-1 will be dealt with later.

Table 19 represents a summary of pseudodominance and additional complementation tests involving four of the crossover lethal stocks and known proximal mutations in chromosome 3. Most of the combinations were non-mutant. However, all tra/st-1 progeny were males, thereby indicating that the st-1 stock fails to complement the tra mutation. In addition, p^p e^s-3 was lethal when combined with Msc, Scx and Antp. There is good evidence that Scx and Antp^{Ns} are alleles (see Denell, 1973; Duncan and Kaufman, 1975), while the relation of both of these with Msc is unclear. Finally, the Ki/p^p e^s-3 combination was viable but the flies displayed an enhanced Kinked phenotype reminiscent of Ki/Ki flies.

Table 19

Summary of Pseudodominance and Complementation Tests Between Selected Crossover Mutants and Known Proximal Mutations

Crossover Mutants \ Proximal Mutants	tra	Pc	pb	Ki	Dfd^r	Msc	Scx	$Antp^G$	roe	dsx
st-1	-*	+	+	+	+	+	+	+	+	+
st in ri eg^2-1	+	+	+	+	+	+	+	+	+	+
Ki p^p e^s	+	+	+	not done	+	+	+	+	+	+
p^p e^s-3	+	+	+	e	+	-**	-**	-**	+	+

* All tra/st heterozygotes were males
** Lethal
e = Ki appeared to be enhanced

Cytological analysis

The results of the cytological analysis of 11 of the 16 crossover mutants are given in Table 20 along with some phenotypic descriptions. Eight of the 11 stocks examined displayed no obvious cytological disruptions. However, the remaining 3 were all abnormal and displayed the following aberrations: st-1 = Df(3L)72F-73A;74BC, in ri eg^2 Ki p^p e^s = In(3L)70C;74A, st in ri eg^2-1 = Df(3L)79E5-6;80 heterochromatin. Note that all of these stocks show low viability as heterozygotes and in addition, the basal deficiency (st in ri eg^2-1) includes a Minute, probably M(3)LS4 (Lindsley et al., 1972). The extent of the heterochromatic deficiency of this stock is not known. The low viability of heterozygotes involving the st in ri eg^2-1 and the Ns^{+R21} or dsx^{D+R2} deficiencies, may be due to the combined effect of the deficiencies in each case. It is worthy of mention that since the recessive lethality of Pc is not exposed by this basal deficiency (see Table 19) and because this gene has recently been localized to the interval between 75A-B to 80 (Puro and Nygren, 1975), these present results further narrow the interval containing Pc to 75A-B to 79D or E in proximal 3L.

The fact that st-1 is a deletion explains its lack of complementation with both st-2 and tra.

Thus, in the case of all 3 aberration-bearing crossover chromosomes, the site of damage for at least one of the breakpoints corresponded to the region where crossing over had occurred.

p^p e^s-3- and Ki p^p e^s-bearing flies were weakly viable and since genetic evidence supports the idea that the former is a deletion which exposes the $Antp^{Ns}$-Scx-Msc homeotic region as well as Ki, it is surprising that neither of these stocks possess chromosome abnormalities.

Table 20

Phenotypic Description and Cytological Analysis of Crossover Mutants

Crossover	Phenotype (other than markers)	
st-1	Low viability (as heterozygote)	Df(3L)72F-73A; 74BC
st-2	-	+
st-3	Low frequency recovery of very small sterile homozygotes	+
in ri eg^2 Ki p^p e^s	Low viability (as heterozygote) and infrequent haltere enlargement	In(3L)70C;74A
st in ri eg^2-2	Minute, low viability (as heterozygote), low female fertility, thick aristae, rough eyes	Df(3L)79E5,6; 80 heterochromatin
Ki p^p e^s	Low viability, gaps in wing vein L2	+
st in ri eg^2 Ki-1	Low frequency recovery of very small homozygotes	+
p^p e^s-1	-	+
p^p e^s-2	-	+
p^p e^s-3	Low viability, sex combs have fewer teeth (5-7) than wild type	+
eg^2	Slight broadening in wing vein L2 near tips, rough eyes	+

Other complementation tests

Since the mutant stock $\underline{eg^2}/\underline{TM3}$ displayed a Delta-like phenotype, it was tested with an allele of Dl. No $\underline{Dl}/\underline{eg^2}$ heterozygotes were scored, thereby indicating that the $\underline{eg^2}$ chromosome carries an allele of Dl.

Tasaka and Suzuki (1973) recovered an EMS-induced ts visible mutation on the third chromosome, which although normal at 22^{o}, when grown at 29^{o} C produced homozygotes that were undersized. When this mutant ($\underline{l(3)ET^{hs18}}$) was retested and crossed to the $\underline{st\ in\ ri\ eg^2\ Ki\text{-}1}$ stock, normal heterozygotes were produced at both 22^{o} and 29^{o} C. It is possible that the small body mutation which is characteristic of the inter se complementation group (d) is due to contamination. However, the recessive lethality carried by both $\underline{st\ in\ ri\ eg^2\ Ki}$ members may be due to a second site which could still correspond to the regions of exchange.

Of the five recessive lethals detected amongst the 30 non-crossover chromosomes tested (from Expt. III), four were distinct (i.e. two did not complement). None of these four was exposed by any of the six deficiencies which were at my disposal (the four described in Table 2 and the two newly synthesized deletions from the present study).

In summary: I detected a total of 16 associated mutations amongst 52 independently-occurring crossovers. Three of the 16 possessed structural abnormalities coinciding with the sites of exchange (st-1, $\underline{in\ ri\ eg^2\ Ki\ p^p\ e^s}$, and $\underline{st\ in\ ri\ eg^2\text{-}1}$) and the lesions of three additional lethals (st-2, $\underline{p^p\ e^s\text{-}3}$ and $\underline{Ki\ p^p\ e^s}$) were cytologically mapped to sites close to or at the regions of exchange. Thus, at least 6 of the 16 mutants show correlations between the position of

lethal sites and sites of crossing over. The failure of st in ri eg^2-2 and st in ri eg^2-3 to complement each other may indicate a similar correlation for these 2 lethal crossover chromosomes, thereby further increasing this total to 8 of 16.

IV. Discussion

It is clear from the present study that selection of induced crossovers between proximal markers in irradiated males, can enrich for proximally-located lethals and more specifically, for aberrations. Furthermore, the more frequent types of aberrations found in this case were deficiencies. Since the cytological positions of both the aberrations and many of the non-aberrant crossover lethals were very similar to the regions of exchange, these data support the contention that at least some of the crossover chromosomes originated from asymmetrical exchange events.

The results of this study reveal that crossovers occurring within proximally-adjacent euchromatic segments are more frequently associated with mutations, than are crossovers occurring within the more proximal segment which spans the heterochromatin. This observation is consistent with the findings of Hannah-Alava (1968) and Mglinets (1972).

At least 3 factors which could contribute to the apparent lower susceptibility of proximal exchanges to the concomitant occurrence of mutations are: (i) relatively few sites capable of being mutated to lethality exist in heterochromatin as compared to euchromatin (Muller et al., 1937; Hilliker and Holm, 1975; Hilliker, 1976), (ii) a mechanism which exists in the testis could allow for the selective elimination of pre-meiotic stem cells possessing chromosome breaks (Puro, 1966), and (iii) stage-specific differences in pairing of homologous chromosomes during spermatogenesis could allow for more complete pairing of homologues in gonia and therefore promote reciprocal crossing over (Hannah-Alava, 1968; Mglinets, 1972).

The relevance of (i) is self-evident. Puro (1966) has found evidence for a regenerative stem cell mechanism in the testes of Drosophila. Therefore it is possible that where breakage of unpaired homologous chromosomes does occur in stem cells, resulting in crossovers which involve chromosome damage, these cells would be lethal, thereby accounting for the relative dearth of crossovers in broods derived from such cells (see Hannah-Alava, 1968). The results of this present study corroborate those of earlier workers in showing that proximal crossovers are more frequently associated with clusters. Such a selective mechanism as described above would likely lead to a situation where fewer proximal crossover chromosomes contained lethals.

Finally, both Hannah-Alava (1968) and Mglinets (1972) have referred to evidence which suggests that homologous pairing occurs to different degrees during gonial stages and meiosis. Thus, more complete somatic pairing prior to meiosis would permit more exact homologous interchange to take place following radiation-induced chromosome breakage, thereby producing a situation analogous to that of meiotic exchange in females.

The stable deficiencies isolated in the course of this study are potentially very useful. Thus, st-1 (Df(3L)st) has permitted the cytological localization of tra and st in ri eg^2-1 (Df(3L)M(3)LS4) has further clarified the position of Pc in the left arm of the chromosome. It is worthwhile to emphasize that the latter deficiency probably lacks some of the heterochromatin in 3L, although the extent of this heterochromatic deletion is unknown. The existence of such a mutant will be an important tool for use in the continuing search for genes within heterochromatin.

The induced crossover technique offers a unique approach for the study of proximal regions of chromosome 3. To increase the resolution of this method, it should be possible to construct a genetic screen whereby induced exchanges would be selected for and non-crossovers eliminated. For example, males containing two ts mutations (spanning the region of interest) linked in trans, could be irradiated and crossed to females homozygous for both ts mutations at 22°C. If the resulting cultures were raised at 29°C, only wild-type crossover progeny would be expected to survive, thereby enriching the selection system for radiation-induced crossover chromosomes.

CHAPTER 5

A GENETIC AND DEVELOPMENTAL STUDY OF Q-III, A TEMPERATURE-SENSITIVE MINUTE MUTATION

I. Introduction

The phenotypic similarities between bobbed, some alleles of suppressor of forked and Minutes have been used to argue that they all represent defects in protein synthesis, since protein synthesis is abnormal in both bb (Ritossa et al., 1966a) and $1(1)su(f)^{ts67g}$ (Dudick et al., 1974; Lambertsson, 1975b).

No evidence has been presented which unequivocally identifies a common mode of action for the various Minute loci. In fact, it has been proposed that although all of these mutants probably do inhibit protein synthesis, mutants of different loci could be acting at different levels in the overall process (White, 1974). Thus, while some Minute genes could code for different iso-accepting species of tRNA, others could code for processing enzymes for tRNA or other molecules, amino-acyl synthetases, ribosomal proteins or any of the multitude of structural and functional components which comprise this system.

The hypothesis that Minute loci code for tRNA (Ritossa et al., 1966b; Atwood, 1968) has attracted considerable interest because it provides a plausible explanation for the identical phenotype of many different loci. Since tRNA genes are redundant, this hypothesis also explains why mutations at M loci are often deletions. Furthermore, the number of loci corresponds roughly to the expected number of tRNA

species. Experimentally, the tRNA-Minute relationship can be explored by correlating biochemical studies (viz. in situ hybridization, chromatographic isolation and nucleotide sequencing etc.) with genetic tests such as segmental aneuploidy (Lindsley et al., 1972) and fine structure mapping.

The importance of further genetic study of Minutes therefore, cannot be overemphasized. In this regard, the recovery of EMS-induced Minutes (Holden and Suzuki, 1973; J. Stone, unpublished results; Huang and Baker, 1975) is particularly noteworthy, as EMS is assumed to induce single base transitions. The assessment of developmental anomalies of Minutes which are associated with cytologically-observable deletions, is fraught with difficulties. However, temperature-sensitive EMS-induced Minutes which are presumed to be point mutations (Suzuki, 1970), should permit more definitive and accurate examination of the ontogenic basis of defects produced by such lesions, especially during embryogenesis in Minute homozygotes. Moreover, there is evidence of the existence of recessive lethal sites which have no dominant visible effects and do not complement standard Minute alleles (A. Datagupta, personal communication). This presents the possibility that Minute loci are complex and that the analysis of different alleles and their biochemical properties will provide an insight into the function(s) of Minutes.

A significant proportion of EMS-induced mutations is temperature-sensitive in Drosophila (Suzuki, 1970). The relevance of such mutants to genetic, biochemical and developmental analysis of this organism should considerably expand the scope of Minute investigations. For

example, ts alleles of Minutes could allow construction of recombination selective systems, thereby increasing the resolving power in fine structure studies of such loci. Furthermore, if definitive evidence concerning the primary gene products of Minutes is forthcoming, the existence of a conditional allele could allow the unequivocal identification of the potentially thermolabile gene product and its in vivo and in vitro biochemical characterization.

Temperature shift experiments during development have provided a wealth of information about the interval(s) when the gene product of a given locus is utilized (i.e. temperature-sensitive period, see Suzuki, 1970, 1974b). Furthermore, heat pulse experiments involving ts mutants or tests of such stocks at middle temperature ranges, have resolved phenotypes that are usually masked by lethality in orthodox TSP shift studies (Poodry et al., 1973; Tasaka and Suzuki, 1973). Heat pulse studies of a ts allele of a Minute locus with respect to the attenuated bristle phenotype and TSPs for any other M effects, would help to delineate the extent of Minute function during development.

It is noteworthy that in their search for DTS lethals on chromosome 3, Holden and Suzuki (1973) isolated two mutants, DTS-1 and DTS-6, which displayed the dominant Minute phenotype at 22°C as well as dominant lethality at 29°C. Neither could be made homozygous at 22°C. Owing to semi-sterility of DTS-6 females at 22°C, an accurate genetic location could not be found. However, the ts dominant lethality seemed to map proximally. Crossover studies involving DTS-1 placed the M and dominant lethal phenotypes at an identical site in a distal segment of

the right arm of the chromosome. Furthermore, the lethal phase (LP) of DTS-1 homozygotes is embryonic and this is also true for other known Minutes (see Brehme, 1939; Farnsworth, 1957a,b). It appears therefore that temperature-sensitive Minutes can indeed be induced and recovered.

Since the autonomous cell-lethal nature of Minutes has been demonstrated (Stern and Tokunaga, 1971) and because many important features of pattern formation in imaginal discs have been described through the study of X-linked, ts autonomous cell-lethals (Russell, 1974; Simpson and Schneiderman, 1975; Arking, 1975), it is likely that a ts Minute could provide similar as well as unique information about the development of Drosophila.

The existence of a ts mutation that interacts phenotypically with a distinct, non-ts mutant has been used to define the time of activity of the non-ts mutant (Dudick et al., 1974). Thus, the ts allele of su(f) was used to determine the time of activity of the forked gene. Since it is known that Minutes interact phenotypically with several non-allelic genes (see Schultz, 1929; Lindsley and Grell, 1968), a ts Minute might be exploited in a similar fashion and the time of the interaction between the Minute locus and the product of another non-ts locus determined.

This chapter is a preliminary report on such genetic and developmental studies of a ts Minute, located near the centromere of chromosome 3, which I recovered. The study was initiated to determine the potential utility of the mutation in exploring both Minute function during development and its interaction with other loci.

II. Materials and Methods

The temperature-sensitive Minute, Q-III was recovered by chance in a screen for EMS-induced, ts alleles of a known third chromosome mutation. The retarded development of Q-III/+ flies at 29°C initially resulted in the misclassification of this mutant as a lethal allele of the test chromosome. Crosses of Q-III with Oregon-R or p^p/p^p resulted in the production of heterozygotes at 29°C. This led to the preliminary classification of Q-III as a ts Minute.

Henceforth, I will simply refer to the temperature-sensitive Minute as Q-III. It should be noted that all experiments involve Q-III linked to p^p. Chromosomes used to balance Q-III include: TM1, CxD, and TM3. All references to TM3 throughout the text signify TM3, Sb Ser unless otherwise indicated. For full descriptions of these balancers, see Lindsley and Grell (1968).

Unless specified otherwise, all mapping, complementation and other crosses for assessing the properties of Q-III were carried out in quarter-pint milk bottles with standard Drosophila medium. Ten pairs of parents were introduced into each bottle and these were usually sub-cultured at least once on fresh medium. Where tests at 29°C were made, the females were allowed to lay for 1 or 2 days at 22°C before they were removed and then the culture bottles were shifted up to 29°C.

A standard method of egg collection on petri dishes was used for the developmental and some of the genetic studies (see Tarasoff and Suzuki, 1970). Generally, the first two 2-hour batches of eggs laid were discarded and the third was used either by counting the eggs and

shifting the plates directly or more usually, by picking required numbers of eggs along with some of the medium and placing them in pre-incubated vials or petri dishes for shifts or other analyses.

To test for viability, lethal phases and lengths of developmental periods of Q-III homozygotes and heterozygotes, 22° and 29°C batches of eggs were collected in two control crosses: I p^P/p^P x p^P/p^P, II CxD/TM3 males x p^P/p^P females; and three experimental crosses: (A) Q-III/TM3 females x p^P/p^P males, (B) Q-III/TM1 x Q-III/TM1, (C) Q-III/TM3 x Q-III/TM3. The eggs were counted and transferred to pre-incubated 50mm petri plates on fresh medium (50 to 100 eggs per plate) which were then placed at 17°, 22° or 29°C. No attempt was made to distinguish between fertilized and unfertilized eggs (Wright, 1973) during this procedure. Stage-specific distribution of lethality was estimated for the control and 2 of the experimental crosses by inspecting cultures intermittently and computing the proportions of expected progeny which successfully survived the egg, larval and pupal stages. In some cases, lengths of developmental periods were estimated by inspecting the cultures at various intervals and noting the time when at least half of the total surviving progeny had eclosed, thus providing the period of time (in hours) from oviposition to eclosion.

1. Genetic Analysis

Genetic mapping

Q-IIIp^P/TM3 males were crossed to Gl Sb H/Payne (see Table 1) or st in ri eg^2/st in ri eg^2 females at 22°C. F1 Gl Sb H/Q-III p^P and st in ri eg^2/Q-IIIp^P females were then crossed separately to p^P/p^P males. All matings were at 22°C. In the former cross, six bottles of

3-day cultures (3 originals, then sub-cultured) were kept at 22°C and 6 were transferred to 29°C. In the latter cross, the 3 originals and 3 subcultures were shifted to 29°C, while two more broods were retained at 22°C. Progeny of all bottles were scored until the twentieth day after the parents had been introduced. As many as possible of the Q-III-bearing recombinants (or putative multiples) recovered at 29°C, and all recombinants from 22°C cultures, were tested to verify their genotypes with respect to Q-III or other recessive markers. In addition, the 22° recombinants were crossed at 29° and 22°C so that their progeny could be scored for other phenotypic traits associated with the Q-III chromosome.

Complementation of Q-III with other proximal mutations on chromosome 3

Df(3L)M(3)LS4 is a Minute mutation associated with a cytologically-observable deletion in a proximally-located segment of the left arm of chromosome 3 (see CHAPTER 4). Thus, males from this stock were crossed to Q-III/TM3 females to test for complementation at 22° and 29°C.

Q-III heterozygotes sometimes display reduced eyes at 29°C. Since this is also a trait characteristic of Dfd and $Antp^{Ns}$, males from the stocks $Dfd\ p^{P}/Dfd\ p^{P}$, Ns/Ns and $ru\ h\ Ki\ Antp^{G}\ e^{s}$/TM3 were crossed to Q-III/TM3 females to test for complementation at 22° and 29°C.

2. Developmental Analysis

Tests for sterility and maternal effects

Fifteen homozygous Q-III/Q-III females (0 to 24 hours in age) were individually mated with 5 p^{P}/p^{P} males in shell vials and transferred for four consecutive 2-day broods to fresh vials at 22°C. Subsequently, fresh males were added and the cultures exposed to 28°C for

two additional 3-day broods. New males were added for the second 28°C brood. The vials were left at their respective temperatures and later examined for the appearance of any developmental stages. A similar study involving 15 Q-III/Q-III males was initiated.

To test for any maternal effects, Q-III/Q-III females were mass mated to homozygous p^P/p^P males at 22°C and 322 eggs were collected over a 2-hour period. Of these, 102 were shifted to 29°C while the remaining 220 were kept at 22°C. All eggs were periodically examined for signs of development.

Regular temperature shift studies

For detailed descriptions of the rationale and experimental procedures for determining TSPs, see Tarasoff and Suzuki (1970) and Suzuki (1970). For the present study, the beginning of the TSP was usually defined as the first point when a culture that was shifted to the permissive temperature produced significant numbers of mutant animals (or decreases in viability), and the end of the TSP was defined as the first point when a culture that was shifted to the restrictive temperature produced non-mutant animals (or significant levels of viability).

Developmental stages present in the cultures were determined either by inspecting cultures at 12-hour intervals (or in a few cases, at 6-hour intervals) for the duration of development at 22° and 29°C or by scoring one of the cultures at the time of each shift. Since all shift experiments involved crosses producing a minimum of two classes, at least 20 (usually 30 to 40) progeny were staged at a given interval. The standard method of scoring larval mouthparts was used to distinguish between the different larval stages (Bodenstein, 1950).

Cultures were shifted from permissive (22°C) to restrictive temperatures (29°C) and vice versa at 12-hour intervals. All cultures were inspected every 12 to 18 hours and progeny scored for up to 25 days after the eggs were collected.

(a) TSP for recessive lethality of Q-III

A total of 400 to 500 eggs (50 to 60 eggs per vial) was shifted at each 12-hour interval after oviposition. Developmental stages reached in the cultures at the time of shifts were assessed by scoring the progeny in one extra vial. Furthermore, a detailed assessment of stages reached in cultures kept at 22° or 29°C was provided by inspection at 6-hour intervals during the larval stages.

(b) TSP for dominant eye and bristle phenotypes of Q-III

Since Q-III heterozygotes at 29°C possess rough and less frequently, reduced eyes as well as short and thin bristles, the TSPs for these phenotypic traits were studied. The reciprocal crosses Q-III/TM3 females x p^p/p^p males and Q-III/TM3 males x p^p/p^p females were used to provide eggs. Since results in the two lines were similar, the samples were pooled. A total of 400 to 500 eggs was shifted (50 to 60 eggs per vial) at each interval. Developmental stages present in the samples were determined at the time of shift. The larval stages present were further assessed by 6-hour inspection of parallel cultures at 22° and 29°C. The adult flies were scored for the occurrence of roughened eye surfaces and bristle disruptions.

(c) TSP for vg-Q-III interaction

The recessive mutation vestigial (vg), on chromosome 2 (at 67.0, see Lindsley and Grell, 1968) causes a marked reduction of wing size. Since it is known that in the presence of Minutes, vg/+ flies exhibit

wing scalloping (Green and Oliver, 1940), a vg stock was crossed to Q-III and the resulting 22° and 29°C progeny examined (see the part on Q-III interactions). At 29°C a high frequency of the double heterozygotes displayed nicked wing margins (particularly distally), while at 22°C, essentially no interaction was apparent. It was therefore decided to study the TSP of this interaction. Only one set of experiments was performed. Eggs were collected from the cross +/+; Q-III/TM3 females x vg/vg;+/+ males, and 100 to 200 (50 to 100 per petri plate) were shifted at 12-hour intervals. Parallel 22° and 29°C cultures were examined every 12 hours to determine the developmental stages present. The adult flies were scored for the scalloped wing phenotype.

(d) TSP for D1-Q-III interaction

Since Schultz (1929) reported that some Minutes exhibit lower viability when combined with different alleles of Delta (see Table 1 and Lindsley and Grell, 1968), it was thought that Q-III might interact similarly with D1. Preliminary crosses of a D1 stock (eg^2 D1 see CHAPTER 4) with Q-III produced no D1/Q-III heterozygotes at 29°C, while normal heterozygotes survived at 22°C (see Q-III interactions). It was therefore decided to study the TSP of this interaction. Eggs were collected from the cross Q-III/TM3 females x D1/TM3 males, and 250 to 300 of these were shifted at 12-hour intervals. Developmental stages present at the time of shift were determined by inspecting the culture in an extra vial for each shift at both temperatures. Q-III/D1 flies could be distinguished by their extremely retarded development at 29°C. The survival of adult flies was scored at both temperatures.

(e) TSP for Scx-Q-III interactions

In testing for Scx-Q-III interactions (these were primarily designed to see if the sex comb phenotype of Scx could be suppressed, see the part on Q-III interactions), it was discovered that Scx/Q-III heterozygotes exhibit low viability at 29°C as well as marked scalloping or nicking of the posterior wing margin, while exhibiting no such phenotypic interaction at 22°C. It was decided to study the TSP of this interaction. Eggs were collected from the cross, Q-III/TM3 females x Scx/TM3 (ru h st ri Scx p^p e^s/TM3) males, and 200 to 300 of these (50 to 60 per vial) were shifted at 12-hour intervals. Developmental stages present in cultures at the time of shift were determined as for (d). The adult flies were scored for the presence of nicks in the wing margin.

It should be mentioned that the distinction between Q-III and other heterozygous progeny classes was considerably facilitated by the retardation of growth of Q-III larvae at 29°C. This was also true of Q-III/Q-III homozygotes at 22°C. Furthermore, both the Q-III homozygotes at 22° and heterozygotes at 29°C frequently possess diagnostic internal melanization which provided an additional marker.

Pulse shift studies

Eggs of Q-III/TM1 x Q-III/TM1 matings at 22°C were collected at 2-hour intervals and at various times during development, shifted to 29°C for a period of 24 to 48 hours. The numbers of eggs tested varied for different intervals (see APPENDICES 2 and 3). Petri plate cultures were used for the shifts (50 to 100 eggs per plate) and owing to the differential rate of development of Q-III, no synchronization

was attempted. Several plates were allowed to develop continuously at 22° and 29°C and the developmental stages reached in these cultures were assessed every 12 hours. The progeny were scored daily until at least 20 to 25 days after the eggs had been collected. The numbers of survivors and the occurrence of various phenotypic traits were noted. In some cases, imagoes incapable of emerging from the pupal cases were dissected and inspected. The TSP of a particular mutant defect was defined as the developmental interval when exposure to 29°C would elicit the mutant phene in a significant proportion of the progeny.

Scanning electron microscopy

Selected flies were anaesthetized with CO_2, mounted on chucks using silver conductant paint and examined alive in a scanning electron microscope (SEM, Cambridge Instruments, Cambridge, England).

Q-III interactions

It has been found recently that some Minutes suppress the expression of the extra sex comb phenotypes of the various sex comb homeotic mutations (R. Denell, personal communication). In order to test whether Q-III is capable of effecting similar suppression, groups of Q-III/TM3 females were crossed separately to ru h Msc e^s/TM3, ru h st ri Scx p^p e^s/TM3 and Pc^3/TM3 males in bottles (this and all subsequent Q-III tests involved 5 to 10 bottles at 22°C, sub-cultured to 29°C), and the progeny grown at 22° or 29°C. The adults were scored for viability and the occurrence of visible phenotypes, particularly the presence of sex combs on the second pair of legs of males. Preliminary tests revealed that none of the recessive markers present on the

test chromosomes (singly or in combination) interacts with Q-III. Henceforth the stocks tested will simply be referred to as Msc, Scx and Pc.

A lethal interaction has been reported for the combination of Lyra and $M(3)h^{33j}$ (Lindsley and Grell, 1968). To test for a similar interaction between Q-III and Ly, Q-III/CxD females were crossed to Ly/CxD males at 22° and 29°C and the offspring were scored for survival.

Tests of other Minutes with homeotics

In order to assess interactions of other Minutes with the sex comb homeotics, Df(3L)M(3)LS4/TM3 males were crossed to females carrying Scx, Msc or Pc. Five bottles of each cross were established and after 3 days, subcultured for another 3 days. Similarly, M(2)173/SM5 males were crossed to Scx and Msc in two bottles which were then each subcultured once after 3 days. The adult male progeny of all crosses were scored for the presence of sex combs on the second pair of legs. Also, viability effects and the occurrence of any other phenotypes were noted in the adults.

III. Results

Only the numbers of eclosing adults were recorded in the studies of viability. In other tests, cultures were examined at least every 24 to 48 hours and the numbers of animals reaching key stages (first instar larva, pupa and adult) were noted. The amount of lethality for each stage was estimated on the basis of the expected frequency of each class, relative to the number of eggs for a given cross. Similarly, percent viabilities were calculated using the expected frequencies from the following crosses: Controls, I all progeny $\underline{p}^{p}/\underline{p}^{p}$; II 0.5 $\underline{CxD}/\underline{p}^{p}$, 0.5 $\underline{TM3}/\underline{p}^{p}$. Experimentals (A) 0.5 $\underline{Q\text{-}III}/\underline{p}^{p}$ 0.5 $\underline{TM3}/\underline{p}^{p}$; (B) 0.5 $\underline{Q\text{-}III}/\underline{TM1}$, 0.25 $\underline{Q\text{-}III}/\underline{Q\text{-}III}$, 0.25 $\underline{TM1}/\underline{TM1}$ (egg lethal); (C) 0.5 $\underline{Q\text{-}III}/\underline{TM3}$, 0.25 $\underline{Q\text{-}III}/\underline{Q\text{-}III}$, 0.25 $\underline{TM3}/\underline{TM3}$ (egg lethal). Percent viabilities were calculated as: (Observed number of live progeny/Expected number of live progeny) x 100, where Expected number = Expected proportion of eggs x Total number of eggs.

1. Genetic Analysis

Viability

The crosses to assess $\underline{Q\text{-}III}$ viability (Table 21A,B and C) show that relative to the controls, $\underline{Q\text{-}III}$ is essentially fully viable at 22°C when heterozygous with $\underline{p}^{p}$, $\underline{TM3}$ (also at 17°C) or $\underline{TM1}$, while at 29°C the percent viabilities for these classes were 49, 13 and 0 respectively (where applicable, homozygotes for $\underline{TM1}$, $\underline{TM3}$ and $\underline{CxD}$ have been considered as egg lethals). The exact reason for the low viability of $\underline{Q\text{-}III}$ in combination with the two balancers is not known. Two of the inversions present in $\underline{TM3}$ have the following breakpoints

Table 21

Relative Viabilities of Q-III Homozygotes and Heterozygotes at Different Temperatures

Crosses	Temperature	Number of eggs	Progeny Genotypes	Number of Adults	Percent Viability
Control I					
$p^p/p^p \times p^p/p^p$	22°C	195	p^p/p^p	151	77.4
	29°C	200	p^p/p^p	149	74.5
Control II					
CxD/TM3 x p^p/p^p	22°C	105	CxD/p^p	38	72.4
			TM3/p^p	42	80.0
	29°C	145	CxD/p^p	54	74.5
			TM3/p^p	55	75.9
(A)					
Q-III/TM3 x p^p/p^p	22°C	739	Q-III/p^p	309	83.6
			TM3/p^p	291	81.5
	29°C	783	Q-III/p^p	192	49.0
			TM3/p^p	329	84.0
(B)					
Q-III/TM1 x Q-III/TM1	22°C	642	Q-III/TM1	256	76.3
			Q-III/Q-III	44	27.4
	29°C	900	Q-III/TM1	0	0
			Q-III/Q-III	0	0

Table 21 (continued)

Crosses	Temperature	Number of eggs	Progeny Genotypes	Number of Adults	Percent Viability
(C)					
	17°C	800	Q-III/TM3	335	88.8
			Q-III/Q-III	62*	31.0
Q-III/TM3 x Q-III/TM3					
	22°C	825	Q-III/TM3	357	86.6
			Q-III/Q-III	51	24.8
	29°C	1034	Q-III/TM3	69	13.4
			Q-III/Q-III	0	0

*22/62 showed scutellar disruptions

(see Lindsley and Grell, 1968): In(3LR)79E;100C and In(3LR)76C;93A. It is noteworthy that M(3)LS4 resides in the segment 79D to 80 or 81, while M(3)S34 is located within the segment 75D to 76C. Thus, both proximal Minute loci could be under the influence of some sort of position effect which potentiates the lethal effects of Q-III. Alternatively, the dominant markers Sb and Ser carried by TM3 could be interacting with Q-III. However, the latter idea is less likely, since tests of TM3 without these dominants resulted in similar low frequencies of Q-III/TM3 progeny at 29°C. TM1 has no comparable inversion breakpoints. However, it is possible that Moiré, a recessive lethal marker carried by this balancer, interacts with Q-III to produce synthetic lethality of TM1/Q-III progeny at 29°C. This possibility was not pursued.

In all crosses the heterozygotes which survived continuous exposure to 29°C, displayed small, thin bristles (see Figure 10), a roughened eye surface (sometimes reduced or malformed eyes), slightly pale body colour and occasionally, leg deformities.

In contrast to complete viability of Q-III heterozygotes, only 25 to 31 percent of the expected number of Q-III homozygotes (crosses B and C) survived to adulthood at 22° and 17°C, while none survived continuous exposure to 29°C throughout development. The homozygous adults surviving at 22° and 17°C had bristles with a thickness that appeared to be intermediate between those of Q-III/+ and +/+ at 29°C. In addition, they exhibited slightly roughened eyes, pale body colour and frequently, internal melanotic masses (particularly within the abdomen). At 17°C, one third or more of the homozygotes also displayed a disrupted

thorax phenotype. In the least severe cases this trait consists of extra or misplaced bristle sockets on the scutellum and in the most severe cases, grossly distorted or malformed scutella. The prolonged-development characteristic of Minute heterozygotes was also seen for Q-III/+ flies at 29°C and Q-III/Q-III flies at 17° and 22°C. Clearly the mutation Q-III is very pleiotropic with a complex of different phenotypic effects.

Mapping

The results of the mapping experiments are presented in Table 22. They show that Q-III is located between Gl and p^p (Table 22a), indeed it is between st and p^p (b). Owing to the variability in survival of Q-III-bearing flies, unequivocal localization was difficult but a tentative position based on the dominant semi-lethal effect was computed. Thus, Q-III maps to 45.4 relative to the recessive markers. The location of Q-III cannot be unambiguously assigned. Its most likely position is between st and in, but the in to p^p interval cannot be completely ruled out. The ambiguity arises from the recovery of st Q-III$^+$ p^p (i.e. st in^+ ri^+ p^p), st in ri Q-III p^p (i.e. st in ri p^p—M) and st in Q-III p^p (i.e. st in ri^+ p^p—M) recombinants at 29°C. If Q-III lies between st and in, the latter two classes could be generated only by triple crossovers, and triple crossovers are again required to explain the occurrence of st Q-III$^+$ p^p recombinants, if Q-III is between in and p^p. In either case, the observation of putative multiple exchange classes extends the report that multiple crossovers occur within short genetic intervals in proximal regions of chromosome 3 (Sinclair,

Table 22

Crossover Data From Crosses Designed to Localize Q-III

a) $\underline{Gl\ Sb\ H}/\underline{Q\text{-}III\ p^p}$ Females x $\underline{p^p}/\underline{p^p}$ Males

Type	Genotype/$\underline{p^p}$	Number of Progeny in Each Class		
		$\underline{22^oC}$	$\underline{29^oC}$	
Parentals	$\underline{Gl\ Sb\ H}$	861	562	
	$\underline{p^p}$	748	30	
SCO 1	$\underline{Gl\ p^p\ Sb^+\ H^+}$	35	2	(1M)*
SCO 2	$\underline{Gl^+\ p^{p+}\ Sb\ H}$	51	25	(1M)
SCO 3	$\underline{Gl\ p^{p+}\ Sb^+\ H^+}$	138	60	
SCO 4	$\underline{Gl^+\ p^p\ Sb\ H}$	123	4	(M)
SCO 5	$\underline{Gl\ p^{p+}\ Sb\ H^+}$	92	85	
SCO 6	$\underline{Gl^+\ p^p\ Sb^+\ H}$	138	1	
DCO 1	$\underline{Gl\ p^{p+}\ Sb^+\ H}$	2	1	
DCO 2	$\underline{Gl^+\ p^p\ Sb\ H^+}$	2	1	(M)
DCO 3	$\underline{Gl^+\ p^{p+}\ Sb\ H^+}$	5	2	
DCO 4	$\underline{Gl\ p^p\ Sb^+\ H}$	2	0	
DCO 5	$\underline{Gl\ p^p\ Sb\ H}$	9	0	
DCO 6	$\underline{Gl^+\ p^{p+}\ Sb^+\ H^+}$	11	3	
TCO 1	$\underline{Gl^+\ p^{p+}\ Sb^+\ H}$	1	0	
Totals		2218	776	

b) st in ri eg^2/Q-III p^p Females x st in ri p^p/st in ri p^p Males

Type	Genotype/st in ri p^p	Number of Progeny in Each Class	
		22°C	29°C
Parentals	st in ri	1315	1405
	p^p	1345	402
SCO 1	st in^+ ri^+ p^p	10 (M)	8 (5M)
SCO 2	st^+ in ri p^{p+}	6	23
SCO 3	st in ri^+ p^p	0	1 (M, sterile)
SCO 4	st in ri p^p	1	9 (1M)
SCO 5	st^+ in^+ ri^+ p^{p+}	4 (M)	4 (M)
Totals		2681	1852

Map position of Q-III: 45.4 map units

M = Minute phenotype (delayed eclosion, thin, small bristles)

1975). If Q-III is located between st and in, it is surprising that no Q-III in ri crossovers were recovered at 22°C.

Progeny tests of the recombinants recovered at 22°C (included in Table 22b) support the suggestion that Q-III lies closely linked to in between st and in. It is important to mention that a Minute locus exists between st and in, while two are located between ri and p^p, one on either side of the centromere.

In addition to the initial progeny tests, several recombinant chromosomes were cloned at 29° and 22°C. Almost all of the pleiotropic phenotypes (to be referred to later) attributable to Q-III, segregated with the mutation.

Two Q-III p^p Sb^+ H recombinants that had been generated at 22°C were used to make stocks of Q-III for use in the shift studies. Thus, the marker H was removed from the chromosome via crossing over and with it a second site cold-sensitive mutation that rendered Q-III lethal at 17°C.

Test of Q-III in triploids

In a preliminary test of Q-III in triploids, Q-III/TM3 males were crossed to C(1)RM, y^2 sc w^a ec/FM6;3A females in shell vials and C(1)RM, y^2 sc w^a ec/X;Q-III/+/+ females isolated at 29°C. Five such females were scored and none displayed the M phenotypes, thereby indicating that Q-III is recessive in triploids.

Complementation tests

The complementation data have been converted to a viability index parameter. Thus, the viability index, V.I. = Number (Q-III/mutant)/

Table 23

Relative Viability of Q-III in Combination With Various Mutations at 22° or 29°C

Mutant	22°C V.I.*	22°C Total Progeny	29°C V.I.	29°C Total Progeny
Df(3L)M(3)LS4	1.95	428	0	231
Dfd	1.25	234	0	200
Ns	1.18	804	0.09	246
$Antp^{G}$	1.44	814	0.19	38

* V.I. (Viability Index) = $\frac{\text{Number of Q-III/mutant}}{\text{Number of TM3/mutant}}$

Number (mutant/TM3). Remember that at 29°C, all Q-III heterozygotes are half as viable as wild-type and therefore some decrease in the viability of heterozygotes is expected. However, the V.I. estimates should not be lower than 0.5, if Q-III is viable with these mutations.

The results of the complementation crosses of Q-III are shown in Table 23. In each case, Q-III was viable in combination with the mutations tested at 22°C, while it was lethal when combined with either Dfd or Df(3L)M(3)LS4 at 29°C. Furthermore, Q-III was semi-lethal in combination with Ns (V.I. = 0.09), and $Antp^{G}$ (V.I. = 0.19) at 29°C (although in the latter case, very few progeny of any class resulted). Any uneclosed Q-III/Dfd pupae (at 29°C) which were dissected showed a complete lack of head (i.e. eye-antennal disc) structures, and sometimes uneverted pigmented ommatidia within their thoraces. The phenotype of these flies resembled Arking's (1975) illustration of l(1)ts480, a sex-linked ts autonomous cell-lethal. No Df(3L)M(3)LS4/Q-III pupae were found at 29°C, thereby showing that an earlier lethal phase exists for these heterozygotes.

Since the most likely map position of Q-III is distal to M(3)LS4, their lethality in combination may reflect the propensity of Q-III to interact with different loci such as Dfd, rather than allelism. Furthermore, the relatively normal viability of Df(3L)M(3)LS4/Q-III flies at 22°C argues against allelism. However, it should be noted that $l(1)su(f)^{ts67g}$ (Dudick et al., 1974) is viable when heterozygous with a deficiency for the su(f) locus at 18° and 25°C, but no heterozygotes of this type survive at 29°C.

Table 24

The Lengths of the Developmental Periods From Egg Deposition to Eclosion in Different Classes at Different Temperatures

Genotypes of Parents	Genotypes of Progeny	Data From Different Temperatures: 17°C Total Eggs	17°C Number Eclosing	17°C T 1/2* (hrs.)	22°C Total Eggs	22°C Number Eclosing	22°C T 1/2 (hrs.)	29°C Total Eggs	29°C Number Eclosing	29°C T 1/2 (hrs.)
Controls										
I. $\frac{p^P}{p^P}$ x $\frac{p^P}{p^P}$	$\frac{p^P}{p^P}$	-	-	-	195	151	300±6	200	149	192±6
II. $\frac{CxD}{TM3}$ x $\frac{p^P}{p^P}$	$\frac{CxD}{p^P}$	-	-	-	105	38	312±6	145	54	192±6
	$\frac{TM3}{p^P}$	-	-	-		42	312±6		55	192±6
Experimentals										
A. $\frac{Q\text{-}III}{TM3}$ x $\frac{p^P}{p^P}$	$\frac{Q\text{-}III}{p^P}$		-	-	200	75	312±6	276	72	240±6
	$\frac{TM3}{p^P}$		-	-		87	312±6		125	192±6
C. $\frac{Q\text{-}III}{TM3}$ x $\frac{Q\text{-}III}{TM3}$	$\frac{Q\text{-}III}{TM3}$	800	335	600±6	500	180	324±6	500	10	300±6
	$\frac{Q\text{-}III}{Q\text{-}III}$		62	672±6		53	372±6		0	-

*T 1/2 (hours) = Time in hours from oviposition to eclosion of half of live progeny.

These data underscore the problems inherent in deciding whether particular mutants are allelic, since the possibility of synthetic lethality should always be considered.

2. Developmental Analysis

Duration of developmental periods

Table 24 summarizes the lengths of developmental periods of controls and Q-III heterozygotes and homozygotes. This interval was defined as the time (in hours) from oviposition (± 2 hours) to the time when half the progeny of a given class had eclosed (since cultures were observed every 12 hours, each time period had an error of about 6 hours). Cross A provides an additional control since TM3/p^p progeny are generated along with Q-III/p^p individuals.

The results of cross C show that the Q-III homozygotes took considerably longer to eclose than did the controls, while the Q-III/TM3 heterozygotes developed at the same rate as the controls. At 29°C only a few heterozygotes survived and the latter took a long time to eclose relative to the controls.

Development of Q-III/p^p progeny in cross A is normal at 22°C but greatly prolonged at 29°C. Thus, in addition to the conditional bristle and eye phenotypes, Q-III/+ heterozygotes also develop more slowly at 29°C than at 22°C. At 22°C the homozygous individuals take longer to eclose than do the controls.

Tests for sterility and maternal effects

Of 15 female homozygotes brooded at 22°C, 5 produced no progeny or eggs while the remainder produced a total of 139 progeny (an average

of 14 offspring per fertile female). Upon shifting to 28°C, two of the above 10 fertile females died during the first brood, while the other 8 produced no progeny. Examination of the 28°C vials revealed a few white (and some brown) eggs. After the second 28°C brood, no eggs were detected. Seven females that had been mated to $\underline{p^p}/\underline{p^p}$ males for five days at 28°C were dissected in Drosophila Ringer's solution and their ovaries examined with a compound microscope. Sperm were abundant in the seminal receptacles. The ovaries contained degenerate oocytes which appeared to be heterogeneous for early stages of oogenesis and in few cases, polytenic nuclei were detected.. The latter were presumably undegenerated nurse cells (Miller, 1950).

The study of fertility of Q-III/Q-III males was abandoned when only 2 of 15 showed fertility at 22°C, and that fertility was very poor. When such homozygotes were mated to $\underline{p^p}/\underline{p^p}$ females at 28°C, eggs were laid but failed to develop, thereby suggesting that both homozygous males and females are completely sterile at 28°C. When homozygous males and females are crossed at 22°C, white eggs are deposited but no development occurs.

Of the 102 eggs which were produced by homozygous Q-III females (mated to $\underline{p^p}/\underline{p^p}$ males) and transferred to 29°C, 70 remained white while 30 turned dark after one or two days but development ceased. In only 2 cases did any larval development ensue and in both cases, the larvae turned black and died shortly after hatching. Of the 220 eggs kept at 22°C, 107 exhibited varying degrees of darkening, 42 remained white and 71 hatched as larvae. Of the 71 larvae, 60 eclosed as phenotypically normal adults. It is worthy of mention that eggs

which remain white could be either unfertilized eggs or embryos in which development was blocked at very early stages. On the other hand, eggs which turn dark after a few days are assumed to be embryonic lethals (Wright, 1973). It therefore appears that Q-III can exert a ts maternal effect, since eggs produced by homozygous Q-III females mated to normal males are essentially incapable of supporting normal development at 29°C, while at least a significant proportion of such eggs incubated at 22°C, develop normally.

Stage distribution of lethality of Q-III homozygotes and heterozygotes

Even as early larvae the Q-III/Q-III progeny could be distinguished from the Q-III/TM1 or Q-III/TM3 heterozygotes due to their slower development and internal melanization at 17° and 22°C. At 29°C, since the homozygotes never progressed past the first larval instar, this distinction was also possible.

Most Q-III/p^p progeny developed considerably more slowly than p^p/TM3 types at 29°C (but not at 22°C) and therefore, Q-III/p^p larvae could be unequivocally classified on this basis (as well as on that of internal melanization). However, some overlap of classes did exist. For this reason, stage-specific lethality was determined for all progeny of the cross of Q-III/TM3 females to p^p/p^p males, without attempting to separate the classes, on the assumption that most ts lethality would be due to the death of Q-III/p^p individuals which would be reflected in the results.

Table 25 illustrates the results of the analysis of stage by stage distribution of lethality during the embryo, larval and pupal stages

Table 25

Lethality of Control and Q-III - Bearing Progeny at Different Developmental Stages at Various Temperatures

Genotype of Progeny	Percent Mortality											
	17°C				22°C				29°C			
	Number of Eggs	E	L	P	Number of Eggs	E	L	P	Number of Eggs	E	L	P
Controls												
p^P/p^P	-	-	-	-	195	14.4	5.6	2.6	200	8.5	16.5	0.5
CxD/p^P												
CxD/TM3	-	-	-	-	105	12.4	4.8	6.7	145	8.3	10.3	6.2
Experimentals												
Q-III/TM1	-	-	-	-	300	0.5	20.5	5.5	450	21.6	71.9	6.5
p^P/TM3												
Q-III/p^P	-	-	-	-	268	8.7	9.4	5.0	250	15.0	23.1	12.1
Q-III/Q-III	100	3.0	26.0	40.0	200	2.0	44.0	22.0	225	52	48(L1)*	0

*L1 = First larval instar E = Embryo L = Larva P = Pupa

of the controls and of the various progeny arising from the crosses: Q-III/TM3 x p^p/p^p (22° and 29°C); Q-III/TM1 x Q-III/TM1 (22° and 29°C); and Q-III/TM3 x Q-III/TM3 at 17°C (see Table 21).

All Q-III/TM1 heterozygotes died when exposed continuously to 29°C. More than 70 percent died as larvae. Larval death was also most common for Q-III/p^p progeny at 29°C. At 22°C, again a high proportion of Q-III/TM1 types died as larvae, while mortality was more evenly distributed between the three stages for Q-III/p^p progeny. In comparison, Q-III homozygotes died equally as frequently as embryos or first instars at 29°, while at 22°C the lethality shifted more towards the later larval and pupal stages with very little egg lethality. Furthermore, at 17°C lethality of Q-III homozygotes was chiefly pupal, although frequent larval lethality still occurred. Thus, at progressively lower temperatures, Q-III homozygotes survived to later stages. Most of the Q-III/Q-III pupae at 17° and 22°C contained pale imagoes with bent legs. This may indicate that lethality results from defective sclerotization of the cuticle which leads to desiccation and lack of the muscular control necessary for eclosion.

At 29°C, homozygous Q-III/Q-III larvae died as first instars. Although this death was often not immediate (they sometimes survived for up to 4 or 5 days), no growth or molting took place. They eventually showed internal discolouration and died.

Considerable variation in patterns of lethality in different stages was observed in the controls at all temperatures and generally, larval death increased upon exposure to higher temperatures.

Temperature-sensitive periods for lethality of Q-III

Percent viability was computed for all cultures on the following basis. Since only 25 to 30 percent of Q-III/Q-III progeny survived at 22°, the 22°C level of viability (see cross B, Table 21) was normalized to 100 percent. Thus, for example, the expected number of Q-III/Q-III progeny at 22°C would be: 0.274 x (total number of eggs x 0.25). This kind of analysis was used whenever calculation of such percent viabilities was necessary.

The TSP of Q-III recessive lethality can be inferred in Figure 6. This TSP extends continuously from the latter part of embryogenesis up to the second half of pupation, and therefore the Q-III$^+$ gene product is clearly required throughout most of development.

Since long exposures to the restrictive temperatures can mask successive, but separate TSPs (Poodry et al., 1973; Suzuki, Kaufman, Falk et al., 1976), 24 and 48-hour pulse shifts were performed.

Although a significant number of Q-III/TM1 heterozygotes usually survived most 48-hour heat pulses, three discrete temperature-sensitive intervals were resolved (Figure 7). One spans the latter half of the first instar and the first half of the second instar, another covers most of the third instar, and the third TSP of lethality spans the middle of pupation. On the other hand, the Q-III homozygotes appear to have a single, continuous TSP. These results re-emphasize the importance of the Q-III$^+$ gene product. A lack of this substance for even relatively short periods of development produces death.

Forty-eight-hour heat pulses of homozygotes from the first to mid second larval instar, often resulted in sluggish larvae, many of which did

FIGURE 6 Results of the shift study to delineate a temperature-sensitive period (TSP) for lethality of Q-III homozygotes. The data are given as percent eclosion of Q-III/Q-III progeny that were shifted from 22° to 29°C (closed circles), or from 29° to 22°C (open circles) at various times during development. Temporal estimates of the different developmental stages at 22°C, are indicated below; E = eclosion.

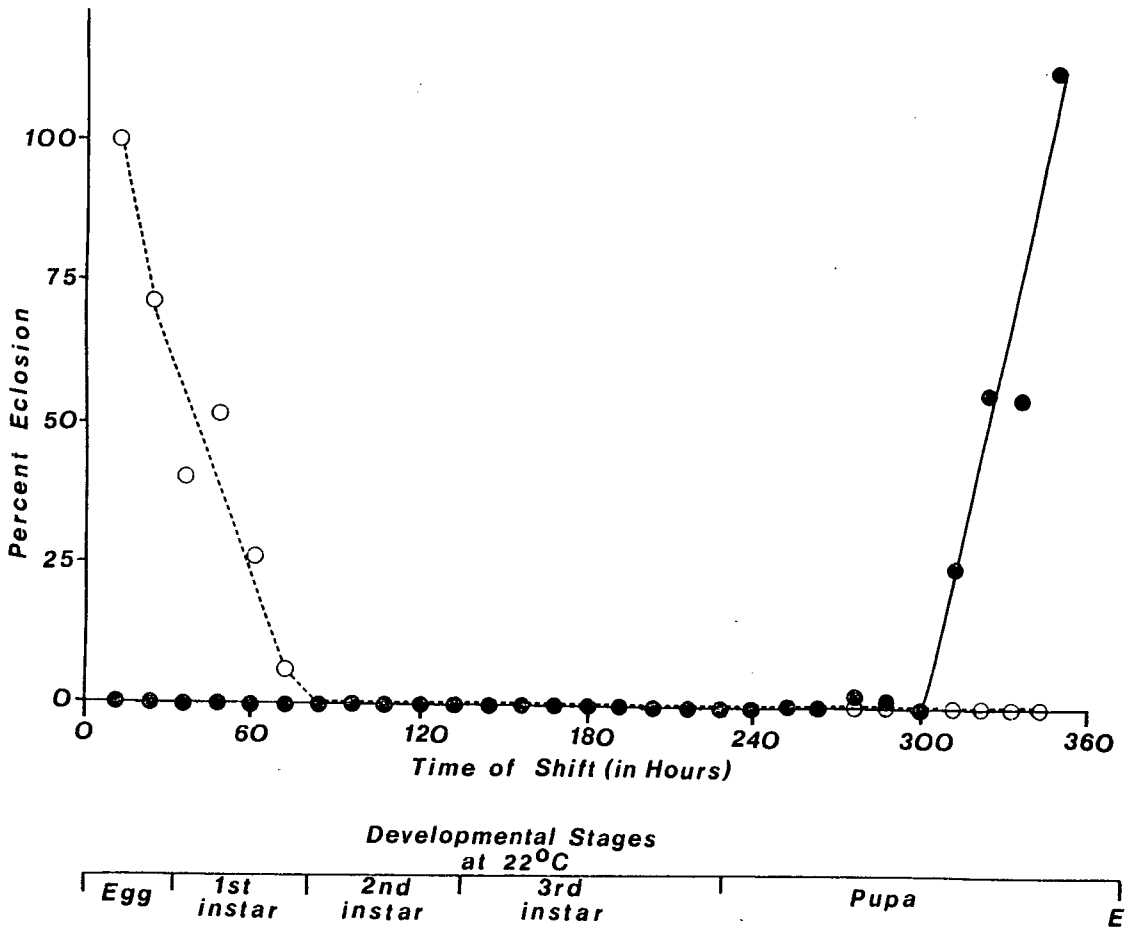
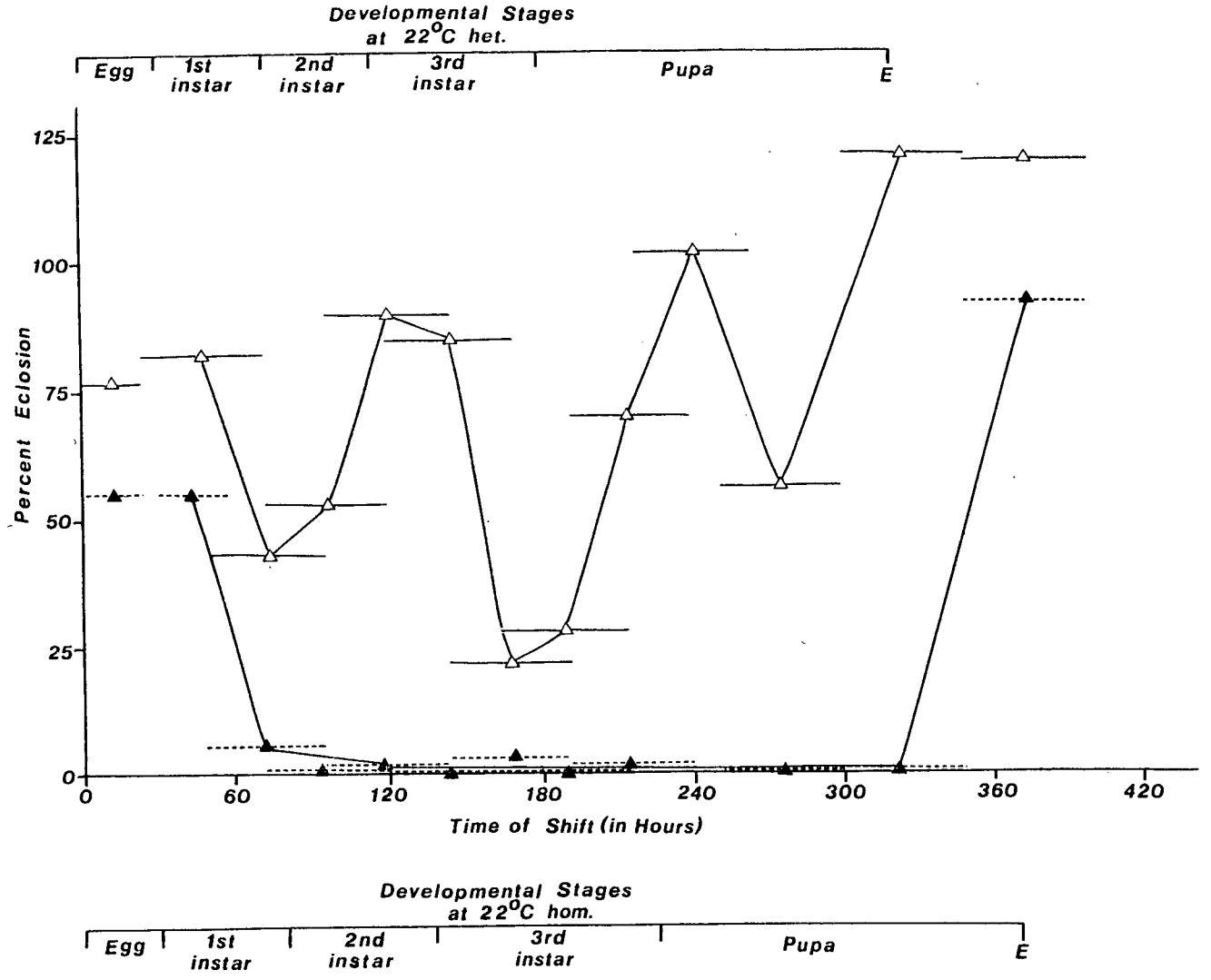

100
75
50
25
0
Percent Eclosion
0
60
120
180
240
300
360
Time of Shift (in Hours)
Developmental Stages
at 22°C
Egg
1st instar
2nd instar
3rd instar
Pupa
E

FIGURE 7 Result of 48-hour pulse shifts to delineate temperature-sensitive periods (TSPs) for lethality of Q-III heterozygotes and homozygotes. The data are given as percent eclosion of Q-III/TM1 (open triangles) or Q-III/Q-III (closed triangles) progeny, that were heat-pulsed for 48 hours, at various times during development (pulses were shorter during embryogenesis). The horizontal bars indicate the duration of the heat pulses to 29°C. Temporal estimates of the different developmental stages of the heterozygotes (het.) and homozygotes (hom.) at 22°C, are indicated above and below, respectively; E = eclosion.

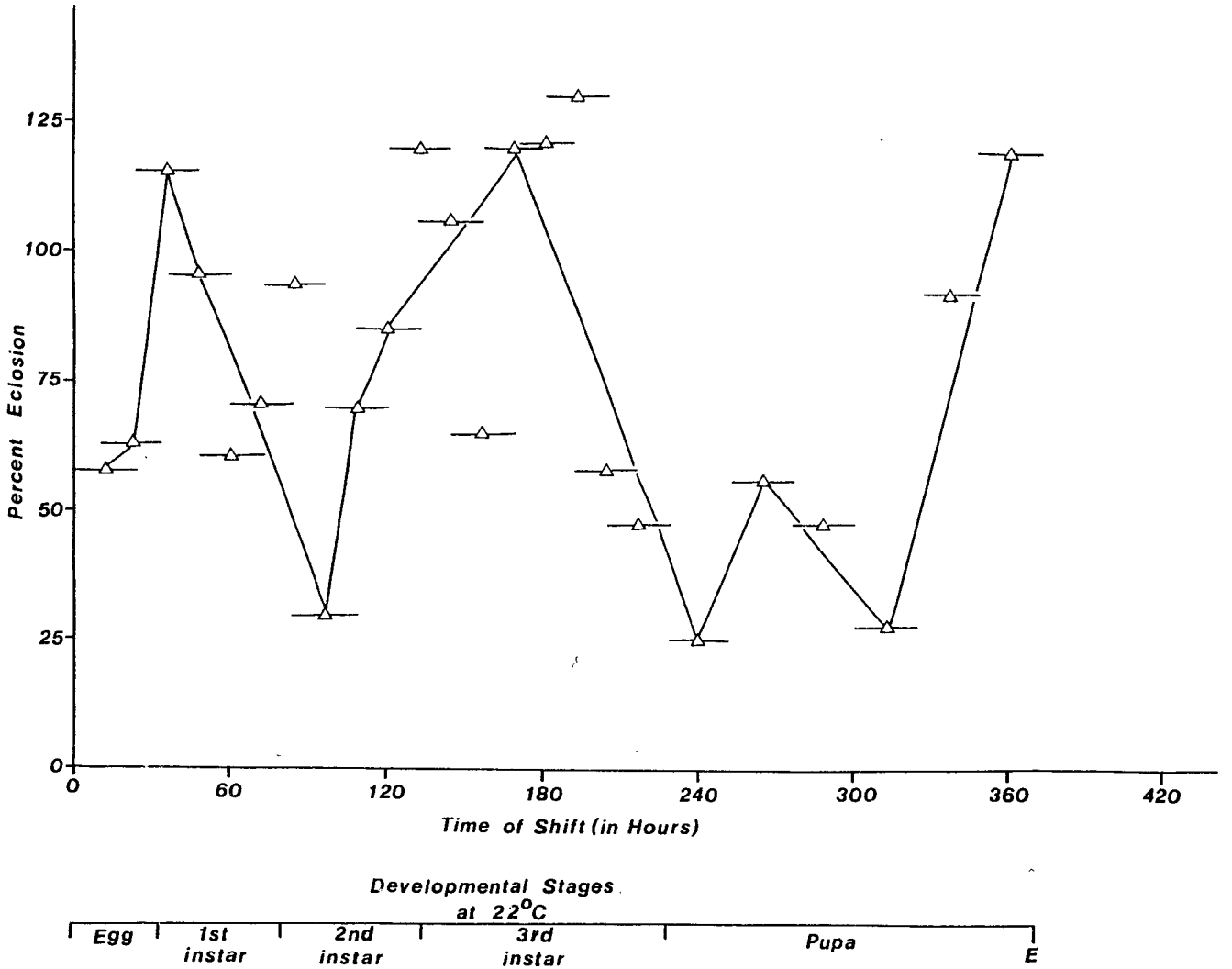

Developmental Stages
at 22°C het.
Egg
1st instar
2nd instar
3rd instar
Pupa
E
Percent Eclosion
125
100
75
50
25
0
0
60
120
180
240
300
360
420
Time of Shift (in Hours)
Developmental Stages
at 22°C hom.
Egg
1st instar
2nd instar
3rd instar
Pupa
E

not grow. These larvae exhibited different degrees of internal discolouration. They sometimes survived for several days after the shift, but most eventually died. Later shifts (i.e. early to late third instar) frequently produced sluggish larvae that never metamorphosed but some also formed incomplete pupae, while still others reached various stages of pupation. The later shifts (late third instar and throughout the first half of pupation) produced pupae which reached various stages including the pharate stage, but never eclosed.

Q-III/TM1 heterozygotes survived 24-hour heat pulses at different stages. However, different lethal TSPs of Q-III homozygotes could be distinguished (Figure 8). The pattern of TSPs is strikingly similar to that resulting from 48-hour heat pulses to Q-III/TM1 heterozygotes (see Figure 7), although the actual position of each TSP is different.

Again, lethality from earlier pulses was primarily larval. For example, a heat pulse from 84 to 108 hours of development (early second instar) produced many dead larvae displaying considerable internal discolouration (probably diagnostic of generalized disruptions). Some pupae were formed but in most cases successful metamorphosis did not occur, although a few managed to complete pupation. Later larval pulses produced progressively more advanced development at the time of lethality. In some cases (for example a pulse from 108 to 132 hours), after surviving homozygous adults had eclosed and were scored, other third instar larvae began to appear in the cultures and these eventually pupated (after several days), but never eclosed. This failure to eclose may have been due to poor leg differentiation, since the legs of dissected pharates were often bent in appearance.

FIGURE 8 Results of 24-hour pulse shifts to delineate temperature-sensitive periods (TSPs) for lethality of Q-III homozygotes. The data are given as percent eclosion of Q-III/Q-III progeny that were heat-pulsed for 24 hours, at various times during development. The horizontal bars indicate the duration of the heat pulses to 29°C. Temporal estimates of the different developmental stages at 22°C are indicated below; E = eclosion.


Percent Eclosion
125
100
75
50
25
0
0
60
120
180
240
300
360
420
Time of Shift (in Hours)
Developmental Stages at 22°C
Egg
1st instar
2nd instar
3rd instar
Pupa
E

The occurrence of this second wave of pupation raises the interesting possibility that larval growth can be reversibly blocked in homozygous Q-III individuals by a brief 29°C exposure during development. This could be further studied by exposing large numbers of synchronously developing larvae to 29°C for even shorter intervals, or alternatively, by employing very short intervals coupled with higher temperatures (e.g. 30° or 31°C).

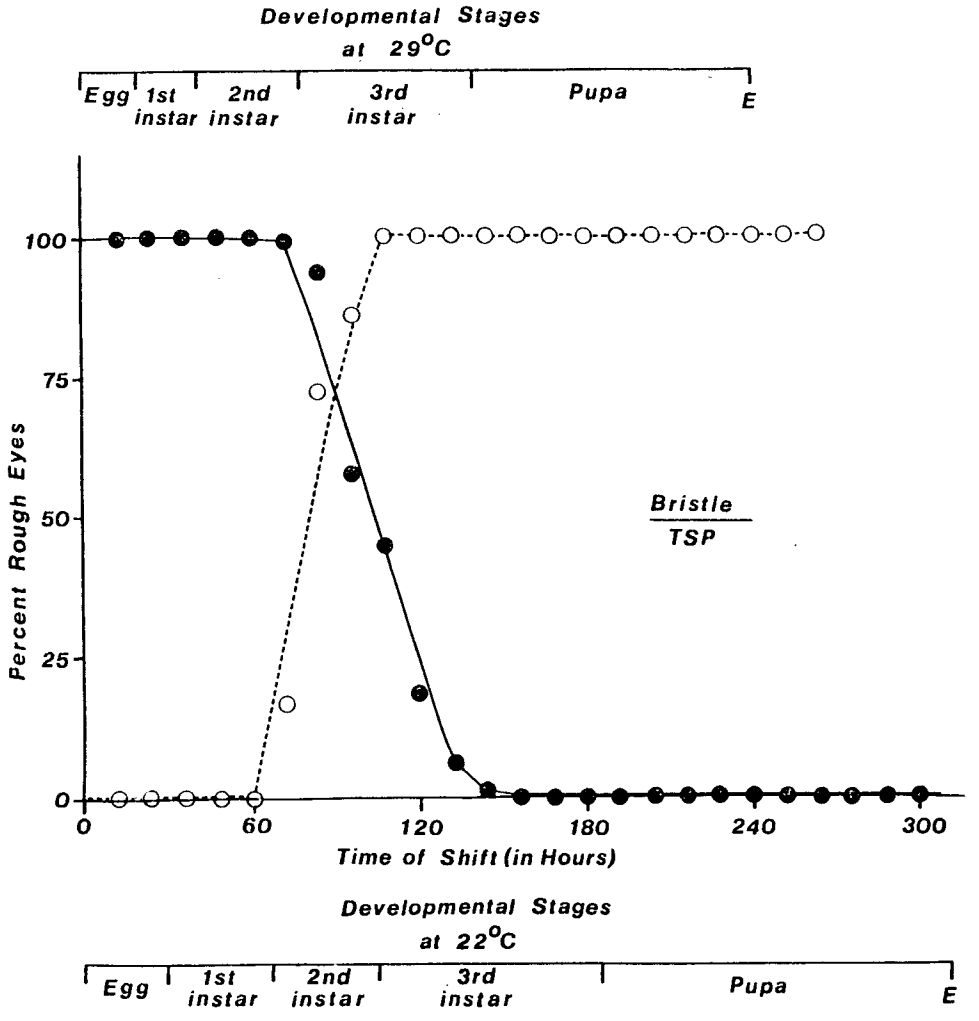
Finally, much of the lethality induced by 24-hour heat pulses during pupation occurred at the pharate stage, with some of the uneclosed flies possessing poorly differentiated legs. All of these were light in colour and many seemed to have been rapidly desiccated (as indicated by collapsed abdomens).

Since only about 30 percent of Q-III homozygotes usually survive at 22°C, conclusions about the patterns of lethality should be viewed with caution. Nevertheless, it does appear that 24-hour heat pulses have resolved three discrete TSPs for inviability: one early in the second instar, one in the late third instar and the early part of pupation, and another just after mid-pupation. Phenotypic descriptions of pulse survivors will be dealt with later.

Temperature-sensitive periods for dominant rough eye and bristle traits

Since heterozygous Q-III (Q-III/+, Q-III/p^p, Q-III/TM3) flies which survive continuous exposure to 29°C have rough eyes and short, thin bristles, TSPs for these phenotypes could be defined (Figure 9). The TSP for the eye phene extends from about a third of the way into the second instar up to the end of this instar. This TSP was also

FIGURE 9 Results of the shift study to delineate temperature-sensitive periods (TSPs) for rough eyes and reduced bristles of Q-III heterozygotes. The eye data are given as percent expression of rough eyes in Q-III/p^p progeny that were shifted from 22° to 29° (closed circles), or from 29° to 22°C (open circles), at various times during development. The TSP for the bristle phene was also provided and is indicated by the horizontal bar. Temporal estimates of the different developmental stages at 22° and 29°C, are indicated above and below, respectively; E = eclosion.


Developmental Stages
at 29°C
Egg
1st instar
2nd instar
3rd instar
Pupa
E
100
75
50
25
0
Percent Rough Eyes
Bristle
TSP
0
60
120
180
240
300
Time of Shift (in Hours)
Developmental Stages
at 22°C
Egg
1st instar
2nd instar
3rd instar
Pupa
E

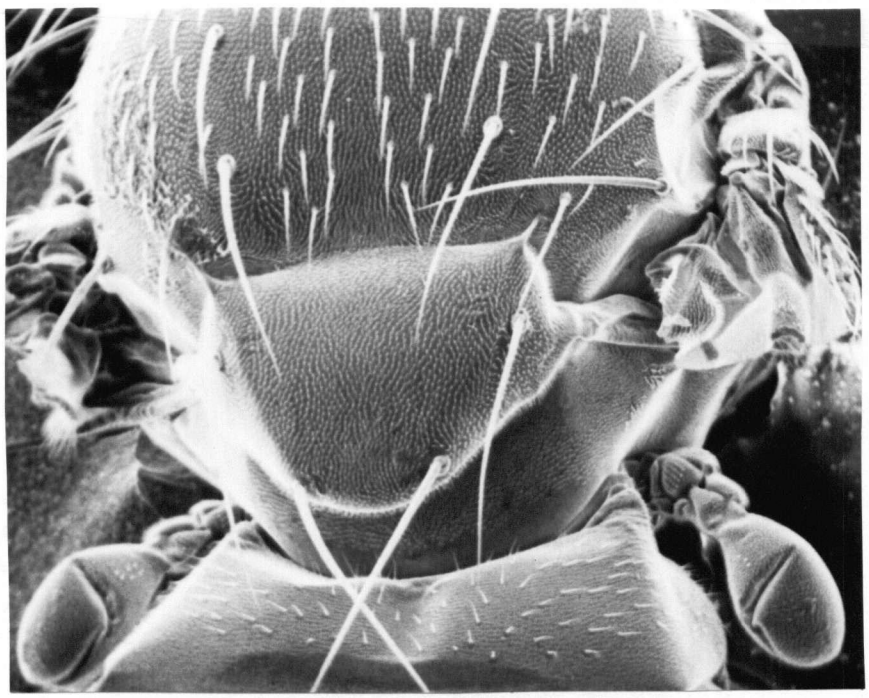
observed after 24 and 48-hour pulses (see APPENDICES 2 and 3) and it overlaps the earlier TSP for inviability that was also resolved by pulse shifts. It is noteworthy that this TSP also coincides with the developmental interval during which intense mitotic activity has been reported for the eye imaginal discs (see Nothiger, 1972). The probable basis for this phenotype will be dealt with later.

The TSP for the bristle phene was initially delineated to a 34-hour period during the first half of pupation. The 24-hour pulse studies of heterozygous Q-III/TM1 progeny allowed further resolution of this TSP to a 16-hour interval prior to mid pupation (before the time when yellow pigment is deposited in the eyes). Moreover, it was possible to verify this TSP in homozygotes surviving 24-hour heat pulses (although the TSP of the bristle trait in the homozygote differs somewhat from that in the heterozygote, see Figure 20). Scanning electron micrographs of the bristle phenotype can be seen in Figure 10. Note the reduction in or absence of thoracic macrochaetae in the homozygote (c), whereas the phenotype is less severe in heterozygotes (b). The abdominal bristles were also slightly reduced in size in the homozygote. It is noteworthy that the bristle TSP corresponds to the pupal interval during which the thoracic and abdominal bristles are formed (Bodenstein, 1950).

Phenotypes revealed by shift experiments

Since nearly all heterozygous phenotypes observed in the shift experiments (both pulse and regular TSP shift studies) were also seen (and were usually more extreme) in Q-III homozygotes, only the phenotypes of the latter will be dealt with in detail. However, reference

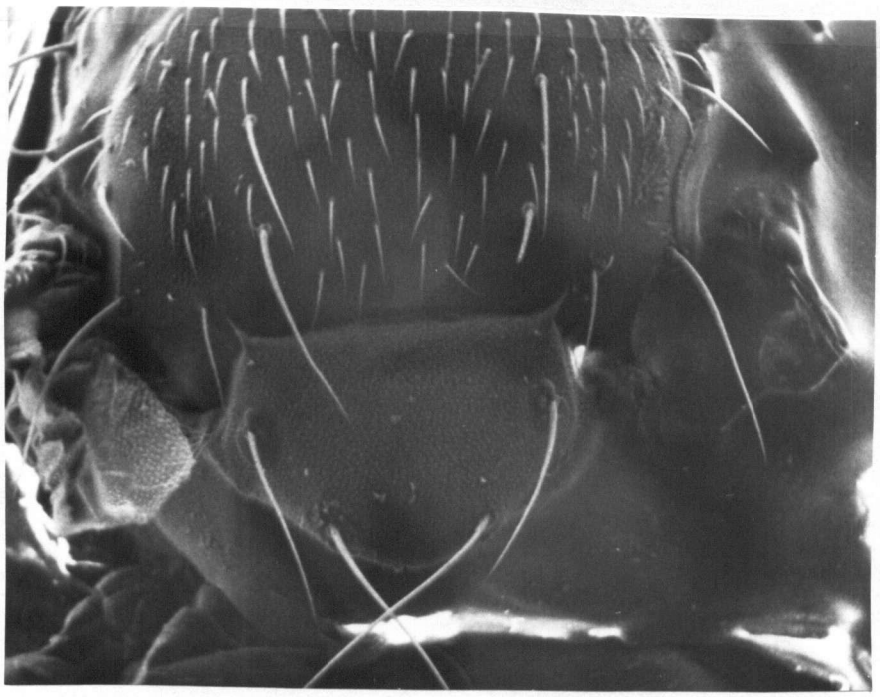
FIGURE 10 Scanning electron micrographs showing the effects of Q-III on macrochaete development; (a) a control (p^P/p^P) fly, grown at 22°C (magnification about x400);



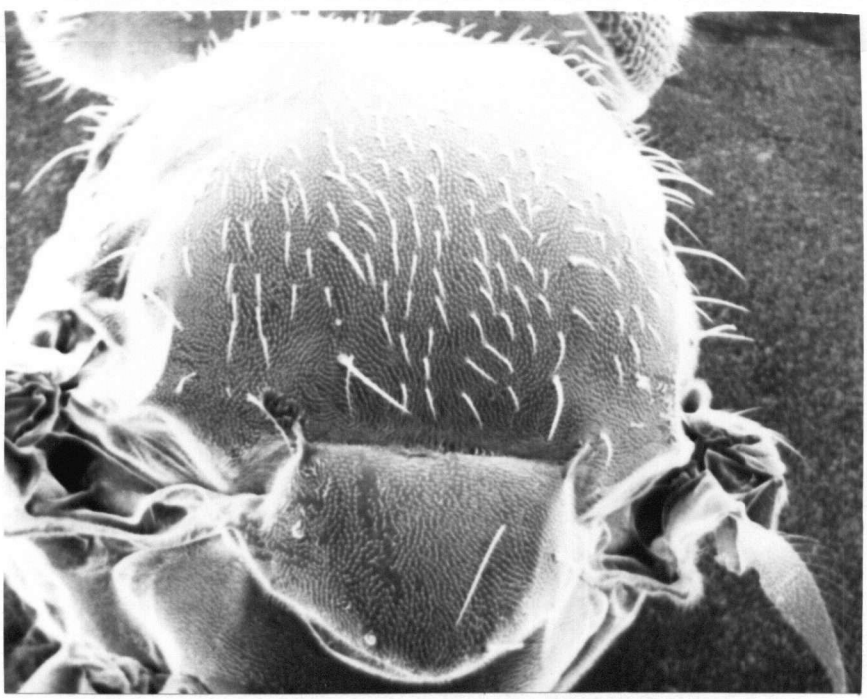
a

FIGURE 10 (continued)

(b) a Q-III/p^P heterozygote, grown continuously at 29°C; (c) a Q-III homozygote, heat-pulsed at 252-276 hours post oviposition (magnification about x400).



b



c

will be made to similar phenotypes of heterozygotes, and any unique traits will be referred to specifically.

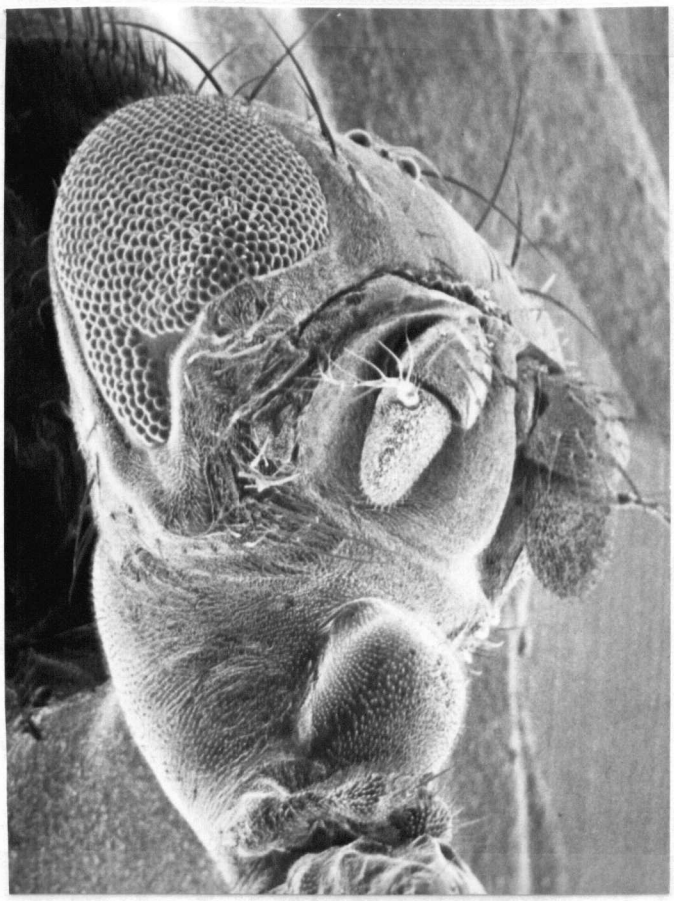
APPENDIX 2 summarizes the types and frequencies of the phenotypes observed in surviving homozygotes after 24-hour heat pulses. These will be dealt with on the basis of the individual imaginal discs from which the affected structures are derived.

(a) pattern defects of the eye-antennal disc

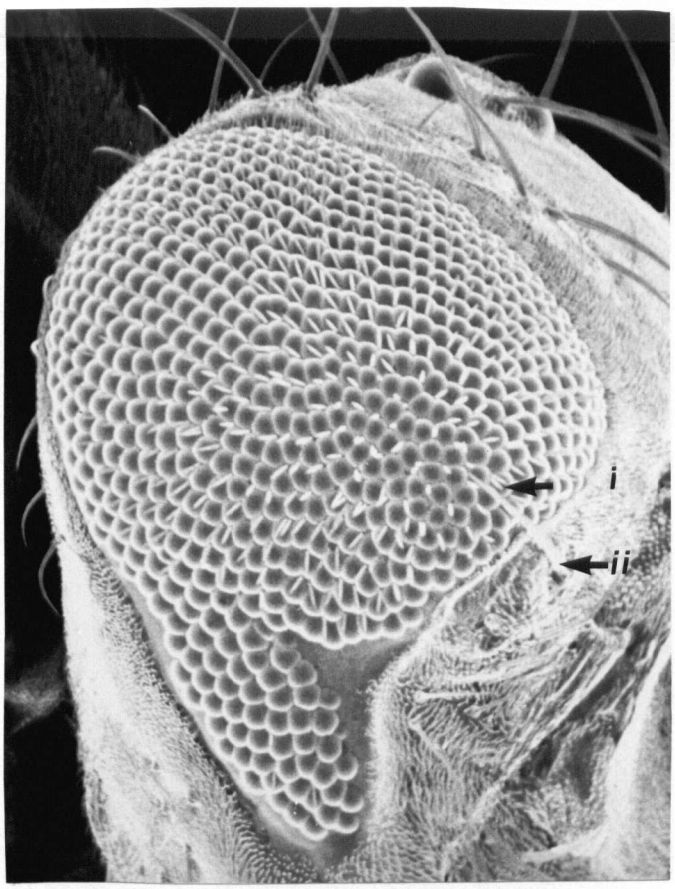
In addition to the roughened eye phenotype, heat-pulsed heterozygous and homozygous (and in the case of heterozygotes from regular shift experiments, those surviving shifts to 29° or 22°C) Q-III flies displayed moderate to severe loss of ommatidial tissue. Figure 11a, b, c and d are scanning electron micrographs of such individuals. Note that there is considerable ventral displacement of eye tissue in the heterozygote (a). High power magnification (b) reveals that although inter-ommatidial bristles are absent in the displaced portion, they are duplicated for many of the other ommatidia. These duplications could be responsible for the rough eye phenotype mentioned earlier (see Poodry et al., 1973). Note also the presence of extra vibrissae which extend across the eye at three o'clock.

In the homozygote, the left eye has been severely reduced (c), while no visible reduction of the right eye has taken place. Thus, although this reduction frequently can be extreme in the homozygote, it does not necessarily always occur for both eyes. Note also that this fly completely lacks the third segment (including the arista) of the left antenna. The highest frequency (83 percent, APPENDIX 2) of ommatidial deficiencies of homozygotes was induced by a heat pulse at

FIGURE 11 Scanning electron micrographs showing the effects of Q-III on eye development; (a) a Q-III/p^p heterozygote, shifted to 29°C at 24 hours post oviposition (magnification about x400); (b) (the same fly), one arrow (i) points out a duplicated interommatidial bristle, while another (ii) points out the enlarged vibrissae (magnification about x800);



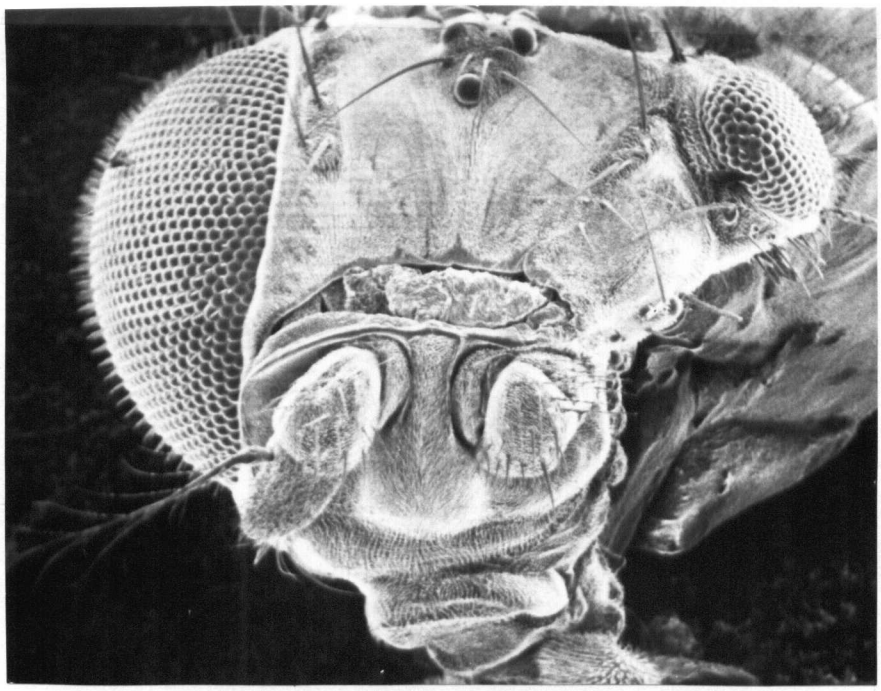
a



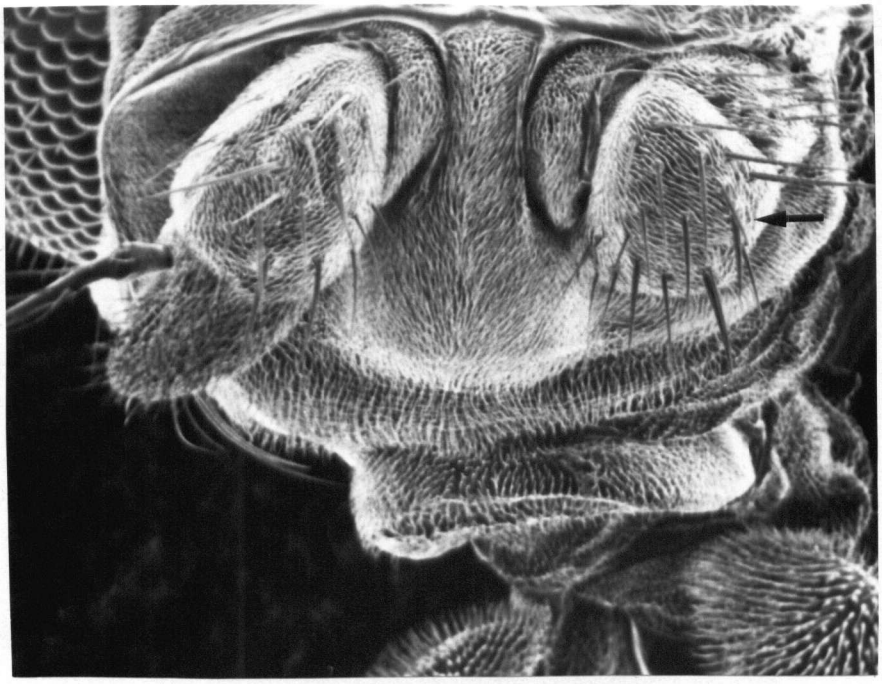
b

FIGURE 11 (continued)

(c) a Q-III homozygote, heat pulsed at 72-96 hours post oviposition (magnification about x400); (d) (the same homozygote), an arrow points out the second antennal segment on the fly's left (magnification about x800).



c

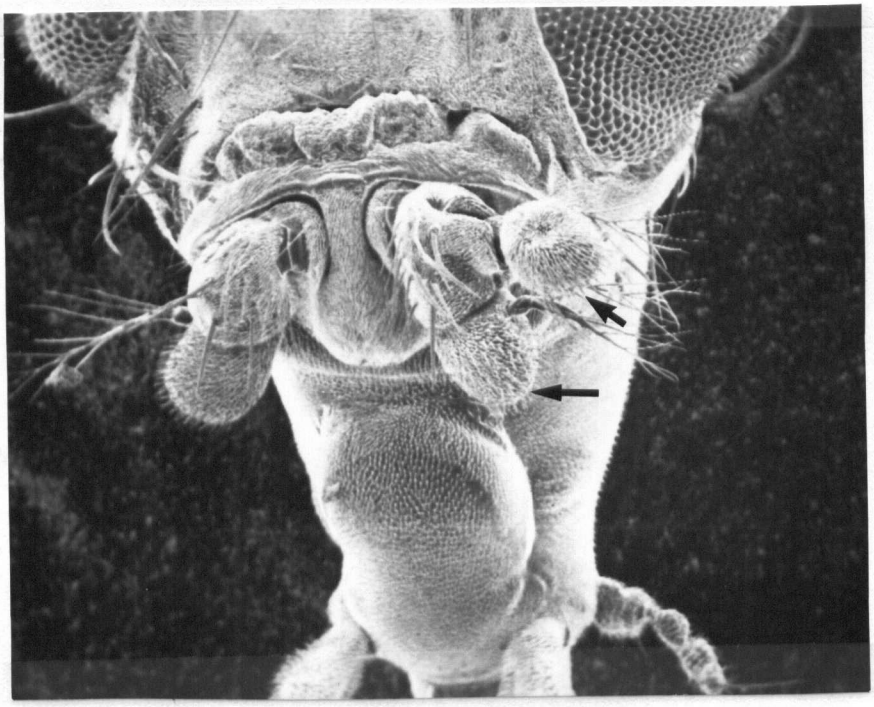


d

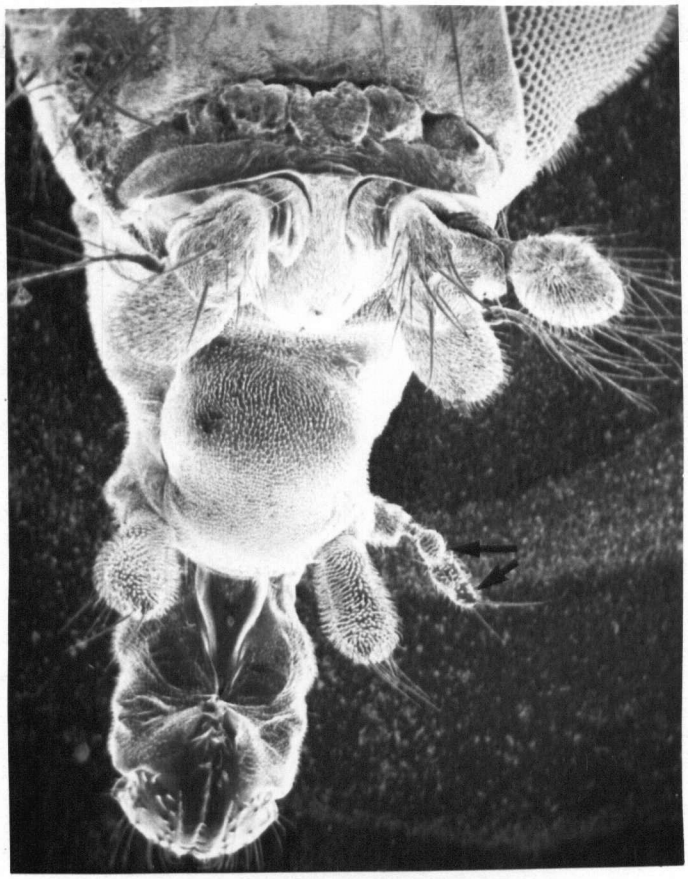
96 to 120 hours (this phenotype was also observed in pulses on either side of this interval). Therefore, the TSP of this phene spans most of the second and the early part of the third instar. Less often, deficiencies and duplications of antennae as well as palps and ocelli occurred within the limits of this TSP. In two cases where the palps were absent, the ipsilateral antennae were also missing. Thick, fleshy aristae were also often induced by heat pulses during this TSP. Since all of the above structures are derived from the eye-antennal disc, the correspondence in TSPs for the different defects is not surprising, but the spectrum of phenotypes is noteworthy.

Dissection of several pharate pupae resulting from heat pulses at 84 to 120 hours revealed that in some cases almost no eye-antennal structures had formed in such pupal lethals. In a few cases, severe deficiencies of the head region were accompanied by complete antennal duplication. A pulse experiment was initiated to further investigate this. Two hundred larvae from a Q-III/TM3 x Q-III/TM3 cross were synchronized in the early part of the second instar and heat-pulsed from about 100 to 140 hours (mid second to early third instars) post oviposition. Only four homozygotes eclosed. Of these, one displayed extreme eye deficiencies on both sides, while the other three exhibited unilateral antennal duplications along with less severe eye deficiencies. Figure 12 is a scanning electron micrograph of one of the latter. The following were observed in this individual: (a) mirror image duplication of the left antenna along with triplication of the arista (compare with the normal right antenna and arista and note also the reduction of the ventral surface of the left eye) and (b) a jointed

FIGURE 12 Scanning electron micrographs showing the eye-antennal pattern defects of a Q-III homozygote, heat pulsed at 100-140 hours post oviposition; (a) arrows point out the duplicated left antenna with triplicated arista; note the ventral reduction of the eye; (b) (the same fly), arrows point out the unidentified jointed structure (magnification about x400).



a



b

structure of unknown origin extending from the proboscis and positioned adjacent to the left palp. Undefined structures have also been frequently observed at the periphery of reduced eyes in other experiments involving Q-III as well as with Dfd (D. Sinclair, personal observations).

Of twenty-one dissections of dead pupae present in the cultures of this heat-pulse experiment, 17 possessed few or no head structures (in many cases, only the proboscis was found) and sometimes in these individuals, eye pigment globules could be seen within the thorax. This phenotype was also observed for Dfd/Q-III heterozygotes grown continuously at 29°C. The other four dead pupae had severely reduced eyes and, in one case, bilateral duplication of the second and third antennal segments (along with the aristae) occurred.

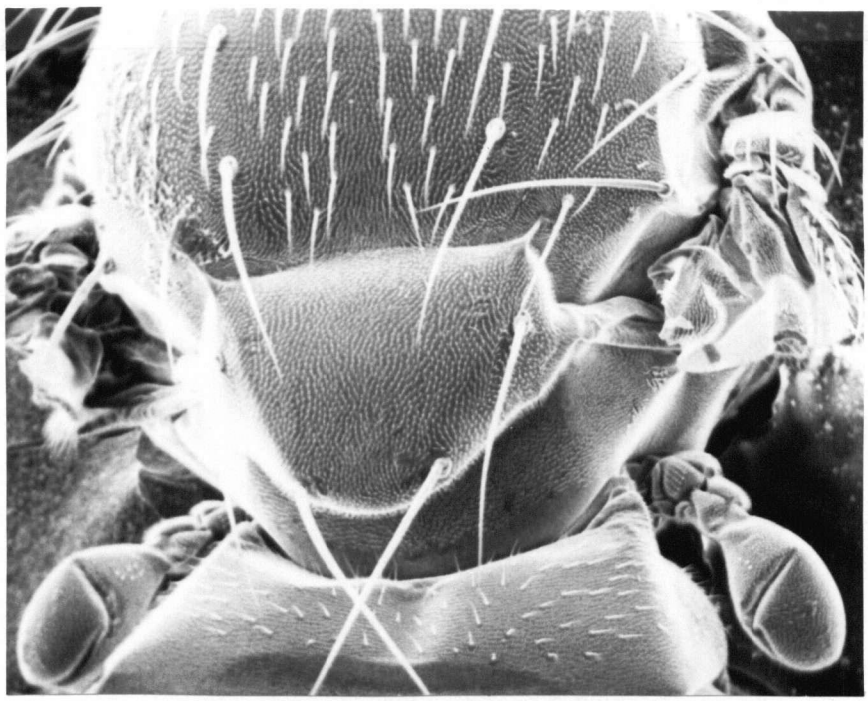
Thus, deficiencies of eye-antennal structures (particularly reduction of the eyes) occur frequently in Q-III/Q-III flies heat-pulsed for brief intervals during the second instar. In its most extreme form, this phene resembles the phenotype of a sex-linked autonomous cell-lethal, 1(1)ts480, which was illustrated and described by Arking (1975).

In heterozygotes, ommatidial deficiencies resembling (but less severe than) those of Q-III homozygotes were found in survivors of 48-hour pulse shifts (see APPENDIX 3) and survivors of shifts up or down. Again the TSP for this phene appears to occur within the second larval instar.

(b) pattern defects of the dorsal mesothoracic (wing) disc

At least thirty percent of homozygotes that were continuously exposed to 17°C, possessed pattern defects of the thorax, particularly of the scutellum. As previously mentioned, expressivity of this trait

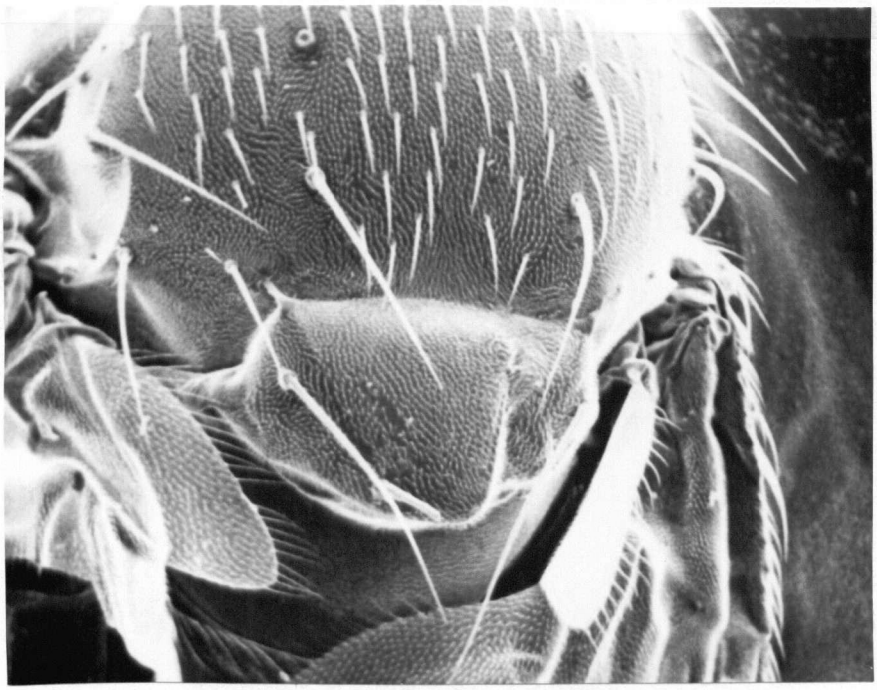
FIGURE 13 Scanning electron micrographs showing the effects of Q-III on the development of the scutellum at 17°C (the effects are similar to those produced by heat-pulsing); (a) a $\underline{p^p}/\underline{p^p}$ fly grown at 22°C (magnification about x400);



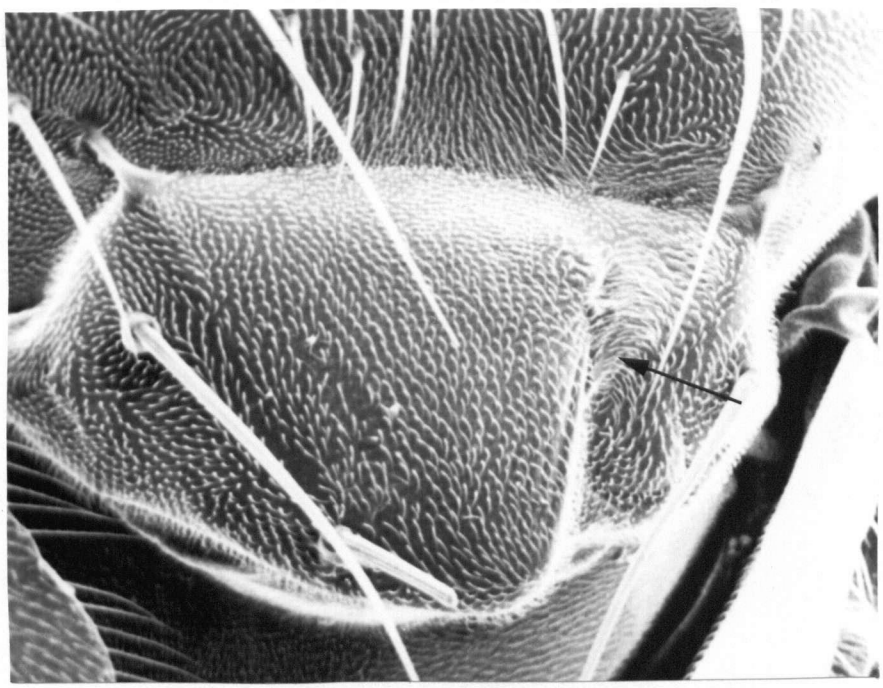
a

FIGURE 13 (continued)

(b) a Q-III homozygote (magnification about x400); (c) (the same fly), at a higher power with an arrow pointing out the hairs in the region of the scar (magnification about x800);

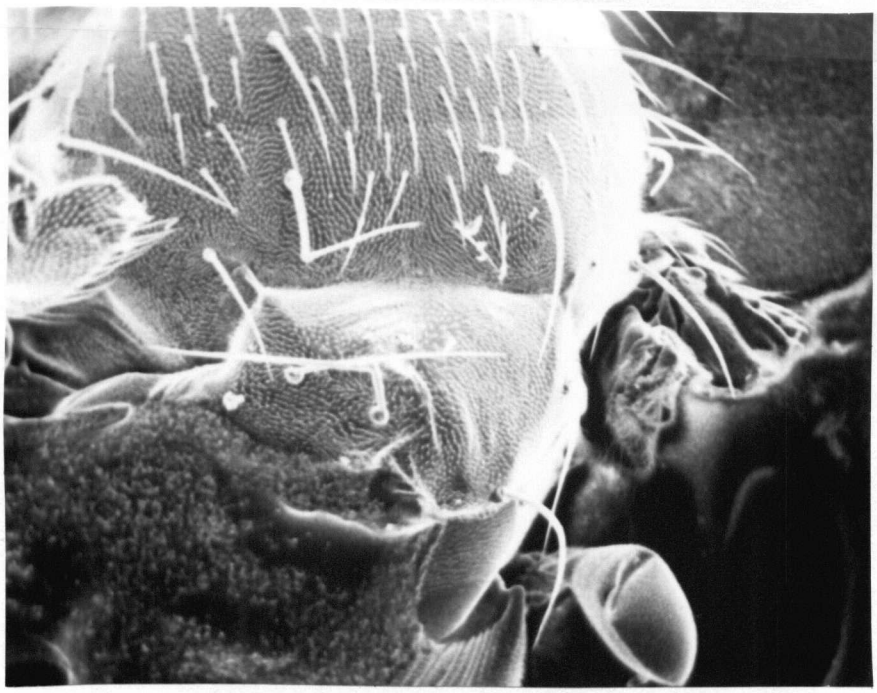


b

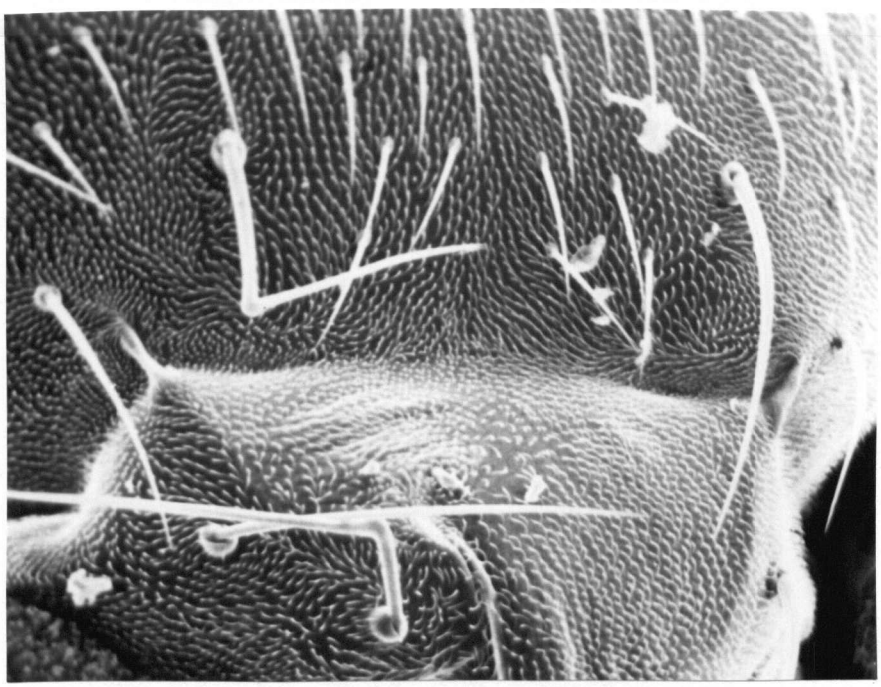


c

FIGURE 13 (continued) (d) a Q-III homozygote (magnification about x400); (e) (the same fly as in d), at a higher power (magnification about x800);



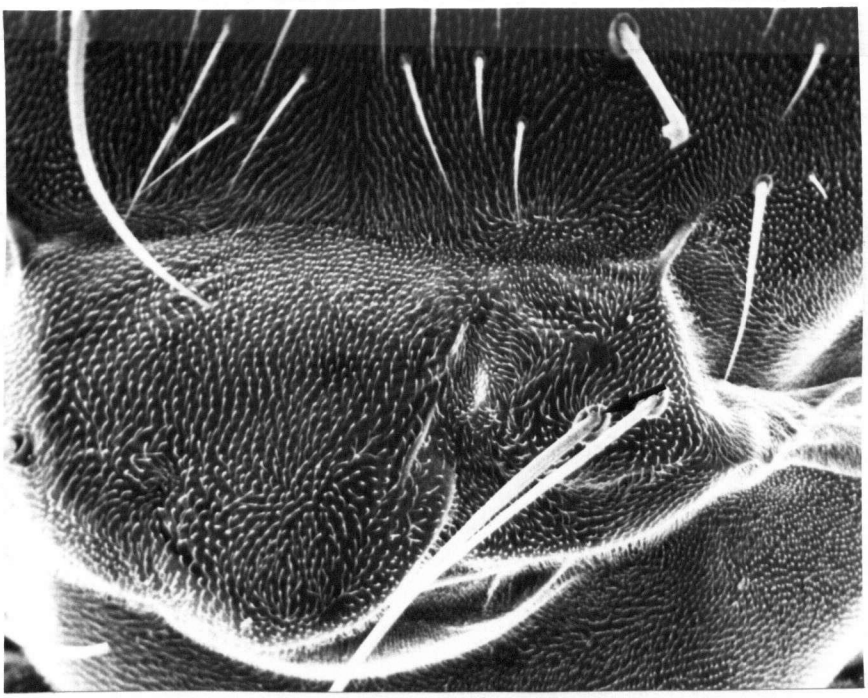
d



e

FIGURE 13 (continued)

(f) a Q-III homozygote with an arrow pointing out a duplicated bristle (magnification about x800). All Q-III homozygotes were grown continuously at 17°C, while (a) was grown at 22°C.



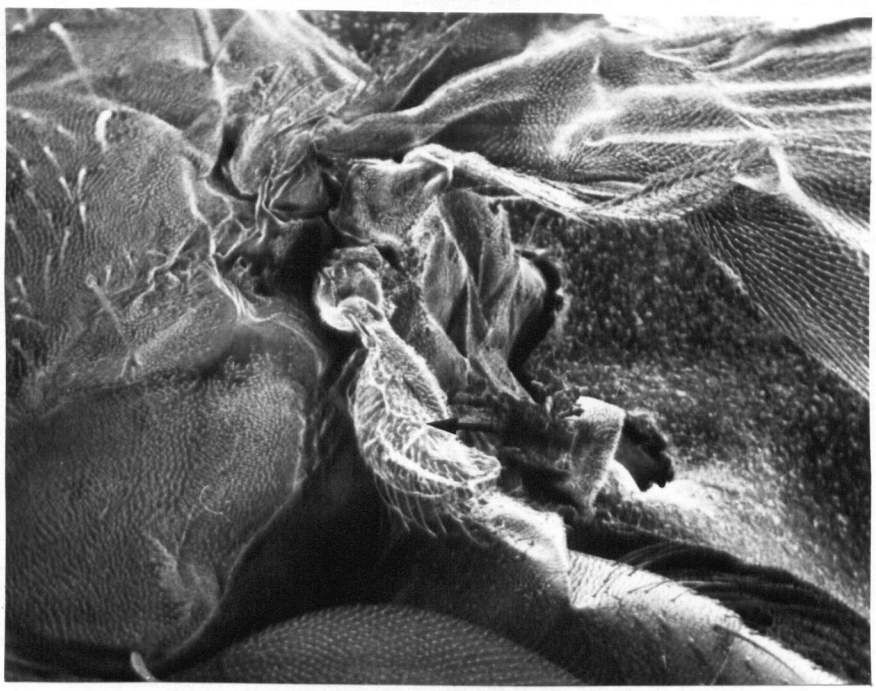
f

ranged from the appearance of extra bristle sockets, randomly placed on the scutellum, to duplicated bristles and even bifurcation of the scutum and prescutum (Figure 13 a,b,c,d,e, and f). Note the normal scutellum and bristle arrangement on the control fly (a). A Q-III/Q-III fly reared at 17°C (b and c) had marked indentation of the scutellum. Higher magnification (c) shows that disarrangement of the small scutellar hairs has occurred in the vicinity of the scar. The fly shown in d and e exhibited an analogous disruption, but it is uncertain whether this represents a duplication of the posterior or anterior scutellar bristles. Finally, the fly shown in (f) possessed a similar scutellar indentation, as well as what appears to be a non mirror-image duplication of a posterior scutellar bristle.

By heat pulsing Q-III/Q-III larvae, similar phenotypes could be induced. When the pulse was applied from 108 to 132 hours after oviposition, all of the progeny displayed the scutellar phenotype, with a minority having only extra bristle sockets at unusual positions on the scutellum (APPENDIX 2). Very rarely, large deficiencies of the scutellum were seen. Their rarity may reflect that any more severe pattern defect in this disc produces death. The TSP for this anomaly thus encompasses the eye TSP in early to late second instar, although the peak for the former was attained just after the middle of this instar.

Heterozygotes (Q-III/TM1, Q-III/TM3 and Q-III/p^p) also displayed the thoracic pattern phenotype at low frequencies, either when heat-pulsed (Q-III/TM1) or when shifted up during the TSP (Q-III/TM3, Q-III/p^p). Occasionally, Q-III/TM3 heterozygotes surviving shifts down possessed wing-like or haltere duplications (Figure 14).

FIGURE 14 A scanning electron micrograph showing a wing-like duplication (arrow) in a Q-III/TM3 fly that was shifted from 29° to 22°C at 156 hours post oviposition (magnification about x800).



It should be mentioned that the above Q-III-bearing heterozygotes which survived shifts up to 29°C also displayed disruption of wing venation, particularly for vein L2. However, this phenotype was not observed in homozygotes pulsed to 29°C and no attempt was made to define its TSP.

Cell death within imaginal discs followed by different degrees of pattern reconstruction, could provide an explanation for most of the above phenotypes. This idea will be more specifically dealt with in the Discussion.

(c) thoracic macrochaetae

To reiterate, thoracic (particularly scutellar) macrochaetae were severely reduced in homozygotes by heat pulses administered during the first half of pupation.

(d) defects involving the leg discs

Twenty-four hour heat pulses of Q-III homozygotes during late second or early third instars induced a low frequency of either missing legs (particularly mesothoracic) or legs with shortened tarsi (APPENDIX 2). This was also observed in heterozygotes that had developed continuously at 29°C or in the shift cultures which had been used to define the eye, bristle and lethality TSPs.

Male homozygotes heat-pulsed from 228 to 276 hours (during pupation) exhibited gaps in their sex combs (Figure 15). Note that the sex combs appear to be incompletely rotated, in that neither the upper nor the lower halves of the comb are aligned with the axis of the leg. Although the TSP for this phenotype corresponds roughly to the time of leg disc eversion and elongation (Bodenstein, 1950), the relationship between these phenomena and sex comb differentiation is unknown.

FIGURE 15 Scanning electron micrographs showing the effects of Q-III on sex comb development; (a) the basitarsus of a normal male ($\underline{p^P}/\underline{p^P}$), grown at 22°C; (b) the basitarsus of a Q-III homozygote, heat-pulsed at 228 to 252 hours post oviposition (magnification about x2000).

a

b

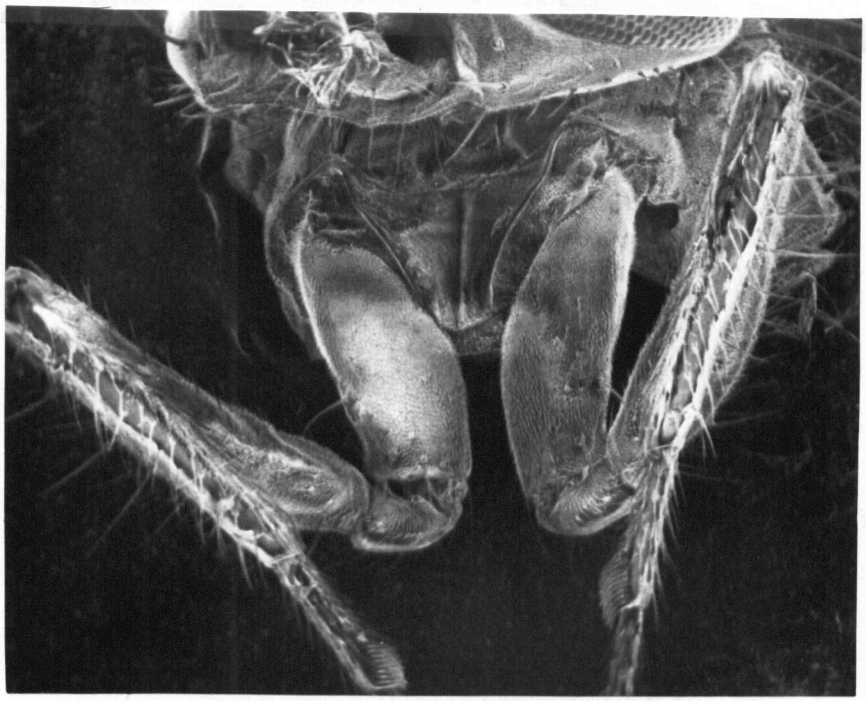
A fused foreleg phenotype was observed only in specific shifts down (or heat pulses at specific times during larval development) involving Q-III/TM3 heterozygotes. This phene was marked by a progressive but variable fusion of the forelegs as seen in Figure 16. At most, only about one third of all heterozygotes in a culture exhibited this phenotype. Although such fusion was seen in shifts down and heat pulses within the interval 84 to 144 hours, it was never observed in flies shifted up during this interval (even in dissections of dead pharate pupae). Furthermore, although the phenotype was repeatedly seen in several independent heat pulse tests using the balancer TM3, SbSer, when a TM3 balancer lacking the Sb and Ser markers was used, leg fusion was never observed. Thus, it appears that this trait may be the result of some ts interaction between Q-III and these or other mutant alleles on the balancer.

It is noteworthy that of the three pairs of discs producing legs, only the pair giving rise to the forelegs remain closely juxtaposed throughout development (Bodenstein, 1950). The fusion may reflect non-autonomous disc overgrowth, resulting from rapid cell proliferation at the medial edges of both discs. Simpson and Schneiderman (1975) described a similar phenotype for 1(1)ts540, an X-linked autonomous cell-lethal.

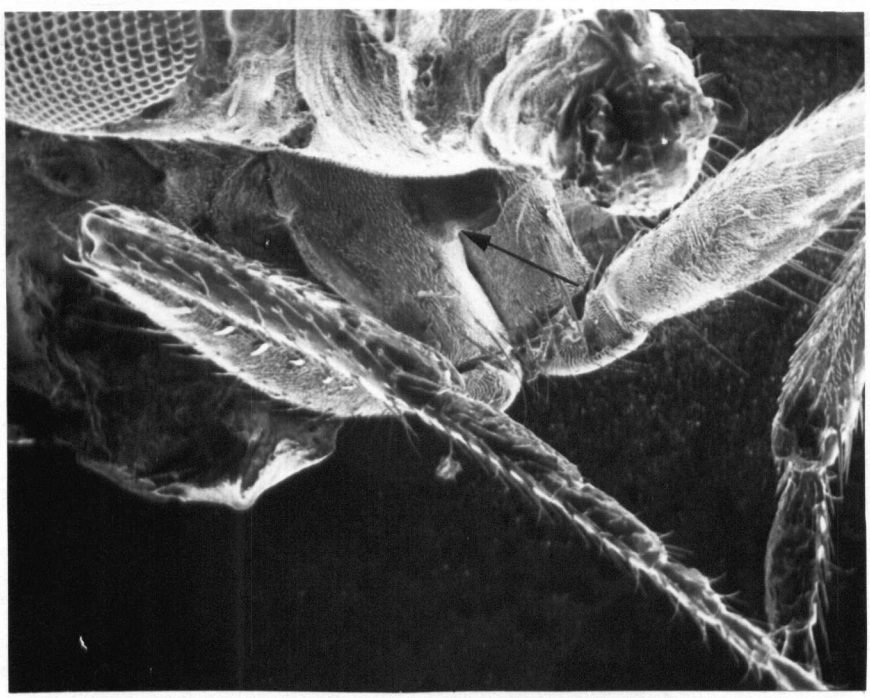
(e) defects involving the genital disc

A variable number of heterozygous (Q-III/TM3 or Q-III/p^p) male progeny displayed malformation of their external genitalia when shifted up or down during development. This phene involved either incomplete rotation of the terminalia (see Miller, 1950) or in extreme cases,

FIGURE 16 Scanning electron micrographs showing the forelegs of: (a) a normal fly ($\underline{p^p}/\underline{p^p}$), grown at 22°C; and (b) a Q-III/TM3 fly, shifted from 29° to 22°C at 120 hours post oviposition, an arrow points out the proximal fusion of the legs (magnification about x400).



a



b

terminalization or lack of the genitalia. The TSP of this phene occurs during the second and part of the third larval instars.

When homozygotes are heat-pulsed, higher proportions (see APPENDIX 2) of males treated during the larval intervals (particularly from 108 to 132 hours, 62 percent) exhibited the genital phene. Thus, the TSP of this phenotype can be more accurately defined. It lies predominantly within the second half of the second instar (although it spans the entire second as well as extending into the early third instar) and it corresponds to the TSPs of many of the other phenotypes discussed heretofore. Miller (1950) claims that cells forming the external male genitalia (i.e. the terminal abdominal segments) normally undergo rotation during development. Whether the lack of Q-III^{+} gene product directly prevents this normal process is unclear. Since female terminalia are not known to rotate, it is not surprising that a comparable phenotype was not observed in female progeny.

(f) defects involving the abdominal histoblasts

Although no homozygotes survived 48-hour heat pulses at 204 to 252 hours (during pupation), many of the dead imagoes (dissected from their cases) exhibited consistent abdominal anomalies including tergite malformation (the segments were missing, uneven, or etched) and incomplete pigmentation. In a few cases, whole patches of tergites were missing from the abdomen. This phenotype was observed at a lower frequency in homozygous survivors of shorter heat pulses at 204 to 228 hours. A few of the dead pharate pupae resulting from a 24-hour pulse showed tergite deficiencies or etching (see APPENDIX 2).

Interactions displayed by Q-III and other Minutes

Some Minutes interact with non-allelic mutations to reduce viability or to enhance the expression of certain phenotypic abnormalities. As previously mentioned, some of the more well known interacting loci include: Delta (Schultz, 1929), vestigial (Green and Oliver, 1940) and Lyra (Lindsley and Grell, 1968). More recently it has been observed that Minutes suppress the sex comb effects on the second or third pairs of legs of some homeotic mutations (R. Denell, personal communication). Consequently, possible interactions between Q-III and some of these mutants were tested for temperature-sensitivity. Although these interactions have both genetic and developmental significance, I have chosen to deal with the results of these tests here.

Heterogeneity of genetic backgrounds in different stocks prevented a meaningful detailed assessment of differences in expressivity of the sex comb phenotypes. Therefore, all male flies were scored strictly for presence or absence of the supernumerary combs (any number of teeth) on either member of the second pair of legs.

Table 26 summarizes the phenotypes and viability of heterozygotes carrying Q-III and Dl, vg, Ly, Scx, Msc, or Pc^3 when raised at 22° or 29°C. While Q-III/Dl, Q-III/Ly, Q-III/Scx and Q-III/Msc flies were fully viable at 22°C (crosses 1,3,4 and 5), they were either totally inviable (Q-III/Dl, Q-III/Ly) or weakly viable (Q-III/Scx, Q-III/Msc) at 29°C. In addition, all Q-III/Scx flies displayed a scalloping of the posterior wing margin at 29°C but not at 22°C. vg/+;Q-III/+ flies were fully viable at both temperatures (cross 2, when compared with Q-III/p^p at 29°C) and more than 80 percent possessed nicked wing

Table 26

Lethal and Visible Phenotypes of Heterozygotes for Q-III and Dl, vg, Ly, Scx, Msc and Pc at Different Temperatures

Surviving Progeny				Visible Phenotypes (Percent Expression)	
		Number			
Parental Genotype	Progeny Genotype	22°C	29°C	22°C	29°C
1. Q-III/TM3 and Dl/TM3	Q-III/TM3	119	5	normal (100)	M (100)
	Dl/TM3	121	175	slight Delta (100)	slight Delta (100)
	Dl/Q-III	127	0	" "	-
2. +/+;Q-III/TM3 and vg/vg;e^s/e^s	vg/+;Q-III/e^s	236	172	normal wings (100)	M (100); nicked wings (81.4)
	vg/+;TM3/e^s	220	359	ebony (100)	ebony (100)
3. Q-III/CxD and CxD/Ly	Q-III/CxD	40	4	Dichaete (100)	severe M (100); Dichaete (100)
	Ly/CxD	30	139	Lyra; Dichaete (100)	Lyra; Dichaete (100)
	Ly/Q-III	50	0	Lyra (100)	-

Table 26 (continued)

Parental Genotype	Progeny Genotype	Number 22°C	Number 29°C	Visible Phenotypes (Percent Expression) 22°C	Visible Phenotypes (Percent Expression) 29°C
4. Q-III/TM3 and Scx/TM3	Q-III/TM3	249	61	normal (100)	M (100)
	Scx/TM3	269	970	*extra sex combs (51.6)	extra sex combs (99.6)
	Scx/Q-III	292	10	normal wings (100); extra sex combs (48.2)	M (100); nicked wings (100); extra sex combs (50)
5. Q-III/TM3 and Msc/TM3	Q-III/TM3	202	7	normal (100)	M (100)
	Msc/TM3	197	893	extra sex combs (58.3)	extra sex combs (91.1)
	Msc/Q-III	203	102	" " " (68.4)	M (100): extra sex combs (10)
6. Q-III/TM3 and Pc/TM3	Q-III/TM3	77	4	normal (100)	M (100)
	Pc/TM3	69	291	extra sex combs (96.4)	extra sex combs (100)
	Pc/Q-III	83	143	" " " (80)	M (100); extra sex combs (4.4)

* Presence of sex combs on second legs of males

margins (particularly in distal margins) at 29°C, but none displayed such a phenotype at 22°C. This ts interaction resembles the non-ts effects of Minutes on vg reported by Green and Oliver (1940). N. Dower (unpublished) discovered that EMS-induced Minutes also enhance the expression of vg in heterozygotes.

Eight of the 10 Scx/Q-III survivors at 29°C were males and 4 of these males had extra sex combs. Owing to the small numbers of these survivors, no further mention will be made of them with respect to the extra sex comb phene.

Q-III/Pc flies were relatively viable at both temperatures. Amongst the male progeny of the Q-III/Pc and Q-III/Msc constitutions, there was a marked reduction in the frequency of individuals displaying the extra sex comb trait at 29°C compared with that at 22°C (Pc/Q-III: 4 percent, down from 80 percent; Msc/Q-III: 10 percent, down from 68 percent).

In summary, Q-III has the following interactions: (1) ts lethality with Dl and Ly (2) reduced viability with Msc and Scx at 29°C (3) ts scalloping of the wing margin with vg and Scx and (4) ts suppression of the sex comb phenotypes of Pc and Msc.

The ts suppression of homeotics by Q-III prompted an appraisal of combinations involving other Minutes and these loci. To this end, Df(3L)M(3)LS4 (hereafter abbreviated as M(3)LS4) and M(2)173 were tested with Scx, Msc and, in the case of the former M, Pc. The results of the crosses performed are shown in Table 27. It should be mentioned that M(3)LS4 flies are poorly viable (undoubtedly due to the deletion), yet M(3)LS4/Scx flies were even less viable, thereby resembling the combination, Scx/Q-III at 29°C. In contrast, viability of

Table 27

Interactions of Known Minutes With Different Homeotic Mutations Affecting Sex Combs

Parental Genotype	Surviving Progeny: Progeny Genotype	Number	(Percent) (Viability)	Number Showing Visible Phenotypes (Percent Expression): Male Sex Comb: Extra	Male Sex Comb: Normal	Wings: Scalloped	Wings: Normal
M(3)LS4/TM3 and Scx/TM3	M(3)LS4/TM3	252	(34.5)	0	136 (100)	0	252 (100)
	Scx/TM3	357	(48.9)	80(44.4)	100 (55.6)	0	357 (100)
	M(3)LS4/Scx	121	(16.6)	3 (3.4)	85 (96.6)	121 (100)	0
M(3)LS4/TM3 and Msc/TM3	M(3)LS4/TM3	129	(15.9)	0	70 (100)	-	-
	Msc/TM3	373	(46.1)	140 (70)	60 (30)	-	-
	M(3)LS4/Msc	307	(37.9)	21 (12)	154 (88)	-	-
M(3)LS4/TM3 and Pc^3/TM3	M(3)LS4/TM3	6	(6.5)	0	4 (100)	-	-
	Pc/TM3	50	(54.4)	20 (83.3)	4 (16.7)	-	-
	M(3)LS4/Pc	36	(39.1)	2 (11.8)	15 (88.2)	-	-
M(2)173/ SM5;+/+ and +/+; Scx/TM3	M(2)173/+;TM3/+	63	(21.6)	0	32 (100)	0	63 (100)
	SM5/+;Scx/+	82	(28.1)	24 (61.5)	15 (38.5)	0	82 (100)
	M(2)173/+;Scx/+	70	(24.0)	11 (29.7)	26 (70.3)	35 (50)	35 (50)
	SM5/+;TM3/+	77	(26.3)	0	35 (100)	0	77 (100)

Table 27 (continued)

Parental Genotype	Progeny Genotype	Surviving Progeny: Number	Surviving Progeny: (Percent) (Viability)	Number Showing Visible Phenotypes (Percent Expression): Male Sex Comb: Extra	Male Sex Comb: Normal	Wings: Scalloped	Wings: Normal
M(2)173/ SM5;+/+ and +/+;Msc/TM3	M(2)173/+;TM3/+	82	(21.5)	0	39 (100)	-	-
	SM5/+;Msc/+	107	(28.1)	51 (76.1)	16 (23.9)	-	-
	M(2)173/+;Msc/+	109	(28.6)	20 (37.7)	33 (62.3)	-	-
	SM5/+;TM3/+	83	(21.8)	0	46 (100)	-	-

M(2)173/+;Scx/+ flies was not decreased. Also no striking viability effects were seen for M(3)LS4/Msc, M(2)173/+;Msc/+ or M(3)LS4/Pc progeny (although the cross which produced the latter yielded few progeny from 4 cultures).

Both M(3)LS4/Scx and M(2)173/+;Scx/+ flies displayed a wing margin phenotype characteristic of Q-III/Scx survivors at 29°C, with penetrance levels of 100 and 50 percent respectively. Expressivity of this phenotype was also greater in progeny of the former class.

It is clear from the data (Table 27, columns 5 and 6) that M(3)LS4 suppresses expression of the sex comb phenotypes of Scx and Msc. Thus, 44 percent of Scx/TM3 males had extra sex combs while only 3 percent of Scx/M(3)LS4 did. Similarly, while 70 percent of Msc/TM3 males had extra sex combs, only 12 percent of M(3)LS4/Msc types did. Penetrance of Pc is also affected, with more than 80 percent of Pc/TM3 males having had the mutant phene, while only 12 percent of M(3)LS4/Pc displayed it. These results indicate that the other third chromosome Minute acts just like Q-III. However, it should be remembered that Q-III might be an allele of M(3)LS4.

The second chromosome Minute, M(2)173 also suppressed the sex comb traits of Msc and Scx (Table 27, columns 5 and 6). Thus, while 62 percent of the SM5/+;Scx/+ males had extra sex combs, only 30 percent of M(2)173/+;Scx/+ males did. Further inspection shows that 76 percent of the SM5/+;Msc/+ males showed the trait, compared to 38 percent of M(2)173/+;Msc/+ males. While suppression of Msc and Scx by M(2)173 is less efficient than by M(3)LS4, it is nevertheless apparent that Minutes in general have a similar effect. Furthermore, Minutes

appear to interact with Scx to produce a phenotype which is characterized by scalloping of the posterior wing margin in both males and females. This further strengthens the classification of Q-III as a genuine Minute mutation.

The existence of interactions between Q-III and vg, Dl and Scx suggested the possibility that specific TSPs could be determined for these interactions. Consequently, regular shift studies were initiated for this purpose.

(a) vg-Q-III wing scalloping

vg/+;Q-III/+ progeny arising from the cross +/+;Q-III/TM3 x vg/vg; +/+ were scored for wing scalloping after shifts (Figure 17). If the beginning of the TSP is taken at the point where a shift down first gives mutant flies, then this is clearly about or just after mid-second instar. The end of the TSP should coincide with the point where a shift up first yields non-mutants. These data indicate a very short TSP that terminates about two-thirds of the way through the second instar. Harnly (1936) exploited the suppression of the vg phenotype by high temperature to determine that the "temperature-effective period" for this gene extends from late second (about the second molt) to the early third instar, a later TSP estimate than that provided by this present study.

(b) Dl-Q-III viability

Adult progeny arising from the cross Q-III/TM3 x Dl/TM3 were counted after shift studies (Figure 18). It can be seen that the reciprocal shifts are not symmetrical, in that in the shifts down, a gradual reduction in viability of Dl/Q-III flies occurred in cultures

FIGURE 17 Results of the shift study to delineate a temperature-sensitive period for the vg-Q-III interaction. The data are given as percent expression of wing nicking in vg/Q-III progeny that were shifted from 22° to 29°C (closed circles), or from 29° to 22°C (open circles), at various times during development. Temporal estimates of the different developmental stages at 29° and 22°C, are indicated above and below, respectively; E = eclosion.

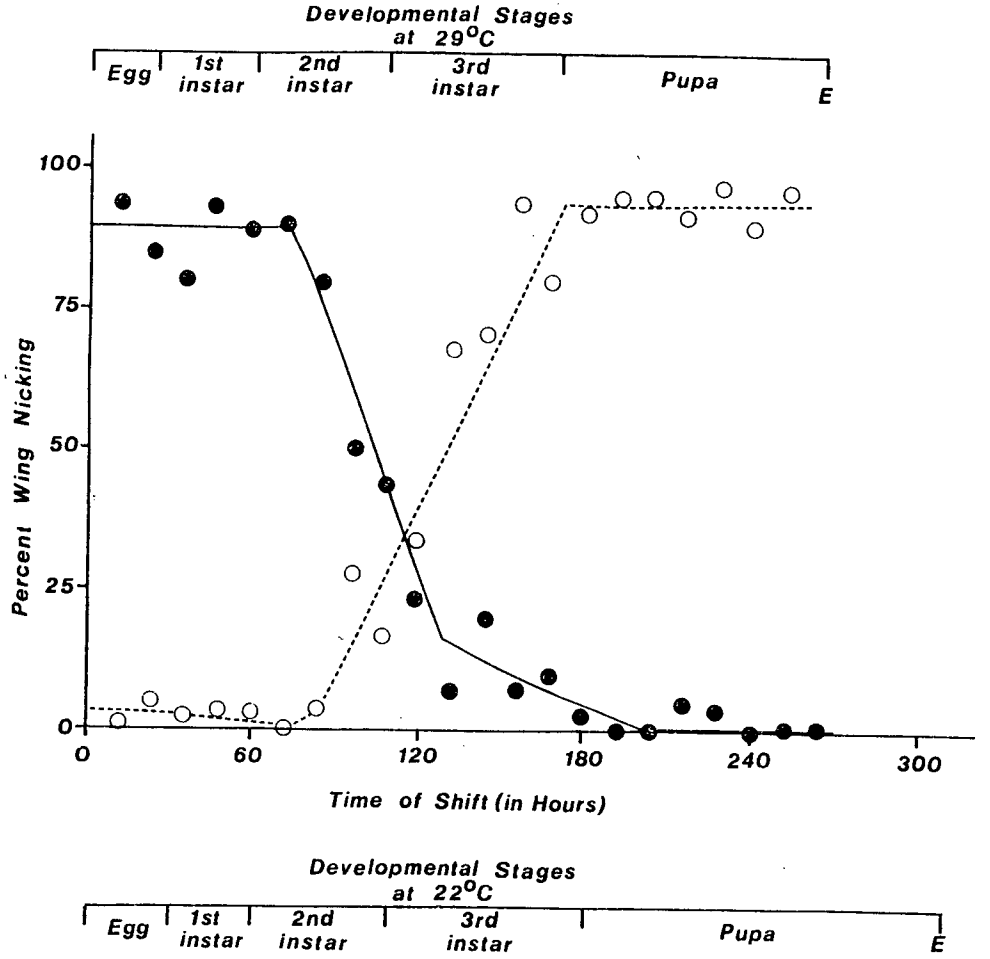
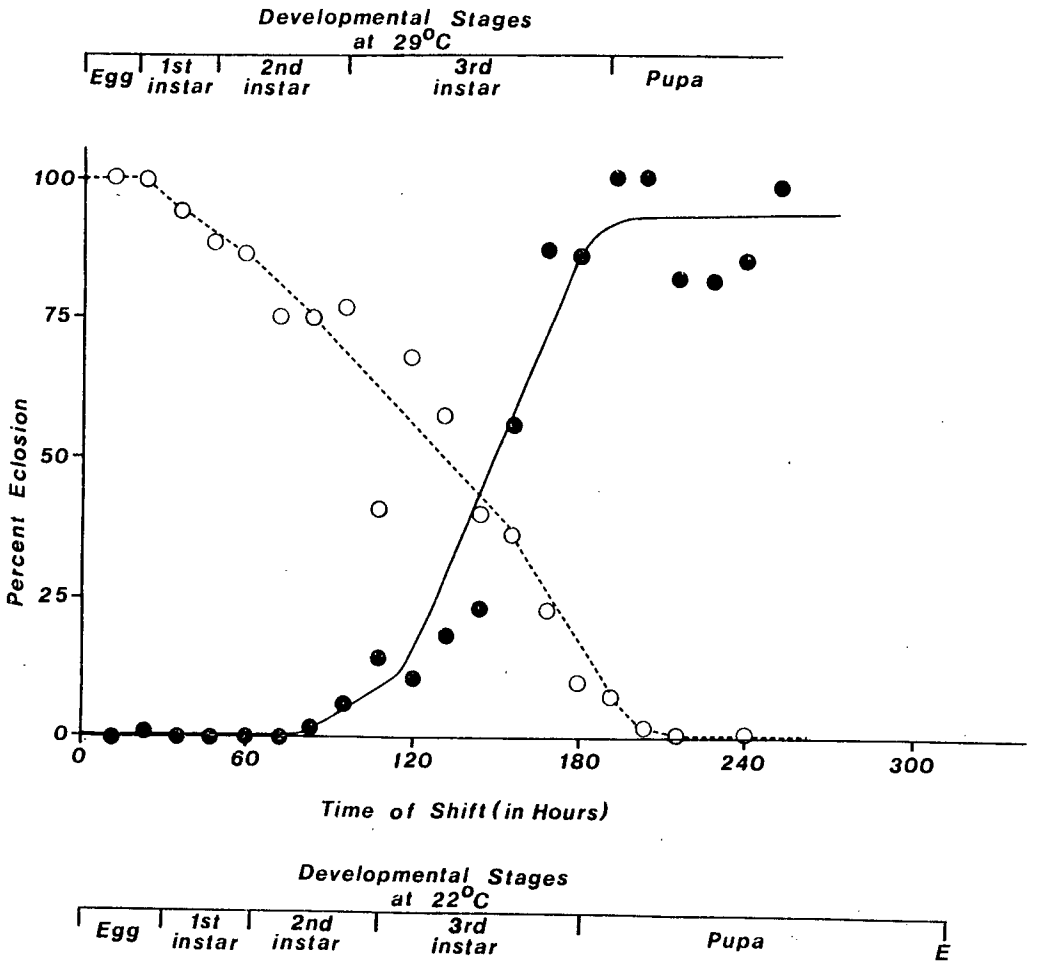

Developmental Stages
at 29°C
Egg
1st instar
2nd instar
3rd instar
Pupa
E
100
75
50
25
0
Percent Wing Nicking
0
60
120
180
240
300
Time of Shift (in Hours)
Developmental Stages
at 22°C
Egg
1st instar
2nd instar
3rd instar
Pupa
E

FIGURE 18 Results of the shift study to delineate a temperature-sensitive period for the Dl-Q-III interaction. The data are given as percent eclosion of Dl/Q-III progeny that were shifted from 22° to 29°C (closed circles), or from 29° to 22°C (open circles), at various times during development. Temporal estimates of the different developmental stages at 29° and 22°C, are indicated above and below, respectively; E = eclosion.


Developmental Stages
at 29°C
Egg
1st instar
2nd instar
3rd instar
Pupa
100
75
50
25
0
Percent Eclosion
0
60
120
180
240
300
Time of Shift (in Hours)
Developmental Stages
at 22°C
Egg
1st instar
2nd instar
3rd instar
Pupa
E

shifted progressively later, while in the shifts up, normal viability was attained fairly rapidly. In this case, if the beginning of the TSP is arbitrarily taken as the point where 75 percent of Dl/Q-III progeny survived shifts down (since shifts down thereafter generally produced more lethality) and similarly, if the end of the TSP is set at the point where a significant increase in viability followed a shift up, then an estimate of the TSP would be from about mid second to mid or late third instar.

One of the problems encountered when collecting data for the above experiments was that Dl/Q-III progeny which emerged in cultures that were shifted down at 12-hour intervals from 92 to 192 hours (post oviposition), did so in a bimodal fashion in that, initially, for a given shift only a few of the flies eclosed, but after 3 or 4 days the bulk of the survivors were scored. This observation, along with the fact that Dl/Q-III larvae showed extremely slow development at 29°C, indicates that even though prolonged exposure of these heterozygotes to 29°C greatly retards their growth, it does not necessarily kill them.

Most of the lethality of Q-III/Dl in cultures kept continuously at 29°C was larval, although a few dead pharate individuals were seen amongst a significant number of heterogeneous (with respect to development) pupae. Schultz (1929) mentioned that some M-Dl combinations died mainly as larvae. Although the Dl wing phenotype was severe in some of the Q-III/Dl individuals surviving shifts down during the TSP, no consistent pattern emerged. Possibly a more precise TSP could be derived from studying Q-III-mediated enhancement of the wing phenotype,

using other Dl alleles that are less susceptible to Q-III-induced lethality.

(c) Scx-Q-III wing scalloping

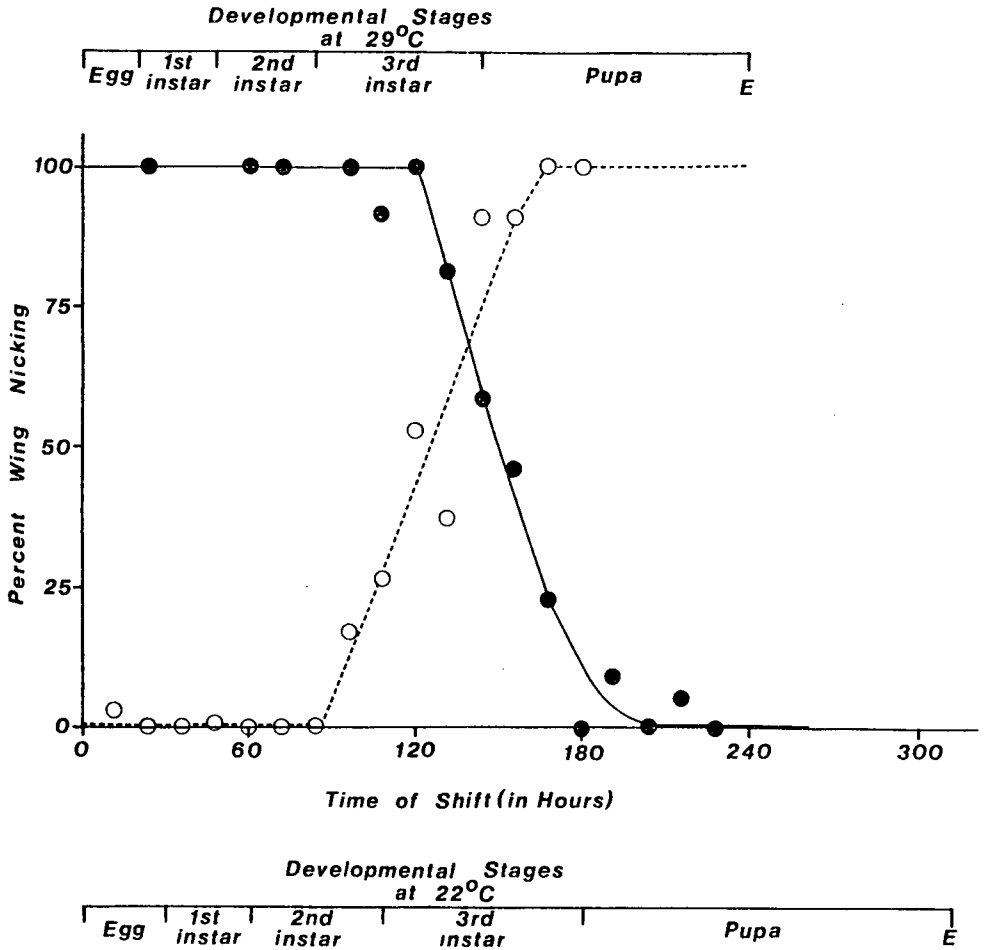
Wings of Scx/Q-III survivors from the cross Scx/TM3 x Q-III/TM3 were scored for the presence of nicks and the results are shown in Figure 19. It should be mentioned that the pattern of semi-lethality of Scx/Q-III individuals is slightly earlier than the TSP for wing nicking. However, this lethality did create a problem. For example, few Scx/Q-III flies survived shifts up at 24, 60 and 72 hours and none survived shifts up at 12, 36, 48 and 84 hours. Nevertheless, the shifts up immediately preceding the end of the TSP (96, 108, and 120-hour shifts) produced significant numbers of Scx/Q-III flies, nearly all of which displayed the scalloped phenotype. It can be seen that the TSP for this interaction is confined to a small developmental period in the early part of the third instar.

Qualitative differences in expression of this phenotype were noted, especially for shifts down at various times during the TSP. For example, many of the Scx/Q-III survivors from earlier shifts down showed anterior and/or posterior scalloping, while those shifted down progressively later (including the 132-hour shift) exhibited distal to proximal incisions in the wing blade. Indeed, some progeny phenotypically resembled flies carrying a less extreme form of apterous^{Xa} (see Lindsley and Grell, 1968).

Scx-ts67 interactions

Since wing scalloping was displayed by flies carrying Scx along with Q-III, M(3)LS4 or M(2)173, it could be postulated that it is a

FIGURE 19 Results of the shift study to delineate a temperature-sensitive period for the Scx-Q-III interaction. The data are given as percent expression of wing nicking in Scx/Q-III progeny that were shifted from 22° to 29°C (closed circles), or from 29° to 22°C (open circles), at various times during development. Temporal estimates of the different developmental stages at 29° and 22°C, are indicated above and below, respectively; E = eclosion.


Developmental Stages at 29°C
Egg
1st instar
2nd instar
3rd instar
Pupa
E
100
75
50
25
0
Percent Wing Nicking
0
60
120
180
240
300
Time of Shift (in Hours)
Developmental Stages at 22°C
Egg
1st instar
2nd instar
3rd instar
Pupa
E

reflection of some general metabolic or developmental effect of Minutes. In that case, other non-Minute mutations such as the ts su(f) allele, 1(1)su(f)^{ts67g} (henceforth known as ts67) (Dudick et al., 1974) with similar biological activity, might also interact with Scx. To test this, a stock of ts67 (the X chromosome also bore a suppressible allele of forked (f^s)) was obtained from T. Wright, and males of the genotype v f^s su(f)/Y;Scx/+ were constructed. These were crossed to ts67/ts67;+/+ females in bottles and the progeny allowed to develop at either 28° or 22°C (3 bottles at each temperature). Since ts67/Y males and ts67/ts67 females die at 30°C (Dudick et al., 1974), cultures were maintained at 28°C in the hope that the males might survive, but at the same time display the 'deficiency' traits (small bristles, rough eyes, see Dudick et al., 1974), phenotypes which are similar to those of Minutes. This would permit a test for interactions between Scx and ts67 to be measured.

The results of this test are summarized in Table 28. At 28°C all (183) of the male progeny were non-forked and displayed the deficiency phenotype. Fifty-five of the 183 males possessed extra sex combs, diagnostic of Scx and 31 of these 55 exhibited slight incisions in the posterior wing margin. In contrast, at 22°C all males (136) had forked bristles (i.e. normal with respect to the 'deficiency' bristle phene) and 52 of these had extra sex combs. However, none of the males reared at 22°C possessed scalloped wings. All of the females (207) reared at 28°C were non-forked, and 58 of these displayed the deficiency phenotype, but only 5 of the 58 had scalloped wings. In contrast, all of the females (150) produced at 22°C had forked bristles. Finally, no

Table 28

Effect of ts67 on Scx Expression in Progeny of the

Cross ts67/ts67;+/+ x v f^s su(f)/Y;Scx/+ at 22° and 28°C

Sex of Progeny (X Chromosome Genotype)		Visible Phenotypes of Surviving Progeny					
		Scalloped	Non-Scalloped	Extra Sex Combs	Normal Sex Combs	Def.	Non-Def.
Males (ts67/Y)	22°C	0	136	52	84	0	136
	28°C	31	152	55	128	183	0
Females (ts67/v f^s su(f))	22°C	0	150	-	-	0	150
	28°C	5	202	-	-	58	149

Def. = Deficiency phenotype i.e. small, thin bristles

Non-Def. = Normal bristles

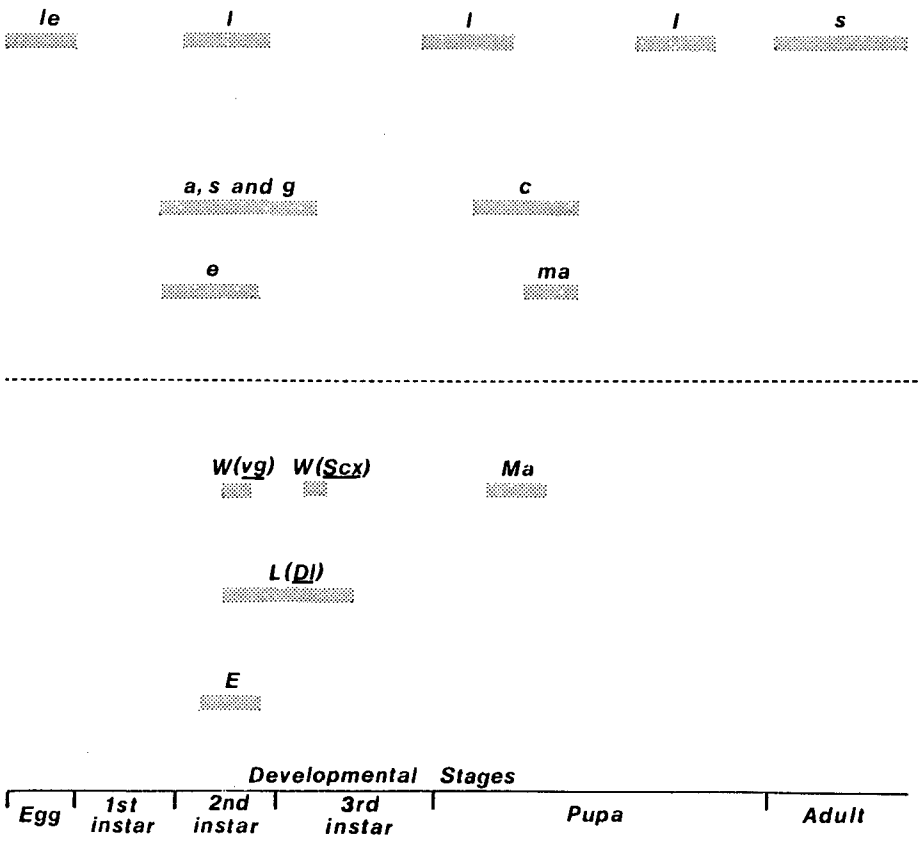
consistent reduction in the penetrance of the extra sex comb phene was noted in ts67/Y;Scx/+ males at 28°C.

These data suggest that the wing scalloping interaction between Minutes and Scx is probably not specific to M loci, since a similar, albeit less marked interaction exists between Scx and su(f).

Summary of TSPs involving Q-III

Figure 20 is a summary of the TSPs that have been provided by the shift experiments of the present study. It is clear that most of the TSPs affecting structures derived from the eye-antennal or wing discs, occur about the second instar (or early third) and that these overlap with the early lethal-sensitive as well as Dl-mediated inviability TSPs. In contrast, the TSPs associated with the differentiation of bristle derivatives (i.e. macrochaetae and sex combs) occur during early pupation, at almost the same time as the second TSP for lethality. This differential undoubtedly reflects a different basis for the origin of these defects. The TSP for female sterility (actually male sterility may be added as well) has been included for reference. The actual extent of this TSP could include pre-eclosion stages, since such females were not tested for fertility. Finally, an embryonic TSP was included and it is likely that the latter could be more accurately delineated by using shorter shifts coupled with higher temperature.

FIGURE 20 Temperature-sensitive periods (TSPs) for lethal, sterile and adult morphological effects of Q-III. Stippled bars indicate the extents of the TSPs relative to developmental stages given below. TSPs for recessive traits are summarized above the dashed line and include those for: embryonic lethality (le); larval and pupal lethality (l); sterility (s); defects involving aristae, scutella and male genitalia (a, s and g); eye reduction (e); sex comb rotation (c); and macrochaete reduction (ma); TSPs for dominant traits are summarized below the dashed line and include those for: rough eyes (E); macrochaete reduction (Ma); the lethal interaction with Dl (L(Dl)); and the wing nicking interactions with vg (W(vg)) and Scx (W(Scx)).


le
l
l
l
s
a, s and g
c
e
ma
W(vg)
W(Scx)
Ma
L(DI)
E
Developmental Stages
Egg
1st instar
2nd instar
3rd instar
Pupa
Adult

IV. Discussion

A temperature-sensitive mutation, Q-III has been found to produce a variety of ts phenes when homozygous and heterozygous. Furthermore, when Q-III is heterozygous with Dfd, vg, Scx, Pc, Msc and Dl, ts interactions specific for each of these mutations are detectable. Unique or overlapping TSPs were determined for several of these attributes. Of the ts mutations known to have multiple, complex patterns of TSPs (Poodry et al., 1973; Grigliatti and Suzuki, 1970; Foster, 1973; Shellenbarger and Mohler, 1975; Holden and Suzuki, 1973), none has been found to be as highly pleiotropic as Q-III.

The pattern of recessive lethality and extreme sensitivity of Q-III homozygotes to 48-hour heat pulses argues that the Q-III locus is indispensible for most of the larval as well as the pupal stage. However, the fact that the organism is relatively refractory to 24-hour exposures to 29°C (except for specific intervals) suggests that irreversible death is not mandatory unless a more prolonged period of deprivation of Q-III^{+} product is involved. Thus, we might expect that larvae for example, might have two types of temperature-sensitivity, one in which a short heat pulse leads directly to death and others where such a pulse stops development but in a semi-reversible fashion. This is supported by the waves of pupation after heat treatment at different times. The temperature-sensitivity (of lethality) of Q-III contrasts with that of shibere, since the latter is extremely susceptible to very short exposures to high temperatures (Poodry et al., 1973).

Preliminary observations show that the presence of functional Q-III^{+} gene product is vital also for embryogenesis and for adult

fertility. Eggs laid by Q-III/Q-III females do not survive at 29°C. Even when Q-III/Q-III females are crossed to normal males, the Q-III/+ embryos fail to survive at the higher temperature, but do survive at 22°C. In contrast, many homozygous Q-III embryos produced by heterozygous females are initially resistant to the lethal effects of high temperature (eventually succumbing after continuous exposure). This difference could indicate that functional gene product is supplied by the mother to the oocyte during oogenesis.

The relationship of oogenesis vis-a-vis embryogenesis is interesting here, with reference to the nature of the Q-III lesion. If the process of oogenesis usually demands a minimum supply of Q-III^{+} gene product, then it is not surprising that partial or complete sterility is manifested at 22° and 29°C. The following 2 alternatives could be offered to explain why Q-III/+ progeny of homozygous Q-III females failed to develop at 29°C, while at least an appreciable proportion developed normally at 22°C: (a) the homozygous females can package enough essential molecules into the egg to support development at 22°C, but not at 29°C and (b) the Q-III gene product is thermolabile. Obviously the latter is the more attractive possibility, particularly with respect to eventual biochemical analysis of the Q-III gene product.

1. The Nature of Minutes and Q-III

Is Q-III a temperature-sensitive Minute?

Certainly Q-III possesses the basic phenotypic characteristics of Minute mutations. At the restrictive temperature, the Q-III heterozygote has lowered viability, lengthened developmental period, and dominant expression of thin, small bristles, rough eyes, and other

less penetrant dominant phenotypes. In addition, the Q-III lesion is recessive lethal at 29°C. These properties have been reported for other mutations called Minutes (Lindsley and Grell, 1968). However, at 22°C no dominant effects are seen. The interactions of Q-III with Dl, Ly and vg have been described for other Minutes (see Schultz, 1929; Green and Oliver, 1940). At 29°C, Q-III/+/+ triploids have wild-type bristles and normal developmental periods, a result which has been reported for other Minutes in triploids (Schultz, 1929). Probably the strongest evidence is that the Q-III interactions led to predictions of Minute interactions that were indeed fulfilled.

Although Q-III is lethal in combination with Df(3L)M(3)LS4, genetic mapping positions Q-III in a region between st and in which does not include the deleted portion of this proximal deficiency. There is a possibility that a Minute point mutant or a cytologically-invisible deficiency of the Q-III locus is present on the deficiency chromosome. At any rate, Q-III amply fulfills the properties of a genuine temperature-sensitive Minute.

The function of Minute genes

The burning question to be answered is, what is the primary function of Minute loci? This study has not provided any definitive answers concerning the specific nature of Minutes. The proposal that some or many of these loci code for tRNA (K.C. Atwood, 1968) is still tenable, although preliminary genetic evidence argues against the idea that they are redundant (Huang and Baker, 1975). Temperature-sensitive mutations of the $tRNA^{TYR}$ locus have been reported for E. coli (Smith et al., 1970; Nomura, 1973). In one case, in vivo

experiments suggested that conformational changes in the mutant tRNA species led to irreversible inactivation of the molecule due to degradation at high temperatures (Nomura, 1973). In yeast, a ts nonsense suppressor has been identified and its genetic properties indicated that a mutant $\underline{tRNA}^{\underline{TYR}}$ was likely involved (Rasse-Messenguy and Fink, 1973). The *in vitro* ability of tRNA isolated from a super-suppressor mutant in yeast to translate nonsense codons, argues that at least for lower eukaryotes, nonsense suppression occurs by tRNA-mediated translational defects (Capecchi *et al.*, 1975). These studies caution us against assuming that thermolabile gene products must be proteins, thereby leaving the possibility open that *Q-III* is a ts mutation in a tRNA gene.

The pleiotropic phenotype of *Q-III* is clearly compatible with the 'tRNA hypothesis', especially in view of the quantitative and qualitative changes that occur for different tRNA species during development in *Drosophila* (White *et al.*, 1973). The disruptive effects of tRNA abnormalities on protein synthesis which might be regulated differently in various cell types and discs, could adequately account for the pleiotropy, although the additional contribution of translational (Ilan, 1968) and post-translational (Jacobsen, 1971) control, is also possible.

In situ hybridization to salivary gland chromosomes permits cytological mapping of loci specifying tRNA transcripts (Grigliatti *et al.*, 1974). If it can be shown that an iso-accepting species maps near the *Minutes* in proximal 3L, the possession of such a mutant will be invaluable (especially since the *Q-III* allele can be made homozygous at $22^{o}C$)

for the isolation and biochemical study of this gene product. It may be possible for example, to study the ability of the homozygote to incorporate labelled amino acids into protein at the restrictive temperature (see Farnsworth, 1970), thereby directly testing for disruption of protein synthesis in Q-III.

Another idea that is mentioned in the literature but seldom considered in detail is that Minute loci might code for ribosomal proteins. Previous evidence suggesting that the basal region of the X chromosome in Drosophila contained a cluster of genes specifying ribosomal proteins (Steffensen, 1973; Finnerty et al., 1973) has been recently disputed (Lambertsson, 1975b; Vaslet and Berger, 1976). Therefore, at least some of the Minute mutations could represent lesions in structural genes for these proteins.

Berger and Weber (1974) found almost no polymorphic electrophoretic variants in ribosomal proteins of several different mutants including su(f) and various Minutes, as well as wild-type strains of Drosophila melanogaster. One protein from the small ribosomal sub-unit in flies bearing a third chromosome Minute did show altered electrophoretic migration. However, such an observation could arise from a second site mutation in a non-Minute locus. The authors did not indicate whether quantitative differences in protein patterns could be measured. The Minutes in which no differences in ribosomal proteins were noted could have been deficiencies or hypomorphs, in which case no qualitative differences would be expected.

Lambertsson (1975a) analysed ribosomal protein patterns electrophoretically at various stages of development in Drosophila. He was

able to detect from 69 (in pupae and adults) to 74 (in larvae) different proteins, numbers which far exceed the estimated number of Minute loci (Lindsley et al., 1972). In addition, he found qualitative and quantitative changes in some of these proteins during development, particularly in the third instar. He (Lambertsson, 1975b) was unable to find evidence for the existence of a mutant ribosomal protein in the $1(1)su(f)^{ts67g}$ strain. However, he did find that pattern changes characteristic of the larval to pupal transition in the wild-type were delayed in ts67 at higher temperatures, and he concluded that the mutation probably causes a severely reduced larval ability to synthesize imaginal ribosomes.

Sussman (1970) proposed a model involving quantitative and qualitative control exerted by ribosomes on translation during development. DeWitt and Price (1974) found differences in ribosomal protein patterns which corresponded temporally with stage-specific appearance of immature erythrocytes in Rana catesbeiana, and they suggested that Sussman's model may have merit. Thus, absence or abnormality of a ribosomal protein could directly (through aberrant translational control), or indirectly (through generalized disruptions in protein synthesis) precipitate a large array of developmental defects.

Future considerations of the nature of Minutes should be concerned with the fundamental genetic organization and control of these genes as well as their biochemical properties. Indeed, it will likely be possible to genetically answer many of the outstanding questions, before their biochemical dissection has been accomplished.

Is Q-III a single site lesion?

The multiple phenes displayed by Q-III could have a trivial basis such as several different mutations on the Q-III chromosome. Cytological examination of the salivary gland chromosomes of Q-III by T. C. Kaufman, revealed no cytologically-visible aberration in chromosome 3. While all EMS-induced ts mutations usually map genetically at single sites (Suzuki, 1970), the occurrence of multiple mutations must be considered as a possibility. Since all experiments of the present study were performed using a recombinant stock in which the distal 3R region of the Q-III chromosome, from Hairless(69.5) to the tip was replaced, this likelihood is diminished. However, this is only a relatively small segment of the chromosome and second site mutations could reside elsewhere.

Three main findings support the notion that defects associated with Q-III represent expression of a single mutant site.

(1) In no case in either of the mapping experiments (see Table 22) were the rough eye, bristle or late-eclosing phenes separated from each other by recombination. For example, all 7 late-eclosing recombinants between Gl and H possessed small bristles and rough eyes as did all 11 late-eclosing crossovers in the st to p^p interval. Furthermore, while many of the late eclosing, eye-bristle recombinants exhibited leg anomalies (e.g. shortened tarsi, gnarled legs) and wing vein disruption, as well as absence of post-verticals, none of the recombinants with normal bristles, eyes and developmental periods displayed any of these defects. This was also verified for recombinants between st and p^p recovered at 22°C and retested at 29°C. While all

late-eclosing recombinants which displayed rough eyes and bristle phenes produced some progeny with different combinations of eye, wing, leg, and genitalia defects, none of these phenotypes was ever seen in recombinant progeny that were $\underline{M}^{+}$.

(2) Some of the ts phenotypes of Q-III such as ommatidial deficiencies and rough eyes have also been observed for ts67 (Dudick et al., 1974). Furthermore, the effects of the combination of Q-III and Scx on the wings at 29°C were also apparent for ts67 males (and a few females) carrying Scx at 28°C. Since Dudick et al. claimed that most of the original X chromosome containing this mutation was replaced, ts67 is likely due to a single lesion. Thus, a single site ts Minute such as Q-III, could mimic the known pleiotropic effects of ts67.

(3) If other third chromosome mutant sites are responsible for some of the mutant phenes expressed by Q-III, either: (i) the second site(s) must be a ts allele of a separate locus or (ii) the other site(s) must interact with Q-III at 29°C but not at 22°C. The probability of (i) would be low since the frequency of EMS-induced third chromosome Minutes is 1 in 3000 tested chromosomes (0.00033) and that of third chromosome recessive ts lesions is 0.055 (including both lethals and visibles) at the same dose of EMS (Tasaka and Suzuki, 1973). The probability of such a double mutant would be $0.00033 \times 0.055 = 1.8 \times 10^{-5}$ or about 1 in more than 55,000 chromosomes. Still, such a possibility cannot be dismissed entirely because Simpson and Schneiderman (1975) reported the recovery of a doubly mutant X chromosome containing a ts allele of scalloped, as well as a ts autonomous cell-lethal mutation.

The second possibility (ii) is difficult to rule out. The best way to counter this is to separate the smallest segment of the original chromosome which still carries the ts Minute locus, or alternatively, to map this locus cytologically using deficiencies. Thus, while it is possible that more than one mutant site is involved in Q-III, it is extremely unlikely.

2. Developmental Characteristics of Q-III

With a few exceptions, shorter (i.e. 24-hour) exposures of Q-III cultures to 29°C did not kill the animals. However, such treatment resulted in the production of many imaginal defects. The type of defects produced, depended on when during development the cultures were pulsed to 29°C. Therefore, the pulse experiments helped to define several TSPs for the pleiotropic phenotype of Q-III.

While most of the adult defects resulted from exposure of Q-III larvae (usually during the second or third instars) to 29°C, a few resulted from exposure during pupation. The former phenes will hereafter be referred to as pattern defects. These are particularly exemplified by deficiencies, duplications or other abnormalities which involve derivatives of the eye-antennal and dorsal mesothoracic discs. Such defects were observed in heat-treated progeny which bore Q-III alone, or in combination with Dfd (eye-antennal disc), vg or Scx (dorsal mesothoracic disc). Pattern defects involving derivatives of other imaginal discs, such as the genital and leg discs were also seen, but at lower frequencies. Since nearly all of the mitotic activity of the imaginal discs of Drosophila is restricted to the larval stages (see Nothiger, 1972), it is not surprising that the pattern defects of Q-III are associated with heat treatment during these stages.

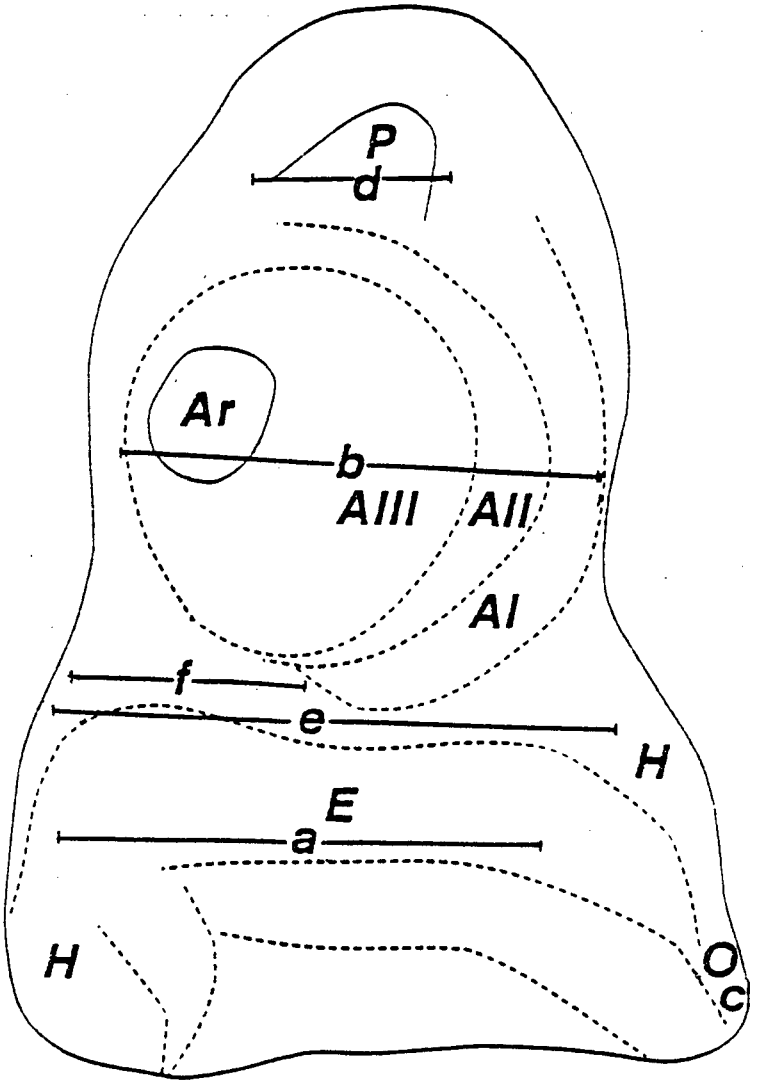
Pattern defects and cell death

Cell death in different parts of an imaginal disc, followed by varying degrees of proliferation could explain most of the eye-antennal phenes of Q-III homozygotes and heterozygotes.

Deficiencies, particularly of eye tissues, were the most frequently encountered pattern defects in derivatives of the eye-antennal discs of Q-III-bearing flies. Figure 21 is a schematic representation of an eye-antennal disc. If Q-III-induced cell death occurred more frequently in region a, lack of adequate cell replacement would produce ommatidial deficiencies. Less frequently, extensive cell death could embrace several regions simultaneously, thereby accounting for the occasional concomitant absence of eye (a), antennal (b), and head (d) structures, etc. (assuming that the presumptive cells were not replaced). More restricted cell death involving specific regions, for example, b, c, and d, could lead to deficiencies of individual antennal structures, ocelli or palps, respectively.

According to Bryant (1971), localized cell death in an imaginal disc could produce deficiencies, as well as regeneration of the missing structures, or mirror-image duplication of structures already present. The type of result depends on the repatterning of the new cells. Bryant suggests that there is a "developmental" gradient in which cells at the 'high' end are totipotent, whereas cells at the 'low' end are more limited. Thus, if cell death occurred and was mitotically compensated for in a region of the disc that is nearer to the top of the developmental gradient, the new cells could be re-patterned to produce structures normally derived from portions of the disc that are

FIGURE 21 A schematic diagram of a mature eye-antennal imaginal disc showing the following structures: presumptive first (AI), second (AII) and third (AIII) antennal segments; presumptive arista (Ar); presumptive eye (E); presumptive head (H); presumptive palpus (P); and the proposed ocellar region (O). Regions a, b, c, d, e and f are hypothetical zones of cell death; (after Gehring, 1966).


P
d
Ar
b
AIII
AII
AI
f
e
H
E
a
H
Oc

further down the gradient and regeneration would result. However, cell death and subsequent replacement in a section of the disc that is low in the gradient would allow only for the 'regeneration' of lower structures and as a result, mirror-image duplication would occur.

In the case of the schematic eye-antennal disc (Figure 21), if cell death was induced in region b to obliterate the antennal portion of the disc, adequate replacement of cells at region e could lead to regeneration of the antenna (Gehring, 1966; Bryant, 1971). On the other hand, if cell death in the area of the disc between e and f was followed by cell divisions near the antennal anlagen (i.e. at f), this could result in mirror-image duplication of the antenna. Finally, if cell death in this same section was followed by simultaneous proliferation at both e and f, concomitant regeneration and duplication could take place, thereby giving rise to a triplication of antennal structures. This present study has demonstrated that all of these eye-antennal pattern defects occur in Q-III-bearing flies exposed to 29°C during the larval period.

Verification of ts induction of cell death by Q-III will depend upon histochemical tests of discs. Such investigations could indicate whether cell death is random or localized. In this regard, the temperature-sensitivity of Q-III should prove amenable to *in vitro* analyses of cell death in isolated discs.

If we can assume that Q-III is an autonomous cell-lethal like other Minutes (Stern and Tokunaga, 1971), then this is the first report of a ts autonomous cell-lethal mutation on an autosome. Since Minutes have been shown to effect cell-autonomous reduction in mitotic rate (Morata and Ripoll, 1975), this may explain the low frequency of

duplications and triplications found in this study. Thus, even brief exposure of the disc to high temperature could prevent cell replacement that might normally follow cell death.

In light of the above, it would not be surprising if some sort of cell death in the wing disc is also responsible for the scutellar pattern defects observed in Q-III-bearing individuals. According to Bryant (1975), fragments isolated from the mesothoracic (wing) discs which include the anlagen of the scutellum, frequently give rise to structural duplications. However, since misplaced bristles or sockets are frequently scattered over the scutella of Q-III flies, clear pattern disruptions, as well as what appear to be duplications, are occurring.

While wing scalloping was not seen in Q-III flies, in adults bearing Q-III in concert with Scx or vg at 29°C, such wing margin effects are quite striking, particularly in Scx/Q-III flies. Fristrom (1969) showed that the wing phenotypes of vg, Beadex (Bx), cut (ct) and apterous^{Xa} are the result of cell death either during the third larval instar, or early pupation (in the case of ct). Santamaria and Garcia-Bellido (1975) used an elegant approach involving clonal analysis to show that the cell death in Bx occurs at about the middle of the third instar. In the present work, the TSP for the Q-III-Scx wing phene occurs during the first half of the third instar and therefore it is likely that cell death occurs at that time or shortly thereafter. It is worthy of mention however, that the TSP for the Q-III-vg interaction seen in this study is considerably earlier than the time when cell death purportedly takes place. Even if Harnly's (1936) TSP for vg at the end of the second instar is quite precise, Fristrom (1969) has

reported that cell death is not detectable until the late part of the third instar in wing discs of vg flies.

Cell death routinely occurs in wild-type discs of Drosophila. For example, Spreij (1971) observed degenerating cells in the wing pouch and other sections of the dorsal mesothoracic disc, at about mid third instar. In addition, Fristrom (1969) detected similar necrosis in the wild-type eye-antennal discs, particularly near the area between the eye and antennal portions, in both the second and third instars.

Since cell death has been offered as a potential contributor to morphogenesis in normal discs (see Nothiger, 1972), one hypothetical mechanism by which mutants such as vg, ap^{Xa}, Bx and ct could produce wing defects, would involve an extension of the boundaries of normally-restricted cell death which is assumed to occur during the formation of the wing margin (Bryant, 1975). A similar explanation could apply to eye mutants such as Bar, eyeless, and Dfd with regard to extensive destruction of presumptive facet tissue promoted by these mutants. Presumably, Q-III alone, or in combination with mutants like Scx (wing disc) and Dfd (eye-antennal disc) might similarly extend the boundaries of cell death upon exposure to 29°C by making more cells susceptible to the genetically programmed, regionally-specific cell death. Further study of these Q-III-mediated interactions should provide considerable information about cell death during development in imaginal discs, and its relationship to pattern phenomena such as duplication formation and regeneration.

Defects resulting from heat treatment of Q-III during pupation

Whereas most of the pattern defects of Q-III-bearing flies (homozygotes) were produced by briefly exposing larvae to 29°C, other imaginal

defects were expressed by Q-III individuals that had been heat-pulsed during pupation. For example, the classical M phene of short bristles, as well as the comb gap phene, were observed in Q-III adults which emerged from cultures treated in this manner. This raises the possibility that these latter abnormalities are directly related to the disruptive effects of the Q-III lesion on protein synthesis at the time of differentiation. This situation contrasts with that of the pattern defects, which probably originate from imaginal cell death at much earlier stages than differentiation (i.e., during the larval stages).

The finding that the TSP for the Minute bristle phenotype occurs at the time of bristle formation during the first half of pupation (Bodenstein, 1950) is consistent with the idea that rapid accumulation of protein is a prerequisite to bristle synthesis (Howells, 1972) and that any disturbance in the translation process (i.e. bb, $su(f)^{ts67}$ or Minutes) directly results in attenuated bristles. My findings are in agreement with those of Dudick et al. (1974) who determined that ts67 suppression of forked occurs at the time of bristle formation. It should be possible to use this phenotype to select for dominant and recessive mutations that affect translation.

While the TSP of the comb gap phenotype also occurs during the first half of pupation, the situation here with respect to the effects of Q-III on protein synthesis, is unclear. Cell lineage studies of the legs of Drosophila males (Tokunaga, 1962) have revealed that cells in the region of the basitarsus which contain the presumptive sex comb tissue (the combs are actually modified macrochaetae) undergo a characteristic shift and rotation of about 90 degrees, so that the formerly transverse row becomes longitudinally placed. Tokunaga makes no mention

of when this shift actually occurs in the presumptive sex comb tissue. If rotation occurs during the early part of pupation, then presumably the lack of Q-III^{+} gene product could prevent successful rotation. For example, the effects of Q-III on translation could kill key cells involved in the rotation. On the other hand, rotation might occur earlier and removal of the Q-III^{+} product at, or just prior to leg eversion could de-stabilize the sex-comb alignment. Remember that rotation of the terminalia in males is also blocked by exposing Q-III progeny to 29^{o}C. However, in this case, the TSP is larval. Further study of this ts Minute will clearly increase our knowledge about such morphogenetic processes.

The interactions of Q-III

This study has shown that lesions such as Q-III can be useful for the developmental study of other genes for which ts alleles are unavailable (see Dudick et al., 1974). Thus, TSPs have been defined for vg, D1 and Scx. The TSP for the vg-Q-III interaction occurs in the second half of the second instar, while that for the Scx-Q-III interaction falls in the first half of the third instar. The D1-Q-III TSP for lethality extends from the mid second to mid third instars. It is interesting that the TSP for Scx is larval since this may also be true for its homeotic effect. However, it should be emphasized that more than one TSP may exist for a given gene product (Grigliatti and Suzuki, 1970; Mglinets, 1975). Therefore, the possibility of additional TSPs should be considered for the above genes.

Similar use of Q-III could permit the delineation of the time of action of the gene products of Dfd and Ly. The suppression of the sex

comb phenotypes of some of the homeotics by Minutes has been demonstrated. Q-III might also provide an estimate of the interval in development when the gene product of Polycomb (the most fully penetrant of the sex comb homeotics) is utilized.

It is noteworthy that of the mutants affected by Q-III at 29°C, both vg and Dl are dosage-sensitive (Lindsley et al., 1972). The basis for the Dfd lesion is unknown and there is some evidence which suggests that at least Pc is an antimorph (Puro and Nygren, 1975). Thus, flies heterozygous for deficiencies for vg and Dl display the respective phenotypes (Lindsley et al., 1972). The interaction of Minutes with these mutations could therefore be due to the inhibitory effects of Minutes on protein synthesis. In other words, the Minutes could be producing a synthetic hypomorphic situation by decreasing the amount of vg^+ or Dl^+ product. If this is true then we might expect that the expression of heterozygous deficiencies for these loci would be enhanced by Q-III at 29°C and this would effectively rule out the possibility that Q-III is interacting with mutant vg or Dl gene products. Other dosage-sensitive loci such as Ultrabithorax, Intersex and Hairless on chromosome 3, and Star, black and Plexate on chromosome 2 (Lindsley et al., 1972) could be tested to see if they are also enhanced by Q-III.

The interaction of Q-III with the homeotics is clearly complex. While Q-III suppresses the sex comb phenes of Pc and Msc, it decreases the viability of Msc-, Scx-, Antp-, and possibly Ns-bearing flies. Furthermore, it interacts with Scx to produce a new mutant phene, wing scalloping. The latter phene is not specific to Minutes, suggesting

that this interaction is metabolic rather than specific. It might be argued that the retarding effects which Minutes have on development actually allow the accumulation of the antimorphic gene products of (some of) the sex comb homeotics, thereby leading to the production of new phenotypes or lethality. The suppression of the sex comb phenes is puzzling since Q-III does not inhibit sex comb formation on the forelegs. This phenomenon clearly merits further study.

The fact that ts67 interacts with Scx in a fashion similar to Q-III, underscores the contention that many of the phenotypic interactions described for different loci in Drosophila (i.e. suppression or enhancement) may be metabolic rather than specific (Kaufman et al., 1973). Therefore, it is important to exercise caution when attempting to interpret interactions in specific terms as has been done with the proposed nature of the suppression of forked by ts67 (Dudick et al., 1974). It may be that this suppression is due to generalized decreases in protein synthesis rather than to ribosomally-mediated, informational suppression.

Additional uses for Q-III in studies of development

Several potential uses for Q-III in the study of development emerge from the fact that Minutes lower mitotic rates in a cell-autonomous fashion (Morata and Ripoll, 1975). For example, it should be possible to produce clones of Q-III homozygous cells in a Q-III heterozygous background at 22°C by somatic crossing over. Since the background cells would be wild-type at 22°C, such an approach may allow more information to be gleaned from studies which exploit this phenomenon. In one instance, Garcia-Bellido et al. (1973) used the tendency for M^+/M^+

clones to overgrow their heterozygous background to investigate developmental compartmentalization in the wing disc. The possession of Q-III should considerably expand the scope of similar investigations, particularly those involving the eye-antennal disc.

The detrimental effects of Q-III on viability and growth of larvae are amply demonstrated in this study. Some of the Q-III-induced larval lethality could be due to the inability of the larvae to metamorphose, which in turn could be related to lack of competence of imaginal disc cells (see Nothiger, 1972) because of death or slow mitosis. However, larval death is undoubtedly also due to the direct effects of Q-III-mediated disruptions in protein synthesis. It is known that larval cells grow by increases in cell size, while imaginal disc grow mitotically (Bodenstein, 1950). It should therefore be possible to specifically probe the growth of imaginal vis à vis that of larval tissue.

CHAPTER 6

OVERVIEW

The research described in this thesis was designed to investigate regions near the centromere of chromosome 3 of Drosophila melanogaster, with the following aims: (a) to investigate proximal recombination in females with a view to examining interference and interchromosomal effects (b) to determine if Deformed, a mutation which maps near the centromere, is recessive lethal and to map this locus relative to Kinked (c) to see if selecting for radiation-induced, proximal crossovers will enrich for deletions in proximal segments and (d) to genetically and developmentally characterize Q-III, a ts allele of a proximally-located Minute. For the most part these objectives have been realized.

The experiments described in CHAPTER 2 have helped to further characterize crossing over near the centromere of chromosome 3. First, some results suggested that much of the increase in proximal crossing over caused by the inverted attached-X chromosome C(1)M3, preferentially takes place within centrically-adjacent euchromatin. Since it is likely that little or no spontaneous crossing over occurs in heterochromatin, such recombinagenesis may be confined solely to proximal euchromatic segments. Second, this study has provided results suggesting that multiple (double and triple) crossovers are detectable at higher than expected frequencies in proximal intervals.

The latter results are of particular interest since they mark a striking departure from two classical rules of intergenic recombination: (a) positive chromosome interference within a given region varies

inversely with the genetic size of that region and (b) crossing over within different arms of the same chromosome is independent, i.e. positive interference does not extend across the centromere. Three possible explanations were offered to account for these results: (1) the Two-Step model (mitotic followed by meiotic crossovers) (2) chromatid interference and (3) gene conversion.

The following experimental evidence supports the first possibility: (i) females producing multiples also showed higher frequencies of crossing over than did those females producing no multiples and (ii) fewer double relative to triple exchange tetrads were observed in a tetrad analysis of the data. However, the predicted clustering of single crossovers which would likely accompany a Two-Step production of multiple crossovers, did not occur for females producing double crossovers.

Two lines of evidence were presented in support of the idea that gene conversion may be involved in the production of putative multiple crossovers. First, equal numbers of reciprocal crossover classes were not recovered. Second, when crossing over was measured in females carrying C(1)M3, negative interference in most proximal intervals showed a relative decrease, in spite of the fact that crossing over was increased in all intervals. Whatever the cause of this observed high negative interference, these findings have introduced a totally new dimension to the consideration of linked exchange in this organism.

CHAPTER 3 described a genetic study of the Dfd locus. The results of the mapping experiment suggest that Ki and Dfd are very close, genetically. The results of the analysis of a large number of recombinant crossover chromosomes and successful synthesis of a homozygous Dfd

stock, confirm the notion that the Dfd lesion is by nature homozygous viable. Therefore, most Dfd stocks must carry at least 1 lethal site in addition to the Dfd mutation. However, the Dfd stock examined in the present study probably carried a minimum of 3 extra lethals.

This information will allow a more complete assessment of developmental and genetic studies of Dfd. It also emphasizes the fact that cytological mapping is preferable to crossover mapping, particularly when the mutation(s) in question lies near the centromere. Thus, the need for a wider inventory of stable proximal deficiencies and duplications is obvious.

The results of the study described in CHAPTER 4 agree with the idea that a large proportion of induced crossovers recovered from irradiated males, arise via asymmetrical exchange. Some of the results also support other workers in their claim that lethals are more common to crossover chromosomes, when the crossovers are produced by induced exchange within proximally-adjacent euchromatic segments.

In future, similar investigations will not only be sources of proximal aberrations, but will also provide considerable information about induced crossing over. Of particular interest will be the comparison of exchange within heterochromatic and proximally-adjacent euchromatic segments of the chromosome.

CHAPTER 5 was a detailed description of the genetic and developmental properties of Q-III, a ts allele of a Minute which is located near the centromere of chromosome 3. This represents the first report of a truly conditional Minute allele i.e. one which elicits the dominant M traits under restrictive conditions, but produces no such effects under permissive conditions.

The phenotypic similarities between Q-III, ts67 (su(f)^{ts67g}) and bobbed, favour the idea that Minute loci are involved at some level(s) in the process of translation. No conclusions about the exact nature of M gene products were reached in this study. In the case of Q-III, the primary product may be thermolabile. However, this would not eliminate the possibility that Minutes code for products as diverse as tRNA species or ribosomal proteins. Indeed, the assortment of M gene products may not be homogeneous with respect to the different components of translation.

The pleiotropy of Q-III is impressive. By exposing cultures to 29°C at various developmental intervals, it was possible to kill Q-III homozygotes as well as to produce homozygous (and heterozygous) adults with a large spectrum of imaginal defects. While the TSP of lethality is polyphasic, TSPs of the different phenotypes are generally monophasic. The patterns of lethality and sterility show that the Q-III^{+} product is essential for nearly all stages of development. However, if the organism is deprived of this substance for shorter periods, viability is more normal, but the phenotypic anomalies are seen. The observation that homozygous Q-III individuals (produced by heterozygous mothers) frequently reach the first larval instar before dying at 29°C, suggests that considerable Q-III^{+} product is supplied to the developing oocyte. This is further supported by the observed ts maternal effects of Q-III.

Sensitivity of Q-III larvae to heat-induced lethality is marked by either a reversible blockage of growth (usually followed by death), or more frequently by fairly rapid death. On the other hand, pupal

sensitivity appears to be related to blockages in differentiation. A parallel difference is apparent for some of the imaginal defects. Thus, most pattern defects,for example, those involving eye-antennal or wing disc derivatives, were produced by exposure of larvae to 29°C (usually during the second or third instar), whereas other defects such as attenuated bristles or abnormal sex combs, resulted from heat exposure during pupation. It is likely that the former phenes are caused by cell death within the respective anlagen in larvae, while the TSPs of the latter defects imply that they are due to the direct disruption of differentiation in Q-III individuals.

The Q-III-mediated translational difficulties can easily account for all of the phenotypic traits of this mutant. All developmental stages of Q-III homozygotes which require protein synthesis would inevitably succumb to heat-induced death. At 29°C, an embryo produced by a Q-III female could survive only as long as its supply of Q-III^{+} product lasted. Cell death, which is likely responsible for pattern defects exhibited by imagoes heat-treated as larvae, could be the result of the cell-autonomous failure of translation. On the other hand, imaginal defects such as the small bristle and comb gap phenes, which are seen in individuals heat treated during pupation, could be produced by impaired differentiation due to translational collapse at critical intervals in Q-III pupae. Finally, a lack of protein synthesis would probably result in the sterility of Q-III (homozygous) adults at 29°C.

Several intriguing ts interactions between Q-III and non-allelic genes were documented in this study. Some of these interactions (but

non ts) had been previously reported for Minutes, viz. synthetic lethality with Dl and Ly, production of wing scalloping with vg and suppression of the sex comb phenes of some of the homeotic mutants. In addition, I was able to show that Q-III is lethal in combination with Dfd at 29°C and that this situation is due to the failure of eye-antennal disc derivatives to develop. Q-III/Scx and Q-III/Msc combinations are less viable than controls at 29°C and the former individuals possess variable nicking of the posterior wing margin.

It was possible to determine TSPs of the gene products of vg, Dl and Scx. In each case the TSP is larval and in the case of Scx and vg, cell death is probably involved in the production of these traits.

The notion that these interactions are specific rather than metabolic in nature, is challenged by the observations that ts67 and other Minutes interact similarly with Scx at 29°C, and also that at least one additional Minute mutation suppresses the sex comb phene of Msc. These findings imply that the reduced translational capacity of Q-III-bearing individuals is sufficient to account for the observed interactions, without invoking the idea of gene-product interactions involving Q-III and the other loci.

The wide spectrum of Q-III properties has thus provided additional scope for the analysis of Minutes in general, and this proximally-located Minute in particular. That Q-III possesses great potential for the study of developmental processes peculiar to itself or other non-allelic mutations, has been amply demonstrated by these experiments.

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APPENDIX 1

Crossover Data and Estimates of Crossing Over

a) Crossover Data from Irradiated Males and Controls

Treatment of Male Parents	Brood Interval (days)	Parental Genotypes		Single Crossover Progeny		
				Region 1		Region 3
		+	in ri eg^2 Ki p^p e^s	st	in ri eg^2 Ki p^p e^s	eg^2 Ki p^p e^s
Control	0-3	1,565	1,584			
	3-6	665	623			
No Radiation	6-9	638	707			
	9-12	822	858			
	12-15	384	429			
Totals		4,074	4,201			
Expt I	0-3	1,518	1,585			
	3-7	552	544			
1000R	7-11	918	920	1	1	1
	11-16	3,699	3,710	3	2	1
	16-21	5,391	8,293	4		
	21-26	1,678	1,716			
Totals		13,756	13,768	8	3	2
Expt II	0-3	140	141			
	3-7	97	109			
2000R	7-11	589	549	2		
	11-16	309	303		1	
	16-21	1,693	1,703			
	21-26	732	760			
Totals		3,560	3,565	2	1	
Expt III	0-3	1,142	1,113			
	3-6	628	680	1		
3000R	6-12	805	848	1	1	
	12-17	3,614	3,586	1		
	17-22	3,881	3,981			
Totals		10,070	10,208	3	1	

APPENDIX 1a (continued)

Treatment of Male Parents	Brood Interval (days)	Single Crossover Progeny: Region 4 st in ri eg^2	Region 4 Ki p^p e^s	Region 5 st in ri eg^2 Ki	Region 5 p^p e^s	Region 6 st in ri eg^2 Ki p^p	Region 6 e^s	Doubles 3,4 eg^2	Doubles 5,6 p^p
Control	0-3						1		
	3-6								
No Radiation	6-9				3				
	9-12								
	12-15								
Totals					3		1		
Expt I	0-3						1		
	3-7								
1000R	7-11				1				
	11-16		6	1	2	1			
	16-21		2	1	1		1		
	21-26	6	5	2	1				
Totals		6	13	4	5	1	2		
Expt II	0-3								
	3-7								
2000R	7-11	1	1						
	11-16		1						
	16-21	7							
	21-26		3						
Totals		8	5						
Expt III	0-3								1
	3-6								
3000R	6-12	5	5	2		1	2	2	
	12-17	5	12	1	2				
	17-22	8	26						
Totals		18	43	3	2	1	2	2	1

b) Summary of Male Crossover Data and Estimation of Crossing Over Between st and p^p.

Treatment of Male Parents	Number of Parentals	Number of Crossovers		Experiment Totals
		st to p^p	p^p to c^s	
Control	8,275	3	1	8,279
Expt I	27,524	41	3	27,568
Expt II	7,125	16	-	7,141
Expt III	20,278	73	3	20,354
Experiment Totals	54,927	130	6	55,063
Control	3,835	3	-	
Expt III*	10,796	64	4	10,864
Estimations of Crossing Over	Control = 0.08 Percent (3838 Progeny) Expt III = 0.59 Percent			

* Progeny of fourteen cultures, days 7 to 22

APPENDIX 2

Summary of Results of 24-Hour Exposures of Q-III Homozygotes to 29°C at Specific Times During Development

Hours of Pulse	Number of Adults	Corrected Percent Viability	Percent Expression of Various Phenotypes of Surviving Progeny				
			Ommatidial Deficiencies	Antennal Deficiencies	Antennal Duplications	Thick Aristae	Palp Deficiencies
0-24	14(300)*	58.3	0	0	0	0	0
12-36	12(242)	63.2	0	0	0	0	0
24-48	28(300)	116.0	0	0	0	0	0
36-60	22(285)	95.7	0	0	0	0	0
48-72	25(515)	60.6	0	0	0	4.0	0
60-84	34(600)	70.8	26.1	8.8	0	11.8	0
72-96	37(504)	93.8	53.3	0	0	13.5	0
84-108	12(500)	30.0	72.3	0	8.3	25.0	8.3
96-120	35(500)	87.5	83.1	0	0	22.9	2.9
108-132	34(500)	85.0	58.3	8.8	8.8	58.8	2.9
120-144	20(200)	125.0	20.0	0	0	50.0	5.0
132-156	17(225)	106.0	5.9	0	0	5.9	5.9
144-168	13(250)	65.0	0	0	0	0	0
156-180	42(300)	120.0	0	0	0	0	0
168-192	29(300)	120.8	0	0	0	0	0
180-204	27(200)	130.0	0	0	0	0	0
192-216	14(300)	58.3	0	0	0	0	0
204-228	20(300)	47.6	0	0	0	0	0
228-252	6(300)	25.0	0	0	0	0	0
252-276	16(360)	55.6	0	0	0	0	0
276-300	13(342)	47.5	7.6	0	0	0	0
300-324	8(360)	27.7	0	0	0	0	0
324-348	22(300)	91.7	0	0	0	0	0

* Number in parentheses = Number of Eggs Shifted

APPENDIX 2 (continued)

Hours of Pulse	Percent Expression of Various Phenotypes of Surviving Progeny							
	Ocellar Duplications or Deficiencies	Small Thoracic Macrochaetae	Sex Comb Gaps	Thoracic Pattern Disruptions	Wing Vein Disruptions	Misrotated Male Terminalia	Leg Deformities	Etched Tergites
0-24	0	0	0	0	0	0	0	0
12-36	0	0	0	0	0	0	0	0
24-48	0	0	0	0	0	0	0	0
36-60	0	0	0	0	0	0	0	0
48-72	0	0	0	12.0	0	4.0	0	0
60-84	0	0	0	32.4	0	10.0	0	0
72-96	5.4	0	0	21.6	0	20.0	0	0
84-108	0	0	0	41.7	10.0	50.0	0	0
96-120	8.6	0	0	94.3	39.1	33.3	0	0
108-132	14.7	0	0	100.0	5.0	61.5	8.8	0
120-144	0	0	0	65.0	0	37.5	30.0	0
132-156	0	0	0	23.5	0	50.0	0	0
144-168	0	0	0	33.3	0	33.3	0	0
156-180	0	0	0	0	0	0	0	0
168-192	0	0	0	0	0	0	0	0
180-204	0	0	0	0	0	26.6	0	0
192-216	0	0	0	0	0	25.0	0	0
204-228	0	0	0	0	0	0	0	5.0
228-252	0	16.5	100.0 (males)	0	0	0	0	16.7
252-276	0	100.0	100.0	0	0	0	0	0
276-300	0	7.7	0	23.1	0	0	0	0
300-324	0	0	0	0	0	0	0	0
324-348	0	0	0	0	0	0	0	0

APPENDIX 3

Summary of Results of 48-Hour Exposures of Q-III Heterozygotes (TMI/QIII) to 29°C at Specific Times During Development

Hours of Pulse	Number of Adults	Corrected Percent Viability	Percent Expression of Various Phenotypes		
			Roughened Eyes	Ommatidial Deficiencies	Antennal Deficiencies
0-48	191	86.6	0	0	0
24-72	152	82.6	0	0	0
48-96	93	42.9	98.8	53.1	0
72-120	308	53.0	98.9	63.3	1.0
96-144	244	90.2	98.4	3.2	0
120-168	58	85.3	16.4	11.5	0
144-192	30	21.8	0	0	0
168-216	26	28.0	0	0	0
192-240	129	70.1	0	0	0
216-264	204	100.0	0	0	0
204-252	265	123.8	0	0	0
252-300	300	56.2	0	0	0
300-348	270	126.0	0	0	0
348-396	304	120.0	0	0	0

APPENDIX 3 (continued)

Hours of Pulse	Percent Expression of Various Phenotypes			
	Antennal Duplications	Wing Duplications	Thoracic Pattern Disruptions	Small Thoracic Macrochaetae
0-48	0	0	0	0
24-72	0	0.7	0.7	0
48-96	0	0	0	0
72-120	0.7	0	0	0
96-144	0.4	0	0	0
120-168	0	0	0	0
144-192	0	0	0	20.0
168-216	0	0	0	100.0
192-240	0	0	0	98.4
216-264	0	0	0	62.0
204-252	0	0	0	25.0
252-300	0	0	0	0
300-348	0	0	0	0
348-396	0	0	0	0