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Effects of a temperature-sensitive *Minute* mutation on gene expression in *Drosophila melanogaster*

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SUMMARY

Minute (*M*) lesions exhibit a striking propensity for interacting with many different mutations. In the past, few attempts have been made to explain these diverse phenomena. This study describes a variety of temperature-sensitive (ts) interactions exhibited by the ts third chromosome *Minute* mutation $M(3)LS4^{Q\text{-}III}$ (*Q-III*). Most of these interactions (i.e. those involving *vg*, *cp*, *Dl*, *Dfd* or *Ly*) reflect *Q-III*-induced enhancement of the respective mutant phenotypes at the restrictive temperature. However, *Q-III* also suppresses the extra-sex-comb phenotypes of *Pc* and *Msc* at 29 °C and evokes lethal and bristle traits when combined with J^{34e} at the restrictive temperature. All of these interactions are characteristic of non-ts *Minute* lesions and thus they appear to be correlated with general physiological perturbations associated with the *M* syndrome. In addition, our findings show that mutations that affect ribosome production and/or function, namely $su(f)^{ts67g}$ and bb^{ts-1}, exhibit interactions comparable to those elicited by *Q-III*. Hence, in accordance with previous findings, we argue that most of the *Q-III* interactions can be attributed to reduced translational capacity at the restrictive temperature. Finally, reciprocal temperature shift studies were used to delineate TSPs for interactions between *Q-III* and *vg* (mid to late second instar), *cp* (about mid-third instar), *Dfd* (early third instar) and *Dl* (late second to mid third instar). We believe that these TSPs represent developmental intervals during which the respective gene products are utilized.

1. INTRODUCTION

Minute (*M*) loci constitute the major class of haplo-insufficient genes in *Drosophila melanogaster*. Flies that are heterozygous for any *M* lesion exhibit a classical syndrome of phenotypic effects, including small and thin thoracic bristles, cuticular defects, prolonged development and reduced viability and fertility (Lindsley & Grell, 1968). In addition, these mutations are usually recessive lethal.

Despite the fact that *M* loci were identified more than 50 years ago, little definitive information about their genetic and functional properties is available. However, several different theories pertaining to the molecular basis of the *M*

phenotype have been proposed (for review, see Sinclair, Suzuki & Grigliatti, 1981). The most attractive is that of Ritossa, Atwood & Spiegelman (1966*a*), who postulated that *M* loci encode components of the cellular translation apparatus. This hypothesis primarily hinges upon the similarity between the *M* syndrome and the phenotype of *bobbed* (*bb*) individuals. The latter possess reduced amounts of ribosomal DNA and presumably are defective in their ability to synthesize proteins.

Atwood subsequently proposed the specific hypothesis that *M* loci are actually the structural genes of tRNAs (Ritossa, Atwood & Spiegelman, 1966*b*). However, this hypothesis is not supported by experimental evidence (White, 1974; Huang & Baker, 1976; Hayashi *et al.* 1980; Larsen *et al.* 1982).

One possibility that has not been actively pursued is that *M* loci encode other specific components of translation, e.g. amino acyl tRNA synthetases or ribosomal proteins (Ritossa *et al.* 1966*a*; Huang & Baker, 1976). Obviously, it is equally reasonable to suggest that *M* genes are functionally heterogeneous with respect to protein synthesis (White, 1974), or alternatively, that their functions are not directly related to the process of translation.

Recently, a third chromosome temperature-sensitive (ts) *Minute* mutation $M(3)LS4^{Q\text{-}III}$ (hereafter referred to as *Q-III*) was recovered and characterized (Sinclair *et al.* 1981). The conditional nature of *Q-III* has enabled us to identify novel developmental properties of *M* gene function and we have argued that all of these properties are consistent with the hypothesis that *M* loci encode components of translation.

One of the most intriguing attributes of *M* lesions is their ability to evoke an increase or decrease in the expressivity or penetrance of a wide variety of unrelated mutations (Schultz, 1929; Lindsley & Grell, 1968). We have observed that *Q-III* exhibits *M*-like interactions, but in a ts fashion (Sinclair *et al.* 1981). In this paper the catalogue of ts interactions exhibited by *Q-III* is extended. Many of these are typical *M*-like interactions, but some are novel. We have established that the novel interactions are not specific to *Q-III*, but apply to *M* mutations in general. Additional experiments are described in which $l(1)su(f)^{ts67g}$ and bb^{ts-1}, mutations that affect ribosomal protein (Dudick, Wright & Brothers, 1974; Lambertsson, 1975) and rRNA production, respectively, were tested for their ability to elicit *M*-like interactions. We have found that these lesions, presumably defective in their capacity for translation, do indeed exhibit interactions comparable to those evoked by *Q-III*. These findings are discussed with reference to proposed functions of *M* loci.

Finally, in analogy with previous studies (Kaufman, Tasaka & Suzuki, 1973; Dudick *et al.* 1974; Cross, 1977), we have utilized temperature shift analysis of these interactions to confirm that *Q-III* will be useful for delineating patterns of gene expression during development.

2. MATERIALS AND METHODS

Mutant strains and special chromosomes used

For a complete description of most of the mutations and special chromosomes used, consult Lindsley & Grell (1968). The following strains require special mention.

car l(1)su(f)^{ts67g} (hereafter referred to as *ts67*): an *X* chromosome line carrying a ts allele of the *suppressor of forked* (*su(f)*) locus (Dudick *et al.* 1974) and marked with the recessive eye-colour mutation *carnation* (*car*). *ts67/su(f)*$^-$ flies display a *bb*-like phenotype (i.e. prolonged development, small and thin bristles, cuticular defects) when raised at 28 °C. *ts67* homozygotes also exhibit an abnormal ribosomal protein profile during development at the restrictive temperature (Lambertsson, 1975).

B^s Ybb^{ts-1}y+ (hereafter referred to as *bb^{ts-1}*): a *Y* chromosome bearing an EMS-induced cold-sensitive *bb* lesion (a deletion that removes 45 % of the normal amount of rDNA), as well as euchromatic fragments from the *X* chromosome containing the *Bar* (*Bar of Stone* or *B^S*) duplication and the *y*$^+$ gene (Procunier & Williamson, 1974). *bb^1/bb^{ts-1}* or *bb*$^-$*/bb^{ts-1}* individuals die when raised at 18 °C and are semi-viable when raised at 25 or 29 °C; survivors exhibit an extreme or moderate *bb* phenotype at 25 and 29 °C, respectively. *bb^{ts-1}/bb*$^+$ individuals appear normal at all three temperatures. This *Y* chromosome is routinely maintained in combination with *XY^L. Y^S, y su-w^a w^a*.

Df(3L)M(3)LS4 (hereafter referred to as *M(3)LS4*): a gamma ray-induced third chromosome deficiency lacking the 79E5, 6; 80 segment of proximal *3L* (Sinclair, 1977). Deficiency heterozygotes exhibit an extreme *M* phenotype due to hemizygosity for the *M(3)LS4* locus (Lindsley *et al.* 1972).

M(3)LS4$^{Q\text{-}III}$ (referred to as *Q-III*): an EMS-induced ts *M* mutation (3–47·4) that is allelic to *M(3)LS4*. *Q-III/+* heterozygotes exhibit dominant *M* traits when raised at 29 °C, but display no obvious phenotype when raised at 22 °C. *Q-III* homozygotes survive and exhibit a moderate *M*-like phenotype at 22 °C, but die when raised at 29 °C; the temperature-sensitive period (TSP) of lethality extends from early first instar to the latter half of the pupal period (see Sinclair *et al.* 1981). All crosses involving *Q-III* utilized a third chromosome containing both *Q-III* and *p^p*.

Dfd p^p: a third chromosome line containing a homozygous viable allele of *Deformed*, linked to the recessive eye-colour mutation *pink-peach* (*p^p*) (Lindsley & Grell, 1968; Sinclair, 1977).

Scr^{Msc} (hereafter referred to as *Msc*) and *Antp^{Scx}* (hereafter referred to as *Scx*): for a complete description of these third chromosome homeotic mutations, see Lindsley & Grell (1968). The new nomenclature reflects the recent work of Lewis *et al.* (1980).

Dl^D: a recently isolated gamma ray-induced allele of *Delta* (Sinclair, 1977; see also Lindsley & Grell, 1968).

Culture conditions

All stocks were maintained and crosses performed on standard cornmeal-sucrose Drosophila medium with tegosept added as mold inhibitor. The standard culture temperature used was 22 °C. Unless indicated otherwise, the permissive and restrictive temperatures used were 22 and 29 °C, respectively.

Standard crosses

Routine crosses were performed in quarter-pint milk bottles. Fifteen pairs of parents were introduced into each bottle and the parents were transferred to fresh medium (at least twice) at 2-day intervals. These cultures were then grown at either the permissive or restrictive temperature and all classes of F_1 progeny were examined for reduced viability or the expression of novel phenotypes until 15 (at the restrictive temperature) or 18 (at the permissive temperature) days after the parents had been introduced. Specific crosses testing for *Minute*, *ts67* and bb^{ts1} interactions will be described in the appropriate section of the RESULTS.

Temperature shift studies.

For a complete description of the rationale and experimental procedures for determining temperature-sensitive periods (TSPs) using reciprocal shifts of cultures between permissive and restrictive temperatures, consult Tarasoff & Suzuki (1970) and Suzuki (1970). In the present work, the beginning of the TSP was usually defined as the first culture that produced a significant increase in the expression of the phenotype associated with gene interaction (visible trait or lethality) when shifted from the restrictive to the permissive temperature. Conversely, the end of the TSP was defined as the first culture that produced a significant reduction in the expression of the interaction phenotype when shifted from the permissive to the restrictive temperature.

TSPs of interactions between *Q-III* and *vg*, *cp*, *Dfd* and *Dl* were determined in four separate temperature shift studies, using the following procedure. First, a standard method of egg collection was used to obtain developmentally synchronous cultures (0–2 h post-oviposition, see Sinclair *et al.* 1981) produced by matings between *Q-III/TM3* females and males of the following genotypes: (*a*) *vg/vg*; (*b*) *cp/cp*; (*c*) *Dfd* p^p/*Dfd* p^p; and (*d*) Dl^D/*TM3*. Groups of petri plates (cross *a*) or vials (crosses *b*, *c* and *d*) containing embryos from the different crosses, were incubated at 22 or 29 °C. Then, subsets of each group (consisting of 4–7 vials or 2–4 petri plates, with 50 individuals per container), were shifted from the permissive to the restrictive temperature and vice versa, at successive 12-hour intervals after oviposition.

Developmental stages present in the cultures were identified at the time of shift and different larval stages were distinguished by examining the morphology of their mouthparts and anterior spiracles (Bodenstein, 1950). At 29 °C, the slow development of *Q-III* versus non-*Q-III* heterozygotes served to distinguish the two larval classes. Shifted cultures obtained from crosses (*a*) and (*b*) were examined for the presence of the nicked wing phenotypes characteristic of *vg*/+; *Q-III*/+ and *Q-III/cp* heterozygotes raised at 29 °C (Sinclair *et al.* 1981). Viability of the various

progeny classes was also noted. Shifted cultures obtained from crosses (*c*) and (*d*) were examined for viability (i.e. percent eclosion) of the progeny classes and enhanced expression of the mutant phenotypes displayed by *Dfd* and *Dl* individuals.

3. RESULTS

Minute-*like interactions displayed by* Q-III

We have previously shown that *Q-III* interacts in a dominant ts fashion with a variety of different mutations (Sinclair *et al.* 1981). Thus, the combination of *Q-III* with *vestigial* (*vg*) or *clipped* (*cp*) at 29 °C results in the manifestation of characteristic apical or posterior nicked wing phenotype, respectively. In addition, when combined with *Dfd*, *Q-III* elicits complete lethality at the restrictive temperature; death occurs at the pharate adult stage and is probably due to lack of eye-antennal disc derivatives. Finally, $Q\text{-}III/Dl^{D}$ heterozygotes fail to survive development at 29 °C. None of these effects occurs at 22 °C.

Two additional dominant mutations, *Lyra* (*Ly*) and *Jammed* (*J*) are known to exhibit reduced viability in combination with *M* lesions (Lindsley & Grell, 1968). Therefore, appropriate crosses were made to test for analogous interactions with *Q-III* and the results of these tests are shown in Table 1. Note that *Q-III/Ly* heterozygotes were inviable and $Q\text{-}III/+\,;\,J^{34e}/+$ individuals were only partially viable, when raised at 29 °C. In contrast, the viability of their 22 °C counterparts appeared normal. Clearly, *Q-III* exhibits ts lethal interactions with both mutations. It is also noteworthy that $J^{34e}/+\,;\,Q\text{-}III/+$ heterozygotes exhibited a scute-like bristle phenotype at 29 °C.

Preliminary observations suggested that *Q-III* may actually reduce the expression of the extra-sex-comb phenotype of the homeotic mutation *Msc*. Therefore, we decided to examine in detail the effects of *Q-III* on the expression of *Msc* and another sex-comb homeotic mutation, *Polycomb* (*Pc*). Accordingly, harems of *Q-III/TM3* females were mated separately to groups of *Msc/TM3* or $Pc^{3}/TM3$ males and the resulting cultures were grown at either 22 or 29 °C. The mesothoracic legs of all male progeny arising from these matings were examined for the presence of one or more sex comb teeth. The resulting data, given as percent expression (penetrance) of the respective mutant phenotypes, are presented in the bottom half of table 1. It is evident that *Q-III* evokes a marked reduction in penetrance of both Pc^{3} and *Msc* at 29 °C. For example, only 10% of the *Msc/Q-III* males exhibited the mutant phenotype at 29 °C, whereas more than 90% of the *Msc/TM3* males from the same cross did so. Similarly, the mutant phenotype was essentially absent in $Pc^{3}/Q\text{-}III$ males (i.e. the penetrance was 4%) at 29 °C, but it was fully expressed in $Pc^{3}/TM3$ males from the same cultures. In contrast, *Q-III* caused no substantial reduction in expression of either phenotype at 22 °C.

It is important to establish that all *Q-III* interactions are due to the *M* defect and do not stem from non-*M* and/or temperature effects. It is clear that most of them (i.e. interactions with *vg*, *Dfd*, *Dl*, *Ly* and *J*) are definitely *M*-like in nature (Schultz, 1929; Lindsley and Grell, 1968). However, the effects manifested by *Q-III* in combination with *cp*, *Pc* and *Msc* are fairly novel *vis á vis* the *M* syndrome.

Hence, we decided to test two non-ts *M* lesions, *viz. Df(3R)M(3)LS4* (note that *Q-III* is an allele at this locus) and *M(2)173*, for analogous interactions. The results of this analysis are presented in Table 2 (these crosses were performed at 22 °C). It can be seen that both unconditional *M* lesions evoke the characteristic nicked wing phenotype in combination with the *cp Scx* chromosome. However, note that they differ quantitatively with respect to their ability to effect this interaction. Thus, whereas all of the *M(3)LS4/cp Scx* heterozygotes displayed this phenotype,

Table 1. *Temperature-sensitive interactions between* Q-III *and* Ly, J, Msc *and* Pc

Genotype of male parent	Progeny genotype	Number		Visible phenotypes* (penetrance)	
		22 °C	29 °C	22 °C	29 °C
ay/In(3LR)CxD†	*Q-III/CxD*	40	4	None	*M* (100 %)
	Ly/CxD	30	139	*Ly* (100 %)	*Ly* (100 %)
	Ly/Q-III	50	0	*Ly* (100 %)	—
J³ᵈᵉ/J³ᵈᵉ‡	*J³ᵈᵉ/+; TM3/+*	82	230	*J* (100 %)	*J* (100 %)
	J³ᵈᵉ/+; Q-III/+	112	19	*J* (100 %)	*J*; *M*; scute-like (100 %)
1sc/TM3‡	*Q-III/TM3*	202	7	None	*M* (100 %)
	Msc/TM3	197	893	Extra sex combs§ (58·3 %)	Extra sex combs§ (91.1 %)
	Msc/Q-III	203	102	Extra sex combs§ (68·4 %)	*M* (100 %); extra sex combs§ (10 %)
ᵇc³/TM3‡	*Q-III/TM3*	77	4	None	*M* (100 %)
	Pc³/TM3	69	291	Extra sex combs§ (96·4 %)	Extra sex combs§ (100 %)
	Pc³/Q-III	83	143	Extra sex combs§ (80 %)	*M* (100 %); extra sex combs§ (4·4 %)

* Exclusive of dominant phenotypes displayed by *CxD* and *TM3* flies.
† Female parent = *Q-III/CxD, D.*
‡ Female parent = *Q-III/TM3, Sb Ser.*
§ Presence of sex combs on second legs of males.

only half of the *M(2)173/+; cp Scx/+* flies did so. Subsequently this effect was confirmed using an unmarked *cp*-bearing chromosome and we have also observed equivalent effects in $M(3)h^{S37}/cp$ flies (data not shown).

The data in Table 2 also indicate that both of the unconditional *Minutes* markedly suppress the extra-sex-comb traits of *Pc* and *Msc*. For example, only about 12 % of the *Msc/M(3)LS4* or *Pc³/M(3)LS4* males displayed the mutant phenotype, whereas 70 and 83 % of their respective *TM3* counterparts did so. A comparable though less dramatic effect was observed from *M(2)173*. Thus, the penetrance of the *Msc* phenotype was only 38 % for *M(2)173/+; Msc/+*, versus 76 % for *SM5, Cy/+; Msc/+* males. Similarly, the penetrance of *Pc* was only 5 % for *M(2)173/+; Pc³/+* versus 53 % for *Cy, SM5/+; Pc³/+* males. From an examination of the progeny of the crosses involving the *cp Scx* chromosome, it was also evident that both *M(3)LS4* and *M(2)173* suppress the extra-sex-comb trait of *Scx*. For example, only 3 % of the *M(2)173/cp Scx* males exhibited the mutant phenotype, whereas more than 40 % of the *cp Scx/TM3* males did so. Likewise the

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penetrance of this phenotype was less than 30% for *M(2)173/+; cp Scx/+*, compared to 62% for *SM5, Cy/+; cp Scx/+* males. Similar observations have been made by R. Denell (personal communication) with respect to other *M* mutations. Therefore, it appears that the effects of *Q-III* on the expression of *cp*, *Msc* and *Pc*

Table 2. *Interactions between two unconditional* Minute *mutations and* cp, Scx, Pc *and* Msc *at 22 °C*

Parental genotype	Progeny genotype	Progeny Number	Visible phenotypes (penetrance) Extra sex combs	Visible phenotypes (penetrance) Nicked wings*
M(3)LS4/TM3† × *cp Scx/TM3*	*cp Scx/TM3*	357	44·4%	None
	M(3)LS4/cp Scx	121	3·4%	100%
M(3)LS4/TM3† × *Msc/TM3*	*Msc/TM3*	373	70%	None
	M(3)LS4/Msc	307	12%	None
M(3)LS4/TM3† × *Pc³/TM3*	*M(3)LS4/TM3*	6	None	None
	Pc³/TM3	50	83·3%	None
	M(3)LS4/Pc³	36	11·7%	None
M(2)173/SM5, Cy‡ × *cp Scx/TM3*	*M(2)173/+; TM3/+*	63	None	None
	SM5, Cy/+; cp Scx/+	82	61·5%	None
	SM5, Cy/+; TM3/+	77	None	None
	M(2)173/+; cp Scx/+	70	29·7%	50%
M(2)173/SM5, Cy‡ × *Msc/TM3*	*M(2)173/+; TM3/+*	82	None	None
	SM5, Cy/+; Msc/+	107	76·1%	None
	SM5, Cy/+; TM3/+	83	None	None
	M(2)173/+; Msc/+	109	37·7%	None
M(2)173/SM5, Cy‡ × *TM1/Pc³*	*M(2)173/+; TM1/+*	62	None	None
	SM5, Cy/+; Pc³/+	155	53%	None
	SM5, Cy/+; TM1/+	11	None	None
	M(2)173/+; Pc³/+	172	5·2%	None

* Exclusive of *Serrate* phenotype displayed by *TM3* flies.
† Cross utilized *M(3)LS4/TM3* males.
‡ Cross utilized *M(2)173/SM5, Cy* females.

are indeed typical of *M* lesions in general and that collectively these interactions probably constitute another facet of the *M* syndrome.

All of the *Q-III* interactions observed to date are summarized in Table 3. Perhaps not surprisingly, most represent enhancement of mutant phenotype. This is obvious for *vg* and *cp*, whose recessive effects on wing morphology are transformed into semi-dominant effects in the presence of *Q-III* at 29 °C. Similarly, the phenotype of uneclosed *Dfd/Q-III* pharate adults (i.e. a lack of eye, antennal

and head structures) at 29 °C is probably due to *Q-III*-mediated enhancement of the *Dfd* phenotype. In addition, the inviability of *Q-III/Ly* and *Q-III/Dl*^{D} heterozygotes at 29 °C, is consistent with the suggestion that *Q-III* enhances the lethal effects of these dominant mutations. In distinct contrast to these enhancing effects, *Q-III* suppresses the penetrance of the mutant sex-comb phenotypes of *Pc* and *Msc* at the restrictive temperature. Finally, the viability of *J*^{3ae}/+; *Q-III*/+ individuals is substantially reduced and they exhibit a novel bristle phenotype

Table 3. *Summary of temperature-sensitive lethal and visible phenotypic interactions evoked by* Q-III

Genotype of heterozygote	Phenotype of heterozygote* 22 °C	29 °C	Type of interaction
vg/+; *Q-III*/+	Normal	Apical wing nicks	Enhancement
J^{3ae}/+; *Q-III*/+	*J*	Low viability; *scute*-like	?
Ly/Q-III	*Ly*	Lethal	Enhancement
cp/Q-III	Normal	Reduced viability; wing nicks in posterior margin	Enhancement
Dfd/Q-III	*Dfd*†	Lethal‡	Enhancement
Dl^{D}/*Q-III*	*Dl*	Lethal	Enhancement
Msc/Q-III	*Msc*	Reduced expression	Suppression
Pc^{3}/*Q-III*	*Pc*	Reduced expression	Suppression

* Exclusive of the standard *M* phenotype.
† Low penetrance (i.e. less than 30%).
‡ Pharate adults lacked eye-antennal disc derivates.

when raised at 29 °C. Since the *J* lesion is not recessive lethal, it would appear that this interaction represents neither simple enhancement nor suppression of the mutant phene.

Do presumptive translational mutants display M-like interactions?

Since all of the interactions described above are not restricted to *Q-III*, it is reasonable to speculate that they stem from a basic metabolic defect common to all *M* lesions. Therefore, further investigation of these phenomena may provide useful information about the molecular nature of the *Q-III* defect, as well as those of other *M* mutations. For example, if we hypothesize that *M* gene products are involved in translation (Ritossa *et al.* 1966*a*), then all of the phenotypes characteristic of *M* interactions should be attributable to reduced rates of protein synthesis. One prediction arising from this hypothesis is that other mutations that affect protein synthesis should display similar interactions. Obvious candidates for translation defective lesions would be *bb* (the *bb* locus encodes rRNA) and *su*(*f*) mutations (*ts67*, a conditional allele of *su*(*f*), alters the ribosomal protein profile during development; Lambertsson, 1975). Therefore, the aforementioned prediction

was tested by examining bb^{ts-1} (Procunier & Williamson, 1974) and *ts67* (Dudick *et al.* 1974) for their ability to exhibit *M*-like interactions.

In the first experiment, harems of *ts67/ts67* females were mated separately to *vg/vg*, *cp/cp*, *Dfd/Dfd* or $Dl^D/TM3$ males. The resulting cultures were raised at 22 and 28 °C (*ts67*/Y individuals do not survive at 29 °C) and the progeny examined for reduced viability and expression of interaction phenotypes. The

Table 4. *Tests for interactions between* ts67 *and* vg, cp, Dfd *and* Dl

Genotype of male parent*	Progeny genotype	Number 22 °C	Number 28 °C	Visible phenotypes† (penetrance) 22 °C	Visible phenotypes† (penetrance) 28 °C
+/*Y*; *vg/vg*	*ts67*/+; *vg*/+	633	1043	None	None
	ts67/Y; *vg*/+	655	615	Nicked wings‡ (1·5%)	*bb*-like§ (100%) Nicked wings‡ (99·3%)
+/Y; *cp/cp*	*ts67*/+; *cp*/+	586	859	None	None
	ts67/Y; *cp*/+	542	232	None	*bb*-like§ (100%) Nicked wings‡ (99·1%)
+/Y; *Dfd/Dfd*	*ts67*/+; *Dfd*/+	778	597	*Dfd*‖	*Dfd*‖
	ts67/Y; *Dfd*/+	750	287	*Dfd*‖	Extreme *Dfd* (100%) duplicated maxillary palps (33·4%)
+/Y; $Dl^D/TM3$	*ts67*/+; Dl^D/+	307	349	*Dl* (100%)	*Dl* (100%)
	ts67/Y; Dl^D/+	262	207	*Dl* (100%)	*bb*-like§ (100%) extreme *Dl* (100%)
	ts67/*Dl*+; *TM3*/+	249	365	None	None
	ts67/Y; *TM3*/+	268	349	None	*bb*-like§ (100%)

* Genotype of female parent = *car ts67/car ts67*.

† Exclusive of dominant phenotypes displayed by *TM3* flies.

‡ Respective wing phenotypes similar to those of *Q-III/cp* and *vg*/+; *Q-III*/+ heterozygotes at 29 °C.

§ *bb*-like phenotype = small bristles, slow development, cuticular defects.

‖ Penetrance less than 30%.

results of these crosses (Table 4) are similar to those observed in the *Q-III* analysis. *ts67*/Y males heterozygous for recessive alleles of *vg* or *cp*, displayed reduced viability as well as the respective nicked wing phenotypes when raised at 28 °C, but they exhibited no such effects when raised at 22 °C. In addition, although *ts67*/Y; *Dfd*/+ males exhibited a normal phenotype at 22 °C, at 28 °C they had extremely reduced eyes and frequently their maxillary palps were duplicated in mirror image symmetry. Moreover, this genotype displayed reduced viability at 28 °C. Thus, *ts67* clearly resembles *Q-III* in its ability to enhance the expression of *vg*, *cp* and *Dfd*. Finally, it appears that *ts67* also enhances the expression of *Dl*, albeit in a less striking fashion than did *Q-III*. This conclusion is based on the slight but perceptible reduction in viability of *ts67*/Y; *Dl*/+ individuals (i.e. compare the relative viability of *ts67*/Y, Dl^D/+ males versus *ts67*/+; Dl^D/+ females at

22 °C, to that of *ts67*/Y; *TM3*/+ males versus *ts67*/+, *TM3*/+ females at 28 °C) and the fact that survivors of this genotype expressed a more severe *Dl* phenotype at the restrictive temperature.

bb^{ts-1} is a Y-linked cold-sensitive *bb* mutation (Procunier & Williamson, 1974). This lesion was tested for its ability to elicit *M*-like interactions with *vg* and *cp* as follows. First, *C(1)DX, y f*/Y; *SM1, Cy/vg* and *C(1)DX, y f*/Y; *TM3/cp* females

Table 5. *Tests for interactions between* bb^{ts-1} *and vg* and *cp*

Genotype of female parent*	Progeny class	Number	Inferred genotype	Novel phenotypes
C(1)DX, y f/Y; *SM1, Cy*/vg	(females)			
	f, B^S, *Cy*†	8	*C(1)DX, yf*/Y/B^S $Ybb^{ts-1}y^+$; *SM1*/+	None
	f, B^S†	7	*C(1)DX, yf*/Y/B^S $Ybb^{ts-1}y^+$; *vg*/+	None
	f, B^S, *bb, Cy*	19	*C(1)DX, yf*/B^S $Ybb^{ts-1}y^+$; *SM1*/+	None
	f, B^S, *bb*‡	3	*C(1)DX, yf*/B^S $Ybb^{ts-1}y^+$; *vg*/+	Nicked wings§
	(males)			
	y, Cy	351	$\widehat{XY}$, *y su-w^a* w^a/Y; *SM1*/+	None
	y	366	$\widehat{XY}$, *y su-w^a* w^a/Y; *vg*/+	None
	B^S†	1	$\widehat{XY}$, *y su-w^a* w^a/B^S $Ybb^{ts-1}y^+$; *vg*/+	None
C(1)DX, y f/Y; *TM3, Sb Ser/cp*	(females)			
	f, B^S, *Sb, Ser*†	28	*C(1)DX, yf*/Y/B^S $Ybb^{ts-1}y^+$; *TM3*/+	None
	f, B^S†	17	*C(1)DX, yf*/Y/B^S $Ybb^{ts-1}y^+$; *cp*/+	None
	f, B^S, *Sb, Ser, bb* ‡	1	*C(1)DX, yf*/B^S $Ybb^{ts-1}y^+$; *TM3*/+	None
	f, B^S, *bb*‡	3	*C(1)DX, yf*/B^S $Ybb^{ts-1}y^+$; *cp*/+	Nicked wings§
	(males)			
	y, Sb, Ser	174	$\widehat{XY}$, *y su-w^a* w^a/Y; *TM3*/+	None
	y	210	$\widehat{XY}$, *y su-w^a* w^a/Y; *cp*/+	None

* Genotype of male parent = XY^L. Y^S, *y su-w^a* w^a/B^S $Ybb^{ts-1}y^+$.

† Progeny probably arose through non-disjunction.

‡ *bb* phenotype = extreme bristle and tergite phenes and prolonged development.

§ Respective wing phenotypes similar to those of *Q-III/cp* and *vg*/+; *Q-III*/+ heterozygotes at 29 °C.

were produced (*C(1)DX* lacks most or all of the rRNA cistrons). Then, in separate crosses, females of each genotype were mated to $\widehat{XY}/Ybb^{ts-1}$ (i.e. XY^L. Y^S, *y su-w^a* w^a/B^S Ybb^{ts-1} y^+) males. The resulting cultures were incubated at 22 °C (a temperature which produces an extreme *bb* phenotype in bb^{ts-1}/bb^- individuals) and the appropriate offspring were examined for viability and the appearance of nicked wing phenotypes. The results of this experiment are presented in Table 5. As expected, because of the lack of rRNA cistrons in *C(1)DX, yf*/Ybb^{ts-1} individuals, there was a paucity of female relative to male progeny. Unfortunately, in both crosses only 3 of the diagnostic *bb* females (presumably *C(1)DX, y f*/$Ybb^{ts-1}y^+$; *vg*/+ and *C(1)DX, yf*/$Ybb^{ts-1}y^+$; *cp*/+) survived. However, the important observation is that these flies displayed nicked wing phenotypes very similar to those of their respective *vg*/+; *Q-III*/+ and *cp*/*Q–III* counterparts. In this context, it is noteworthy that none of the putative *C(1)DX, y f*/Y/$Ybb^{ts-1}y^+$; *vg*/+ and *C(1)DX, y f*/Y/Ybb^{ts-1} y^+; *cp*/+ individuals (probably derived from non-disjunction in the female parent) displayed wing phenotypes.

Taken together, the results of the foregoing experiments show that the presumptive translation mutants, bb^{ts-1} and *ts67*, mimic *M* lesions in their ability to elicit interactions when combined with certain mutations. These observations are entirely consistent with the hypothesis that products of *M* loci are involved in protein synthesis.

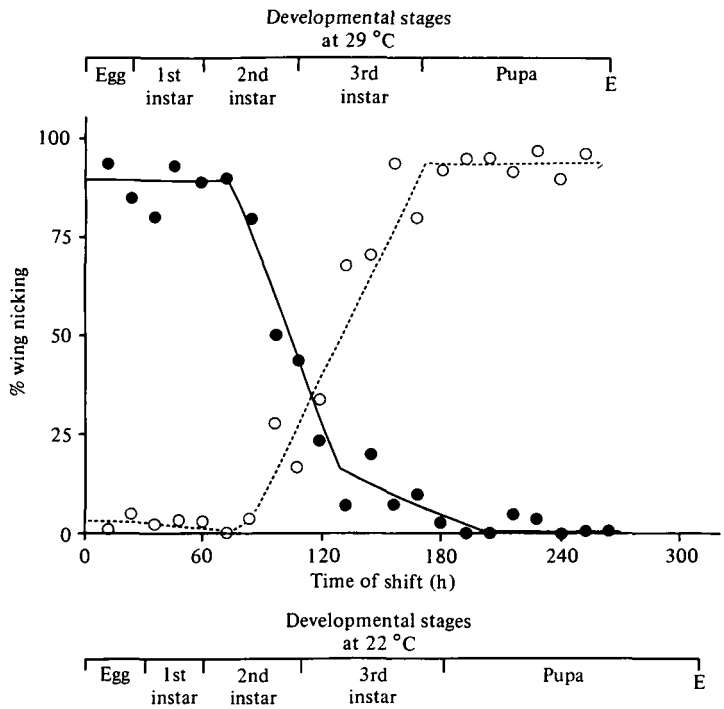

Fig. 1. Results of the reciprocal shift study to delineate a TSP for the wing phenotype of *vg*/+ ; *Q-III*/+ heterozygotes. The data are given as percent expression of the nicked wing trait among *vg*/+ ; *Q-III*/+ individuals shifted from 22 to 29 °C (●—●) and from 29 to 22 °C (○---○), at various times during development. The duration of each developmental stage at 29 and 22 °C, is indicated above and below, respectively. E, eclosion.

TSPs of Q-III *interactions*

The utility of ts interactions for the investigation of gene expression in *Drosophila melanogaster* is well documented (Kaufman *et al.* 1973; Dudick *et al.* 1974; Cross, 1977). Therefore, specific temperature shift studies were undertaken to establish whether *Q-III* interactions with *vg*, *cp*, *Dfd* and *Dl* were amenable to similar analysis. The results of these studies are presented in Figs. 1–4.

An examination of Fig. 1 reveals that the TSP of the wing phenotype of *vg*/+ ; *Q-III*/+ individuals occupies a short developmental interval in the latter half of the second larval instar. Perhaps not surprisingly, earlier studies utilizing different ts alleles of *vg* had established that the TSP for *vg* occurs during the larval stage (for review, see Suzuki *et al.* 1976). However, it is interesting that the TSP for the interaction with *Q-III* is reasonably similar to the *vg* TSP observed by Harnly (1936).

The TSP of the wing phenotype of *Q-III/cp* heterozygotes spans a developmental interval just prior to the middle of the third instar (Fig. 2). These heterozygotes also exhibited reduced viability at 29 °C and the TSP for this effect coincided precisely with that of the wing trait (data not shown). In the course of these experiments, we also observed that *Q-III/cp* survivors from cultures shifted up or down during the TSP frequently exhibited extreme reduction in wing size and/or loss of dorsal mesothoracic structures.

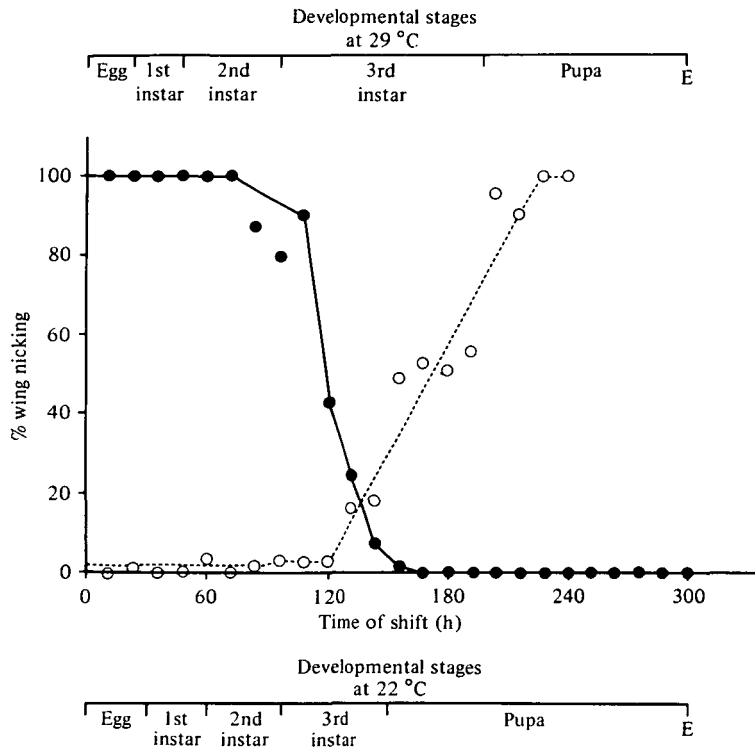

Fig. 2. Results of the reciprocal shift study to delineate a TSP for the wing phenotype of *cp/Q-III* heterozygotes. The data are given as percent expression of the nicked wing trait among *cp/Q-III* individuals shifted from 22 to 29 °C (●—●) and from 29 to 22 °C (○---○), at various times during development.

The TSP for lethality of *Q-III/Dfd* heterozygotes is a relatively short developmental interval corresponding to the first quarter of the third instar (Fig. 3). However, *Q-III/Dfd* heterozygotes resulting from cultures shifted from 29 to 22 °C during the early part of the second instar (i.e. prior to the lethal TSP) exhibited extreme eye reduction. This observation indicates that the lethal TSP is only a minimal estimate of the developmental interval during which the *Q-III/Dfd* interaction occurs. It is also of interest that *Q-III/Dfd* survivors of shifts up or down during the lethal TSP frequently displayed pattern defects, such as loss of antennal and head derivatives and antennal duplications.

The results of the experiments to delineate the TSP of lethality of *Q-III/Dl*^{D}

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heterozygotes are shown in Fig. 4. In the shift-down experiment, the effects of the restrictive temperature on viability were additive. Cultures shifted down prior to the third instar were mildly affected, whereas cultures shifted down during the third instar exhibited progressively more marked reductions in viability. Thus, it is difficult to define the precise onset of the TSP. However, the shift-up experiment indicates that the TSP for the lethal interaction terminates in the latter half of

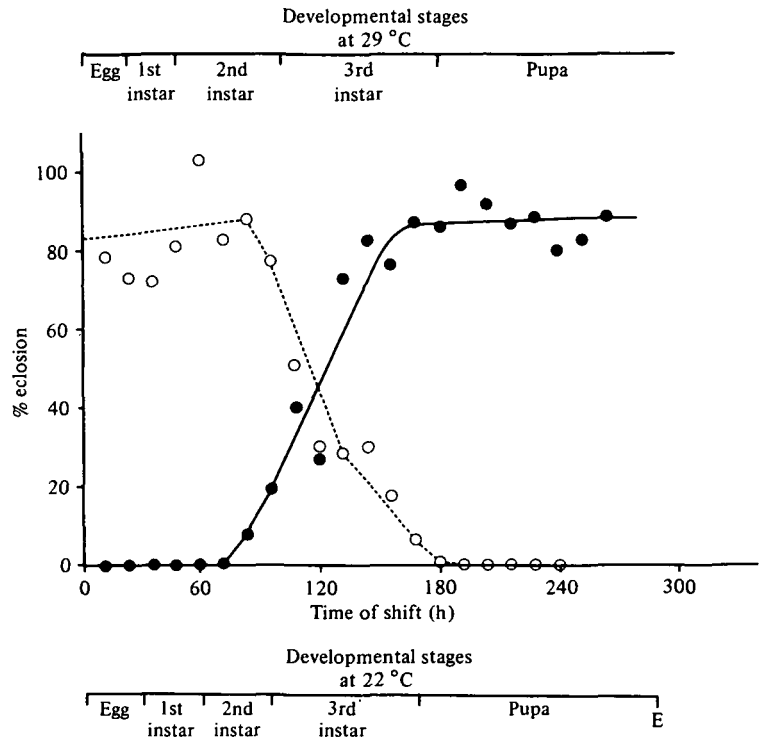

Fig. 3. Results of the reciprocal shift study to delineate a TSP of lethality for *Q-III/Dfd* heterozygotes. The data are given as percent eclosion of *Q-III/Dfd* individuals shifted from 22 to 29 °C (●—●) and from 29 to 22 °C (○---○), at various times during development.

the third instar. If the onset of the TSP is arbitrarily designated as the developmental interval when the viability of $Q\text{-}III/Dl^{D}$ individuals shifted from 29 to 22 °C was reduced to 75%, then the TSP of this lethal interaction extends from late second to about mid third instar.

4. DISCUSSION

The results of the present study, coupled with those of our earlier work (Sinclair *et al.* 1981), provide a striking illustration of the propensity of *Q-III* to exert dominant ts effects on the expression of a wide variety of mutations. At 29 °C, *Q-III* transforms the recessive effects of *vg* and *cp* on wing development into

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semi-dominant phenotypes. In addition, $J^{34e}/+$; *Q-III*/+, *Q-III/Ly*, *Q-III/Dfd* and *Q-III*/Dl^D genotypes are either lethal, weakly viable when raised at 29 °C. With the exception of the *J* interaction, all of these effects appear to involve *Q-III*-induced enhancement of mutant phenotypes at the restrictive temperature. Finally, *Q-III* exhibits ts suppression of the extra-sex-comb phenotypes of *Pc* and *Msc*.

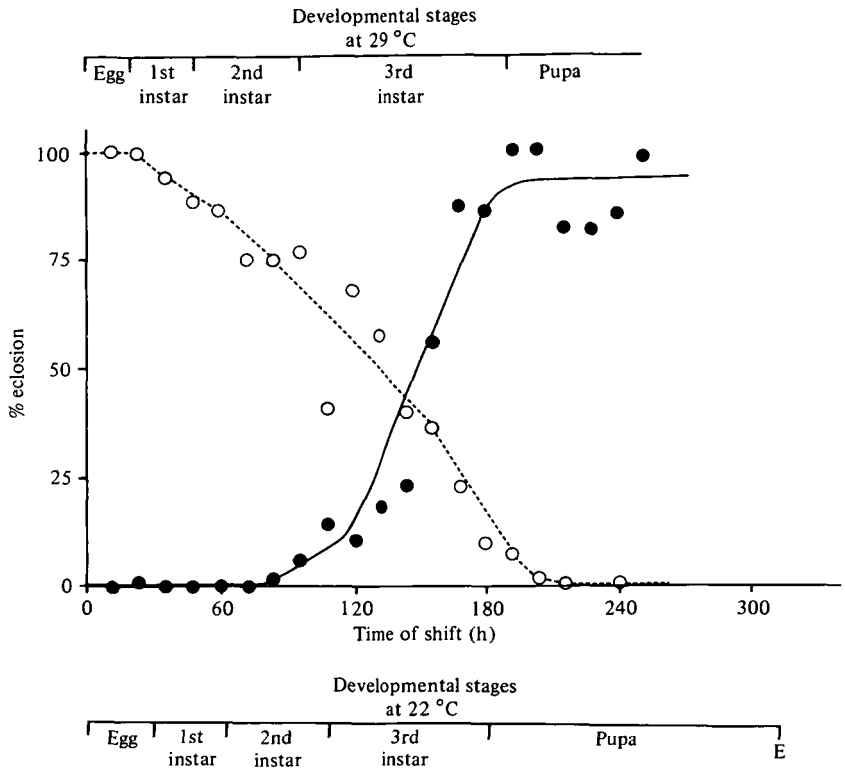

Fig. 4. Results of the reciprocal shift study to delineate a TSP of lethality for *Q-III*/Dl^D heterozygotes. The data are given as percent eclosion of *Q-III*/Dl^D individuals shifted from 22 to 29 °C (●—●) and from 29 to 22 °C (○---○), at various times during development.

It is clear from our work and previous studies that all of the interactions discussed here are exhibited by one or more *M* lesions and therefore they undoubtedly constitute a property of the *M* syndrome. The present work has also revealed that other mutations that produce a *M*-like phenotype, namely *ts67* and bb^{ts-1}, evoke similar interactions. Hence, our findings suggest that these phenomena do not stem from the specific interaction of one gene with another, but rather from indirect or metabolic perturbations (Gorini & Beckwith, 1966; Kaufman *et al.* 1973) common to all *Minute*, *bb* and *su(f)* lesions.

It is possible that many of these interactions represent independent but additive developmental phenomena. For example, it is likely that *Q-III*, *vg* and *cp* all cause cell death in the mesothoracic anlagen. Therefore, it is conceivable that enhancement of wing defects in *Q-III/cp* and *vg*/+; *Q-III*/+ heterozygotes results from the

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additive effects of the mutant combinations on cell death. A similar explanation could account for the *Q-III/Dfd* interaction. However, an important corollary of this hypothesis is that *Q-III* should exhibit comparable interactions with most if not all mutants that cause similar kinds of cuticular defects. A variety of observations (data not shown) indicate that this is not the case. For example, *Q-III* does not enhance the dominant notched wing phenotype of *Serrate*; nor does it enhance the severe reduced eye phenotype of *Lobed*. Moreover, *Q-III* exhibits no phenotypic interactions (e.g. extensive pattern abnormalities) with other *M* mutations or with *ts67*. Finally, the results of tests with several different unconditional recessive mutants that probably cause cell death in specific anlagen (e.g. *eyegone*, *gespleten* and *cut*), indicate that none of these lesions interacts with *Q-III* in the predicted fashion. Thus, although the cell death hypothesis cannot be disproven unequivocally, we feel that without special assumptions, it is inadequate to explain the aforementioned interactions.

Since *ts67* and bb^{ts-1} are presumptive translation mutants, it is reasonable to speculate that all of the *M* interactions are also attributable to reduced capacity for protein synthesis. This hypothesis is consistent with nearly all of the other aspects of the *Q-III* phenotype. However, it begs the question: how could reduced translational capacity produce such diverse effects on gene expression? Some *Q-III* interactions resemble the effects of prolonged pre-imaginal development on the expression of certain mutations and hence their relationship to the translation hypothesis may be self-evident. For example, we have observed that the *Msc* and *Pc* phenotypes are less extreme at lower temperatures (e.g. Table 1: compare the expression of *Msc* in *Msc/TM3* males at 29 °C, with that of their 22 °C counterparts). A similar effect has been observed for *Pc* under crowded culture conditions (Hannah-Alava, 1958). In addition, some of the *Pc* traits are less extreme in males than females (Lindsley & Grell, 1968) and development proceeds more slowly in males. Furthermore, the eye reduction produced by *Dfd* is more severe at lower temperatures (Lindsley & Grell, 1968). Therefore, the simplest explanation is that the *Pc*, *Dfd* and perhaps, *Msc* interactions result from prolonged pre-imaginal development, due to reduced rates of protein synthesis, in *Q-III* individuals.

The interactions involving *Dl*, *Ly*, *vg* or *cp* are less straightforward. One observation that may shed some light on the etiology of these interactions, is that the *vg* and *Dl* loci are haplo-abnormal; that is, flies hemizygous for the wild-type allele at either locus exhibit the corresponding mutant phenotype (Lindsley *et al.* 1972). Presumably, rapid synthesis and/or accumulation of vg^+ and Dl^+ gene products is required during development, and it is likely that most *vg* and *Dl* alleles are either hypomorphic or amorphic, mutations. Hence, a decreased rate of protein synthesis in *Minute* individuals bearing these alleles, could result in enhancement of the mutant *vg* or *Dl* phenotypes, by reducing the availability of the respective wild-type products. A simple prediction arising from this hypothesis is that the combination of a *vestigial* deficiency (an amorph) with *Q-III* would produce an even more extreme phenotype than that displayed by $vg^1/+$; *Q-III*/+ heterozygotes at 29 °C (presumably vg^1 is hypomorphic). A comparison of the wing phenotypes exhibited by flies of these two genotypes (Plate 1) confirms this prediction. At 22 °C, most $Df(2R)vg^B/+$; *Q-III*/+ heterozygotes exhibit the

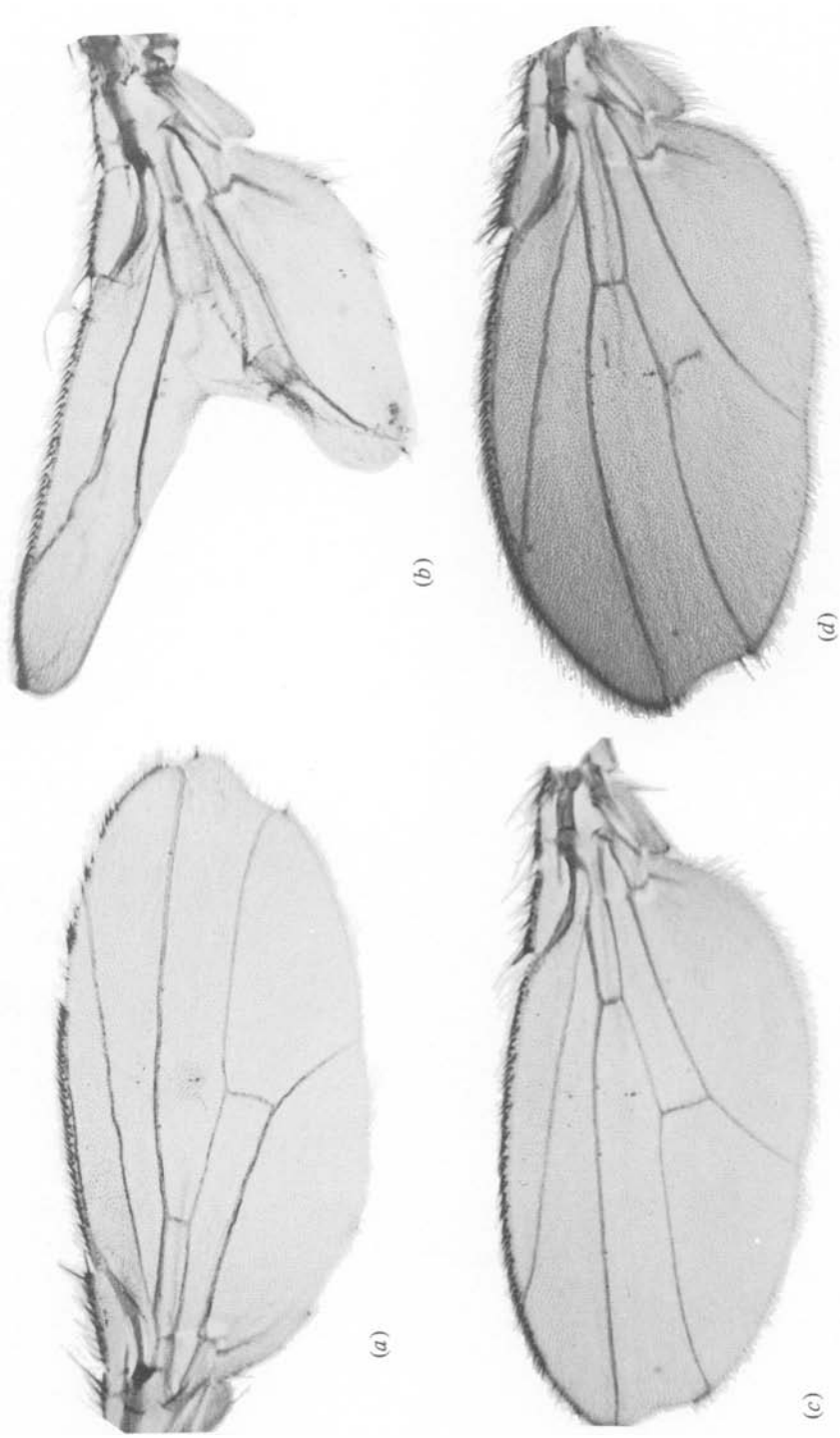
classical haplo-abnormal phenotype of *vg*, i.e. slightly nicked wings (Plate 1*a*). This phenotype is very similar to that of $vg^1/+$; *Q-III*/+ individuals raised at 29 °C (Plate 1*d*). However, at 29 °C, $Df(2R)vg^B/+$; *Q-III*/+ flies exhibit a strikingly reduced wing (Plate 1*b*). These observations are in complete accord with the contention that *Q-III* potentiates the effects of reduced *vg* gene function by decreasing the rate of synthesis of the vg^+ product. Similar reasoning would explain the *Q-III* interaction with *Dl*. Furthermore, if both *Ly* and *cp* were either hypomorphic or amorphic, then an analogous interpretation could also be extended to the interactions between these mutations and *Q-III*.

The basis of the *Q-III* interaction with *J* remains obscure. The viability and scute-like phenotypes displayed by $J^{34e}/+$; *Q-III*/+ individuals at 29 °C are novel and bear no resemblance to the *J* phenotype (i.e. J^{34e} is homozygous viable and affects only wing morphology). Furthermore, there is convincing evidence that *J* alleles are neomorphic or antimorphic mutations (Schultz, 1934; Mange & Sandler, 1973). Therefore, neither of the aforementioned explanations apply in this case and thus no obvious information about *Q-III* (or *Minute*) function can be gleaned from this observation.

In summary, like most of the developmental properties of *Q-III* (Sinclair *et al.* 1981), nearly all of the *Q-III* interactions can be explained on the basis of reduced protein synthesis. This hypothesis is further supported by the observation that the *ts67* (an allele of *su(f)*) and bb^{ts-1} mutations, both of which appear to effect translation, elicit comparable interactions. In this context, it should be mentioned that it has yet to be established unequivocally that *su(f)* mutations affect translation. Finnerty *et al.* (1973) have reported that polysomes isolated from *su(f)* larvae were defective in their ability to support polypeptide chain elongation in an *in vitro* assay. However, another group of workers (Hansson, Lineruth & Lambertsson, 1981) has claimed that *ts67* affects primarily glue protein production, rather than protein synthesis in general. We believe that it is extremely difficult to reconcile the latter argument with the highly pleiotropic phenotypes (e.g. larval and pupal lethality, sterility, imaginal disc and cuticular defects, small bristles, prolonged development, interactions with certain mutations etc.) of *ts67* and other *su(f)* alleles (Schalet, 1972; Dudick *et al.* 1974; Russell, 1974; Wilson, 1980). Clearly this pleiotropy points to a more fundamental defect, such as reduced translational capacity.

Ultimately, the validity of the hypothesis that *Q-III* affects translation must be substantiated by demonstrating a reduced ability of *Q-III* individuals to support protein synthesis at 29 °C. Recent preliminary experiments in our laboratory indicate that this is indeed the case. The level of protein synthesis in homozygous *Q-III* adults exposed to 29 °C is about half that of wild-type flies at the same temperature, whereas at 22 °C, protein synthesis in the two genotypes is equivalent. We are currently attempting to confirm this preliminary observation and to quantify these effects more precisely in both larval and adult stages.

Although the translation hypothesis is attractive, it is obvious that the present data are also compatible with other hypothetical *Minute* functions. For example, defects at the level of transcription, RNA processing, or even post-translational modification could elicit developmental effects similar to those observed here.



Comparison of the wing phenotype exhibited by $Df(2R)vg^B/+;Q\text{-}III/+$ with that of $vg^1/+;Q\text{-}III/+$ heterozygotes. (*a*) $Df(2R)vg^B/+;Q\text{-}III/+$ heterozygotes raised at 22 °C. (*b*) $Df(2R)vg^B/+;Q\text{-}III/+$ heterozygotes raised at 29 °C. (*c*) $Df(2R)vg^B/+;TM1/+$ heterozygotes raised at 29 °C. (*d*) $vg^1/+;Q\text{-}III/+$ heterozygotes raised at 29 °C.

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Indeed, even if specific *M* mutations (e.g. *Q-III*) do directly affect protein synthesis, it is possible that other *M* loci are involved in some of these other general processes. Clearly, the resolution of the question of *Minute* function will require in-depth genetic, developmental and molecular analyses of a variety of different *M* genes.

The present study also demonstrates the value of *Q-III* as a tool for investigating gene expression. Thus, we have used reciprocal temperature shift studies to delineate specific TSPs for several *Q-III* interactions: *vg* (the latter half of the second instar), *cp* (just prior to the middle of the third instar), *Dfd* (first quarter of the third instar) and *Dl* (late second to mid third instar).

The important question is do the interaction TSPs actually represent developmental intervals during which the *vg*, *cp*, *Dfd* and *Dl* gene products are required? Assuming that the interactions stem from *Q-III*-mediated effects on the expression of these genes and given that the *Q-III* gene product is required continuously throughout development (Sinclair *et al.* 1981), we feel that this is a reasonable interpretation. It is strengthened considerably by the similarity between the TSP for *vg* obtained from an earlier temperature-shift study (Harnly, 1936) and that obtained for the *vg* interaction in the present study. Moreover, it is consistent with conclusions of comparable studies that involved ts interactions between *zeste* and *bithorax* (Kaufman *et al.* 1973), *ts67* and *forked* (Dudick *et al.* 1974), as well as *ts67* and *lozenge* (Cross, 1977). Although we favour this interpretation, we recognize the complexity of these developmental phenomena. Thus, even if interaction TSPs are equivalent to TSPs of the genes in question, it is unlikely that in every instance they constitute the entire profile of expression. Indeed, it is clear from our study that the TSP for *Q-III/Dfd* lethality is only a minimal estimate of the developmental requirement for the *Dfd* product. Furthermore, one would expect the scope of the interaction and thus the TSP, to vary according to the strength of the allele tested. This could influence not only the temporal but also the tissue-specific pattern of gene expression obtained from such studies. In summary, we believe that the TSPs for the interactions between *Q-III* and *vg*, *cp*, *Dfd* and *Dl* identify intervals during development when the respective gene products are utilized.

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