GENETIC AND DEVELOPMENTAL ANALYSIS OF A TEMPERATURE-SENSITIVE MINUTE MUTATION OF *DROSOPHILA MELANOGASTER*[1,2]

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ABSTRACT

A temperature-sensitive (ts) third chromosome Minute (*M*) mutation, designated *Q-III*, has been recovered and characterized. *Q-III* heterozygotes raised at 29° exhibit all of the dominant traits of *M* mutants including small bristles, rough eyes, prolonged development, reduced viability and interactions with several unrelated mutations. *Q-III* homozygotes raised at 29° are lethal; death occurs primarily during the first larval instar. When raised at 22°, *Q-III* heterozygotes are phenotypically normal and *Q-III* homozygotes display moderate *M* traits. In addition, *Q-III* elicits ts sterility and maternal-effect lethality. As it true of *M* lesions, the dominant traits of *Q-III* are not expressed in triploid females raised at 29°. Complementation tests suggest that *Q-III* is a ts allele of *M(3)LS4*, which is located in *3L* near the centromere.——Reciprocal temperature-shift experiments revealed that the temperature-sensitive period (TSP) of *Q-III* lethality is polyphasic, extending from the first instar to the latter half of pupation. Heat-pulse experiments further resolved this into two post-embryonic TSPs: one occurring during the latter half of the second larval instar, and the other extending from the larval/pupal boundary to the second half of pupation. In addition, heat pulses elicited a large number of striking adult phenotypes in *Q-III* individuals. These included pattern alterations such as deficiencies and duplications and other morphological defects in structures produced by the eye-antennal, leg, wing and genital imaginal discs and the abdominal histoblasts. Each defect or pattern alteration is associated with a specific TSP during development.——We favor the interpretation that most of the major *Q-III* defects, particularly the structural duplications and deficiencies, result from temperature-induced cell death in mitotically active imaginal anlagen, while the small macrochaete phene probably results from the direct effects of *Q-III* on bristle synthesis. The hypothesis that the *Q-III* locus specifices a component required for protein synthesis is discussed, and it is concluded that this hypothesis can account for the pleiotropy of *Q-III*, and that perhaps it can be extended to *M* loci in general.

ONE of the most intriguing phenotypic syndromes in eukaryotes is displayed by mutants bearing lesions in any of the multitude of Minute (*M*) loci in *Drosophila melanogaster* (LINDSLEY and GRELL 1968). While this group of

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phenotypically similar mutants has been studied in detail for more than half a century, a definitive molecular characterization of the *M* defect has remained elusive. Over the years, it has been postulated that the *M* phenotype stems from defective production or utilization of ecdysone (BREHME 1939), respiratory enzyme defects (FARNSWORTH 1965), or lesions in the structural genes of tRNAs (RITOSSA, ATWOOD and SPIEGELMAN 1966a). However, none of these ideas has survived experimental scrutiny, and as yet, no workable hypothesis has emerged to explain the *M* syndrome. This paper presents a novel analysis of the *M* function. Taken together with other evidence, our findings are consistent with the hypothesis that the phenotypic characteristics of *M* mutations as a group are attributable to alterations in the translation process leading to a reduction in the rate of protein synthesis. It is our belief that, while this notion encompasses a rather broad spectrum of genetically determined functions, the hypothesis that *M* loci encode a single class of gene products is an unwarranted oversimplification.

Minute mutants characteristically display several dominant traits, including thin and small bristles, rough eyes, abnormal wings, defective abdominal segmentation, reduced viability and fertility, small body size and prolonged developmental period (LINDSLEY and GRELL 1968). They also act as recessive lethals, with death usually occurring during embryogenesis or the first larval instar (BREHME 1939; FARNSWORTH 1957a,b). Moreover, *M* lethality is known to be cell autonomous (STERN and TOKUNAGA 1971). Minute loci are haplo-insufficient; that is, flies bearing only one dose of a chromosome segment containing a wild-type *M* gene display the same phenotype as *M* point mutations (LINDSLEY *et al.* 1972). By the criterion of haplo-insufficiency, it has been estimated that 40 to 50 *M* loci exist throughout the genome (LINDSLEY *et al.* 1972).

In his pioneering studies of *M* genes, SCHULTZ (1929) found that different *M* mutations were complementary with respect to lethality and did not interact phenotypically; that is, the effects of the different lesions were not additive. Furthermore, he determined that a duplication that included a given *M* locus suppressed only mutations involving that locus. Thus, he concluded that, while the function of each *M* gene is unique, different *M* genes might encode products responsible for similar cellular processes. During the subsequent 37 years, this idea produced only two proposals for *M* function: (1) *M* gene products are involved in the production or metabolism of, or response of target cells to, the moulting hormone, ecdysone (BREHME 1939) and (2) *M* loci code for enzymes involved in oxidative phosphorylation (FARNSWORTH 1965). However, no firm experimental evidence exists to support either of these proposals.

More recently, ATWOOD has postulated that *M* genes specify the various isoaccepting species of tRNA molecules (RITOSSA, ATWOOD and SPIEGELMAN 1966a). This hypothesis provides a plausible explanation for the *M* syndrome and the phenotypic similarity between this group of mutations and those that presumably affect protein synthesis. The latter include bobbed (*bb*) mutations (which involve partial deficiencies of ribosomal RNA genes; RITOSSA, ATWOOD and SPIEGELMAN 1966b), *min* mutations (which involve partial deficiencies of

5S RNA genes; PROCUNIER and TARTOF 1975; PROCUNIER and DUNN 1978), alleles of the suppressor of forked [*su(f)*] locus (which alter the developmental profile of ribosomal proteins; DUDICK, WRIGHT and BROTHERS 1974; LAMBERTSSON 1975), and various *X*-linked cold-sensitive mutations (many of which appear to be defective in ribosome assembly; MAYOH and SUZUKI 1973; WRIGHT 1973; FALKE and WRIGHT 1975). It has also been suggested that the known 10- to 12-fold redundancy of tRNA genes may account for the fact that *M* mutations are frequently associated with chromosome deficiencies (RITOSSA, ATWOOD and SPIEGELMAN 1966a).

The hypothesis that *M* sites are equivalent to sites of tRNA genes has provoked a great deal of interest. While this hypothesis has not been critically tested, several independent lines of evidence strongly suggest that, in general, *M* loci do not correspond to tRNA genes. First, levels of different tRNAs were assayed in several *M* strains, and in only one case was the quantity of a specific tRNA significantly reduced (WHITE 1975). Second, in most instances, the coding sites of tRNA species that have been localized by *in situ* hybridization do not reside near known *M* sites (GRIGLIATTI *et al.* 1977; HAYASHI *et al.* 1980). Third, recent evidence suggests that ethyl methanesulfonate (EMS) readily induces *M* mutations (STONE 1974; HUANG and BAKER 1975; SHELLENBARGER and DUTTAGUPTA 1978). Since EMS is thought to induce primarily single-site lesions, HUANG and BAKER (1976) have concluded that *M* genes are not redundant. Finally, the salivary gland chromosome segments 84D and 92B (on the right arm of chromosome 3) contain about 90% of the haploid number of genes coding for $tRNA^{Val}_{3b}$ (DUNN *et al.* 1979), and we have observed that flies bearing deficiencies that lack both segments are viable and phenotypically non-Minute (unpublished observations).

A significant proportion of EMS-induced mutations in Drosophila are temperature sensitive (SUZUKI 1970). The utility of temperature-sensitive (ts) mutations for the temporal and functional analysis of genes is well documented (SUZUKI 1970; SUZUKI 1974; SUZUKI *et al.* 1976). It occurred to us that the existence of a ts Minute mutation would expand the scope of the functional dissection of *M* loci considerably and that such a mutant might be used to delineate the role of *M* loci throughout the entire developmental period of the organism. Recently, we recovered an EMS-induced mutant, *Q-III*, which on the basis of several criteria appears to be a ts *M* mutation. This paper is a report of the genetic and developmental properties of *Q-III*.

MATERIALS AND METHODS

Stocks and culture conditions: For a complete description of most mutations and balancer chromosomes used in the present study, consult LINDSLEY and GRELL (1968). The mutant *Q-III* was recovered by chance from among 3,000 EMS-treated third chromosomes that were screened for allelism with a lethal-bearing third chromosome. The slow development of *Q-III* heterozygotes at 29° resulted in the initial misclassification of this mutant as a lethal allele of the locus under study. However, subsequent examination revealed that the mutation responsible for the developmental delay involved a distinct locus, and that heterozygotes for *Q-III* were pheno-

typically normal at 22°, but exhibited dominant *M*-like traits at 29°. This preliminary evidence suggested that *Q-III* was a ts Minute mutation.

All experiments reported herein utilized third chromosomes in which *Q-III* was linked to the markers p^p or *red* (the latter chromosome was synthesized in the laboratory of T. C. KAUFMAN). All stocks were maintained and crosses performed on standard cornmeal-sucrose Drosophila medium, with tegosept added as the mould inhibitor. Unless otherwise indicated, the permissive and restrictive temperatures used were 22° and 29°, respectively.

Standard crosses and egg collection: Routine crosses were carried out in quarter-pint milk bottles. Ten to 15 pairs of parents were introduced into each bottle and were usually subcultured at least once on fresh medium. For experiments performed at 29°, the females were allowed to lay for 1 or 2 days at 22° before they were removed and the cultures shifted to 29°.

A conventional method of egg collection on petri plates was used for developmental studies (see TARASOFF and SUZUKI 1970). The first productive 2-hr egg collection was discarded and the second was used. Sections of food with the required numbers of eggs were removed and placed in preincubated vials or petri dishes for temperature shifts or other analysis. In all experiments, cultures were examined for a minimum of 18–20 days after they had been established (*i.e.*, from the time of oviposition).

Viability: To measure *Q-III* viability, groups of eggs were collected from the following crosses (the expected frequencies of the various progeny genotypes among the total of each egg collection are shown in parentheses): Controls: (1) $p^p/p^p \times p^p/p^p$ (all p^p/p^p); (2) *CxD/TM3, Sb Ser males* (hereafter the balancer *TM3*, *ri* p^p *sep Sb* bx^{34e} e^s *Ser* will be referred to as *TM3*) $\times$ p^p/p^p females ($CxD/p^p = 0.5$; $TM3/p^p = 0.5$); Experimentals: (3) *Q-III* $p^p/TM3$ females $\times$ p^p/p^p males (*Q-III* $p^p/p^p = 0.5$; $TM3/p^p = 0.5$); (4) *Q-III red/TM3* $\times$ *Q-III red/TM3* [*TM3/TM3* (egg lethal) = 0.25; *Q-III red/TM3* = 0.5; *Q-III red/Q-III red* = 0.25]. The eggs were counted and transferred to pre-incubated 50 mm petri dishes containing fresh medium (50–100 eggs per plate), and these were placed at 22° or 29°. The various cultures were then examined for adult eclosion. In some cases, cultures were examined to identify the developmental stage at which death occurred (effective lethal phase).

Routine examination of Q-III *properties*: Other genetic properties of *Q-III* were explored, using several routine tests including conventional mapping of *Q-III*, complementation tests between *Q-III* and *M* mutations located on the third chromosome, determination of the phenotype of *Q-III* in triploid females, examination of interactions between *Q-III* and mutations whose expression is known to be affected by *M* mutations, and tests for *Q-III* sterility and maternal-effect lethality. These tests required straightforward mating procedures and will be described in appropriate sections in the RESULTS.

Temperature-shift studies: A detailed description of the rationale and experimental procedures for determining temperature-sensitive periods (TSPs) by reciprocal shifts of cultures between permissive and restrictive temperatures, or by heat pulses, is pesented in TARASOFF and SUZUKI (1970) and SUZUKI (1970). In the present work, the beginning of the TSP was defined as the first culture that produced significant numbers of mutant animals (or decreased viability) when shifted from the restrictive to the permissive temperature. The end of the TSP was defined as the first culture that produced significant numbers of wild-type animals (or increased viability) when shifted from the permissive to the restrictive temperature. In pulse-shift experiments, the TSP of a given trait was defined as the developmental interval during which exposure to the nonpermissive temperature produced the mutant phenotype. The TSPs of lethality were defined as developmental intervals during which heat pulses produced very low levels of viability.

Developmental stages of individuals present in the cultures were determined either by inspecting cultures intermittently (at 6- to 12-hr intervals) for the duration of development at 22° or 29°, or by examining a portion of each culture at the time of shift. The developmental stages of at least 20 progeny were determined at any given inspection. Different larval instars were distinguished by the morphology of their mouthparts and anterior spiracles (BODENSTEIN 1950). In some experiments, the recessive mutation *red*, linked to *Q-III*, was used to identify homozygous larvae. In cultures where it was not possible to use *red* to identify *Q-III*-bearing individuals (*i.e.*, in embryonic or first-instar larval cultures and in experiments utilizing *Q-III* p^p homo-

zygotes or *Q-III* heterozygotes), slow development of *Q-III*-bearing larvae was used to distinguish them from non-*Q-III* individuals in the same cultures. This proved to be a reliable phenotype.

In reciprocal shift studies, cultures were established from synchronously collected eggs and groups of 8 to 10 vials, each with 50 to 60 individuals per vial, were shifted from permissive to restrictive temperatures, and *vice versa*, at successive 12-hr intervals after oviposition. Developmental stages present in the sample were identified at the time of shift, and in some cases a detailed assessment of larval stages present in 22° or 29° culture was provided by inspection at 6-hr intervals.

Two different reciprocal shift studies were performed. First, to determine the TSP of *Q-III* lethality, offspring of matings between *Q-III* p^p/*TM3* males and females were shifted at successive developmental intervals. These cultures were examined for eclosion of *Q-III* p^p homozygotes. Second, TSPs of the dominant rough eye and small bristle (macrochaete) phenes were established by similar shift experiments, using offspring of matings between *Q-III* p^p/*TM3* females and p^p/p^p males, or *Q-III* p^p/*TM3* males and p^p/p^p females. Adults from these cultures were examined for the presence of rough eye surfaces and small, thin thoracic macrochaetae. Since results from the reciprocal crosses used for the latter were similar, they have been pooled.

Two heat-pulse studies were performed. In the first, developmentally synchronous eggs were collected from matings between *Q-III* p^p/*TM1* males and females, transferred to petri plates with fresh medium, and then these cultures were exposed to 29° during various overlapping or successive developmental intervals for periods of 48 hr. The cultures were then returned to 22° for the remainder of development. At least 200 individuals were treated in each shift. To monitor development in the cultures, some eggs were allowed to develop continuously at 22°, and the stages present were assessed every 12 hr. The cultures were examined for eclosion of *Q-III* p^p homozygotes.

In the second pulse-shift study, cultures containing developmentally synchronous *Q-III red* homozygotes were subjected to heat treatments lasting 24 or 36 hr during various developmental intervals in two separate experiments. At least 100 individuals were treated per shift. All homozygotes used were produced by matings between *Q-III red/TM3* males and females. Since the *red* phenotype is generally not clearly visible until the second instar (*i.e.*, about 72 hr post-oviposition for *Q-III*), in earlier shifts (0–72 hr after oviposition), mixed populations of *Q-III red* heterozygotes and homozygotes were treated. During subsequent developmental intervals, *Q-III red* homozygotes (identified and selected as larvae at 72 or 96 hr after oviposition) were treated. Eggs and larvae used for these experiments were collected from petri plates, placed in vials and allowed to develop for prescribed intervals prior to heat treatments. Several cultures were allowed to develop continuously and the developmental stages assessed every 12 hr. The shifted cultures were examined for eclosion of *Q-III red* homozygotes, as well as to determine the lethal phases. In addition, live progeny or dead pharate adults dissected from pupal cases, were examined for mutant phenotypes.

Selected flies from heat-treated cultures were anaesthetized with CO_2, mounted on chucks using silver paint and examined in a scanning electron microscope (SEM, Cambridge Instruments, Cambridge, England). In some cases, flies or pharate adults were preserved in 70% ethanol for further examination with a dissecting microscope.

RESULTS

Genetic analysis of Q-III

(1) *Viability and Minute-like phenotypes*: The results of the crosses to test the viability of *Q-III* heterozygotes and homozygotes are presented in Table 1. Note that the relative viability of *Q-III* at 22° is essentially normal in homozygotes and heterozygotes, in contrast to the striking reduction in viability of *Q-III* p^p/p^p individuals and complete lethality of *Q-III red/Q-III red* individuals at 29°. Note also that the viability of *Q-III red/TM3* individuals is very low at 29°.

TABLE 1

Relative viabilities of Q-III *homozygotes and heterozygotes at 22° and 29°*

Crosses	Temperature	Number of eggs	Progeny genotype	Number of adults	Percent viability
Controls					
1. $p^p/p^p \times p^p/p^p$	22°	195	p^p/p^p	151	77.4
	29°	200	p^p/p^p	149	74.5
2. *CxD/TM3* $\times$ p^p/p^p	22°	105	*CxD*/p^p	38	72.4
			TM3/p^p	42	80.0
	29°	145	*CxD*/p^p	54	74.5
			TM3/p^p	55	75.9
Experimentals					
3. *Q-III* p^p/*TM3* $\times$ p^p/p^p	22°	739	*Q-III* p^p/p^p	309	83.6
			TM3/p^p	291	78.8
	29°	783	*Q-III* p^p/p^p	192	49.0
			TM3/p^p	329	84.0
4. *Q-III red/TM3* $\times$ *Q-III red/TM3*	22°	400	*Q-III red/TM3*	157	78.5
			Q-III red/Q-III red	83	83 0
	29°	300	*Q-III red/TM3*	28	18.7
			Q-III red/Q-III red	0	0

This could be due to interactions between *Q-III* and mutations carried by the *TM3* balancer. *Q-III* heterozygotes eclosing at 29° displayed characteristic dominant *M*-like traits (rough eyes, thin and small macrochaetae, prolonged development), while heterozygotes grown at 22° were phenotypically normal. Furthermore, *Q-III* homozygotes grown at 22° displayed moderate small bristle and rough eye phenes, as well as prolonged development.

(2) *Mapping*: The genetic position of the dominant *Q-III* macrochaete phenotype was established using standard mapping procedures. Using the dominant chromosome 3 markers *Gl*, *Sb* and *H*, *Q-III* was initially localized to the right of *Gl*. Then, using the recessive markers *st*, *in*, *ri* and p^p, *Q-III* was localized to a map position of about 47.4.

More than 100 non-*Q-III* and *Q-III*-bearing recombinant individuals were isolated and used to establish lines at 22° and 29°. Examination of these recombinant lines revealed that the dominant rough eye, small macrochaete and late eclosion traits (as well as other less penetrant phenotypes) were inseparable from each other. It should be mentioned that analysis of salivary gland chromosomes of *Q-III* (kindly performed by T. C. KAUFMAN) revealed no structural abnormalities.

(3) *Complementation*: As a result of the mapping study, *Q-III* was tested for allelism at 22° and 29° with two known *M* loci: *M(3)S34* and *M(3)LS4* (the latter locus is included in the chromosome segment deleted by *Df(3L)79E5,6;80*; SINCLAIR 1977). Both *M* loci are located in the proximal region of the left arm of chromosome 3 (LINDSLEY *et al.* 1972). *Q-III/M(3)S34* heterozygotes survived at either temperature. In contrast, *M(3)LS4/Q-III* heterozygotes (*i.e.*, *Df(3L) 79E5,6;80/Q-III*) survived only when grown at 22°. A subsequent test revealed

that individuals heterozygous for *Q-III* and *M(2)173* also survive at both temperatures, suggesting that *Q-III* generally does not exhibit lethality in combination with other *M* lesions. It should be mentioned that the phenotype of *M(3)LS4/Q-III* heterozygotes at 22°, while more extreme than that of *Q-III* homozygotes, was similar to that of *M(3)LS4/+* heterozygotes. However, on the basis of the conditional lethality of *M(3)LS4/Q-III* individuals, *Q-III* appears to be a ts allele of the *M(3)LS4* locus.

(4) *Test for* Q-III *expression in triploid females*: Dominant *M* traits are known to be recessive in triploids (SCHULTZ 1929). To examine *Q-III* expression in triploids, *Q-III* p^p*/TM3* males were crossed to *C(1)RM*, y^2 *sc* w^a *ec/FM6*; +/+/+ triploid females at 29° and several *C(1)RM*, y^2 *sc* w^a *ec/X*; *Q-III* p^p/+/+ female progeny from this cross were recovered. None displayed *M*-like phenotypes. Therefore, *Q-III* further resembles *M* lesions in that its dominant phenotypes are recessive in triploid females.

(5) Q-III *interactions*: Minute mutations are known to interact with a variety of unrelated mutations (LINDSLEY and GRELL 1968). For example, it has been reported that *M* lesions act to reduce the viability of Delta (*Dl*)-bearing individuals, and produce a nicked-wing phenotype in individuals heterozygous for the recessive mutation vestigal (*vg*). Moreover, we have observed enhancement of the wing phenotype of clipped (*cp*) by *M(2)173*, and enhancement of the eye phenotype of Deformed (*Dfd*) by $M(3)h^{S37}$ (unpublished observations; SCHULTZ 1929). Thus, it is probable that these phenomena are typical *M* interactions.

In order to test *Q-III* for analogous interactions, appropriate crosses were performed at 22° and 29°, and the results of these crosses are shown in Table 2

TABLE 2

Temperature-sensitive lethal and novel visible phenotypes expressed by heterozygotes between Q-III *and* vg, cp, Dfd, *and* Dl

Genotype of male parents*	Genotype of progeny	Number of survivors		Novel visible phenotypes (percent expression)	
		22°	29°	22°	29°
vg/vg;+/+	*vg*/+;*TM3*/+	220	359	—	—
	vg/+;*Q-III* p^p/+	236	172	—	apical wing notches (81.4)
cp/cp	*cp/TM3*	157	123	—	—
	cp/Q-III p^p	135	12	notches in posterior wing margin (0.7)	notches in posterior wing margin (100.0)
Dfd p^p/*Dfd* p^p†	*Dfd* p^p*/TM3*	149	155	—	—
	Dfd p^p*/Q-III* p^p	137	0	—	—‡
Dl/TM3	*Dl/TM3*	121	175	—	—
	Q-III p^p*/TM3*	119	5	—	—
	Q-III p^p*/Dl*	127	0	—	—

* Genotype of female parents: *Q-III* p^p*/TM3*.
† *Dfd* is now known to be homozygous viable (SINCLAIR 1977).
‡ No viable adults but pharate adults displayed headless phenotype.

(note that *vg* is located on chromosome *2*; whereas, *Dfd*, *Dl* and *cp* are located on chromosome *3*). These results clearly demonstrate that *Q-III* exhibits ts *M*-like interactions with all four mutants. Thus, while *vg/+;Q-III* p^p/+ and *Q-III* p^p/*cp* adults displayed no novel phenotypes at 22°, at 29° they possessed characteristic wing nicking (*vg/+*; *Q-III* p^p/+: apical wing notches; *Q-III* p^p/*cp*: nicks in posterior wing margins). Furthermore, *Q-III* p^p/*Dl* and *Q-III* p^p/*Dfd* p^p heterozygotes survived when grown at 22°, but died when raised continuously at 29°. It is worthy of mention that at 29°, most *Q-III* p^p/*Dl* individuals failed to undergo metamorphosis; whereas, *Q-III* p^p/*Dfd* p^p individuals pupated, but the pharate adults failed to eclose and most lacked head structures. We have established that the aforementioned interactions can be used to delineate TSPs for the various mutations (SINCLAIR 1977; and manuscript in preparation).

Finally, we have observed that two different known *M* mutations [*M(2)173* and *M(3)LS4*] evoke suppression of the extra sex-comb phenotypes of Multiple sex comb and Polycomb; *Q-III* exhibits analogous suppression, but in a ts fashion (SINCLAIR 1977). These observations agree with the findings of DENELL and DALEY (personal communication), who have tested other *M* lesions, and it appears that this suppression phenomenon is also a typical *M*-like interaction.

(6) *Sterility and maternal effects*: In addition to its characteristic dominant phenotypic and dominant and recessive viability effects, *Q-III* also elicits ts sterility. Matings between *Q-III/Q-III* females and wild-type males produced significant (albeit reduced) numbers of progeny at 22°, but none at 28°. These females stopped producing eggs within 1 or 2 days after being shifted to 28°. Examination of females maintained at 28° for several days revealed that sperm were abundant in their seminal receptacles and that their ovaries contained oocytes characteristic of earlier stages of oogenesis. Matings between homozygous *Q-III* males and wild-type females produced few progeny at 22°, and none after a 1- to 2-day exposure to 28°. Thus, it was not possible to obtain a pure breeding stock of *Q-III*.

The ts sterility of *Q-III/Q-III* females suggested that maternally induced lethality might be associated with the lesion. To examine this possibility, a group of synchronously fertilized eggs was collected from *Q-III/Q-III* females mated to wild-type males at 22°. Some of these eggs were maintained at 22°, while the remainder were shifted to 29°. Of 220 eggs cultured at 22°, 32% hatched into larvae, and 85% of these larvae developed into phenotypically normal adults. Most of the unhatched eggs displayed varying degrees of pigmentation. Of 102 eggs shifted to 29°, only 2% hatched into larvae. These larvae died shortly thereafter. At 29°, most of the unhatched eggs remained unpigmented. In control crosses between heterozygous *Q-III* females and wild-type males, about 80% of the heterozygous *Q-III* progeny survived to the first instar, and the majority of these reached adulthood at 29°. Furthermore, 77% of the homozygous offspring produced by matings between heterozygous *Q-III* parents survived to the first instar stage at 29° (see below). These observations suggest that ts maternal-effect lethality is indeed a property of the *Q-III* lesion.

Developmental analysis of Q-III

(1) *Lethal phase*: The effective lethal phase (LP) of *Q-III* was determined by examining 29° cultures of *Q-III* red homozygotes (Table 1, cross 4) at key developmental intervals: first-larval instar, pupariation and the adult stage (the *red* marker served to distinguish the appropriate classes). Seventy-seven % of the expected homozygous *Q-III red* embryos hatched into first-instar larvae at 29°. While none of these homozygotes exhibited subsequent growth or development, they all eventually displayed the *red* phenotype and died within several days. Similar examination of 29° cultures also revealed that lethality of *Q-III* heterozygotes (*i.e.*, *Q-III* p^p/p^p and *Q-III red/TM3* individuals, Table 1, crosses 3 and 4), occurred primarily during the larval stages.

To determine if the TSP and LP of *Q-III* extended beyond embryonic and early first-instar stages, groups (numbers of individuals per group are given in parentheses) of early to mid-first (83), mid-second (34) and mid-third (40) instar larvae were selected from 22° cultures and immediately shifted to 29°. None of the first and few of the second-instar larvae moulted, and they all died within 4–5 days from the time of the shift. None of the larvae shifted as third instars formed pupae, and they all died within several days at 29°. These results indicate that the TSP and LP of *Q-III* do indeed extend throughout the larval phase.

(2) *Temperature-sensitive period of lethality*: Relative viability of *Q-III* homozygotes in all temperature-shift experiments was computed by designating the 22° level of viability as 100%. Thus, in each case, the number of adult homozygotes expected to result from matings between heterozygous *Q-III* (*Q-III/TM3* or *Q-III* $p^p/TM1$) parents was based on this scheme of normalized viability as follows: (1) expected number of homozygous adults from eggs = (% viability at 22°) × [(total number of eggs) × (0.25)]; (2) expected number of homozygous adults from selected *red* larvae = (% viability of selected larvae at 22°) × (total number of selected larvae). Percent viability (*i.e.*, percent eclosion) was then calculated as: (observed number of adults/expected number of adults) × 100.

TSP of lethality defined by reciprocal shift studies: The results of the reciprocal shift study to determine the TSP of *Q-III* lethality are shown in Figure 1. This TSP extends continuously from the first larval instar to the latter half of the pupal period. Thus, it appears that the *Q-III* gene product is required throughout the major portion of development.

TSPs of lethality defined by heat-pulse experiments: Since exposure to the restrictive temperature for long periods of time can mask successive but separate TSPs (Poodry, Hall and Suzuki 1973; Suzuki *et al.* 1976), the TSP of *Q-III* lethality was subjected to further analysis by using brief heat treatments (heat-pulses) of 48, 36 and 24 hours duration at various developmental intervals after oviposition. The patterns of *Q-III* lethality delineated by heat pulses are shown in Figure 2. Note that the 48-hour pattern (Figure 2A) closely parallels that obtained in the reciprocal shift study (Figure 1), thus emphasizing the importance of the *Q-III* gene product during development.

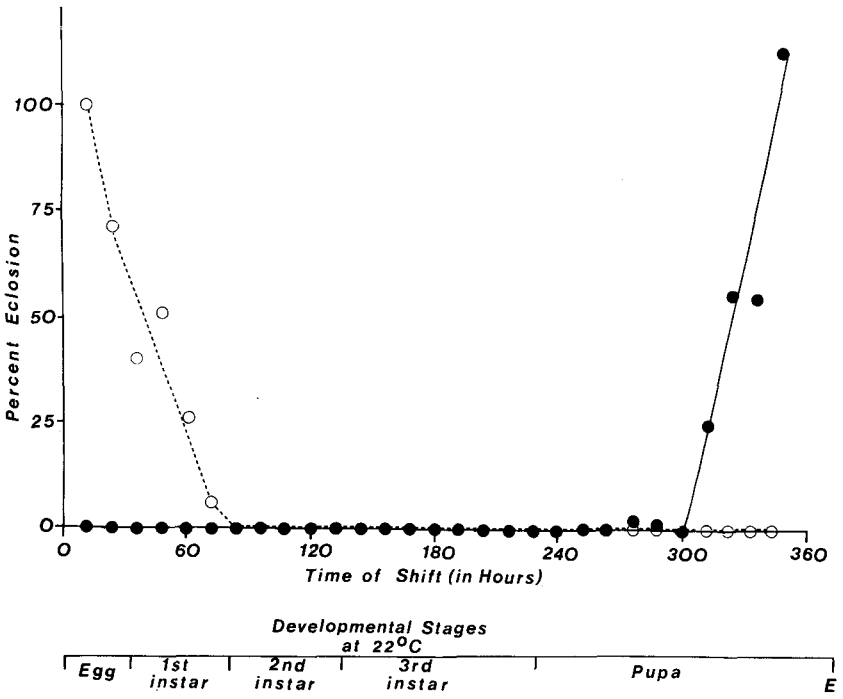

FIGURE 1.—Results of the reciprocal shift study to delineate a TSP of lethality for *Q-III*. The data are given as percent eclosion of *Q-III* homozygotes (from matings between *Q-III* p^p/*TM3* parents) shifted from 22° to 29° (closed circles, solid line), and from 29° to 22° (open circles, dashed line) at various times during development. The duration of each developmental stage of *Q-III* homozygotes at 22° is indicated below. (E = eclosion.)

The 24- and 36-hour heat-pulse experiments delineated two discrete post-embryonic TSPs of lethality (Figure 2B); one in the second half of the second larval instar, and the other extending from the late third instar/early pupal boundary to the latter half of the pupal period. Thus, while the *Q-III* individual is relatively refractory to shorter heat-pulses during most of the larval stages, it is markedly sensitive during most of the pupal period. Moreover, in the 24-hour experiment, *Q-III* homozygotes exposed to heat pulses during the larval TSP exhibited greater viability than did those treated during the pupal TSP. These observations suggest that larvae are less sensitive than pupae to the loss of *Q-III* function.

The application of 36-hour heat pulses to *Q-III red* homozygotes during most larval stages resulted in a relatively low level of larval death and, more frequently, incomplete metamorphosis. However, 24- or 36-hour heat pulses during the second half of the second instar generally produced pharate adults that failed to eclose. Only rarely did these heat pulses produce larval death or undifferentiated pupae. Examination of the pharate adults revealed a striking loss of eye, antennal and head structures, as well as other phenotypes (see below). The fact that most deaths resulting from heat pulses during the larval TSP did not occur

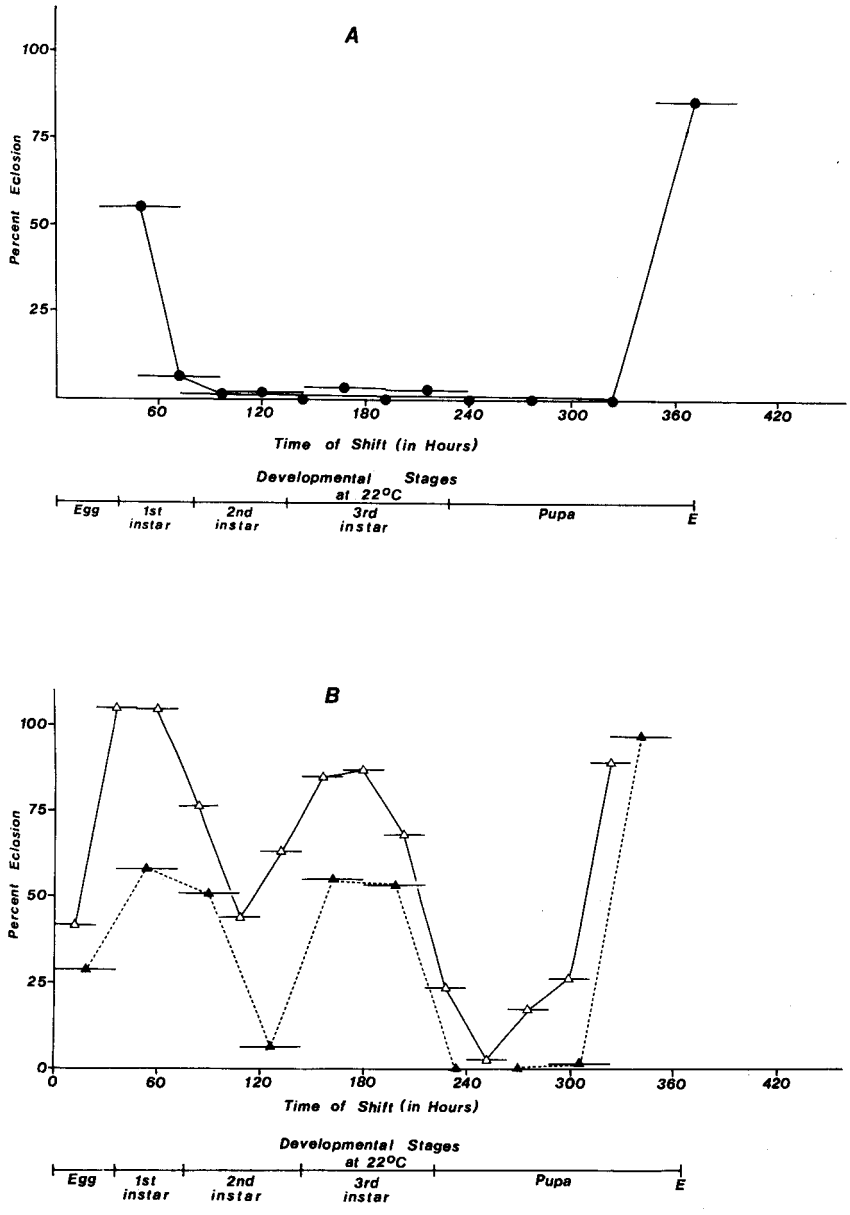

FIGURE 2.—Results of heat-pulse studies to delineate TSPs of lethality for *Q-III*. The data are given as percent eclosion of *Q-III* homozygotes (produced by either *Q-III* p^p/*TM1* parents (A) or *Q-III red* parents (B) heat-pulsed at various developmental intervals. (A) 48-hour heat pulses (closed circles, solid line); (B) 36-hour heat pulses (closed triangles, dashed line) and 24-hour heat pulses (open triangles, solid line). The horizontal bars indicate the duration of the heat pulses. The duration of each developmental stage of *Q-III* homozygotes at 22° is indicated below.

until after pupation is consistent with the aforementioned suggestion that larvae are less sensitive to the viability effects of *Q-III*.

By and large, *Q-III red* homozygotes heat-treated for 24 or 36 hours during the early part of the second TSP of lethality (third instar/early pupal boundary) either failed to complete metamorphosis or gave rise to dead pharate adults with several characteristic phenotypes (see below). Many individuals that were heat-pulsed for 24 hours near the end of the third instar exhibited striking partial metamorphosis, during which the anterior half of the body (head, legs and thorax) differentiated, but the abdomen remained undifferentiated. The application of 24- or 36-hour heat pulses during most of the remainder of the pupal period (up to 324 hours after oviposition) resulted in progressive pupal development, but few homozygotes eclosed. Pharate adults resulting from the latter heat pulses frequently displayed markedly deformed legs (see below).

The application of 24- or 36-hour heat pulses to *Q-III red* homozygotes during embryogenesis (Figure 2B) resulted in somewhat reduced levels of viability. Most heat-pulsed embryos hatched into first-instar larvae, many of which ultimately died. Note that in the case of the 36-hour heat-pulse experiment, this lethality was much less marked than that observed for the larval and pupal TSPs. Thus, no clear-cut TSP of embryonic lethality has been defined by these experiments. This is probably due to the fact that the homozygous *Q-III red* embryos were derived from heterozygous *Q-III red* females.

(3) *Temperature-sensitive periods of dominant eye and macrochaete phenotypes*: The results of the reciprocal temperature-shift experiments used to delineate the TSP of the dominant rough eye and small macrochaete phenotypes are shown in Figure 3. Thus, the TSP of the eye phene spans the latter half of the second instar and coincides with the earlier TSP of *Q-III* lethality. Close examination of the eyes of mutant individuals revealed disrupted eye facets and duplicated inter-ommatidial bristles. The TSP of the dominant small macrochaete phene (also indicated in Figure 3) occurs during the first half of the pupal period. Comparable TSPs of both the eye and macrochaete traits were observed when *Q-III* heterozygotes were subjected to 48-hour heat pulses.

TSPs of recessive adult morphological defects: Examination of survivors and dead pharate adults from 24-hour heat-pulse cultures revealed a large number of phenotypes, most of which were incompletely penetrant. These included head, thoracic/wing, leg, macrochaete and abdominal defects (some of which were also observed in *Q-III* heterozygotes at low frequencies). Both the penetrance and expressivity of these phenes were markedly increased among survivors and pharate adults resulting from the 36-hour heat-pulse experiment. The various phenotypes and their times of induction observed in this experiment are summarized in Table 3. A wide spectrum of defects affecting most of the major cuticular derivatives was observed. These defects frequently involved loss or duplication of specific structures. Each trait possesses a characteristic TSP during development. While most defects are produced by heat-pulses during the larval stages, a few have TSPs that occur during the pupal stage.

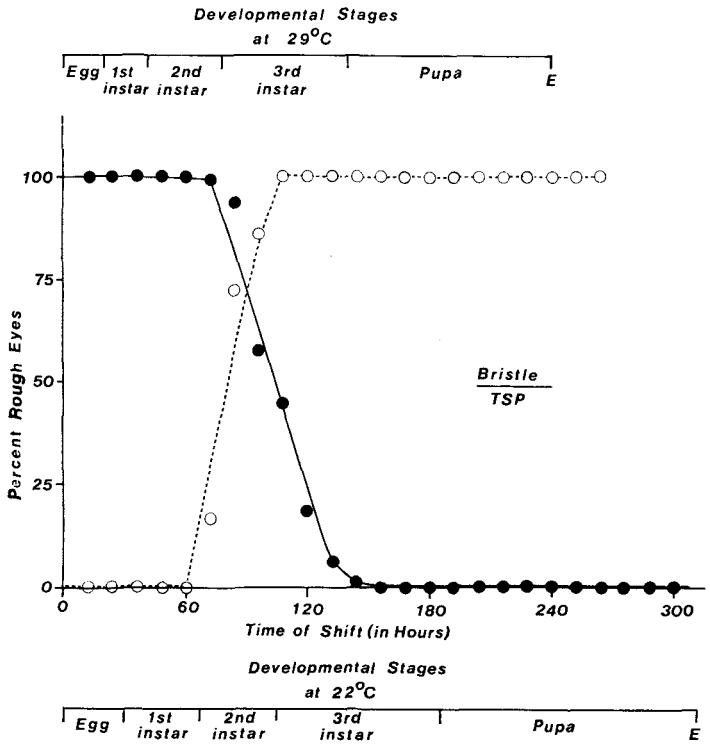

FIGURE 3.—TSPs of dominant rough eye and small bristle phenotypes of *Q-III*. The data for the eye phenotype are given as percent expression of rough eyes in *Q-III* p^p/p^p heterozygotes (from reciprocal matings between *Q-III* $p^p/TM3$ and p^p/p^p parents) shifted from 22° to 29° (closed circles, solid line), and from 29° to 22° (open circles, dashed line) at various developmental intervals. The TSP of the bristle (macrochaete) phenotype is indicated by the horizontal bar and is positioned relative to the appropriate developmental stages at 22°. The duration of each developmental stage of *Q-III* p^p heterozygotes at 29° and 22°, is indicated above and below, respectively.

A more detailed description of *Q-III* defects observed in the 36-hour heat-pulse experiment is presented in Table 4. The defects are listed according to the imaginal anlagen from which they are derived (Column 1); and the range of expression of each phenotype, and its corresponding TSP, are presented in Columns 2 and 3, respectively. Striking phenotypes affecting derivatives of the eye-antennal, leg, wing and genital imaginal discs are produced by heat pulses during the second and/or third larval instar. These defects include loss of eye-antennal-head structures (frequently resulting in a headless phenotype), see Figure 4, duplicated antennal structures (Figure 5); loss, duplication (Figure 6) and fusion (Figure 7) of leg elements; moderate leg deformities; incomplete formation of wing veins; abnormal thoraces (usually involving structural loss, see Figure 8) and rare loss of wings; and rotation or loss of external male genitalia (Figure 9). The derivatives of eye-antennal, leg and genital discs appear to be most sensitive during the

TABLE 3

Summary of results of 36-hour heat-pulse experiment

Hours of pulse	Number of survivors*	Corrected percent viability	Percent expression of adult and pharate adult phenotypes														
				e-h		ant		Thorax			Leg					Abdomen	
			n	df	dp	df	dp	abn	wv	m	df	dp	f	def	cg	seg	gen
0–36	15(250)	28.9	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36–72	169(1400)	58.2	169	0	0	0	0	0	0	0	0	0	0	0	0	0	0
72–108	321(710)	51.0†	321	37.1	0.9	0.3	0.6	3.1	0	0	0	0	0	0	0	0	83.1
108–144	38(681)	6.3	401	88.5	0	79.1	27.2	15.7	‡	0	19.7	12.0	21.9	89.3	0	0	80.0
144–180	134(274)	55.2	134	44.0	0.7	0	3.7	73.1	11.2	0	0	0	0	0	0	0	52.1
180–216	259(546)	53.5	297	26.6	0	0.3	0	33.2	77.3	0	0.3	0	0	0	0	0	43.3
216–252	0(566)	0	318	0	0	0	0	0	‡	98.4	0	0	0	0	73.0	99.4	0
252–288	1(665)	0.2	32	0	0	0	0	0	‡	12.5	0	0	0	96.9	0	3.1	0
288–324	4(299)	1.5	50	0	0	0	0	0	‡	0	0	0	0	96.0	0	0	0
324–360	175(204)	96.8	175		0	0	0	0	0	0	0	0	0	0	0	0	0

* Number of individuals (collected as either eggs or larvae at 22°) used to establish cultures for each treatment interval is shown in parenthesis.

† The *red* phenotype is recognizable reliably after 72 hours; therefore, from this stage on, relative viability was based on survival of *Q-III red/Q-III red* larvae selected at 22° (88.6% of untreated larvae reach adulthood).

‡ Wings could not be scored.

Abbreviations: *n* = number of live adults and/or pharate adults scored; e-h = eye-head; df = structural deficiencies; dp = structural duplications; ant = antenna; abn = abnormal thorax; wv = incomplete wing veins; m = small thoracic macrochaetae; f = fused forelegs; def = leg deformities; cg = sex comb gaps; seg = defective abdominal segmentation; gen = abnormal male genitalia.

TABLE 4

Detailed description of Q-III *defects revealed in 36-hour heat-pulse experiment*

Imaginal analagen affected	Defect*	TSP†
Eye-antennal discs	eye-head-antennal df	late 1st to late 3rd instar
	(reduced eyes/headless)	(mid to late 2nd instar)
	ocellar dp	late 1st to mid-3rd instar
	antennal dp	late 1st to mid-3rd instar
	(aristal dp/total antennal dp)	(mid to late 2nd instar)
Wing discs	abnormal thorax	late 1st to late 3rd instar
	(aberrant scutellum/severe mesothoracic df)	(early to mid 3rd instar)
	incomplete wing veins II and IV	entire 3rd instar
		(mid to late 3rd instar)
	size of macrochaetae reduced	early to mid pupal period
		(first quarter of pupal period)
Leg discs	leg df (distal df/total leg df)	mid to late 2nd instar
	leg dp (distal dp/total leg dp)	mid to late 2nd instar
	foreleg fusion	mid to late 2nd instar
	(moderate proximal/extreme distal)	
	moderate leg deformities	entire 2nd instar
	(bent tarsi and femurs)	(mid to late 2nd instar)
	severe leg deformities	middle half of pupal period
	(extremely gnarled elements)	
	sex comb gaps	first quarter of pupal period
Genital disc	abnormal male genitalia	late 1st to late 3rd instar
	(rotation/partial or complete loss)	(late 1st to late 2nd instar)
Abdominal histoblasts	incomplete abdominal segments	first half of pupal period
		(first quarter of pupal period)

* Range of phenotypic expression (least extreme/most extreme) is given in parentheses.
† Segment of TSP during which most extreme expression and/or highest penetrance occurs is given in parentheses.

second instar, while thoracic and wing structures are most sensitive during the third instar.

Several prominent *Q-III* phenotypes resulting from pupal heat pulses are also detailed in Table 4. These include small thoracic macrochaetae (Figure 10) (a less striking reduction in the size of abdominal microchaetae also occurred); severe leg deformities; sex comb gaps (Figure 11); and incomplete formation of abdominal segments (Figure 12). Note that, whereas macrochaetae, sex combs and abdominal segments are most sensitive during an early part of the pupal period, the legs are most sensitive during a later pupal interval.

Summary of TSPs of Q-III *properties*: A summary of the TSPs of the lethal, sterile and morphological phenotypes of *Q-III* is presented in Figure 13. In addition to illustrating the extreme pleiotropy of *Q-III*, this figure serves to emphasize three important observations. First, pupae are more sensitive to *Q-III*-induced lethality than are larvae. Not only is the pupal TSP longer than the larval TSP,

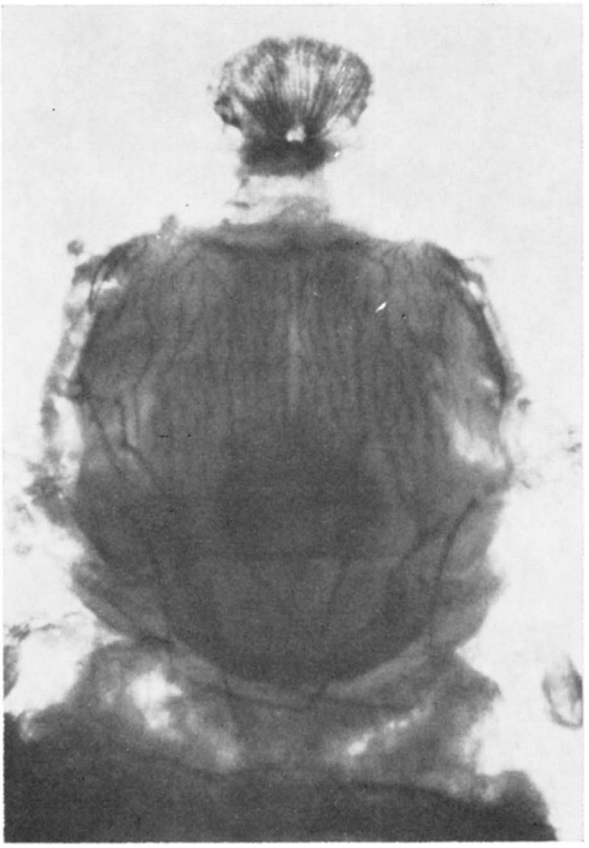

FIGURE 4.—Micrograph showing the *Q-III* headless phenotype resulting from a 36-hour heat pulse applied during the latter half of the 2nd larval instar. All of the eye-antennal disc derivatives are absent; only the proboscis (labial disc derivative) was produced. (40×.)

but as noted previously, pupae are more susceptible to lethality resulting from short heat pulses. Second, while most *Q-III* defects result from heat treatment during the second and third larval instars, a few result from pupal heat treatments. Third, TSPs of lethality exhibit marked overlap with TSPs of extreme morphological defects. Thus, the TSPs of severe pattern defects (*e.g.*, duplications and deficiencies involving eye-antennal and leg structures) coincide with the earlier (larval) TSP of lethality; whereas, the later TSP of lethality coincides with other striking phenotypes (*e.g.* abnormal abdominal segmentation, extreme leg deformities, etc.).

DISCUSSION

We have demonstrated that the autosomal ts mutation *Q-III* is associated with a wide spectrum of fascinating developmental and adult defects. It is crucial to establish that all of these phenotypes are the consequence of a single-site mutation. This conclusion is based on the following lines of evidence: (1) *Q-III* was induced

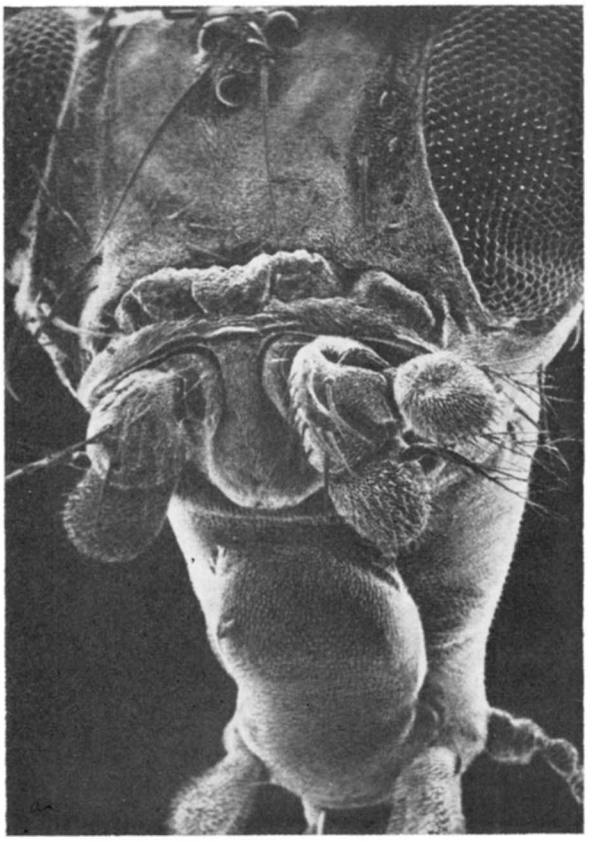

FIGURE 5.—Scanning electron micrograph showing a *Q-III* antennal duplication resulting from a 36-hour heat pulse applied during the latter half of the 2nd larval instar. In this individual, the left antenna is duplicated at the third segment and three aristae are present. (100×.)

with EMS, which causes a preponderance of point mutations rather than deletions (SUZUKI 1970); (2) essentially all *Q-III* phenes are ts and the probability of simultaneous induction of two or more ts lesions on a single chromosome is low (TASAKA and SUZUKI 1973); (3) mapping experiments have revealed consistent co-segregation of all dominant *Q-III* traits; and (4) other ts mutations known to be single-site lesions exhibit complex pleiotropic phenotypes (FOSTER 1973; POODRY, HALL and SUZUKI 1973; DUDICK, WRIGHT and BROTHERS 1974; RUSSELL 1974; SHELLENBARGER and MOHLER 1978). Thus, all the evidence is consistent with the interpretation that *Q-III* is a single-site mutation.

The most intriguing aspect of *Q-III* is its resemblance to the well-studied, but little understood, *M* mutations. We propose that *Q-III* is a ts allele of a *M* locus, based on the following observations: (1) *Q-III* exhibits ts expression of the classical *M* phenotype (*i.e.*, the dominant small-bristle trait, delayed development and reduced viability as a heterozygote, as well as recessive lethality); (2) by analogy with *M* lesions, dominant *Q-III* traits are recessive in triploid females; and (3)

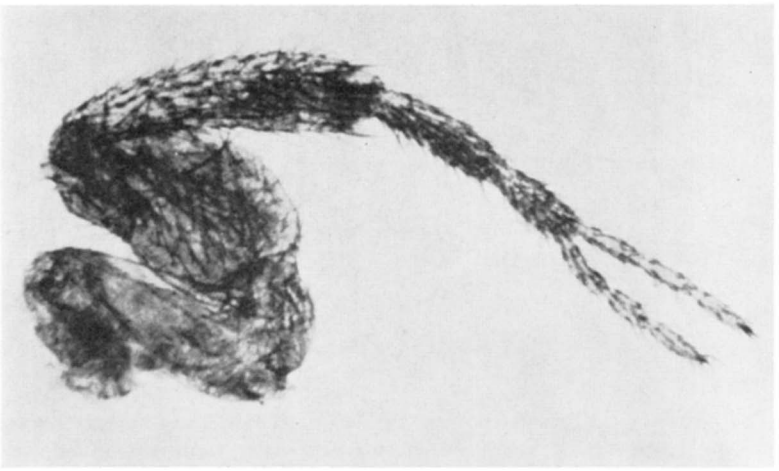

FIGURE 6.—Micrograph showing a *Q-III* leg duplication resulting from a 36-hour heat pulse applied during the latter half of the 2nd larval instar. Note that 3–4 tarsal segments of this foreleg are duplicated. (200×.)

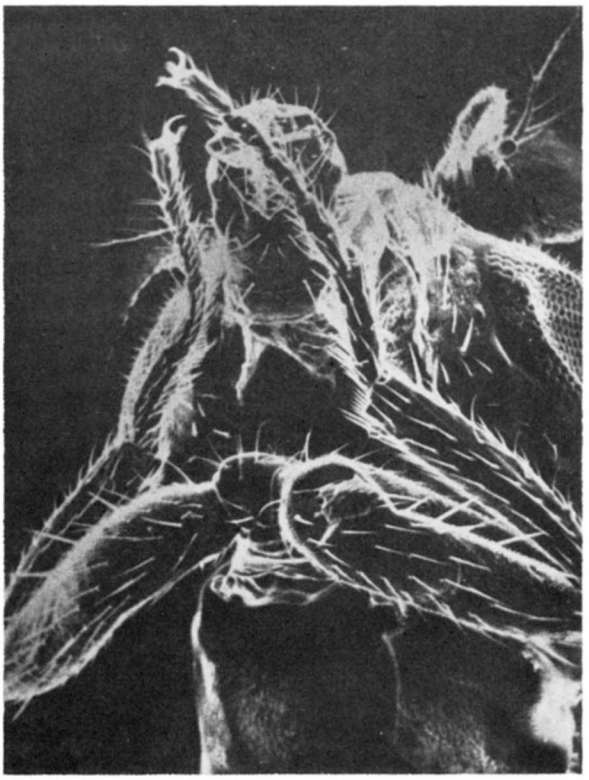

FIGURE 7.—Scanning electron micrograph showing *Q-III* foreleg fusion resulting from a 36-hour heat pulse applied during the latter half of the 2nd larval instar. Note that the forelegs are fused at the coxae. (50×.)

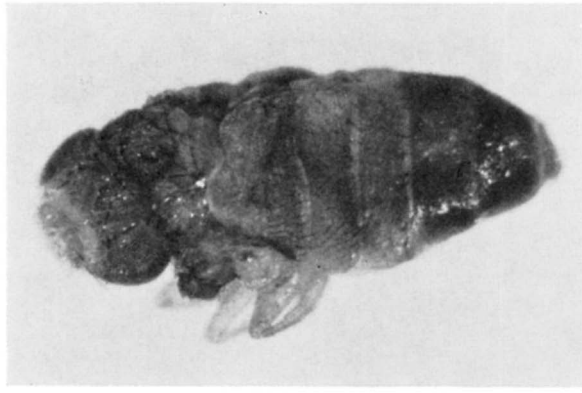

FIGURE 8.—Micrograph showing *Q-III* thoracic abnormalities resulting from a 36-hour heat pulse applied during the first half of the 3rd larval instar. Note the striking loss of thoracic structures. (20×.)

Q-III exhibits typical *M*-like interactions with several different mutations. The failure of *Q-III* to complement the lethality of *Df(3L)M(3)LS4* at 29° suggests that *Q-III* is a ts allele of *M(3)LS4*.

The conditional nature of the *Q-III* mutation provides a means of experimental manipulation never before possible with *M* mutants. Shift experiments between permissive and restrictive temperatures have resolved a polyphasic TSP of *Q-III* lethality extending throughout most of development. Moreover, heat-pulse studies (24- or 36-hour exposures to 29°) show that the organism is markedly sensitive to brief lapses in *Q-III* function during discrete intervals in the larval and pupal stages of development. Thirty-six-hour exposures to the restrictive temperature

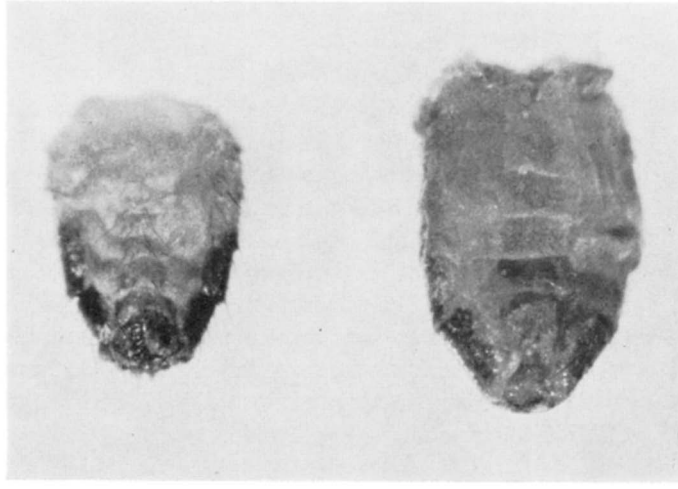

FIGURE 9.—Micrograph showing *Q-III* male genital defects resulting from a 36-hour heat pulse applied during the 2nd larval instar. The abdomen of the treated *Q-III* male (shown on the right) lacks the external genitalia present in the wild type (shown on the left). (20×.)

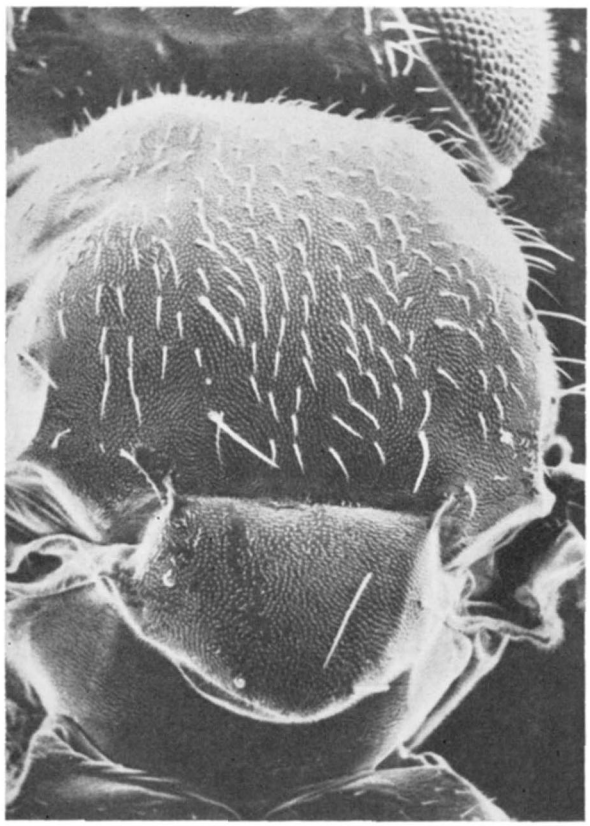

FIGURE 10.—Scanning electron micrograph showing the *Q-III* macrochaete phenotype resulting from a 36-hour heat pulse applied during the first quarter of the pupal period. This striking reduction in size of the thoracic macrochaetae is typical of individuals heat-pulsed during this developmental interval (200×.)

have revealed a panorama of striking phenotypes, each with a specific TSP during development. In addition, our findings indicate that functional *Q-III* gene product is required for both embryogenesis and gametogenesis. Finally, we have demonstarted that *Q-III* interacts with several different developmental mutations. The essential nature of the *Q-III* gene product and the temperature sensitivity of *Q-III* can be exploited to delineate TSPs of these other genes (SINCLAIR 1977, and manuscript in preparation). Thus, *Q-III* can be used as a probe to determine the developmental intervals during which specific unrelated genes function. Of the mutations known to possess multiple complex patterns of TSPs (GRIGLIATTI and SUZUKI 1970; FOSTER 1973; POODRY, HALL and SUZUKI 1973; SHELLENBARGER and MOHLER 1978), *Q-III* is one of the most pleiotropic.

That dividing cells are particularly sensitive to the loss of *Q-III* activity is suggested by two observations. First, the TSPs of specific *Q-III* defects coincide with developmental intervals during which rapid cell division occurs in the affected imaginal anlagen. For example, defects involving eye-antennal, head, leg, tho-

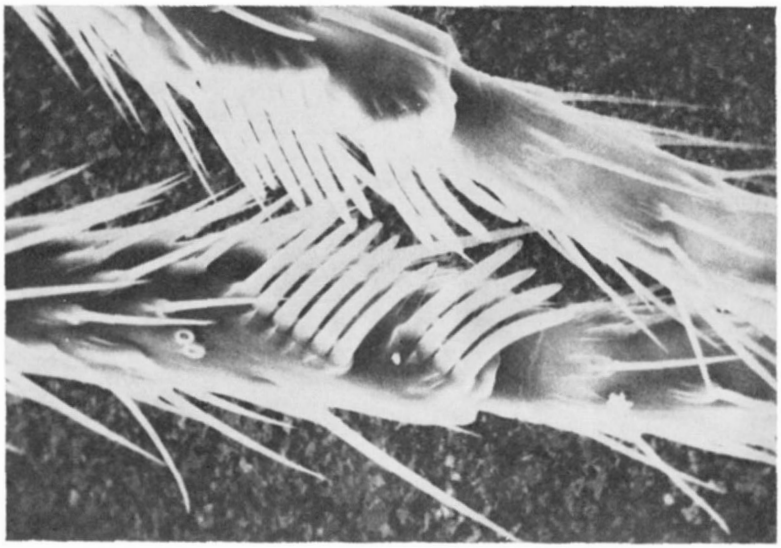

FIGURE 11.—Scanning electron micrograph showing the *Q-III* sex-comb gap phenotype resulting from a 36-hour heat pulse applied during the first quarter of the pupal period. (500×.)

racic and genital structures all have TSPs in the second or third larval instars. During these stages, the imaginal discs responsible for the production of these structures are engaged in rapid growth, with cell division occurring every 6–15 hours (see NÖTHIGER 1972). Furthermore, the pupal TSP for abnormal abdominal segmentation coincides precisely with the initiation of rapid cell division in the abdominal histoblasts, which are mitotically dormant during the larval stages

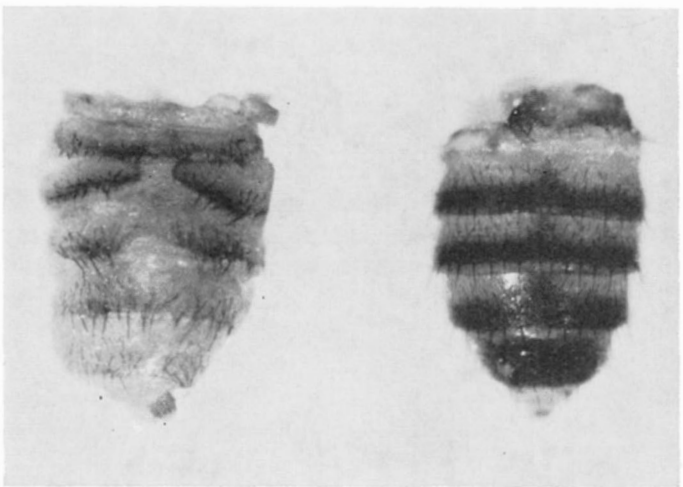

FIGURE 12.—Micrograph showing abnormal *Q-III* abdominal segmentation resulting from a 36-hour heat pulse applied during first quarter of the pupal period. Note that the abdominal segments of the treated *Q-III* individual (shown on the left) are incompletely formed, in contrast to the normal segments of the wild-type individual (shown on the right). (20×.)

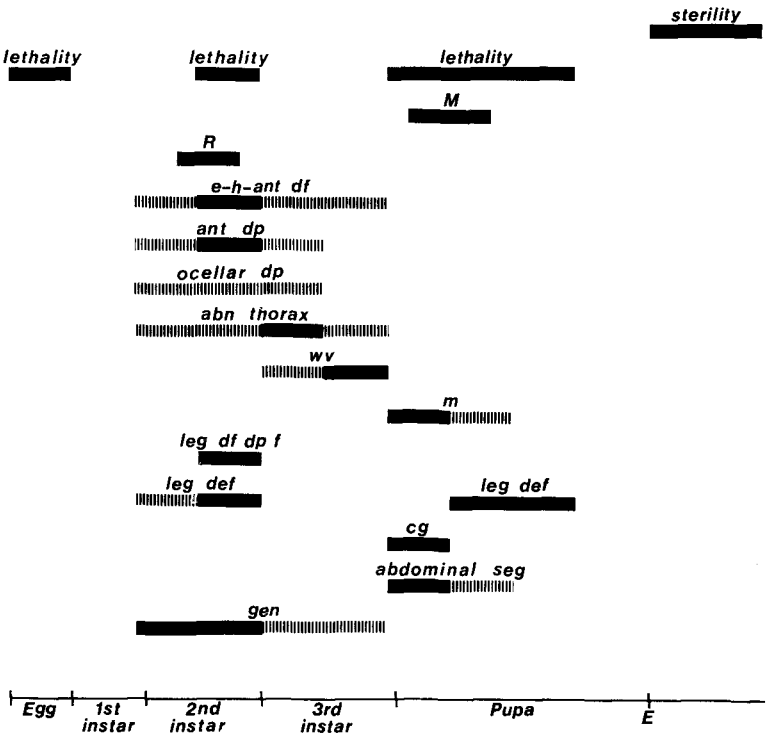

FIGURE 13.—Summary of the TSPs of lethal, sterile and adult morphological phenotypes of *Q-III* observed in temperature shift studies. Horizontal bars indicate the actual extent of TSPs relative to the developmental stages (at 22°) given below. The embryonic TSP is expressed in progeny of homozygous *Q-III* females. The black segments denote that portion of the TSP during which heat treatment elicited the most extreme effects. Abbreviations are identical to those in Table 3, except: M = dominant small macrochaete trait; R = dominant rough eye trait.

(ROBERTSON 1936; GARCIA-BELLIDO and MERRIAM 1971; reviewed by NÖTHIGER 1972). Second, our studies indicate that larvae are relatively refractory to transitory loss of *Q-III* activity, and it is known that most larval cells are mitotically inactive (BODENSTEIN 1950).

The properties of *Q-III* clearly indicate that this gene has an essential role in the cell, but what is this role? The phenotypic similarity between *Q-III* and mutations known to affect protein synthesis [*e.g.*, *bb*, *min*, *su(f)*] is compelling, albeit circumstantial, evidence in favor of the hypothesis that the *Q-III* locus specifies a component required for the translation process. This interpretation is compatible with the observed properties of *Q-III*. Clearly, lethality and sterility may result from the loss of such a basic cellular process. Moreover, a drastic interruption of protein synthesis could produce most, if not all, of the adult morphological defects observed in our experiments with *Q-III*. For example, since bristle synthesis occurs during the pupal period (BODENSTEIN 1950; MITCHELL, LIPPS and TRACY 1977) and because rapid accumulation of protein is undoubtedly a prerequisite to bristle assembly (HOWELLS 1972), the effects of temperature shifts

on macrochaetae could stem from reduced levels of protein synthesis (as predicted by RITOSSA, ATWOOD and SPIEGELMAN 1966b). In addition, analogous to the observed effects of ts cell-lethal mutations on imaginal tissue (RUSSELL 1974; ARKING 1975; SIMPSON and SCHNEIDERMAN 1975), *Q-III* may destroy rapidly dividing cells in imaginal anlagen. Since cell division in the anlagen occurs every 6 to 15 hours, then 36-hour heat pulses (and to a lesser extent, 24-hour heat pulses) could induce loss of tissue resulting in structural deficiencies. Similarly, removal of certain cells within anlagen, followed by the rapid proliferation of surviving determined neighboring cells, could result in structural duplications (BRYANT 1971; 1975). This hypothesis is strengthened by the observation that *M* mutations are autonomous cell lethals (STERN and TOKUNAGA 1971). The autonomous cell-lethal mutation *l(1)ts726* exhibits a spectrum of effects similar to that of *Q-III* (RUSSELL 1974). *l(1)ts726* is a ts allele of *su(f)*, and recently it was determined that this locus affects the developmental profile of ribosomal proteins (LAMBERTSSON 1975) and, by inference, protein synthesis.

While providing evidence in support of the hypothesis that the *Q-III* mutation affects protein synthesis, our experiments have not directly addressed the question of *Q-III* (or Minute) function. We feel that the multiple phenotypes of *Q-III* and those of *M* mutants as a class are consistent with the broad interpretation that they affect various components required for translation (see WHITE 1974). Thus, there is no *a priori* reason to favor a specific class of translational components (*e.g.*, tRNAs *versus* ribosomal proteins) as putative *M* gene products.

The homozygous viability and single-site status of the *Q-III* lesion should facilitate attempts to delineate its molecular basis. A first approach would be to monitor amino acid incorporation in mutant individuals (*e.g.*, see FARNSWORTH 1970), to determine if their translational capacity is impaired at the restrictive temperature. If this proves to be the case, the next step would be to assay the competence of various components of the *Q-III* translation apparatus in *in vitro* translation systems (*e.g.*, see CAPECCHI, HUGHES and WAHL 1975). Such experiments are in progress.

Q-III should also be useful for the study of a variety of contemporary problems in developmental biology. For example, the effects of *Q-III* on imaginal structures will provide a new system for examining current models of pattern formation in Drosophila (*e.g.*, FRENCH, BRYANT and BRYANT 1976). In addition, one could use *Q-III* to explore the effects of short-term changes in *M* gene expression on cellular dynamics within imaginal anlagen (MORATA and RIPOLL 1975). Finally, the effect of *Q-III* on the relationship between larval functions and the growth and maturation of imaginal anlagen could be examined using transplantation experiments.

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