THE DEVELOPMENTAL GENETICS OF THE TEMPERATURE-SENSITIVE LETHAL ALLELE OF THE SUPPRESSOR OF FORKED, *l(1)su(f)^{ts67g}*, IN *DROSOPHILA MELANOGASTER*[1]

MARIANNE E. DUDICK[2], THEODORE R. F. WRIGHT AND LYNDA LEE BROTHERS[2]

Department of Biology, University of Virginia, Charlottesville, Va. 22901

Manuscript received August 4, 1973
Revised copy received December 6, 1973

ABSTRACT

A temperature-sensitive lethal allele of suppressor of forked, *l(1)su(f)^{ts67g}* (*ts67*), has been discovered and characterized as follows: Flies which are hemizygous for *ts67* live at 18° and 25° but die at 30° primarily as larvae. The temperature-sensitive period for *ts67* homozygotes or hemizygotes begins in second instar and ends at pupation. *ts67* is lethal at 30° when heterozygous with suppressor of forked (*su(f)*), a deficiency for suppressor of forked (*su(f)*$^-$), and a non-conditional lethal allele of suppressor of forked (*3DES*). It is viable at 30° when heterozygous with the wild-type allele of suppressor of forked. At 25° but not at 18° forked bristles are suppressed in flies of the following genotypes: *f^{s}ts67/Y*, *f^{s}ts67/f^{s}ts67*, *f^{s}ts67/f^{s}su(f)*, *f^{u}ts67/f^{s}3DES*, *f^{u}ts67/f^{s}su(f)*$^-$, *f^{u}ts67/f^{s}su(f)*. There is some suppression of forked bristles at 25° in the heterozygote, *f^{s}ts67/f^s+$^{su(f)}$*. The forked bristle phenotype is not suppressed at either temperature in flies of the genotypes *f^{u}ts67/Y*, *f^{u}ts67/f^{u}ts67/* (*f^s* and *f^u* indicating suppressible and unsuppressible alleles of forked). The temperature-sensitive period for suppression of forked bristles begins at pupation and extends through the period of bristle synthesis. The deficiency phenotype (bristles reduced in size or absent, wing wrinkled or blistered, eyes rough) typical of flies of the genotype *f^{s}su(f)/f^{s}su(f)*$^-$ at 18° and 25°, is exhibited by flies of the genotypes *f^{s}ts67/f^{s}su(f)*$^-$ at 25° and *f^{u}ts67/f^{s}su(f)* at 29°. An allele of lozenge (*lz^1*) which can be suppressed by *su(f)* is suppressed at 25° but not at 18° in *lz^{1}ts67/Y* males. *ts67* homozygous females are fertile at 25° but sterile at 30°. The hypothesis is discussed that the *su(f)* locus codes for a ribosomal protein and that suppression and enhancement are affected by mutations at the locus by mutant ribosome-induced misreading. The possibility is presented that *ts67* may be used to determine the translation time in development of any gene.

SUPPRESSOR of forked, *su(f)*[1], is a recessive, *X*-linked, allele-specific suppressor of a number of mutations in *Drosophila melanogaster*. As its name implies, it suppresses the bristle phenotype produced by some of the alleles at the forked locus, *f* (1–56.9) (GREEN 1956). The suppressible alleles of forked (*f^s*) include *f^1*, *f^5*, and *f^X* while the unsuppressible alleles (*f^u*) are *f^3*, *f^{3N}*, *f^{51a}*, *f^H*, *f^{X1}*, *f^{X2}*, *f^{X3}*, and *f^{X4}* (LINDSLEY and GRELL 1968). In addition to forked, *su(f)*[1]

[1] Research supported by National Science Foundation Grants GB7707 and GB20910 and National Institutes of Health Grant GM19242.

[2] Supported by National Institutes of Health Training Grant GM01450.

partially suppresses dusky, dy^1 (1–36.2), and one allele of lozenge, lz^1 (1–27.7), while it enhances lz^{s7h} and white apricot, w^a (1–1.5) (SCHALET 1970). The enhancement of w^a is temperature-dependent, being most extreme at 17–18°, intermediate at 24–25°, and non-existent at 29–30° (SCHALET 1972). Enhancement of the lozenge phenotype is accompanied by a reduction in phenol oxidase activity, while suppression is accompanied by an increase in this enzyme activity (SNYDER and SMITH 1972). PEEPLES, BARNETT and OLIVER (1968) had previously shown that lozenge decreases phenol oxidase activity. GREEN (1959) found that *su(f)* was ineffective in suppressing or enhancing a large number of allelic mutants at the white locus. In addition, at 22–25° suppressor of forked has no effect on the visible phenotypes of the following mutant alleles: lz^s, lz^{BS}, lz^{s4K}, lz^{48f}, lz^{50e}, lz^{E1}, m, m^D, v^1, v^2, v^{s6f}, v^K, v^{48a}, sn^3, sn^{X2}, ct^6, car, ras^2, w^{Bwx}, w^{cp}, and w^{a2} (SCHALET, personal communication).

The map position of *su(f)* is given as 1–65.9 (LINDSLEY and GRELL 1968). Its position to the left of bobbed, *bb* (1–66.0), was confirmed through genetic analysis of grossly deleted *X* chromosomes (LINDSLEY and SANDLER 1958). The mapping of X-ray and chemically-induced lethal and visible mutants in the proximal portion of the *X* chromosome, along with a careful cytological analysis, so far indicates that *su(f)* is the closest standard *X*-linked gene to *bb* (SCHALET and LEFEVRE 1973).

The analysis of these lethals for the purpose of mapping the proximal region of the *X* chromosomes has revealed some interesting alleles of $su(f)^1$. One of these lethals, $su(f)^{pb}$ at 17° in uncrowded cultures gives viable progeny which show the following characteristics: pale yellow and thread-like bristles and hairs, dark pigment on the thorax, and curled or wrinkled wings (SCHALET 1972). At 17–18° it suppresses forked in homo- and hemizygotes, and at this temperature lozenge is enhanced and w^a partially enhanced in $lz^{s7h}su(f)^{pb}$ and $w^asu(f)^{pb}$, respectively. The heterozygote $su(f)^{pb}/su(f)^1$ suppresses forked at all temperatures and enhances w^a in $w\ su(f)^{pb}/w^asu(f)^1$ females at 24–25°. Another of the induced lethal alleles, $su(f)^{sDES}$, when heterozygous with $su(f)^1$ at 25° does suppress forked but does not enhance w^a. The heterozygous females, $su(f)^{pb}/su(f)^{sDES}$, "are fully viable, show a good but not complete suppression of *f*, and are normal for the $su(f)^{pb}$ phenotype at all temperatures" (SCHALET 1972), indicating partial intracistronic complementation. Two additional lethal alleles of $su(f)^1$, $su(f)^{X-2}$ and $su(f)^{D-13}$, along with their interactions at various temperatures in heterozygotes with $su(f)^1$ and $su(f)^{pb}$, are also described by SCHALET (1972).

Suppressor of forked when heterozygous with deficiencies for the locus results in flies which exhibit a collection of mutant phenes giving them a characteristic phenotype—hereafter in this paper referred to as the "deficiency phenotype". The characteristics include bristles which are reduced in size or are absent, rough eyes with an anterior indentation, and in some flies wings which may be wrinkled, blistered, broad, held up or out or which may have extra veins (SCHALET 1968) (designated as Minute-like in SCHALET 1972). The abnormal phenotype was reported to occur in *su(f)* homozygotes at 29° (LINDSLEY and GRELL 1968). The reduced bristle phenotype is similar to the bristle phenotype of bobbed.

It has been suggested (SCHALET 1970; DUDICK 1973; WRIGHT 1973; FINNERTY *et al.* 1973) that suppressor of forked mutations affects protein synthesis. More specifically the hypothesis is that altered ribosomes may ultimately be responsible for effecting suppression and enhancement and for producing the deficiency phenotype and lethality associated with mutant alleles at the *su(f)* locus.

This paper is a report of a new and potentially very useful temperature-sensitive lethal allele of *su(f)*, *l(1)su(f)^{ts67g}* (preliminary report by DUDICK and WRIGHT 1973). It is characterized with respect to its temperature-dependent effects on viability and to its maternal temperature effects, maternal dosage effects, and female sterility. Its temperature-sensitive periods for lethality and suppression of forked bristle are established, and the effects of heterozygosity with other *su(f)* alleles on viability and suppression are described. The bearing of the results on the hypothesis that the *su(f)* gene product is a ribosomal protein is discussed, and the suggestion is made that the *ts67* could be used to determine the developmental stage(s) during which translation takes place for any gene for which a *su(f)^{ts67g}* suppressible allele is available.

MATERIALS AND METHODS

Mutations, chromosomes, and stocks: Unless otherwise indicated, the symbols and balancer chromosomes are the same as those found in LINDSLEY and GRELL (1968).

l (1)su(f)^{ts67g}, abbreviated to *ts67* in this paper, was recovered as one of two temperature-sensitive lethals induced in the same *X* chromosome by ethylmethane sulfonate (EMS) in a screen for temperature-sensitive lethal alleles of *l(1)mys* (WRIGHT 1968), the other being *l(1)mys^{ts2}*. In all *ts67g* stocks used most of the EMS-treated *X* chromosome was replaced; at least everything to the left of *f*(56.9) in *f ts67* chromosomes and at least everything to the left of *car* (62.5) in the *car ts67* chromosome.

The wild-type, *+/FM6*, *+/C(1)DX,y f/Y*, *car ts67/car ts67*, *car ts67/FM6*, *car ts67/C(1)DX,y f/Y* stocks used to generate the data in Tables 2 and 3, Figures 4, 7 and 8 and in the maternal temperature effect experiment (see below) were all made coisogenic with each other for the second and third chromosomes (and the wild-type *X* chromosome if present) by making the appropriate series of crosses with the multiple balancer stock *FM6/FM6;SM5/Sp bw^{D}*; *TM3,Ser/Sb* and with a wild type Oregon R-Inbred stock originally obtained from Yale University, New Haven, Conn. (for details see BROTHERS 1971).

Listed below are the derivations and complete genotypes of the abbreviated chromosome designations used in this paper (for details see DUDICK 1973).

ts67 chromosome = *car ts67* chromosome constructed by BROTHERS (1971).

f^{u}ts67 chromosome contains the unspecified, unsuppressible allele of forked derived from the *sc ec cv ct^{6}v g^{2}f* chromosome.

f^{u}+$^{su(f)}$ chromosome = *v g f^{u}* derived from the *sc ec cv ct^{6}v g^{2}f* chromosome also.

f^{s}su(f) chromosome = *f^{s}car su(f)^{1}* which contains a suppressible allele of forked, presumably *f^{1}*, derived from the *y ct^{6}v f^{s} car su(f)* chromosome obtained from the Bowling Green, Ohio Stock Center.

f^{s}ts67 chromosome derived by crossing over to the left of *car* between the *f^{u}ts67* and *f^{s}car su(f)^{1}* chromosomes listed above.

f^{s}+$^{su(f)}$ chromosome = *w m f* carrying a suppressible allele of forked, presumably *f^{1}*.

f^{s}su(f)$^{-}$ chromosome = *Df(1)mal^{3},y ct^{6}f^{1}·Dp sc^{V1},y+* carries a suppressible allele of forked, presumably *f^{1}*. The breaks defining the deficiency are on the left between *l(1)34* (56.7) and short wing, *sw* (64.0), and on the right between *su(f)* and *bb* (SCHALET and FINNERTY 1968). This chromosome and the next one listed were obtained in the stock *Df(1)mal^{3},y ct^{6}f^{s}/Basc,bb/y+Yma-1+* from the University of Connecticut, Storrs, Conn.

Ysu(f)+ chromosome = *y+Ymal+* contains the $+^{su(f)}$ locus (SCHALET and LEFEVRE 1973) The source was the same as for *f^ssu(f)$^-$* above.

f^s3DES chromosome = *y v f^sl(1)su(f)^{3DES}* obtained from DR. A. SCHALET, Dept. of Radiation Genetics, Univ. of Leiden, The Netherlands in the stock; *C(1)DX,y f/B^SY × y^2v f^sl(1)su(f)^{3DES}/ B^SY.*

B^SYsu(f)+ chromosome = *B^SY* which carries the $+^{su(f)}$ *locus.* The source was the same as *f^s3DES* above.

lz ts67 chromosome = *lz^1car ts67* derived by crossing over to the left of *car* between a *lz^1* chromosome and the *car ts67* chromosome listed above.

The above chromosomes were used in the crosses listed in Table 1 which produced the progeny and data presented in Table 4 on viability, Table 5 on the deficiency phenotype, and Figures 1, 2, and 3 on the suppression of forked. Although no attempt was made to make the stocks carrying the above chromosomes coisogenic, their background genomes were not completely unrelated since in all cases the *FM4* balancer chromosome was introduced by crossing to *FM4/Y* males from the same *FM4/l(1)mys* stock.

Procedures: For all experiments and crosses timed eggs were collected, precise numbers counted and placed in ¾-oz. culture containers (creamers) by a method previously described (WRIGHT 1973). The method for determining the number of "fertilized" eggs and calculating stage distributions of mortalities has also been described previously (WRIGHT 1973). In those experiments where the timing or temperature regime was a factor a standard four-hour egg collection period was used so that the stated ages of all individuals is ± 2 hours. For the crosses and experiments reported in Tables 2 and 3, Figures 4, 7 and 8 and for the maternal effect experiments a total of 150 eggs were picked per cross per temperature or treatment either as 5 creamers with 30 eggs per creamer or as 3 creamers with 50 eggs per creamer. For all *C(1)DX,y f/Y* crosses 5 creamers with 50 eggs per creamer were used. In Table 1 are listed the numbers of "fertilized" eggs scored to produce the viability data presented in Table 4.

For the temperature shift experiments on viability, Figures 5 and 6, eleven creamers of 30 eggs/creamer for each shift time were picked from the cross *f^uts67/f^uts67* × *f^uts67/Y*. At the time of every shift one creamer was sacrificed to determine the stage of development at the time of the shift. Therefore, each shift was performed on the individuals that developed from 300 eggs.

The data in Table 4 on viability and Figures 1 and 2 on suppression of forked were collected on the same individuals produced by the series of crosses listed in Table 1. The numbers of non-*FM4* males and females scored for forked bristles are presented in Table 1. Suppression of forked bristles was quantified by scoring the number of forked bristles found among the eight thoracic bristles, the anterior and posterior scutellars and the anterior and posterior dorsocentrals. Bristles were considered to be forked if under 30X magnification a sharp bend or split was visible. The statistical comparisons of the mean number of forked bristles per fly were done by converting the means to proportions by dividing them by 8, i.e. the number of bristles scored. The resulting proportions (p) were tested with the statistic, $t_s = \dfrac{\arcsin\sqrt{p_1} - \arcsin\sqrt{p_2}}{\sqrt{820.8\left(\dfrac{1}{n_1}+\dfrac{1}{n_2}\right)}}$ (see page 607, SOKAL and ROHLF 1969). The Chi-square test was used to compare the frequency distributions of the number of forked bristles per fly.

Female sterility was tested by collecting homozygous virgins less than 8 hours old from stocks of *car ts67*, *f^sts67*, and *Ore-R* kept at 23°. Each female was mated immediately to three 3–7-day-old, wild-type males in a vial containing medium preincubated at 23° or 30°. These vials were placed at these two temperatures and the flies transferred daily for eight consecutive days to fresh preincubated vials, replacing the males if necessary. It was noted whether any eggs had been laid in the old vial, which was then kept at 23° for two weeks to determine whether any adults would emerge.

TABLE 1

Crosses performed to determine viability and suppression of forked bristles of the resulting progeny

Cross no.	Female parent	Male parent	"Fertilized eggs" 18°	25°	30°	Scored for forked bristles non-*FM4*♂♂ 18°	25°	non-*FM4*♀♀ 18°	25°
1	$\frac{f^{u}ts67}{FM4}$	× $\frac{f^{u}ts67}{Y}$	261	289	292	58	56	57	45
2	$\frac{f^{s}ts67}{FM4}$	× $\frac{f^{s}ts67}{Y}$	226	225	224	10	21	20	27
3	$\frac{f^{s}su(f)}{FM4}$	× $\frac{f^{s}su(f)}{Y}$	244	193	286	53	42	46	43
4	$\frac{f^{s}su(f)}{FM4}$	× $\frac{f^{u}ts67}{Y}$	246	187	172	40	37	61	41
5	$\frac{f^{s}su(f)}{FM4}$	× $\frac{f^{s}ts67}{Y}$	223	201	172	25	41	32	39
6	$\frac{f^{s}su(f)^{-}}{FM4}$	× $\frac{f^{s}su(f)^{-}}{Ysu(f)^{+}}$	144	142	135	26	23	—	—
7	$\frac{f^{s}su(f)^{-}}{FM4}$	× $\frac{f^{s}su(f)^{-}}{Ysu(f)^{+}}$	141	188	195	27	33	—	—
8	$\frac{f^{u}ts67}{FM4}$	× $\frac{f^{s}su(f)^{-}}{Ysu(f)^{+}}$	187	138	138	33	25	40	28
9	$\frac{f^{s}ts67}{FM4}$	× $\frac{f^{s}su(f)^{-}}{Ysu(f)^{+}}$	153	135	149	34	23	40	—
10	$\frac{f^{u}ts67}{FM4}$	× $\frac{f^{s+su(f)}}{Y}$	192	189	146	33	46	45	54
11	$\frac{f^{s}ts67}{FM4}$	× $\frac{f^{s+su(f)}}{Y}$	229	215	224	48	42	45	58
12	$\frac{f^{s}su(f)}{FM4}$	× $\frac{f^{s+su(f)}}{Y}$	167	167	183	31	41	32	29
13	$\frac{f^{s}su(f)^{-}}{FM4}$	× $\frac{f^{s+su(f)}}{Y}$	139	143	154	—	—	19	28
14	$\frac{f^{s+su(f)}}{FM4}$	× $\frac{f^{s+su(f)}}{Y}$	171	184	197	41	46	49	39
15	$\frac{+}{FM4}$	× $\frac{f^{s+su(f)}}{Y}$	187	192	191	39	58	42	30
16	$\frac{f^{u}ts67}{FM4}$	× $\frac{f^{s}3DES}{B^{s}Y,su(f)+}$	183	189	173	—	—	53	31
17	$\frac{f^{u+su(f)}}{FM4}$	× $\frac{f^{u+su(f)}}{Y}$	225*	222*	—	45	39	43	52

* Hatched eggs.

Viabilities in Table 4 were determined on the number of "fertilized" eggs listed above. Also listed above are the number of non-FM4 male and female progeny scored to determine the numbers of forked bristles per fly presented in Figures 1 and 2.

RESULTS

Temperature-dependent effects on viability: It is clear from the results presented in Table 2 and illustrated in Figure 4 that *ts67* is a temperature-sensitive lethal mutation. A comparison with the wild-type strain coisogenic for the second and third chromosomes indicates that *ts67* homo- and hemizygotes are, if anything, more viable than wild type at 16° and 20° and in Cross D at 25°. However, at 30° *ts67* exhibits 100% mortality, while the wild type exhibits at most 30% mortality. The data in Table 3 show that when heterozygous with a wild-type allele *ts67* does not affect viabilities at the higher temperatures. The decrease in viability of the heterozygote at 16° in Cross E (45%) is apparently not indicative of a detrimental effect of *ts67* as a heterozygote in the cold, since in Cross G (Table 3) at 18° 91% of the *ts67/FM4* heterozygotes survive.

Interaction between ts67 *and* su(f) *on viability:* Two attempts to map *ts67* on the basis of its temperature-sensitive lethality at 30° gave a map position well to the

TABLE 2

Viability of ts67 *homo- and hemizygotes exposed continuously to five different temperatures throughout development*

Cross	Percent of expected* 16°	20°	25°	30°	33°
A. $+/+ \times +/Y$	26.6	81.0	69.8	71.3	0
B. $+/FM6 \times +/Y$	63.6	77.4	89.1	76.7	0
C. $C(1)DX,y\ f/Y \times +/Y$	81.6	78.4	84.8	80.0	0
D. $ts67/ts67 \times ts67/Y$	31.9	76.1	76.5	0	0
E. $ts67/FM6 \times ts67/Y$	82.7	88.3	81.1	0	0
F. $C(1)DX,y\ f/Y \times ts67/Y$	99.2	83.2	62.4	0	0

Parents raised and 4 hr long egg collections made at 23°.

* Percent of expected: Crosses A and D $\dfrac{\text{total adult progeny}}{\text{"fertilized eggs"}} \times 100$

Crosses B and E $\dfrac{\text{non-FM6 ♀ ♀ and ♂ ♂}}{\text{.5 "fertilized eggs"}} \times 100$

Crosses C and F $\dfrac{\text{total ♂ ♂}}{\text{.25 total eggs}} \times 100$

TABLE 3

Viability of ts67/FM6 *heterozygotes at five different temperatures*

Cross	Percent of expected* 16°	18°	20°	25°	30°	33°
B. $+/FM6 \times +/Y$	80.6	—	84.7	64.2	90.2	0
E. $ts67/FM6 \times ts67/Y$	45.1	—	71.7	81.1	94.4	0
G. $ts67/FM4 \times ts67/Y$	—	91.1	—	82.2	—	—

* Percent of expected $= \dfrac{\text{heterozygous ♀ ♀}}{\text{.25 "fertilized" eggs}} \times 100$

right of *car* (62.5). A subsequent attempt with mutant markers closer to the centromere, including *su(f)* (65.9), also proved to be unsuccessful because all *ts67/ su(f)* heterozygotes died. This led directly to the discovery that *ts67* is an allele of *su(f)*.

That *ts67* produces temperature-sensitive lethality when heterozygous with *su(f)* or with deficiencies that delete the *su(f)* locus ($su(f)^-$) can be seen by the absence of female progeny of the genotypes *su(f)/ts67* or $su(f)^-/ts67$ in Crosses 4, 5, 8, and 9 at 30° in Table 4. Viability of $ts67/su(f)^{3DES}$ females is also greatly reduced at 30° (Cross 16). Those females which did survive exhibited the deficiency phenotype. For all three genotypes *ts67/su(f)*, $ts67/su(f)^-$, and *ts67/3DES*, viability at 18° and 25° is quite variable, but in all cases is over 50% of expected. $ts67/+^{su(f)}$ females are viable at all three temperatures (Crosses 10 and 11). Confirming the results presented in Table 2, *ts67/Y* males do not survive at 30° (Crosses 1, 2, 10, 11 in Table 4), but $ts67/Ysu(f)^+$ ($ts67/y^+Yma\text{-}1^+$) and $ts67/B^sY,su(f)^+$ males are viable at 30° (Crosses 8, 9, 16), indicating that *ts67* must be covered by these *X;Y* translocations which do include the + allele of *su(f)* (SCHALET 1968; LINDSLEY and GRELL 1968).

Since none of the stocks used for the crosses (Table 1) performed to provide the data presented in Table 4 were coisogenic, comparisons of viabilities between crosses should be made with caution.

Suppression of forked bristles: The mutant *ts67* is a temperature-dependent suppressor of the forked bristles produced by a suppressible allele of forked in that it suppresses forked bristles at 25° but not at 18°. In Figures 1 and 2 the average number of forked bristles per male (Figure 1) and per female (Figure 2) are graphically represented. The genotypes of the flies which show an obvious decrease in the number of forked bristles at 25° in comparison to 18° include f^sts67/Y (Cross 11, Figure 1), f^sts67/f^sts67 (Cross 2, Figure 2), $f^sts67/f^ssu(f)$ (Cross 5), $f^uts67/f^ssu(f)$ (Cross 4), f^uts67/f^s3DES (Cross 16), and $f^uts67/f^ssu(f)^-$ (Cross 8). These decreases in the numbers of forked bristles are significant even when differences between 18° and 25° in the controls, $f^s+^{su(f)}/Y$ (Cross 14, Figure 1) and $f^s+^{su(f)}/f^s+^{su(f)}$ (Cross 14, Figure 2), are taken into account.

Although there is no apparent effect of *ts67* as a heterozygote on viability, *ts67* does effect some temperature-dependent suppression of forked bristles when heterozygous. At 18° $f^sts67/f^s+^{su(f)}$ females (cross 11, Figure 2) have a mean of 4.96 forked bristles/fly, which is significantly different from the mean of 3.31 forked bristles/fly found at 25° ($p = .0376$), while control $f^s+^{su(f)}/f^s+^{su(f)}$ females (Cross 14, Figure 2) with 4.67 forked bristles/fly at 18° do not differ significantly from the 4.74 forked bristles/fly found at 25° ($p = .9362$). Furthermore, while a statistical comparison of the mean forked bristles/fly for heterozygous $f^sts67/f^s+^{su(f)}$ females with the mean for homozygous $f^s+^{su(f)}/f^s+^{su(f)}$ females shows that at 18° they do not differ from each other ($p = .7264$), at 25° the difference between means is significant at the 10% level but not the 5% level ($p = .0818$). Figure 3 compares the distribution frequencies of forked bristles per fly for these two genotypes at 18° and 25°. At both temperatures the

TABLE 4

Effects of su(f) *alleles on viability*

	Non-*FM4* male progeny				Non-*FM4* female progeny			
		Percent of expected*				Percent of expected*		
Cross no.	Genotype	18°	25°	30°	Genotype	18°	25°	30°
1	$\frac{f^{u}ts67}{Y}$	88.9	77.5	0	$\frac{f^{u}ts67}{f^{u}ts67}$	87.4	62.3	0
2	$\frac{f^{s}ts67}{Y}$	17.7	37.3	0	$\frac{f^{s}ts67}{f^{u}ts67}$	35.4	48.0	0
3	$\frac{f^{s}su(f)}{Y}$	86.9	87.0	51.8	$\frac{f^{s}su(f)}{f^{s}su(f)}$	75.4	89.1	44.8
4	$\frac{f^{s}su(f)}{Y}$	65.8	75.5	58.1	$\frac{f^{u}ts67}{f^{s}su(f)}$	100.4	83.7	0
5	$\frac{f^{s}su(f)}{Y}$	44.8	81.6	9.3	$\frac{f^{s}ts67}{f^{s}su(f)}$	57.4	77.6	0
6	$\frac{f^{s}su(f)^{-}}{Ysu(f)+}$	72.2	64.8	71.7	$\frac{f^{s}su(f)^{-}}{f^{s}su(f)^{-}}$	0	0	0
7	$\frac{f^{s}su(f)}{Ysu(f)+}$	76.6	70.2	67.7	$\frac{f^{s}su(f)}{f^{s}su(f)^{-}}$	69.4	87.2	4.1
8	$\frac{f^{u}ts67}{Ysu(f)+}$	70.6	72.5	60.9	$\frac{f^{u}ts67}{f^{s}su(f)^{-}}$	87.7	81.2	0
9	$\frac{f^{s}ts67}{Ysu(f)+}$	88.9	68.2	64.4	$\frac{f^{s}ts67}{f^{s}su(f)^{-}}$	104.6	56.3	0
10	$\frac{f^{u}ts67}{Y}$	68.8	99.5	0	$\frac{f^{u}ts67}{f^{s}+^{su(f)}}$	93.8	112.2	95.9
11	$\frac{f^{s}ts67}{Y}$	85.6	71.8	0	$\frac{f^{s}ts67}{f^{s}+^{su(f)}}$	78.6	111.6	78.6
12	$\frac{f^{s}su(f)}{Y}$	74.3	98.2	48.1	$\frac{f^{s}su(f)}{f^{s}+^{su(f)}}$	69.5	76.7	72.1
13	$\frac{f^{s}su(f)^{-}}{Y}$	0	0	0	$\frac{f^{s}su(f)^{-}}{f^{s}+^{su(f)}}$	54.7	78.3	57.5
14	$\frac{f^{s}+^{su(f)}}{Y}$	98.3	100.0	81.2	$\frac{f^{s}+^{su(f)}}{f^{s}+^{su(f)}}$	114.6	84.8	97.5
15	$\frac{+}{Y}$	83.4	120.8	88.0	$\frac{+}{f^{s}+^{su(f)}}$	89.8	62.5	88.0
16	$\frac{f^{u}ts67}{B^{s}Y,su(f)+}$	89.6	91.0	85.6	$\frac{f^{u}ts67}{f^{s}3DES}$	115.9	65.6	9.3
17†	$\frac{f^{u}+^{su(f)}}{Y}$	80.0	70.4	—	$\frac{f^{u}+^{su(f)}}{f^{s}+^{su(f)}}$	76.4	93.5	—

The crosses producing these offspring are listed by Cross no. in Table 1.

* Percent of expected $= \frac{\text{Non-}FM4\ ♂\ ♂ \text{ or } ♀\ ♀}{.25\text{ “fertilized” eggs}} \times 100$

† Percent of expected $= \frac{\text{Non-}FM4\ ♂\ ♂ \text{ or } ♀\ ♀}{\text{Hatched eggs}} \times 100$

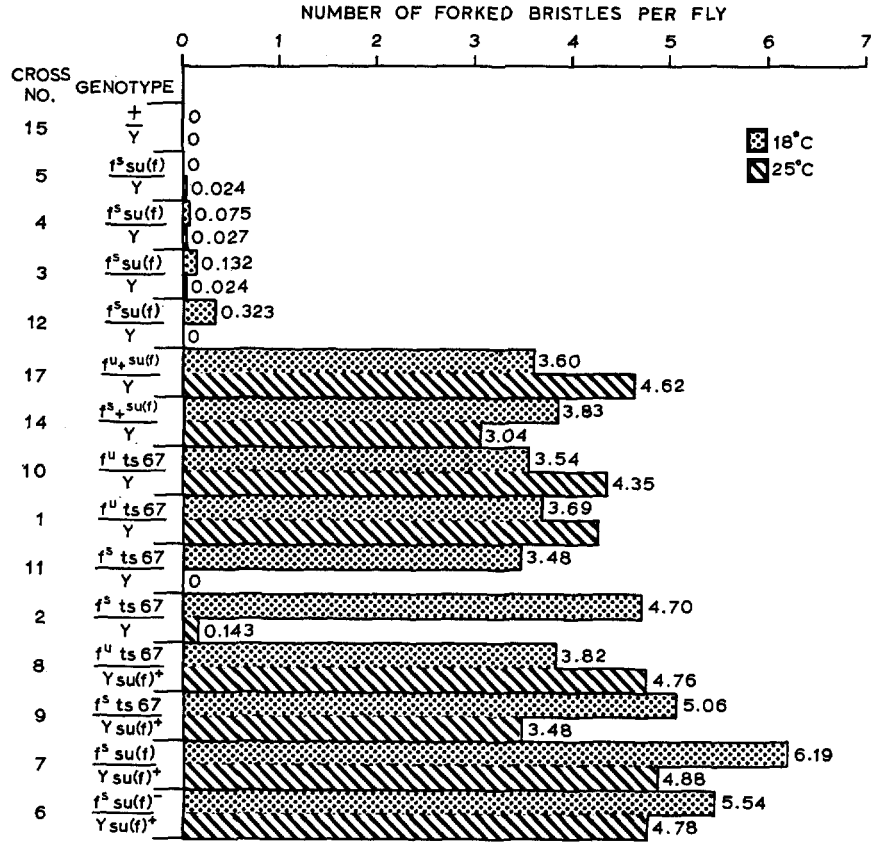

FIGURE 1.—Number of forked bristles per male grown at 18° and 25°. Eight bristles, the anterior and posterior scutellars and the anterior and posterior dorsocentrals were scored as forked or non-forked. The flies scored, designated by the genotype and cross number, were progeny of the crosses listed in Table 1 where the numbers of flies scored are presented.

distributions differ significantly from each other; at 18° $\chi^2 = 11.8549$, df = 3, $p<.01$ and at 25° $\chi^2 = 110.3202$, df = 3, $p \ll .001$. Since at 18° the mean for the distribution for the heterozygote is larger (4.96) than the mean for the homozygote (4.67) and since the reverse is true at 25°, the distributions for the two genotypes differ in opposite directions from each other at the two temperatures, emphasizing that temperature-dependent suppression does take place in *f^s ts67/f^s+^su(f)* heterozygotes.

Since *f^s ts67/Ysu(f)^+* males (Cross 9, Figure 1) are heterozygous mutant/non-mutant at the *su(f)* locus one might expect to observe temperature-dependent suppression in these heterozygous males also. The means at 18° (5.06) and 25° (3.48) are not significantly different ($p = .1388$) although the two frequency distributions do differ significantly ($\chi^2 = 55.2072$, df = 2, $p<.001$). The absence of an *f^s+/Ysu(f)^+* control permits the speculation that the *Ysu(f)^+* chromosome itself may have a temperature effect on the frequency distribution of forked bristles. This possibility is enhanced by the observations that neither the means

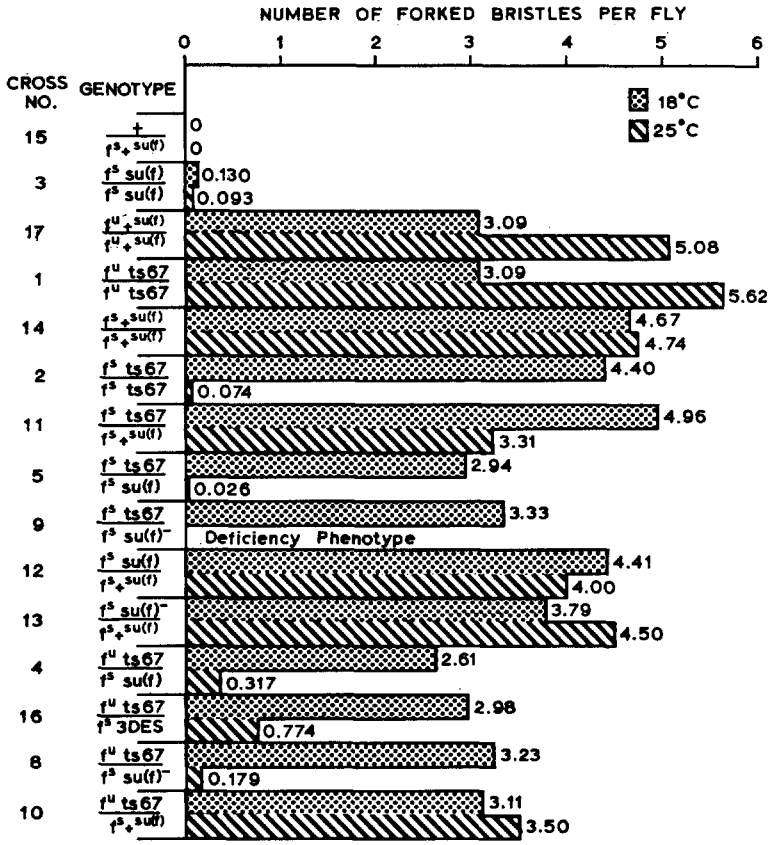

FIGURE 2.—Number of forked bristles per female grown at 18° and 25°. For details see Figure 1 legend.

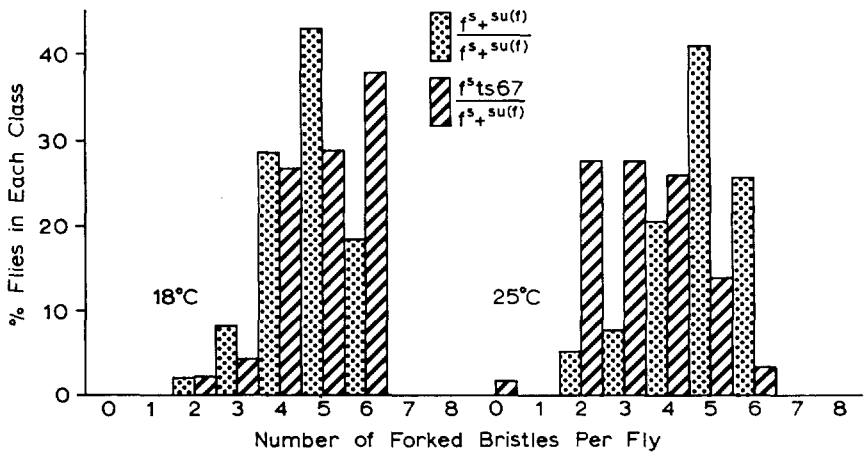

FIGURE 3.—Frequency distribution of the number of forked bristles per fly. The $f^{s}+^{su(f)}/f^{s}+^{su(f)}$ females were offspring of Cross 14, Table 1 with $n = 49$ at 18° and $n = 39$ at 25°. The $f^{s}ts67/f^{s}+^{su(f)}$ females were offspring of Cross 11, Table 1 with $n = 45$ at 18° and $n = 58$ at 25°.

for $f^s su(f)/Y su(f)^+$ (Cross 7, Figure 1) at 18° and 25° (6.19 *vs.* 4.88, p = .1706) nor the means for $f^s su(f)^-/Y su(f)^+$ (Cross 6) (5.54 *vs.* 4.78, p = .4902) differ significantly, yet the frequency distributions for the number of forked bristles per fly at these two temperatures do differ significantly for both genotypes ($f^s su(f)/Y su(f)^+$; $\chi^2 = 63.7650$, df = 1, p<.001 and $f^s su(f)^-/Y su(f)^+$; $\chi^2 = 12.1806$, df = 1, p<.001).

A comparison of the number of forked bristles per fly for $f^u ts67/f^u ts67$ females (Cross 1, Figure 2) at 18° and 25° (p = .0012) might suggest that *ts67* is a temperature-dependent enhancer of this f^u allele. However, since virtually identical results are obtained with $f^u{+}^{su(f)}/f^u{+}^{su(f)}$ females (Cross 17) (p = .015) and the means for the two genotypes at 25° do not differ significantly (p = .4778), it is clear that this is a temperature effect on the phenotype of this f^u allele and occurs whether *ts67* is present or not. The $f^u{+}^{su(f)}/Y$, $f^u ts67/Y$, and $f^u ts67/Y su(f)^+$ males (Crosses 17, 10, 1 and 8 in Figure 1) all show an increase in the forked bristles of 25° relative to 18°, but these increases are not significant (p = .2460, p = .3788, p = .4354, and p = .3734 for the crosses, respectively). No temperature effect is apparent for the forked bristle phenotype of f^s, e.g. $f^s{+}^{su(f)}/f^s{+}^{su(f)}$ females (Cross 14, Figure 2) (p = .9362) and $f^s{+}^{su(f)}/Y$ males (Cross 14, Figure 1) (p = .3524).

One should note that the forked bristle phenotype of f^u/f^s heterozygotes is suppressed (see $f^s su(f)/f^u ts67$ Cross 4, $f^u ts67/f^s 3DES$ Cross 16, and $f^u ts67/f^s su(f)^-$ Cross 8 in Figure 2). This suggests that a functional product is produced by f^s in the presence of the *su(f)* mutations, rather than that f^u does something negative which is unaffected by the presence of mutants at the *su(f)* locus.

One other aspect of *ts67* which was tested was its suppression of lz^1. At 18° males hemizygous for lz^1 and *ts67* showed the lozenge eye phenotype, at 25° they had almost wild-type eyes, and at 30° they died as expected.

The "deficiency" phenotype: Several of the genotypes generated by the crosses used to score suppression of forked bristles exhibited some or most of the characteristics associated with the deficiency phenotype described in the introduction. The phenotype at 18°, 25°, and 30° of those flies which exhibited this phenotype are included in Table 5. The following is a list of those genotypes and the characteristics shown by most of the individuals:

$f^s su(f)/Y$ at 30°—small and thin or missing bristles, rough oval eyes, dark pigment at leg joints occasionally.

$f^s su(f)/f^s su(f)$ at 30°—small and thin or missing bristles, dark pigment at leg joints and rough oval eyes occasionally.

$f^s su(f)/f^s su(f)^-$ at 18° and 25°—small and thin or missing bristles, rough oval eyes, wings which were wrinkled, blistered, outheld or indented at inner margin, and disrupted abdominal segmentation occasionally.

$f^s ts67/f^s su(f)^-$ at 25°—small and thin or missing bristles.

$f^u ts67/f^s 3DES$ at 30°—small and thin or missing bristles, rough oval eyes, crumpled legs.

$f^u ts67/f^s su(f)$ at 29°—small and thin or missing bristles.

Those bristles which were present in the flies listed above were usually not forked, i.e., probably suppressed forked bristles.

Differences between f^sts67 *and* f^uts67: In addition to suppressibility of their forked bristle phenotype the chromosomes *f^sts67* and *f^uts67* differ from each other in other parameters. For instance, a comparison of the viability of *f^uts67* homo- and hemizygous progeny from Cross 1 (Table 4) with those of *f^sts67* from Cross 2 shows a very obvious difference in viability, with the latter being considerably less viable. Furthermore, a phenotypic difference was observed between these two chromosomes when they were heterozygous with the *f^ssu(f)$^-$* chromosome. At 18° flies of both genotypes, *f^sts67/f^ssu(f)$^-$* and *f^uts67/f^ssu(f)$^-$*, had forked bristles, but at 25° *f^uts67/f^ssu(f)$^-$* females had wild-type bristles while *f^sts67/f^ssu(f)$^-$* exhibited characteristics of the deficiency phenotype (small and thin or missing bristles) and were also less viable (Crosses 8 and 9, Table 4). *Female sterility and maternal effects:* In addition to being a temperature-sensitive lethal, *ts67* is a conditional female sterile mutation. In all those cases (more than 73) when very young *car ts67/car ts67* and *f^sts67/f^sts67* virgins were mated at 30° to 3–7-day-old wild-type males, no eggs were laid. At 18°, 23° and 25° homozygous *ts67* are perfectly fertile. Very young wild-type (Oregon-R) virgins were perfectly fertile when mated at 30° to 3–7-day-old wild-type males (sibs of the above males). When very young *car ts67/car ts67* virgins mated immediately at 30° continuously for four days were dissected, normally motile sperm were observed to be present in the female seminal receptacles when examined at 440X with a compound microscope. The ovaries of these well-yeasted females were bloated with degenerating oocytes of all stages, which included only one or two Stage 14 oocytes per ovary and between 5 and 10 Stage 11 through Stage 13 oocytes per ovary (see KING, RUBINSON and SMITH 1956 for staging of oocytes). Virtually the entire range of degenerating Stage 1 through Stage 10 oocytes were found backed up in the ovarioles behind the Stage 11–14 oocytes.

A vast majority of the *ts67/ts67* females which had been laying fertile eggs at 25° (virgin females aged 3 days at 25° and mated for 24 hours at 25°) become completely sterile and stop laying eggs within 68 hours after being shifted up to 30°, and all females become completely sterile within 96 hours. When these females were shifted back down to 25° after 11 days at 30° they remained completely sterile. However, with shorter exposures to 30° sterility is not irreversible, for homozygous *ts67* females incubated at 30° for 3 days become fertile when brought to room temperature.

Since *ts67* is a conditional female sterile mutation, the possibility that there might be some maternal effects on the viability of zygotes was investigated (BROTHERS 1971). The data in Table 2 indicate that except at 16° there is little difference in the viabilities of *ts67* homo- and hemizygous progeny produced by *ts67/ts67* females (2 doses *ts67* : 0 $+^{ts67}$ doses), *ts67/FM6* females (1 dose *ts67* : 1 $+^{ts67}$ dose), and *C(1)DX,y f/Y* females 0 *ts67* doses : 2 $+^{ts67}$ doses). At 16° there was an apparent increase in viability as the number of wild-type doses were increased. However, inspection of data for the crosses with the coisogenic strains not carrying *ts67* (Crosses A, B, and C in Table 2) shows a similar increase in viability from the homozygous cross (Cross A) to the heterozygous cross (Cross B), and to the attached-*X* cross (Cross C), indicating that there is no real maternal dosage effect of *ts67* on progeny viabilities even at 16°.

Newly emerged, virgin females of the genotype *ts67/ts67* and *ts67/FM6* raised at 23° and aged 6–8 days at 18° and 25° were mated to wild-type males in laying bottles at 23°. Four-hour-long egg collections were made, and the eggs were placed at 20°, 25°, and 30°. For neither genotype did the maternal temperature have a significant effect on the viability of *ts67/Y* male progeny or on the stage distribution of mortalities (for details see BROTHERS 1971). No attempt has been made to investigate the possibility that a maternal temperature effect could be demonstrated if females were raised at different temperatures rather than just aged at different temperatures after eclosion or if stage distribution of mortalities were more precisely determined than just the gross embryonic, larval, and pupal stages used.

Stage distribution of mortalities and temperature-sensitive period: On continuous exposure to 30° during development *ts67* homo- and hemizygotes are lethal in the larval stage, as illustrated in Figure 4. Of the 100% mortality of *ts67* at 30°, almost 90% is larval mortality with no individuals surviving to form puparia. At 33° half of this larval mortality appears to have been shifted to embryonic mortality, but at 33° the wild-type control has a large component of embryonic mortality also.

The temperature-sensitive period (TSP) at 30° for *ts67* homo- and hemizygotes was established by shifting timed cultures up from 25° to 30° or down from 30° to 25° during progressively later stages of development. As can be seen from Figure 5 (shift-up) and Figure 6 (shift-down) the temperature-sensitive period begins during the second instar (Figure 6) and ends at pupation (Figure

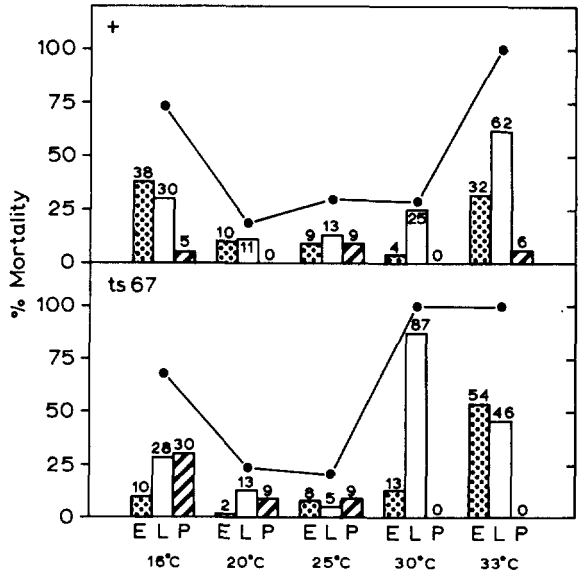

FIGURE 4.—Stage distribution of mortalities and total mortality, ●———●, for zygotes raised continuously at the indicated temperatures. E = embryonic mortality, L = larval mortality, and P = pupal mortality. Eggs were laid at 23° by homozygous +/+ or *ts67/ts67* females mated to +/*Y* or *ts67/Y* males.

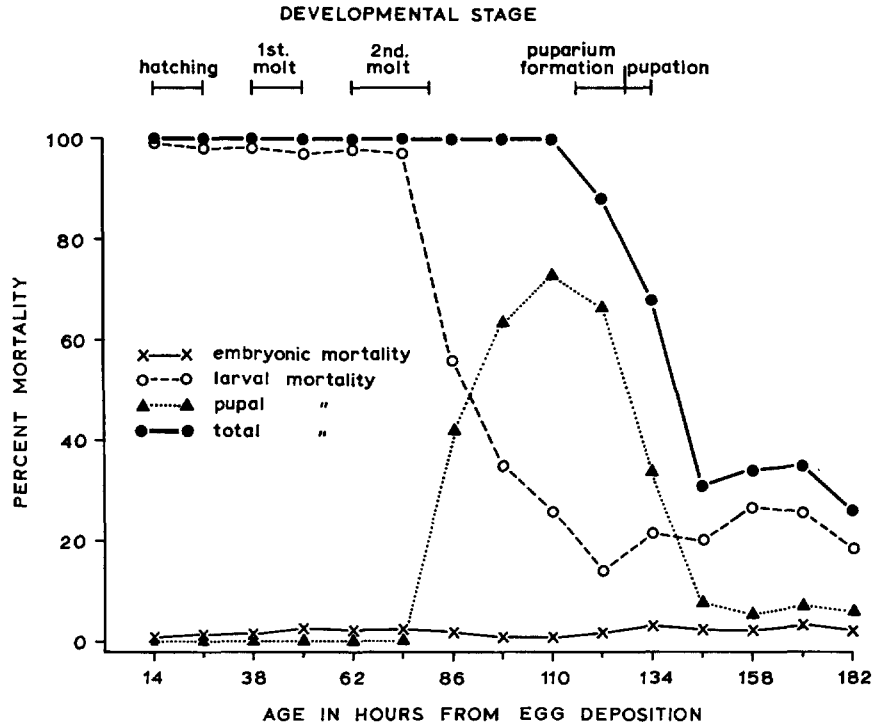

FIGURE 5.—Shift-up experiment for viability. Stage distribution of mortalities and total mortalities resulting from the shift of zygotes up from 25° to 30° at twelve-hour intervals progressively later in development. The developmental stage at the time of the shift is indicated at the top of the figure. Eggs were laid at 25° by $f^{u}ts67/f^{u}ts67$ females mated to $f^{u}ts67/Y$ males.

5). Larvae shifted up to 30° any time during the first, second, or early third instar failed to pupate, as indicated by the high larval mortalities at those times (Figure 5). Those shifted up later in the third instar did pupate and developed to the stage of red eye pigment formation, but they failed to eclose. This resulted in the high pupal mortality illustrated at 110 hours in Figure 5. Eggs placed at 30° but shifted to 25° before the end of the second instar were able to develop into adults. Those grown at 30° through the second instar appeared to be undergoing degeneration: the tracheal trunks were disrupted and the anterior spiracles and mouthparts were not typical of any larval stage; the last showed an extra, long spike projecting from the base of the mandibular hooks.

Heat shocks at higher temperatures, 33° and 35° have produced additional information on the TSP of *ts67*. It is clear from Figure 7 that *ts67* individuals are susceptible to 33° heat shocks at various times during development. An 18-hour heat shock from 6–24 hours of embryogenesis increases total mortality significantly, with most of it being embryonic mortality. Forty-eight-hour-long heat shocks at 33° during the first and second larval instars (24–72 hr) and early during metamorphosis (120–168 hr) produce total mortalities of 80% and 90%, respectively. Most of the former occurs during the larval stages, whereas the

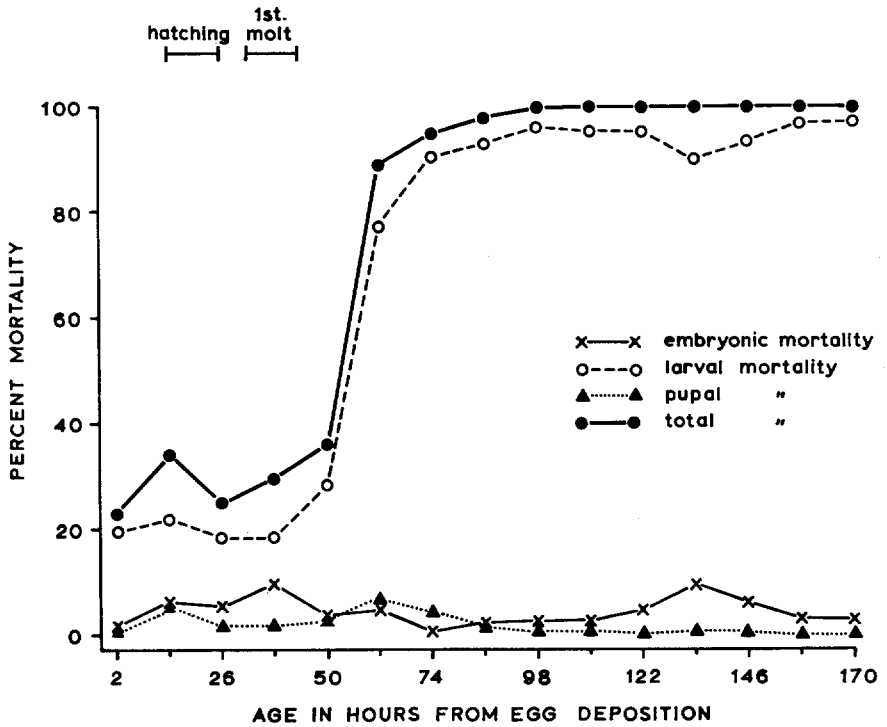

FIGURE 6.—Shift-down experiment for viability. Shift down from 30° to 25° For details see Figure 5 legend.

latter occurs in the pupal stage as expected. The third larval, instar appears to be relatively insensitive to 48-hour-long 33° heat shocks, and since a heat shock from 168 to 216 hours of development during the later stages of metamorphosis kills 97% of the wild type control, no inferences can be made on the temperature sensitivity of *ts67* individuals during this stage from this data.

Figure 8 illustrates the mortalities obtained when 35° heat shocks were administered for only 24-hour periods to larvae and early pupae. Except for the pupae, there is very little if any increase in mortality, indicating that larvae are capable of recovering from drastic heat shocks of short duration, but that the early pupae are not.

Temperature-sensitive period for the suppression of forked bristles: The temperaature-sensitive period for the suppression of forked bristles was determined for $f^{s}ts67/f^{s}ts67$ females by shifting cultures up from 18° to 25° and down from 25° to 18° at progressively later stages in development. The TSP for suppression of forked bristles is different from the TSP for lethality. The former occurs after pupation (eversion of the cephalic imaginal discs), as can be seen in Figure 9, whereas the TSP for the latter does not extend beyond puparium formation (Figures 5 and 6). It is apparent in Figure 9 that as cultures are shifted up to 25° progressively later a high percentage of flies emerge which have wild-type bristles, until a period of time after pupation when shifting to 25° is too late to cause

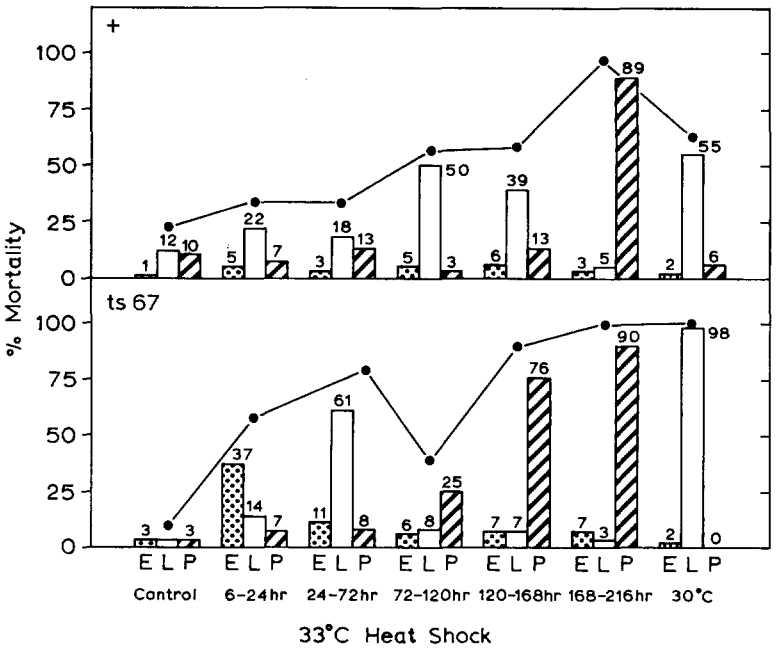

FIGURE 7.—33° heat shock experiment: Stage distribution of mortalities and total mortalities, ●——●; for zygotes subjected to 33° heat shocks for the durations and times in development indicated. E = embryonic mortality, L = larval mortality, P = pupal mortality. Eggs were laid at 23° by *ts67/ts67* females mated to *ts67/Y* males. For the control and 30° data zygotes were maintained continuously at 23° and at 30°, respectively.

suppression. The curve for the shift-down experiment is almost a mirror image of the one for the shift-up experiment, and the two curves together delineate a rather precise TSP of approximately 24 hours. Shifts at much shorter intervals, 2 or 4 hours, might delineate the TSP even more precisely. It appears that the TSP begins just prior to the initiation of bristle formation at 30 hours after the white puparium stage at 25° (BODENSTEIN 1950).

DISCUSSION

Temperature-sensitive period and the duration of gene activity: In addition to establishing the existence of a temperature-sensitive allele of the suppressor of forked, the results reported here indicate functions of that gene other than suppression of forked bristles. First, functional gene product is necessary for survival with the TSP for the *ts* allele beginning in the second instar and ending at pupation. This does not preclude functioning of gene product before or after this period. It only establishes that activity of gene product or accumulation of active gene product must occur during the TSP. That at least the gene product functions subsequent to the TSP for viability is demonstrated by the fact that the TSP for the suppression of forked bristles occurs post-pupation after the end of the TSP for viability.

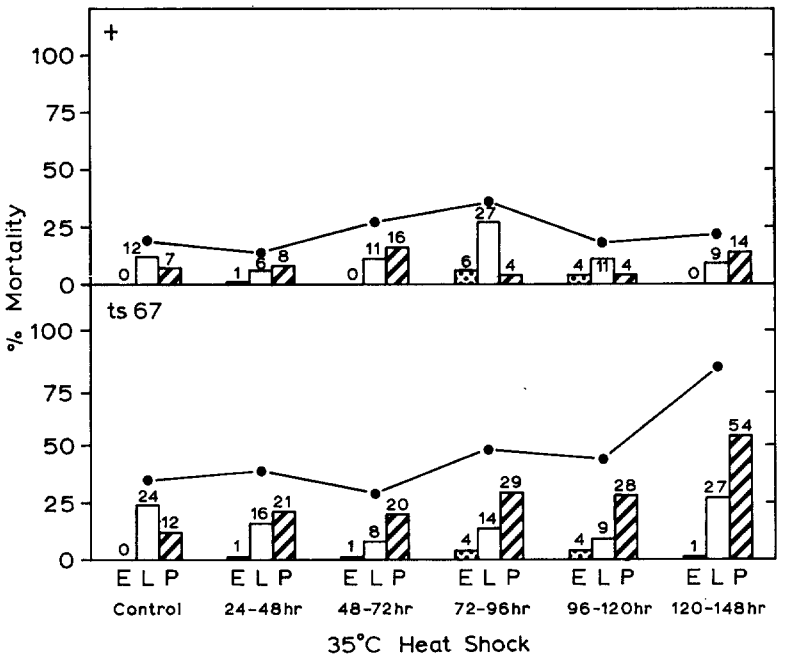

FIGURE 8.—35°-24 hr heat shock experiment. Stage distribution of mortalities and total mortalities for zygotes subjected to 24-hour 35° heat shocks at the times in development indicated. For details see Figure 7 legend.

Furthermore, although the viability shift experiments show that the TSP begins at the second instar, the fact that a 33° heat shock from 6–24 hours of embryogenesis increases embryonic mortality 32% above the control wild-type stock and the fact that the non-conditioned lethal allele *l(1)su(f)*^{sDES} is an embryonic lethal clearly indicate that the wild-type *su(f)* gene product must function during embryogenesis prior to the initiation of the TSP. That *ts67* homo- and hemizygotes can survive until the second instar when shifted down from 30° to 25° suggests that enough functional *su(f)* gene product is deposited in the egg by the mother at the permissive temperature to permit zygotic development to proceed through the first larval instar at 30° in the absence of the synthesis of more functional gene product. The failure to establish a predictable maternal effect has been considered earlier.

Another aspect of wild-type *su(f)* gene function which has been exposed for the first time by the analysis of *ts67* is that it is necessary for the production of eggs—i.e. oogenesis is another process for which functional gene product is critical. That a female sterile phene for mutant alleles at the *su(f)* locus has not previously been reported is understandable, for if a non-conditional mutation of the *su(f)* locus was severe enough to cause female sterility, it probably would be lethal to the organism at an earlier critical period in development before such an effect on oogenesis could be observed. It is obvious that an advantage of *ts* alleles is

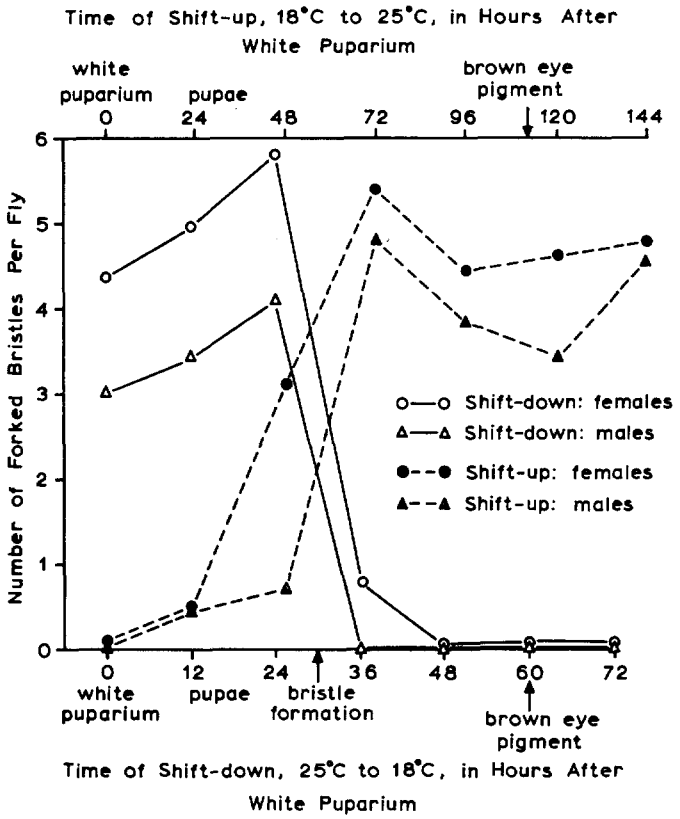

FIGURE 9.—Temperature-sensitive period for the suppression of forked bristles. Mean number of forked bristles per fly for individuals shifted up from 18° to 25° and shifted down from 25° to 18° at progressively later stages in development after white puparium formation. Individuals homo- and hemizygous for $f^{s}ts67$ were raised to the white puparium stage (approximately 1 hour duration) at 25° when they were transferred to culture vials and placed at 18° or 25° until shifted up or down. Approximately 25 flies of each sex were scored for each shift time. The time for development from the white puparium stage to brown eye pigment stage at 18° (established in this and other experiments) relative to that time at 25° was used to normalize the time scale at 18° to coincide with time scale at 25°.

that critical periods of gene activity other than the initial one in development may be discovered.

Expressivity of the phenotypes associated with the su(f) *and forked loci*: It is evident from the data presented here and previously (SCHALET 1968 1972) that in the presence of mutations at the forked locus, mutations at the *su(f)* locus can effect in essence four levels of expressivity which are shown not to be discontinuous. These are in ascending order of severity: unsuppressed forked bristles < suppressed forked bristles < the deficiency phenotype < lethality. It is also apparent that just which of these levels of expressivity will be produced is dependent on three variables: the genotype at the *su(f)* locus, temperature, and the genotype at the forked locus. Our observations on the phenotypes produced by various

TABLE 5

Expressivity and the influence of temperature and genotype at the su(f) *and forked loci*
(Forked bristle counts were not made at 30°)

Cross no.*	Genotype	18°	25°	30°
14	$\frac{f^{s}\ +}{f^{s}\ +}$	forked	forked	viable blunt bristles
12	$\frac{f^{s}su(f)}{f^{s}\ +}$	forked†	forked¶	viable
13	$\frac{f^{s}su(f)^{-}}{f^{s}\ +}$	forked†	forked¶	viable
11	$\frac{f^{s}ts67}{f^{s}\ +}$	forked†	partial suppression¶,‖	viable blunt bristles
3	$\frac{f^{s}su(f)}{f^{s}su(f)}$	suppression	suppression	deficiency phenotype
5	$\frac{f^{s}ts67}{f^{s}su(f)}$	forked†	suppression	lethal
2	$\frac{f^{s}ts67}{f^{s}ts67}$	forked†	suppression	lethal
9	$\frac{f^{s}ts67}{f^{s}su(f)^{-}}$	forked†	deficiency phenotype	lethal
7	$\frac{f^{s}su(f)}{f^{s}su(f)^{-}}$	deficiency phenotype	deficiency phenotype	lethal
6	$\frac{f^{s}su(f)^{-}}{f^{s}su(f)^{-}}$	lethal	lethal	lethal
10	$\frac{f^{u}ts67}{f^{s}\ +}$	forked†,§	forked	forked some blunt bristles
16	$\frac{f^{u}ts67}{f^{s}3DES}$	forked‡,§	suppression (22°)	semi-lethal (def. phenotype)
4	$\frac{f^{u}ts67}{f^{s}su(f)}$	forked‡,§	suppression	lethal (29° def. phenotype)
8	$\frac{f^{u}ts67}{f^{s}su(f)^{-}}$	forked†,§	suppression	lethal
17	$\frac{f^{u}\ +}{f^{u}\ +}$	forked	enhanced forked	———
1	$\frac{f^{u}ts67}{f^{u}ts67}$	forked	enhanced forked	lethal

TABLE 5—Continued

Cross no.*	Genotype	18°	25°	30°
	$\frac{f^s su(f)}{+\ su(f)^-}$	———	deficiency** phenotype	———
	$\frac{+\ ts67}{+\ ts67}$	non-forked	non-forked	lethal
	$\frac{+\ ts67}{+\ su(f)^-}$	———	non-forked	lethal

* The crosses producing these offspring are listed by Cross no. in Table 1.
** Data from SCHALET (1968b).
† Number of forked bristles per fly not significantly different from Cross 14 ♀ ♀ at 18° at the 5% level. (Cross 5, p = .0545; Cross 10, p = .0574.)
‡ Significantly different from Cross 14 ♀ ♀ at 18° at the 5% level.
§ Not significantly different from Cross 17 ♀ ♀ at 18°.
¶ Not significantly different from Cross 14 ♀ ♀ at 25°. (Cross 11, p = .0818).
‖ Significantly different from Cross 11 ♀ ♀ at 18°, p = .0376.

combinations and permutations of these variables are summarized in Table 5. Expressivity increases as the temperature increases and at the *su(f)* locus as *su(f)* substitutes for *su(f)*$^+$, *ts67* substitutes for *su(f)*, and *3DES* substitutes for *ts67*. At the forked locus expressivity generally increases as f^+ is replaced by f^u and f^u by f^s.

The ribosomal protein hypothesis: It has previously been suggested that mutants at the *su(f)* locus affect protein synthesis (SCHALET and SINGER 1971; SCHALET 1973), and more specifically that the locus may be directly involved in translational events (FINNERTY *et al.* 1973; WRIGHT 1973) and even further that suppression may be effected by the misreading of specific codons by modified ribosomes (DUDICK 1973). The working hypothesis concerning the suppressor of forked locus might now be stated as follows: (1) that the *su(f)* locus codes for a ribosomal protein which when partially altered by mutation leads to the production of ribosomes modified such that a low level of misreading of specific codons takes place, resulting in the suppression or enhancement of specific mutant alleles at other loci in the genome; (2) that when this ribosomal protein is more drastically modified or is available at a reduced level, suboptimal, but non-lethal, levels of protein synthesis ensue producing flies which exhibit the deficiency phenotype; (3) finally, that lethality occurs when complete inactivity of the mutant ribosomal protein or its drastically reduced availability leads to either the production of fatally reduced numbers of ribosomes or to normal numbers of ribosomes with fatally reduced functional efficiency. The possibility also exists that increased levels of misreading are responsible in part for the deficiency phenotype and lethality. Some of the supporting observations and arguments which have been presented in the past (SCHALET 1970, 1973; SCHALET and SINGER 1971; WRIGHT 1973; DUDICK 1973; FINNERTY *et al.* 1973) and others which derive from the work on *ts67* are discussed below.

In various combinations with each other and under various temperature regimes mutants at the suppressor of forked locus produce the deficiency pheno-

type with its small, thin, or absent bristles, rough, oval eyes, and sometimes abnormal wings and legs. The similiarity of the deficiency bristle phene with the bristle phenotype of bobbed suggest that these phenes may arise from a reduced rate of protein synthesis. In the case of bobbed this origin of the mutant phenotype is interpreted to result from modified protein synthesis because *bb* flies have fewer than normal numbers of 18S and 28S ribosomal RNA genes (RITOSSA, ATWOOD and SPIEGELMAN 1966). The fact the *su(f)* locus is immediately adjacent to the bobbed locus presents the possibility that genes with related function are clustered and that *su(f)* might code for a ribosomal protein. Since there is clustering of rRNA and ribosomal protein genes in prokaryotes (DAVIES and NOMURA 1972), the hypothesis is most attractive. STEFFENSEN (1973) presents evidence which he interprets as being indicative of clustering of ribosomal protein genes toward the proximal end of the *X* chromosome in Drosophila.

Some other phenes generated by mutations at the *su(f)* can most easily be explained by the reduced production of ribosomes or the reduced efficiency of ribosomes in protein synthesis. That lethality produced by the non-conditional lethal alleles of *su(f)* and the conditional allele, *ts67*, could be the result of defective ribosomes or defective ribosome synthesis is obvious, yet it is relevant that normally the accumulation of ribosomes occurs during the third instar and stops at pupation (HOWELLS 1972), a period equivalent to the TSP of *ts67*. Furthermore, since protein synthesis is necessary for oogenesis to proceed and since it is mandatory that competent ribosomes be incorporated into oocytes during oogenesis, one would predict that any conditional mutant that affects ribosome structure or function should be a conditional female sterile mutation or a conditional maternally-influenced zygotic lethal. That *ts67* imparts temperature-sensitive female sterility is consistent with the hypothesis that mutations at the *su(f)* locus affect ribosomes in some way.

Reduced protein synthesis above could explain the reduced bristle size in the deficiency phenotype, the failure of *ts67* homozygotes to produce eggs at 30°, and the larval lethality of *ts67* homo- and hemizygotes. The fact that in addition *ts67* and *su(f)* suppress and enhance certain mutant genes suggests that their effects may be more specific than just reducing levels of protein synthesis. There is a precedent in bacterial systems for suppression by increased misreading caused by altered ribosomes. A similar phenomenon could also explain suppression of *f*, *lz*, and *dy* by a mutation at the *su(f)* locus. The fact that only certain alleles of those mutant genes are suppressed can be explained by further analogy to work done with bacterial systems. In a study of missense mutations in the gene of the histidine operon in Salmonella, only 33% of the mutations tested were suppressible by addition of streptomycin to the growth medium (WHITFIELD, MARTIN and AMES 1966). It is known that streptomycin causes suppression by altering the ribosomes and increasing misreading. *In vivo* work with streptomycin-induced misreading indicates that some codons are much more likely to be misread than other (DAVIES *et al.* 1964). This might imply that *in vivo* the misreading of only certain codons could occur, and if this misreading resulted in an acceptable amino substitution in the product of a mutant gene, functional product would result.

Larval death and female sterility at 30° of *ts67* flies could also be explained in terms of ribosomal-modified misreading. It might be that at 18° normally functioning ribosomes are produced which support the development of normal flies. At 25° ribosomal function may be altered in such a way that mistakes are made to suppress the forked bristle phenotype, but the mistake level may not be high enough to affect viability or female fertility significantly. At 30° all of the ribosomes may contain a highly distorted protein and many mistakes in translation may be made. At critical periods in development, when high levels of protein synthesis are required, the presence of a great many nonfunctional proteins would result in aborted development. A similar series of events was observed in a temperature-sensitive mutation in *E. coli* which caused suppression at the permissive temperature and death at the restrictive temperature (APIRION 1966). While these effects were correlated with altered ribosomes, they were not demonstrated to be due to ribosomal modification of translation.

Many of the observations can be interpreted to mean that *su(f)* and *ts67* produce ribosomal mutations which cause informational suppression. However, there remain some unanswered questions. One problem is that nothing is known about the products of those genes which are suppressed. One argument which cannot be resolved because of lack of information is that informational suppression is not compatible with the extremely high degree of phenotypic suppression effected by *su(f)* and *ts67*. Informational suppression is usually very inefficient. This has been established by determining enzyme levels in bacteria which contain mutations in genes for enzymes and which also contain informational suppressors (GORINI, JACOBY and BRECKINRIDGE 1966). At the morphological level suppression of forked bristles by *su(f)* and *ts67* is very efficient. However, since the product of the forked locus is unknown, it is possible that a small amount of wild-type product formed by translational misreading of the mutant gene would result in the wild phenotype. If this were so, one might expect *su(f)* and *ts67* to be dominant to the wild-type allele and the genotypes $f^{s}su(f)/f^{s}+^{su(f)}$ and $f^{s}ts67/f^{s}+^{su(f)}$ to produce flies with normal bristles. That there is such a heterozygous effect of *ts67* has now been demonstrated, albeit rather small.

In spite of all the above indirect evidence and arguments, the hypothesis would be strengthened immeasureably by some direct biochemical evidence that mutation at the *su(f)* locus alters ribosomes. In a recent preliminary communication FINNERTY *et al.* (1973) report that "acrylamide gel electrophoresis of 80S ribosomal proteins, isolated from *su(f)* homozygotes, are deficient for a ribosomal protein found in a number of wild type strains" and "polysomes isolated from *su(f)* larvae, examined in an *in vitro* protein synthesizing system . . . show a dramatically lowered ability to elongate their nascent polypeptide chains when compared to wild type polysomes". Confirmation of this report and the eventual biochemical demonstration of ribosomal misreading should establish the validity of the hypothesis that the *su(f)* locus codes for a ribosomal protein.

Finally, it is entirely possible that *ts67* may turn out to be an extremely powerful tool in the analysis of gene action in the development of Drosophila. For if *su(f)* does code for a ribosomal protein, and if *ts67* suppression and enhancement

of other mutant genes can be effected at 25° with ribosomes pre-formed at 18° with *ts67*-defective protein, then it should be possible by temperature shift experiments to determine that stage or those stages in development when translation occurs for any gene for which a *ts67*-suppressible allele is available. That a very closely delineated TSP for the suppression of forked bristles in $f^{s}ts67/f^{s}ts67$ individuals has been established subsequent to the TSP for lethality enhances the probability that *ts67* will meet the specifications necessary for such a powerful developmental genetic probe.

We would like to thank DR. ABRAHAM SCHALET, Laboratory of Radiation Genetics, University of Leyden, The Netherlands, for numerous informative and most helpful personal communications during the course of this work, for a number of stocks, and most importantly for making the initial observation that *ts67* suppresses the forked bristles produced by f^{s}.

LITERATURE CITED

APIRION, D., 1966 Altered ribosomes in a suppressor strain of *Escherichia coli*. J. Mol. Biol. **16**: 285–301.

BODENSTEIN, D., 1950 The postembryonic development of Drosophila. pp. 275–367. In: *Biology of Drosophila*. Edited by M. DEMEREC. John Wiley and Sons, New York.

BROTHERS, L. L., 1971 Maternal effects of some temperature-sensitive lethal mutations in Drosophila. Master of Science thesis, University of Virginia, Charlottesville, Virginia.

DAVIES, J., W. GILBERT and L. GORINI, 1964. Streptomycin, suppression and the code. Proc. Natl. Acad. Sci. U.S. **51**: 883–890.

DAVIES, J. and M. NOMURA, 1972 The genetics of bacterial ribosomes. Ann. Rev. Genetics **6**: 203–234.

DUDICK, M. E., 1973 A temperature-sensitive allele of a suppressor gene in Drosophila. Master of Arts thesis, University of Virginia, Charlottesville, Virginia.

DUDICK, M. and T. R. F. WRIGHT, 1973 A temperature-sensitive allele of suppressor of forked in Drosophila. Genetics **74**: s67.

FINNERTY, V. G., L. M. BARTON, A. SCHALET, W. A. ELMER and P. D. SMITH, 1973 The *suppressor of forked* mutation: a putative protein synthesis mutant in *Drosophila melanogaster*. Genetics **74**: s79–s80.

GORINI, L., G. A. JACOBY, and L. BRECKINRIDGE, 1966 Ribosomal Ambiguity. Cold Spring Harbor Symp. Quant. Biol. **31**: 657–664.

GREEN, M. M., 1956 A further analysis of the forked locus in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. **42**: 73–77. ——, 1959 Spatial and functional properties of pseudoalleles at the *white* locus in *Drosophila melanogaster*. Heredity **13**: 303–315.

HOWELLS, A. J., 1972 Levels of RNA and DNA in *Drosophila melanogaster* at different stages of development: a comparison between one bobbed and two phenotypically non-bobbed stocks. Biochem. Genet. **6**: 217–230.

KING, R. C., A. C. RUBINSON and R. F. SMITH, 1956 Oogenesis in adult *Drosophila melanogaster*. Growth **20**: 121–157.

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

LINDSLEY, D. L. and L. SANDLER, 1958 The meiotic behavior of grossly deleted X chromosomes in *Drosophila melanogaster*. Genetics **43**: 547–563.

PEEPLES, E. E., D. R. BARNETT and C. P. OLIVER, 1968 Phenol oxidases of a lozenge mutant of Drosophila. Science **159**: 548–552.

RITOSSA, F. M., K. C. ATWOOD and S. SPIEGELMAN, 1966 A molecular explanation of the *bobbed* mutants of *Drosophila melanogaster* as partial deficiencies of "ribosomal" DNA. Genetics **54:** 819–874.

SCHALET, A., 1968 Evidence for a different function associated with the locus of *suppressor of forked.* Drosophila Inform. Serv. **43:** 125. ——, 1970 Some observations on the proximal euchromatic-heterochromatic region of the X chromosome in *Drosophila melanogaster.* Genen en Phaenen **14:** 16–17. ——, 1972 Report of A. SCHALET. Drosophila Inform. Serv. **49:** 36–37. ——, 1973 The *suppressor of forked* locus: seven characteristics in search of a molecular author. Third European Drosophila Res. Conf. Abstracts.

SCHALET, A. and V. FINNERTY, 1968 Report of A. SCHALET and V. FINNERTY. Drosophila Inform. Serv. **43:** 65–66.

SCHALET, A. and G. LEFEVRE, JR., 1973 The localization of "ordinary" sex-linked genes in Section 20 of the polytene X chromosome of *Drosophila melanogaster.* Chromosoma **44:** 183–202.

SCHALET, A. and K. SINGER, 1971 A revised map of genes in the proximal region of the X chromosome of *Drosophila melanogaster.* Drosophila Inform. Serv. **43:** 131–132.

SNYDER, R. D. and P. D. SMITH, 1972 Phenol oxidase activity in various lozenge-suppressor of forked combinations of *Drosophila melanogaster.* Genetics **71:** s61.

SOKAL, R. R. and F. J. ROHLF, 1969. *Biometry.* W. H. Freeman and Co., San Francisco.

STEFFENSEN, D. M., 1973 Mapping genes for ribosomal proteins of Drosophila. Nature New Biol. **244:** 231–234.

WHITFIELD, H. G., JR., R. G. MARTIN and B. N. AMES, 1966 Classification of aminotransferase (C gene) mutants in the histidine operon. J. Mol. Biol. **21:** 335–355.

WRIGHT, T. R. F., 1968 The phenogenetics of temperature sensitive alleles of *lethal myospheroid* in Drosophila. Proc. of the XII Inter. Congr. Genetics **1:** 141.— —, 1973 The recovery, penetrance, and pleiotropy of X-linked, cold sensitive mutants in Drosophila. Molec. Gen. Genet. **122:** 101–118.

Corresponding editor: A. CHOVNICK