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A Novel Behavior, Bang-Sensitive Paralysis, Associated With The *shibire* Locus of *Drosophila melanogaster*

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The *Drosophila* temperature-sensitive mutant *shibire (shi)* is paralyzed at restrictive temperature by a reversible block in synaptic transmission. To explore the functional relationship among *shi* gene products, viability and temperature-sensitive paralytic behavior were quantitatively analyzed for four *shi* alleles, shi^{ts1}, shi^{ts2}, shi^{ts4}, and shi^{ST139}, and their heteroallelic combinations. The hemizygous combination of *shi* alleles over deficiency was not completely lethal. shi^{ts2} exhibited distinctively higher viability than other alleles. A novel behavior, bang sensitivity, was also found in *shi/Df(1)*. This bang-sensitive paralytic behavior was compared with that of the typical bang-sensitive mutant flies. Heterozygotes, *shi/+*, are more severe in temperature sensitivity than deficiency hemizygotes, *Df(1)/+*. Heteroallelic combinations of *shi* were less sensitive to high temperature than homozygotes. Among all allelic combinations, shi^{ts2}/shi^{ts4} showed an unexpected extreme reduction in temperature sensitivity. The results of allelic interactions among 4 *shi* alleles suggest that the *shi* mutations examined behave as antimorphic alleles and that the gene product of *shi* are likely to function in multimeric forms.

INTRODUCTION

The behavior of an animal is the result of the combined action of sensory input, integration and motor output. These functions are mediated by the basic units of the nervous system. Nervous system components are constructed under the control of genetic information. In *Drosophila,* mutational analysis has been employed to identify the genes whose products are crucial to the function of the nervous system (Suzuki, 1970; Benzer, 1973). Among the various kinds of behavioral mutants of *Drosophila* (Hall, 1982), the temperature-sensitive paralytic mutations are of particular interest (Suzuki, 1974, 1976). Thermolabile gene products of these mutants lose function within a specific range of temperatures thus, circumventing the difficulty of their otherwise lethal effects.

The mutant, *shibire (shi),* gradually causes paralysis at temperatures above

29°C. This effect can be reversed by returning the flies to the permissive temperature (Grigliatti *et al.*, 1973; Poodry, Hall and Suzuki, 1973). Paralysis is due to defective synaptic transmission at the neuromuscular junction. Synaptic vesicle depletion has been proposed as the cause of this paralysis (Ikeda, Ozawa and Hagiwara, 1976; Koenig, Saito and Ikeda, 1983). In addition, *shi* produces defects in the endocytic mechanism of membrane recycling in various types of tissues such as nerve cells (Poodry and Edgar, 1979; Koenig *et al.*, 1983; Masur, Kim and Wu, 1990), nephrocytes (Kosaka and Ikeda, 1983), muscles (Hummon and Costello, 1988), and oocytes (Kessell, Holst and Roth, 1989). Furthermore, retarded neuronal development and growth cone formation at restrictive temperatures, possibly due to loss of adhesion to the substratum resulting from blockage of membrane recycling, have been reported for *shi* neurons in culture (Kim and Wu, 1987). Currently there are 22 *shi* alleles available (Lindsley and Zimm, 1990). Although general descriptions of behaviors of some alleles have been reported (Grigliatti *et al.*, 1973; Siddiqi and Benzer, 1976), there has been no extensive behavioral study comparing the kinetics of paralysis and recovery among the different alleles and heterozygotes for different alleles until very recently (Kim and Wu, 1990).

Another group of behavioral mutations are "bang-sensitive" paralytic mutants which become paralyzed for several minutes following a sudden jolt or mechanical vibration of the culture vials (Judd *et al.*, 1972; Grigliatti *et al.*, 1973; Jan and Jan, 1978; Hall, 1982; Ganetzky and Wu, 1982). The five X-linked mutations which confer this behavior are *bas (bang sensitive,* Grigliatti *et al.*, 1973), *bss (bang senseless,* Jan and Jan, 1978; Ganetzky and Wu, 1982), *eas (easily shocked,* Ganetzky and Wu, 1982), *kdn (knock-down),* and *tko (technical knockout,* Judd *et al.*, 1972). Interestingly, all of these mutants exhibit paralysis at either high or low temperatures (Burg, 1987). Although these bang-sensitive flies have similar behavioral phenotypes, it is not known whether all of these mutations have a similar cellular defect. Complementation tests along with recombinational and cytological mapping have revealed that the bang-sensitive mutants comprise five distinct loci (Ganetzky and Wu, 1982).

A detailed behavioral analysis of the *shi* mutation by using heteroallelic combinations is attempted in the current study. The results indicate that different *shi* alleles display a considerable range in the degree of severity. Heterozygotes of different *shi* alleles over a deficiency, previously believed to be lethal, were successfully constructed. The viability of different *shi* alleles over this deficiency exhibited a different order of severity from that of paralysis. Surprisingly, these *shi* hemizygotes also displayed a novel behavior, bang-sensitivity, which has not previously been detected in any homozygous alleles nor in any combination of two different alleles. The bang-sensitive paralytic behaviors of *shi* hemizygotes were compared with those of the typical bang-sensitive mutants flies. Furthermore, heterozygous combinations of alleles provide an opportunity to analyze interactions between

different alleles. The results of allelic interactions among 4 *shi* alleles suggest that the *shi* mutations examined behave as antimorphic alleles and that the gene product of *shi* is likely to function as a multimer.

MATERIALS AND METHODS

Fly Stocks

All fly stocks were raised at room temperature (21 ± 1°C) on a standard *Drosophila* corn meal medium. The strain *Canton-Special (CS)* was used as a wild type. Chromosome markers are described in Lindsley and Grell (1968). The mutations used are listed below.

- shi^{ts} : *shibire*^{ts}. Ethyl methane-sulfonate(EMS)-induced temperature-sensitive paralytic mutant located at 1-52.2 (Grigliatti *et al.*, 1973; Siddiqi and Benzer, 1976). The alleles used were shi^{ts1}, shi^{ts2}, shi^{ts4}, and shi^{ST139}.
- *bas* : *bang sensitive.* EMS-induced bang-sensitive mutant mapped at 1-49.5 (Grigliatti *et al.*, 1973; Ganetzky and Wu, 1982).
- *bss* : *bang senseless.* EMS-induced bang-sensitive mutant located at 1-54.0 (Jan and Jan, 1978; Ganetzky and Wu, 1982).
- eas^2 : *easily shocked.* EMS-induced bang-sensitive mutant allele mapped at 1-53.2 (Benzer, 1971; Ganetzky and Wu, 1982).
- tko^{25t} : *Technical knockout.* Isolated and mapped at 1-1.0 by Judd *et al.* (1972).
- *Df(1)* : *Df(1)* sd^{72b26}. X-chromosome deficiency which is deficient for *sd (scalloped,* 1-51.5) but not for *bas* (Ganetzky and Wu, 1982; Lindsley and Zimm, 1987).
- FM7 : The X-chromosome balancer which carries the markers, *y (yellow body,* 1-0.0), *w (white eye,* 1-1.5), sn^{x2} *(singed bristle,* 1-21.0), and *B (bar eye,* 1-57.0).

The hemizygote with deficiency chromosome was generated by mating *FM7/Df(1)* with shi^{ts}/Y.

Mechanically Induced Paralysis

Since older bang-sensitive flies are paralyzed for longer time than younger flies (Ganetzky and Wu, 1982), flies between 4 to 10 days old were tested to determine the duration of paralysis induced by mechanical vibrations. Single flies were placed into empty glass vials (9.3 cm in length, 2.4 cm in diameter) and kept undisturbed at least for 30 minutes prior to tests. The flies were vibrated for 10 seconds using a Vortex-Genie mixer (Scientific Products, No. S8223) set at the highest speed. To record detailed behavioral responses of each fly, single flies in the vial were viewed using a dissecting microscope. Flies were considered to be paralyzed when they were no longer standing. For each fly the duration of paralysis was determined by using a stopwatch. The duration of paralysis was defined as the interval of time between the end of the mechanical shock and the moment of recovery. Recovery was defined as regaining the ability to stand and walk.

Temperature-Induced Paralysis

All temperature testing was performed using a temperature-controlled water bath (modified from a 12-liter aquarium). Temperature sensitivity tests at high temperatures were performed using an immersion thermoregulator (Thermomix B; B. Braun Melsungen

AG, W. Germany). The temperature of the bath was monitored and calibrated using at least two different thermometers including one installed digital thermometer, allowing a temperature reading within ± 0.1°C. Ten flies of a specific genotype and sex at the age of 2 to 7 days after eclosion were placed in an empty vial positioned in the water bath. Flies were observed using a magnifying glass equipped with an illuminating lamp. The number of flies paralyzed at each temperature and the time required for paralysis were recorded. Flies were allowed to recover for a day prior to testing at the next temperature. A total of 20 to 60 flies for each genotype were tested at each temperature. The temperature range tested was 24°C to 41°C.

Kinetics of Temperature-Induced Paralysis

Individual flies were introduced into pre-warmed vials using a mouth aspirator. To prevent the possibilities of rapid mechanically-induced paralysis, care was taken not to mechanically disturb the flies excessively. Twenty to 60 flies for each genotype were tested at 27°C. The time required for the fly to become paralyzed was recorded.

RESULTS

Construction of shi Deficiency Hemizygote

The results of hemizygote mutant construction between the 4 *shi* alleles and the deficiency chromosome are shown in Table 1. Since *Df(1)sd*^{72b26} is known to uncover the *shi* locus, it was previously believed to be impossible

Table 1. Viability of Different *shi* Alleles in Deficiency Hemizygotes

Alleles	F1 Progeny from the Cross of $\frac{shi}{Y}$ X $\frac{Df(1)}{FM7}$			
	$\frac{shi}{Df(1)}$	$\frac{shi}{FM7}$	$\frac{FM7}{Y}$	non-disjunction
shi^{ts1}	8 (0.6%)	755 (59.3%)	495 (38.9%)	15 (1.2%)
shi^{ts2}	382* (15.3%)	1328 (53.1%)	696 (27.8%)	96 (3.8%)
shi^{ts4}	0* (0.0%)	451 (65.3%)	230 (33.3%)	10 (1.4%)
shi^{ST139}	3 (0.3%)	540 (57.7%)	376 (40.2%)	17 (1.8%)

Df(1) : Df(1) sd^{72b26}

FM7 : FM7; y w sn^{X2B}

Numbers given represent the number of flies from at least 12 crosses.

* Note clear difference from other alleles in the viabliity of shi/Df(1).

to generate a $shi^{ts}/Df(1)$ hemizygotes. Of the 4 possible constructions, only $shi^{ts2}/Df(1)$ was recovered at substantial rate (15.3%, Table 1) allowing detailed behavioral analysis. Deficiency heterozygotes of shi^{ts1} and shi^{ST139} were rare but were seen (0.6% and 0.3%, respectively); however no heterozygotes with shi^{ts4} were found. All the hemizygous flies were very weak, thus they were kept at 17°C until the behavioral tests were performed. The other difference between the alleles is the higher rate of non-disjunction for shi^{ts2} allele (compare 3.8% for shi^{ts2} to 1.2-1.8% for other alleles). *Df(1)/FM7* female parents occationally produce exceptional eggs containing both X chromosomes and nullo-X eggs (primary non-disjunction). Fertilization of eggs bearing 2X chromosomes by the male X and Y gametes leads to XXX females which usually do not emerge from the pupal case and XXY females which are fertile and have bar eyes because of marker (*B*) on *FM7*. When nullo-X eggs are ferilized by male X (*shi*), *shi/O* males which are fully viable and temperature sensitive but sterile are produced. The XXY females can further produce non-disjunctional gametes containing two X in some eggs and a single Y in others (secondary non-disjunction). When Y eggs are fertilized by male X (*shi*), *shi/Y* male offspring which are viable, temperature sensitive, and fertile are produced. Since males in this case represent the genotypes of *shi/O* and *shi/Y*, this also indicates higher viability of shi^{ts2} than other alleles. According to the viability of *shi* alleles in hemizygous combination with a deficiency, the 4 alleles can be placed in a descending order of severity: $shi^{ts4} \gg shi^{ST139} > shi^{ts1} \gg shi^{ts2}$.

Bang Sensitivity in shi/Df(1) Hemizygotes

Mechanical Vibration-Induced Paralysis: In addition to their typical temperature-sensitive paralytic behavior, $shi^{ts}/Df(1)$ hemizygote flies were found to exhibit a novel behavior, bang-sensitive paralysis. After a mechanical vibration, $shi^{ts}/Df(1)$ flies become paralyzed and are unable to move with coordination. The number of flies used in this behavioral study was 60 $shi^{ts2}/Df(1)$, 1 $shi^{ts1}/Df(1)$ and 1 $shi^{ST139}/Df(1)$. Out of 60 $shi^{ts2}/Df(1)$ flies tested, 8 flies showed either brief recovery within 10 seconds or no paralysis at all. Remaining $shi^{ts2}/Df(1)$ flies showed various lengths of paralysis ranging from 12 seconds to 12 min 46 seconds. The length of paralysis induced by mechanical vibration in $shi^{ts2}/Df(1)$ flies and other typical bang-sensitive mutants are compared in Table 2. The lengths of paralysis in $shi^{ts2}/Df(1)$ vary widely (109 ± 166 seconds; mean ± S.D., n=20). In average lengths of paralysis, $shi^{ts2}/Df(1)$ is similar to *bas* and eas^2, which exhibited 111.9 and 122.3 seconds of paralysis respectively.

Behavioral Repertoires: When paralyzed by a mechanical vibration, *shi/Df(1)* flies usually exhibited a stereotyped sequence of behaviors before they reached complete recovery as diagrammed in Table 3. Table 3 also compares the behavioral repertoires of typical bang-sensitive mutant flies. Even though there was an occasional repeat or omission of certain behaviors, the overall

Table 2. Duration of Paralysis induced by a Mechanical Vibration

Genotype	N	Duration of Paralysis [mean ± S.D. (sec)]
$\frac{shi^{ts1}}{Df(1)}$	1	26
$\frac{shi^{ts2}}{Df(1)}$	20	109.0± 166.0
$\frac{shiST139}{Df(1)}$	1	295
bas	20	111.9± 71.1
bss	21	145.0± 78.0
eas^2	20	122.3± 102.7
tko^{25t}	20	26.0± 20.7

Df(1) : Df(1) sd^{72b26}

sequence of specific behaviors was always maintained. These behaviors were: lying motionless, brief leg pulsation, asynchronous leg movement, leg cleaning, wing buzz, wing stretch, and jump. Although the leg shaking movement was periodic, alternating with periods of motionlessness, most mutant flies demonstrated a characteristic sequence of behaviors prior to full recovery (Table 3). Some $shi^{ts2}/Df(1)$ flies exhibited 14 cycles of alternating periodic movements.

Temperature-Sensitive Paralysis in shi/Df(1) and shi Heterozygous Flies

By using the 4 *shi* mutant alleles 6 heteroallelic combinations were constructed [e.g., from the mating of shi^{ts1}/shi^{ts1} X shi^{ts2}/Y, all the female progeny carried a heterozygous combination of the two alleles (shi^{ts1}/shi^{ts2})]. In addition, heterozygotes of *shi* and + alleles were also made from the cross of either shi^{ts}/Y X +/+ or shi^{ts}/shi^{ts} X +/Y.

Table 4 summarizes the temperature- and bang-sensitive behavior of *shi* alleles in homozygous and heteroallelic combinations. $shi^{ts2}/Df(1)$ flies were distinctive in the temperature-sensitivity when compared to other *shi* homozygotes and heterozygotes. They became paralyzed at 24°C, a temperature at which no other *shi* flies were found to paralyze. If exposed long enough, they became very weak and sluggish even at room temperature (22°C).

Table 3. Behavioral Repertoires of *shi/Df(1)* and Bang-Sensitive Mutants induced by a Mechanical Vibration

Genotype	Sequence of Behaviors	
	Vibration	Recovery
$\frac{shi^{ts1}}{Df(1)}$	ALM	
$\frac{shi^{ts2}}{Df(1)}$	BLP--> ALM-->ML--> wing buzz --> ALM --> ML --> (multiple cycles of ALM --> ML) --> wing stretch --> leg cleaning --> sudden jump	
$\frac{shiST139}{Df(1)}$	ALM --> ML --> (5 cycles of ALM --> ML) -- > ALM	
bas	ML -- > leg cleaning --> wing buzz --> ALM --> ML --> tibial shaking	
bss	ML --> leg retract --> wing buzz --> wing stretch --> ALM --> tibial shaking	
eas^2	ML --> wing stretch & leg retract --> jump --> wing buzz --> ALM --> proboscis extend	
tko^{25t}	ALM--> wing buzz --> ALM --> ML --> tibial shaking	

Df(1) : Df(1) sd^{72b26}
ALM : asynchronous leg movement
BLP : brief leg pulsation
ML : motionless

While wild-type flies and 4 heterozygotes (shi^{ts1} / +, shi^{ts2} / +, shi^{ts4} / +, and shi^{ST139} / +) were not paralyzed by exposure to 29°C, all homozygous and heteroallelic *shi* flies were paralyzed with different degree of sensitivity (Kim and Wu, 1990). The flies exhibit rapid movement of legs, wing flicking, flipping and falling on back prior to total paralysis. To examine whether the behavioral differences among different combinations of *shi* alleles found at 29°C are more pronounced when the temperature is lowered, the kinetics of paralysis at 27°C was examined. Table 4 shows the time required for half of the flies in a sample to become paralyzed at 27°C. All flies of the genotype $shi^{ts2}/Df(1)$ became paralyzed within 74 seconds at 27°C. Only one fly each for $shi^{ts1}/Df(1)$ and $shi^{ST139}/Df(1)$ was tested at 27°C due to their extreme

Table 4. Behavior of *shi* Alleles in Homozygous, Hemizygous and Heteroallelic com Combinations

	shi^{ts1}	shi^{ts2}	shi^{ts4}	shi^{ST139}	*Df(1)*	+
shi^{ts1}	No B.S. 26°C 67				B. S. lower limit $t_{½}$(27, P)	
shi^{ts2}	No B.S. 26°C 105	No B.S. 26°C 118				
shi^{ts4}	No B.S. 26°C 100	No B.S. 26°C 240*	No B.S. 26°C 130			
shi^{ST139}	No B.S. 26°C 70	No B.S. 26°C 65	No B.S. 26°C 85	No B.S. 26°C 60		
Df(1)	B. S.* ≪25°C (67)	B. S.* 24°C* 51	lethal (?)	B. S.* ≪25°C (33)	lethal	
+	Bo B.S. 36°C N/P	No B.S. 38°C N/P	No B.S. 38°C N/P	No B.S. 36°C N/P	No B.S. 40°C N/P	No B.S. 41°C N/P

As indicated in the inset, given in each cell of the matrix are:

B. S. : Bang Sensitivity

lower limit : lowest temperature at which flies demonstrated paralysis.

$t_{½}$ (27,P): Time in seconds at which 50% of flies were paralyzed at 27°C.

Asterisks indicate the distinctive behaviors described in the text.

rarity. $shi^{ts1}/Df(1)$ was paralyzed at 67 seconds and $shi^{ST139}/Df(1)$ was paralyzed at 33 seconds.

Among six heteroallelic combinations, shi^{ts2}/shi^{ts4} displayed distinctive behavior uopn shift to 27°C. shi^{ts2}/shi^{ts4} flies became paralyzed after 1 minute at 27°C, but 9 out of 50 flies were not paralyzed even after 10 minutes. The time for paralysis of 50% of shi^{ts2}/shi^{ts4} population was 4 minutes. No other experimental flies were able to stand for 4 minutes at 27°C. The rate of paralysis in shi^{ts2}/shi^{ts4} was again confirmed to be much slower than that of the two mutant homozygotes, shi^{ts2}/shi^{ts2} and $shi^{ts4}/$

shi^{ts4}.

The 4 heterozygotes for *shi* and + remained active at temperatures at which all homozygotes were paralyzed. Although a previous study (Suzuki, 1974) reported that *shi* heterozygotes were paralyzed at 40°C, there have been no systematic studies on temperature-induced paralysis of different alleles of *shi* in heterozygous combinations. In the current study, four heterozygotes for *shi* and + were tested for temperature-induced paralysis at 35°C to 38°C. Table 4 shows the lowest temperature at which heterozygous flies were paralyzed. All 4 heterozygotes failed to pass out at 35°C during 15 minutes of observation. However, at a temperature of 36°C, differences between the 4 alleles became conspicuous. For shi^{ts1}/+ and she^{ST139}/+, it took less than 10 minutes to paralyze 50% of population, and less than 13 minutes for 100% at 36°C. In contrast, shi^{ts2}/+ and shi^{ts4}/+ flies remained active up to 37°C during 15 minutes of observation. Although some passed out at 38°C, others remained active for 15 minutes. At the temperatures tested above, wild-type flies were never paralyzed. Surprisingly, the lowest temperature at which +/*Df(1)* flies were observed to paralyze was 40°C, while that of x/x was 41°C. According to the results shown in Table 4, the four *shi* alleles can be placed in a descending order of temperature-sensitivity:

$$shi^{ts1}/+ \geq shi^{ST139}/+ > shi^{ts2}/+ \geq shi^{ts4}/+.$$

DISCUSSION

Bang Sensitivity of shi/Df(1)

Heterozygotes between *shi* allele and a deficient chromosome, $Df(1)sd^{72b26}$, can be obtained only in an allele-dependent manner. Only $shi^{ts2}/Df(1)$ flies emerged at substantial rate (15.3%, compare with 0-0.6% for 3 other alleles; Table 1). When viability of *shi/Df(1)* combinations are compared, the order of allele severity ($shi^{ts4} \gg shi^{ST139} \geq shi^{ts1} \gg shi^{ts2}$) is different from that given by homozygous paralytic behavior ($shi^{ST139} \cong shi^{ts1} > shi^{ts4} \cong shi^{ts2}$).

Hemizygous $shi^{ts2}/Df(1)$ flies exhibited a novel "bang-sensitive" paralytic behavior. Despite the similarity of paralysis between the bang-sensitive and temperature-sensitive behaviors, close observations on the behavioral repertoires of the two paralyses revealed that they were distinct. Duration and sequence of behavioral repertoires observed in *shi/Df(1)* were very similar to those of *bas* and eas^2 (Tables 2 and 3). The physical locations of three bang-sensitive loci are distinct in the cytogenetic map (Fig. 1). The *bas* locus is known to be not uncovered by $Df(1)sd^{72b26}$ which conversely uncovers *shi* locus (Ganetzky and Wu, 1982). The same is true for eas^2 locus (Ganetzky and Wu, 1982). Another bang-sensitive mutant allele *bss* is also found to be not uncovered by $Df(1)sd^{72b26}$ (Ganetzky and Wu, 1982). Are *shi/Df(1)* flies bang-sensitive because of some property inherent in the *shi* mutation or

because of defects originating from *Df(1)*? A partial answer can be drawn from the result of *+/Df(1)* flies. Since *+/Df(1)* flies were not bang-sensitive (Table 4), bang sensitivity of *shi/Df(1)* might result from some inherent property of *shi* mutation. However, I cannot exclude the possibility that bang sensitivity might arise from some other locus than *shi* since the deletion segment of *Df(1)sd*^{72b26} is relatively long (Fig. 1; Lindsley and Zimm, 1987). In addition, hemizygous *shi/Df(1)* flies exhibited extreme temperature-sensitivity. They were paralyzed at 24°C at which no other *shi* allelic combinations were found to become paralyzed.

Possible Functional Interaction Among shi Gene Products

Gene-dosage effects of the *shi* alleles on behavioral phenotypes may yield useful information about the product of this gene. So far, the gene product of *shi* has not yet been identified. However, we can infer properties of the *shi* gene product from the above findings.

Although no simple explanations appear to completely account for all of the above results, different lines of evidence from this study suggest that *shi* allele encodes a polypeptide which functions in a multimeric form. Alleles of the *shi* mutation are obviously not "amorphic" alleles (*e.g.* nonsense mutations). This is supported by the observation that *shi/Df(1)* are not the same as *shi/shi*, and *shi/+* are not the same as *Df(1)/+*. Instead, these *shi* alleles are more likely to produce altered gene products which may work

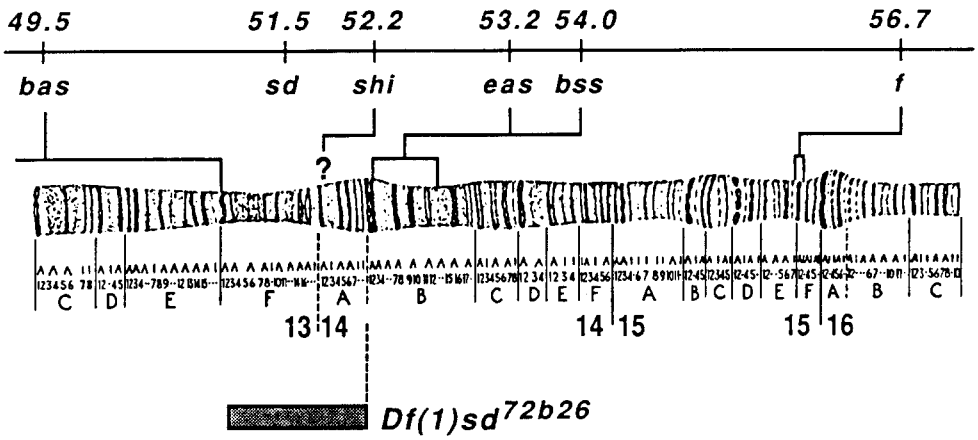

Fig. 1. **Recombination and cytological map of mutant loci in the proximal region of the X-chromosome that cause paralysis. Top line: recombination map. Below: diagram of salivary chromosome banding pattern. The break point of deletion used to determine the cytological positions of the mutants are shown. Probable cytological position of *shi* is indicated as ?. *bas*: bang sensitive, *bss*: bang senseless, *eas*: easily shocked, *sd*: scalloped wing, *shi*: shibire, *f*: forked bristle.**

as a "poison product" or an "inhibitory factor".

Table 4 shows that the *shi* mutation has a semi-dominant effect on paralytic behavior. A semi-dominant effect is indicated when the phenotype of the heterozygote is intermediate to the phenotype of the homozygous mutant and the wild type. Some mutant alleles can partially complement each other to some extent. That is, mutant heteroallelic flies are not as bad as their respective homozygotes (as is the case of shi^{ts2}/shi^{ts4} compared to either shi^{ts2}/shi^{ts2} or shi^{ts4}/shi^{ts4}). It is as if two doses of the same "poison" (altered gene product) are worse than two doses of different "poison", each of which can make up for the deficits of the other to some extent.

Another example which supports the current hypothesis is that *shi*/+ are more severe in temperature sensitivity than *Df(1)*/+. *shi*/+ which yields 50% of normal gene product and 50% of "poison" passed out at 38°C while *Df(1)*/+ which produces only 50% of normal gene product passed out at 40°C. "Poison" in *shi*/+ might interfere with the activity of the normal gene product, resulting in less than 50% of normal activity. The 4 *shi* alleles may produce proteins of different degrees of alteration and/or different levels of altered gene product. The varying severity found in 4 *shi/Df(1)* hemizygotes suggests that the effect of reducing gene products of some alleles is worse than others. $shi^{ts4}/Df(1)$ is most likely lethal (Table 1), but shi^{ts4}/shi^{ts4} is the least severe in temperature sensitivity among the four *shi* mutant homozygotes (Table 4). An interesting possibility is that the shi^{ts4} product may function quite well in the multimeric assembly but has a poor affinity for aggregation among themselves. 50% of shi^{ts4} thus fail to produce a threshold level of functional aggregates. It has the smallest poisoning effect among the heterozygous combinations. Further studies of the *shi* gene product by using biochemical and molecular techniques will be necessary to further elucidate the molecular mechanisms of the *shi* protein and its role in the membrane recycling process.

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노랑 초파리의 *shibire* 돌연변이 유전자에서 발견된 충격 감응 마비성

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노랑 초파리의 온도 감응 마비성 돌연변이종의 하나인 *shibire*(*shi*)는 제한온도인 29°C이상에서 마비현상을 일으켜 날지 못한다. 이 유전자는 제한조건하에서 신경전도저해, 신경전도저해, 내포작용저해, 근섬유연합 등 여러종류의 세포에서 결함을 나타낸다.

본 연구에서는 *shi*의 gene product의 특성을 조사하기 위하여 4개의 대립형질－shi^{ts1}, shi^{ts2}, shi^{ts4}, shi^{ST139}－간의 상호교배를 통해 생존율과 온도감응 마비성을 정량적으로 분석하였다. *shi*유전자와 결손유전자의 조합(*shi*/*Df*)은 부분치사현상을 나타냈으며, 대립형질간의 생존율도 차이를 보여주었다. *shi*/*Df*개체에서는 새로운 표현형인 충격감응 마비성(bang-sensitive paralysis)이 발견되었다. 이 돌연변이 행동의 양상을 이미 알려져 있는 충격 감응계열의 돌연변이종(bang-sensitive mutants)들의 행동과 비교하여 고찰하였다. 본 실험에서는 여러 종류의 표현형에 따른 대립 형질간의 감응도 차이를 밝혀냈으며, 연구결과는 *shi*돌연변이 유전자가 antimorph로 작용하며 그 gene product는 multimer의 형태로 기능함을 시사한다.