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The *Passover* Locus in *Drosophila melanogaster*: Complex Complementation and Different Effects on the Giant Fiber Neural Pathway

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ABSTRACT

Drosophila melanogaster bearing the *Passover* mutation fail to jump in response to a light-off stimulus. *Pas* also disrupts some of the synapses between the neurons of the giant fiber system which mediate this escape behavior. We have mapped *Pas* to the 19E subdivision of the polytene *X* chromosome. Our genetic analyses reveal that deletions of either of two nonoverlapping regions fail to fully complement *Pas*. Heterozygotes of *Pas* with chromosomal deletions in the vicinity of polytene band 19E3 exhibit the full set of neuronal defects shown by *Pas* homozygotes. Alleles of the *R-9-29* complementation group, which maps to band 19E3, exhibit a complex pattern of complementation with *Pas*. Heterozygotes combining the lethal *R-9-29* alleles with *Pas* are all viable, some complement the neuronal defects of *Pas*, but most exhibit these defects. The viable *shaking-B*^{2} mutation also fails to complement *Pas*, the *R-9-29* alleles or the 19E3 deficiencies. The *R-9-29* locus may contain two functional domains, one required for viability the other for normal neuronal phenotype. *trans*-Heterozygotes bearing mutant alleles or a deficiency of the first region (19E3) together with deficiencies of the second region (19E5–6) also exhibit some of the neuronal defects shown by the *Passover* mutant. Deficiencies which delete the entire 19E3 to 19E6 interval do not produce this phenotype when heterozygous with a normal *X* chromosome. Thus normal function requires a *cis*-interaction between the two regions. These findings raise the possibility that the gene mutated by *Pas* is split or separated from a *cis*-activator by at least one other gene.

WHEN startled by a light-off stimulus, wild-type flies jump into the air and fly away. This escape response is mediated by the giant fiber system (GFS), a small group of neurons that channel visual input from the head to some of the muscles of the thorax (KING and WYMAN 1980; TANOUYE and WYMAN 1980; KING and TANOUYE 1983; TANOUYE and KING 1983). A schematic representation of the giant fiber (GF) is shown in Figure 1. The GFs are the axons of a bilaterally symmetrical pair of interneurons with their cell bodies in the posterior, lower protocerebrum of the brain (KOTO *et al.* 1981). Each GF descends from the brain and enters the mesothoracic neuromere of the thoracic ganglion, where it synapses with two identified neurons. One of these cells, named the peripherally synapsing interneuron (PSI), has an axon which exits the ganglion and connects, in the peripheral nerve, with the motor neurons of the dorsal longitudinal muscles (DLMs). The dorsal longitudinal muscles are the wing depressor muscles that power flight along with the wing elevating dorsal ventral muscles. The GF also synapses directly with the motor neuron of the tergotrochanteral muscle (TTM) which extends the mesothoracic leg during jumping.

A fixed pattern of spikes in the motor neurons and muscles (Figure 2A) is seen during a light-off induced escape response (THOMAS and WYMAN 1984; WYMAN *et al.* 1984, 1985). Intracellular or extracellular electrical stimulation of the GF in the brain of wild-type flies activates the circuit described above and produces the same sequence. This set of spikes is termed the "GF response." In wild-type flies the circuit functions with extreme rapidity and reliability.

Mutations which disrupt synaptic connections in the GFS have been isolated in *D. melanogaster* by screening for flies lacking the escape response (THOMAS and WYMAN 1982, 1984). These mutants fail to jump in response to a light-off stimulus and correspondingly the TTM and DLM respond abnormally to GF stimulation. The *Passover* mutation disrupts the pathway to the dorsal longitudinal muscles, as well as the pathway to the tergotrochanteral muscles (THOMAS and WYMAN 1984). The *Passover* mutation was originally denoted *non-jumping* (*nj*) *156* (THOMAS 1980) and then *passover* (THOMAS and WYMAN 1984). We capitalize the name due to the dominant behavioral effects described in this paper. In homozygous *Pas* flies brain stimulation elicits no response from the DLMs and produces only a delayed and intermittent TTM response. The defect does not lie in the motor axons, neuromuscular junctions or muscles since the re-

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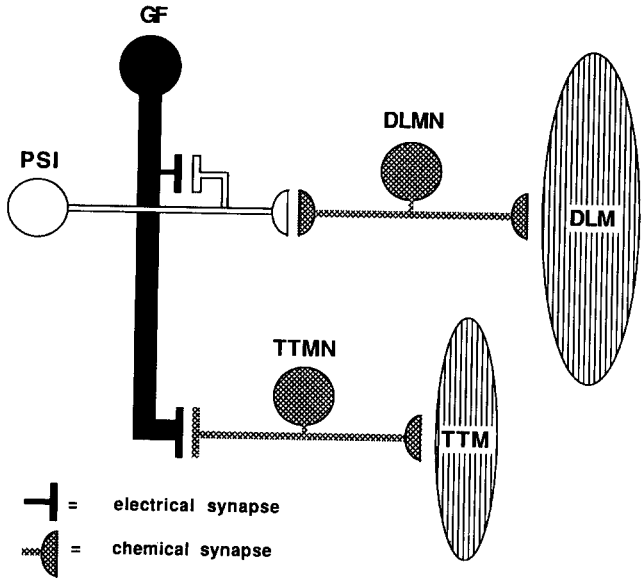


FIGURE 1.—Schematic representation of the neurons and muscles of the GFS. GF = giant fiber, PSI = peripherally synapsing interneuron, DLMN = dorsal longitudinal motor neuron, TTMN = tergotrochanteral motor neuron, DLM = dorsal longitudinal muscle (an indirect flight muscle), TTM = tergotrochanteral muscle (or "jump" muscle).

sponses of the muscles to electrical stimulation of the dorsal longitudinal motor neurons (DLMNs) and the tergotrochanteral motor neuron (TTMN) are normal. These results indicate that there are abnormalities in the synapses between the GF and the neurons it activates (THOMAS and WYMAN 1984). The anatomical nature of the defects are currently under investigation (EGGER *et al.* 1989; SWAIN, WYMAN and EGGER 1990).

THOMAS and WYMAN (1984) originally mapped *Pas* proximal to *forked* by recombination and then established that the *Pas* mutation is located at the base of the *X* chromosome in the region deleted by deficiency *16-3-22*. This deficiency removes approximately 18 polytene bands extending from the *maroonlike* locus in subdivision 19D through the *extra organs* locus in subdivision 20A (SCHALET and LEFEVRE 1976). The proximal *X* chromosome has been the focus of intensive cytogenetic analysis (LIFSCHYTZ and FALK 1968, 1969; SCHALET and LEFEVRE 1973, 1976; LIFSCHYTZ and YAKOBOVITZ 1978; KRAMERS *et al.* 1983; PARADI, VOGEL and SZILAGYI 1983; EEKEN *et al.* 1985; ZUSMAN, COULTER and GERGEN 1985; LEFEVRE and WATKINS 1986; SCHALET 1986; GREEN, YAMAMOTO and MIKLOS 1987; MIKLOS *et al.* 1987, 1988; YAMAMOTO and MIKLOS 1987). These studies provide an extensive base of genetic data with which to define and analyze the *Passover* locus.

That *Passover* lies in a region with abundant genetic variants and affects an easily assayed circuit of identified neurons affords unique experimental advantages in linking genetic and neuronal defects. The combination of high genetic and neuronal resolution have allowed us to demonstrate complex complementation and selective disruption of different parts of the GFS by different genetic lesions.

MATERIALS AND METHODS

Stocks: *Drosophila melanogaster* stocks were maintained at 22–26° on standard food medium consisting of 82.5% water, 6.5% corn meal, 0.74% agar, 1.6% yeast, 8.7% molasses. Either 0.56% propionic acid, or 0.87% Tegosept was added to prevent the growth of mold. Control and experimental genotypes were constructed using crossing procedures similar to those of MIKLOS *et al.* (1987). Some of the deficiency-bearing chromosomes as well as the lethal and visible alleles of the genetic complementation groups in subdivision 19E have been previously described (LIFSCHYTZ and FALK 1968, 1969; SCHALET and LEFEVRE 1973, 1976; MIKLOS *et al.* 1987). Many others are from the unpublished collections of GEORGE LEFEVRE and ABRAHAM SCHALET and have been extensively tested both *intra* and *inter se* in genetic complementation tests. Balancer *X* chromosomes, such as *FM6* (LINDSLEY and ZIMM 1990), as well as *Y* chromosomes bearing translocated portions of the base of the *X* ($y^{+}Ymal^{106}$) have been described earlier (SCHALET and LEFEVRE 1976).

Behavioral test of the escape response: THOMAS and WYMAN (1984) found that flies with phenotypically white eyes respond more often than flies with wild-type eye color; of the combinations of mutations that cause white eyes, the *brown; scarlet* double homozygote responded most reliably. Therefore, all testing was performed on flies homozygous for the autosomal mutations *brown* and *scarlet*, along with the *X* chromosome mutation(s) under study. A modified version of the testing methods of THOMAS and WYMAN (1984) were used to assess the escape response to a light-off stimulus. Flies were placed individually under transparent plastic Petri dishes in a box made of white poster board. The top of the box was removed to admit light, and one side of the box was removed to view the fly's response. A sudden change in the fly's position within the dish after a brief light-off stimulus was taken to indicate a response. If the fly was walking or jumping immediately before the stimulus, the response to that stimulus could not be reliably scored, and was therefore not counted. The number of responses to 20 such stimuli was recorded to measure the response probability of a single fly. To test the effect of a given genotype, several individuals were examined, usually interspersed with control flies of genotype *brown; scarlet* which responded about 90% of the time.

Electrical stimulation of the neurons of the GFS and monitoring of muscle potentials: Most of the results in this paper are descriptions of the muscular response to stimulation that activates the GF. Electrical stimulation of the GFS produces the same escape response elicited by the light-off stimulus described above (THOMAS and WYMAN 1984). The motor outputs of the GFS were recorded using a modified version of the methods of TANOUYE and WYMAN (1980) and THOMAS and WYMAN (1984). The flies were lightly etherized and held in place by suction applied through fine polyethylene tubing and were positioned so that the dorsal insertions of the DLMs and TTMs were accessible for electrode penetration. Sharpened tungsten wire electrodes were used for stimulating and recording. The recording circuits were completed with an electrode inserted between the fourth and fifth abdominal tergites and connected to ground. The GF neurons were stimulated in the brain using a pair of electrodes inserted through the cuticle of the eye margin near the supraorbital bristles. Voltage pulses of 0.1 msec duration were then applied to the stimulating electrodes using a Grass S44 stimulator. The recording electrodes were inserted into left and right DLMs and TTMs.

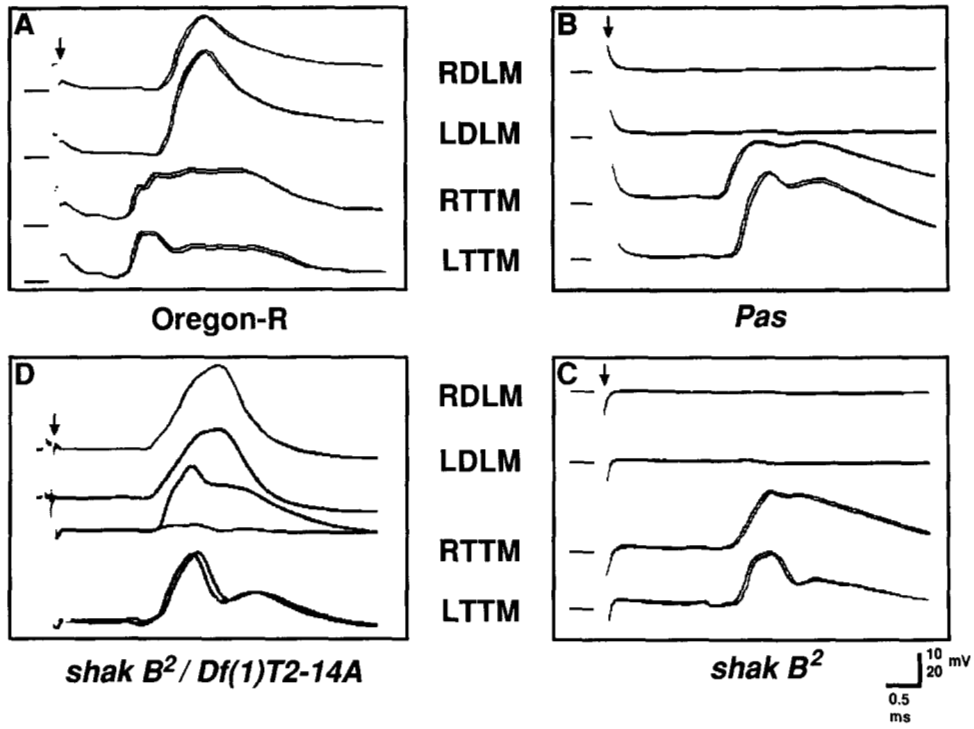


FIGURE 2.—Electrophysiology of the GF pathway: the reponse to brain stimulation of the jump and flight muscles. The fly is impaled with four recording electrodes, two stimulating electrodes and a ground. The four traces of each panel are records from the right and left wing depressor muscles (DLMs) (top two traces) and the right and left jump muscles (TTMs) (bottom two traces). Each record consists of the superimposed responses to two stimuli; in some records slight shifts allow both response traces to be seen separately, in others the two responses superimpose completely. Down arrows indicate the end of the stimulus pulse, the artefact of which can be seen beneath and to the left of the position of the arrow in each trace. Oscilloscope traces were digitized into a MacIntosh II computer for preparation of the figures. Horizontal scale bar is 0.5 msec. Vertical scale bar is 20 mV for the top two traces of panel A, 10 mV for all other traces. A, Oregon-R. Example of normal response of DLM and TTM muscles to brain stimulation. Note how TTM response precedes DLM response, and all four muscles respond to both shocks; there are no failures. B, *Pas*. Example of severe phenotype. Note the absence of response from the DLM muscles. The response of the TTM is delayed from that seen with *O-R* in panel A. C, *shakB*^{2}. Note similarity of response to *Pas* in panel B, *i.e.*, absence of DLM response and delayed TTM response. D, *shakB*^{2}/*Df(1)T2–14A*. Example of "mild" phenotype. DLMs respond to both stimuli with approximately normal latency. The TTMs do not always respond: in this record, the right TTM (third trace) responded only once to the two shocks (double- headed arrow). When it does respond, the TTM is delayed. Its latency is now about the same as the DLM latency rather than shorter as in Oregon-R (panel A).

The DLM electrodes were placed just medial to the anterior dorso-central bristles, whereas the TTM electrodes were placed just dorso-medial to the supra-alar bristles. The motor neurons of the GFS in the thoracic ganglion were stimulated by applying voltage pulses to electrodes inserted into the left and right dorsal ventral muscle (DVM II, TANOUYE and WYMAN 1980). Stimulus electrodes in this position were found to produce the short latency response characteristic of direct motor neuron stimulation using a low stimulus voltage. In either stimulus configuration, the recording electrodes were connected to the vertical amplifier of a storage oscilloscope (Tektronix 5111 with 5A14N four channel amplifier) and measurements were taken directly from the screen of the oscilloscope. Muscle response latencies were measured from the end of the stimulus pulse to the first voltage deflection of the evoked muscle response. In Figure 2, the latency is the time from the arrow indicating the end of the stimulus pulse to the upward deflection indicating the beginning of the muscle response. Maximum response rates were determined by applying a brief train (5–10 pulses) of stimuli at different frequencies. At least one flat trace in response to a train indicated a stimulus rate above the maximum response rate of the pathway. In Figure 2, examples of muscle response failures are seen in panels B, C and D.

Along with maximum response rates, the percentage of muscles that respond to brain stimulation is a measure of the reliability of the response. Each muscle was tested with the stimulus current in both polarities, and the presence or absence of a response noted. Thus the "percent muscles responding" values in Figures 4, 5 and 6 and Tables 3 and 4 reflect a number of tests twice the "*N*" indicated. The genotypes of Figures 4, 5 and 6 were separated into four groups as described in Figure 7 and used as four levels of a single manipulated variable in one way analysis of variance tests. For each of the four dependent (measured) variables (DLM latency and maximum response rate and TTM latency and maximum response rate) a separate analysis of variance was performed using the StatView 512+ program for the MacIntosh computer. The Fisher protected least significant differences (PLSD) test and the Scheffe *F*-test (KEPPEL 1982) agreed on all significance levels in the results except the comparison of the data of Figure 7H with 7L where the former test yielded a 99.999% significance and the latter a 99.99% significance.

Isolation of recombinants between two deficiency chromosomes: To produce females heterozygous for deficiencies *16-3-35* and *A118*, males of the genotype *A118/y*$^+$*Ymal*^{106} were mated to virgin females of genotype *16-3-35/FM6*. F_1 virgin females of genotype *16-3-35/A118* lack the dominant *Bar* mutant phenotype, and were selected on this basis. Paternal non-disjunction rarely produces F_1 *XXY* females

carrying the y^+Ymal^{106} chromosome. The few nondisjunction F_1 females produced were identified and eliminated by checking for the extra hairs caused by the additional y^+ region of the y^+Ymal^{106} chromosome (SCHALET and LEFEVRE 1976). Ten to 20 virgin females of genotype *16-3-35/A118* were mated to *FM6* males in bottles of standard medium at 22–26°. The parents were removed after 7 days, and the resulting adult progeny collected daily between 11 and 18 days. The progeny were searched for phenotypically wild-type males and the number of F_1 females was counted. The putative male recombinants were mated to *C(1)A,y* attached *X* chromosome virgin females to produce stocks of flies bearing the putative recombinant *X* chromosomes. In this way, the fertility of the males of interest could be assessed, and the GF response of several flies of the resulting stock could be recorded. Completely analogous procedures were used to isolate putative recombinant progeny from mothers heterozygous for the deficiency combinations *16-3-35/HC279* and *16-3-35/LB7*. In an additional experiment to confirm the recombinant nature of the F_1 males recovered in the above experiments, we generated *car Df(1)16-3-35 Dp(1;1)sc*^{V1}*/Df(1)A118* females which included markers that closely flank the deficiencies. These females were mated to balancer males that carried the *Bar* marker. Again F_1 progeny were searched for the rare F_1 males.

Measurement of the percentage of eggs hatching: A control rate of hatching was determined from the eggs laid by Oregon-R/*FM6* females mated to *FM6* males. The rate of hatching of a known lethal in the region was determined from the eggs laid by *Df(1)16-3-22/FM6* females mated to *FM6* males. Since *16–3–22* deletes *runt*, a known embryonic lethal (GERGEN and WIESCHAUS 1986), the *16-3-22* male progeny will die, leaving an expected hatching rate of approximately 75%. The experimental group was the offspring of mothers heterozygous for a lethal *R-9-29* allele and *FM6* which had been mated to *FM6* males. For each genotype, roughly 100 to 200 parental flies were placed in a plastic beaker which was inverted over a Petri dish of medium smeared with a paste made from yeast and water. After 3 hr at room temperature the plate was removed. After 30–40 hr, well beyond normal hatching time, the numbers of unhatched eggs and empty, chorionic egg shells were recorded.

RESULTS

Genetic analysis of single complementation groups: Our first task was the assignment of mutations to complementation groups in 19E so that *Pas* could be analyzed with respect to a set of defined loci. There are eight known genetic complementation groups in subdivision 19E (Figure 3). They are *melanizedlike* (*mell*), *runt* (*run*), *R-9-29*, *R-9-28*, *EC235*, *little fly* (*lf*), *varied outspread* (*vao*) and *uncoordinated* (*unc*) (SCHALET and LEFEVRE 1976; MIKLOS *et al.* 1987). The lethal alleles of the complementation groups of subdivision 19E have been extensively tested *inter* and *intra se*, as well as against a panel of published and unpublished lethals, duplications and deficiencies whose breakpoints impinge on this region (SCHALET and LEFEVRE 1976; GREEN, YAMAMOTO and MIKLOS 1987; MIKLOS *et al.* 1987). Tests have also been performed with other rearrangements and alleles of complementation groups outside the 19E region but within the larger *maroonlike* (19D3) to *suppressor of forked* (20F) interval (PERRIMON, SMOUSE and MIKLOS 1989). Whereas most of the complementation groups in subdivision 19E have lethal or viable alleles, *varied outspread* and *melanizedlike* have only been defined by the phenotype of individuals heterozygous for certain overlapping deficiencies. We were also unable to obtain any alleles of *little fly* (19E6) that we could reliably score. All other alleles can be unambiguously assigned to the remaining six complementation groups and are so displayed in Table 1.

The cytological mapping of deficiency breakpoints and loci (Figure 3) is based on the work of SCHALET and LEFEVRE (1973, 1976), who find that "all of section 19 is sufficiently amenable to cytological analysis that in most cases breakpoint determinations are accurate within an error of no more than one band." The association of genes with cytological bands in this paper is subject to this same uncertainty. The association of each gene with a different band is not a necessary conclusion from the cytological data.

Genetic analysis of multilocus deficiencies: The 13 deficiency-bearing *X* chromosomes used in our analyses are depicted in Figure 3. The figure includes four deficiencies (*HC279, LB7, 17-489 and A53*) not reported earlier in the cytogenetic analyses of SCHALET and LEFEVRE. The cytological extent of *A53*, as determined by Lefevre, was reported in LINDSLEY and ZIMM (1986). The cytological limits of the three remaining deficiencies are inferred from genetic analyses. The genetic breakpoints of all 13 deficiencies have been determined using the alleles of the lethal complementation groups listed in Table 1 as well as by complementation testing of the deficiencies *inter se* and with various duplication bearing chromosomes such as *mini-2*, *mini-ring* and *mini-77* (GREEN, YAMAMOTO and MIKLOS 1987; PERRIMON, SMOUSE and MIKLOS 1989). The mode of induction and genetic extents of these deficiencies are shown in Table 2. The previous cytological uncertainties associated with the breakpoints of deficiency *16-3-35* have now been resolved by direct cytological analysis (YAMAMOTO and MIKLOS 1987). *16-3-35* is missing bands 19D3 through 19E3 inclusive and this cytological result is therefore in complete agreement with the genetic extent of this deficiency. Following convention, we have depicted the genetic breakpoints of the deficiencies in Figure 3 as occurring between the various complementation groups. The molecular breakpoints of any of the deficiencies could, of course, be in the genes themselves.

Electrophysiological phenotype of normal and *Passover* flies: In wild-type flies excitation is transmitted rapidly through the elements of the GFS. After excitation of the GF by a stimulus in the brain, a spike is seen in the TTM muscle about 1.0 msec later (Figure 2A). This 1 msec (termed the latency) is the time required for spike conduction and synaptic trans-

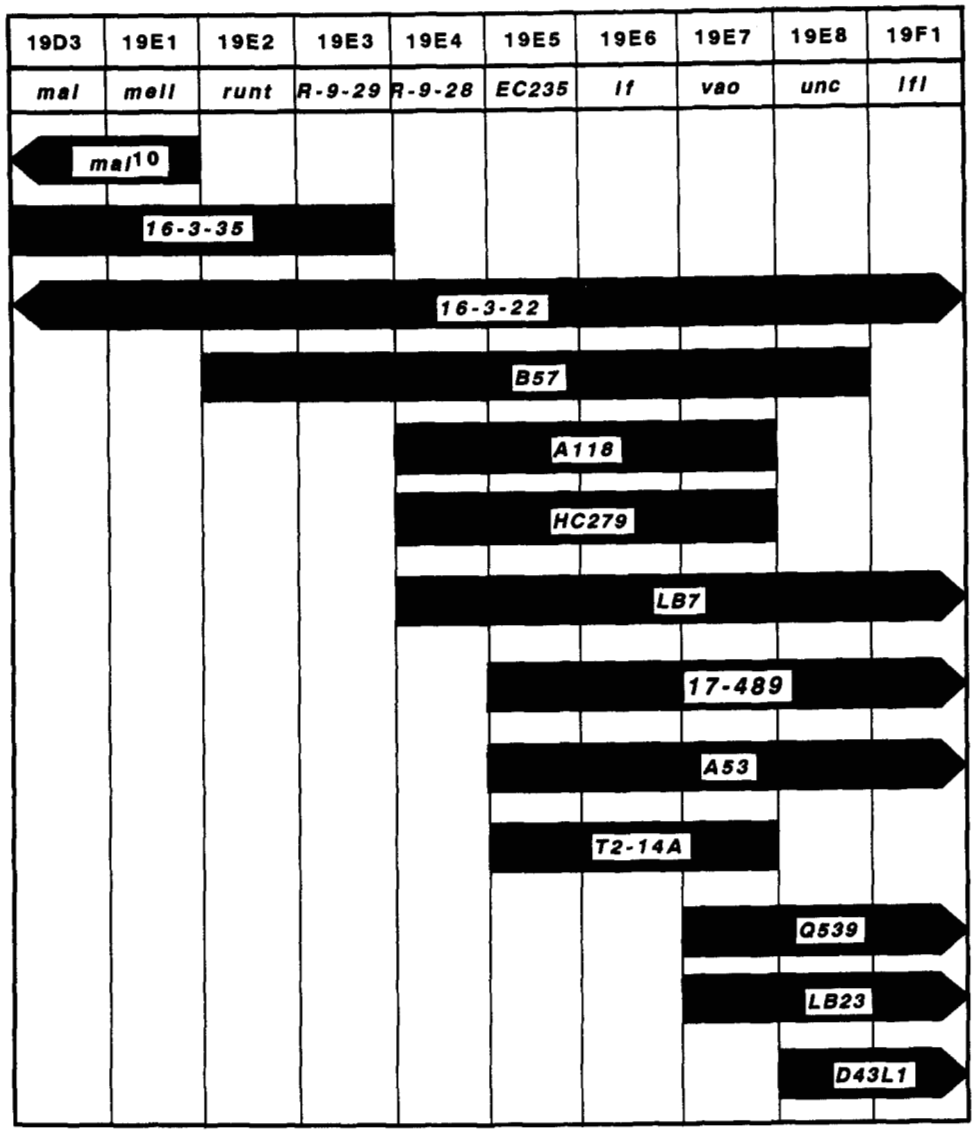


FIGURE 3.—Cytogenetic map of the 19D3 to 19F1 *X* chromosome interval illustrating the genetic complementation groups and chromosomal deficiencies used in the analysis of mutants. The genetic extent of each deficiency relative to the complementation groups listed above is indicated by the black bars. Deficiencies which extend beyond the boundaries of this map are drawn with an arrowed end.

mission through the elements of the pathway. The pathway to the DLM muscle involves one extra chemical synapse (the PSI to DLM synapse, see Figure 1) and therefore responds with a somewhat longer latency, about 1.4 msec. The TTM can respond to each of a series of stimuli delivered at 100/sec (100 Hz) and the DLM can respond nearly as rapidly. Failures to respond are almost never seen.

In homozygous *Pas* flies the DLMs do not respond at all (Figure 2B; Table 4). The TTM muscles do not respond about 50% of the time. When they do respond their latency is longer and more variable (1.6 ± 0.8 msec SD). Whereas in wild type, muscles on the right and left sides of the fly both respond to each stimulus, in *Pas* flies one side may respond without the other. In those cases, reversing the polarity of the stimulating electrodes may change which GF is stimulated and thus affect whether a side responds. Therefore flies were stimulated with both polarities. One of the most sensitive tests of synaptic function is the rapidity with which spikes can be transmitted. In *Pas* flies the maximum rate at which the TTM muscle can respond to each stimulus is less than 1 Hz (0.6 ± 0.3 Hz SD).

The *Pas* allele shows partial dominance: The *Pas* allele is dominant in its elimination of the escape response. Flies of the genotype +; *bw*; *st* respond to a light-off stimulus by jumping nearly 90% of the time. In *Pas*/+; *bw*; *st* flies, however, the jump response is almost totally abolished (Table 3). The absence of a jump response in *Pas*/+ flies, while the electrophysiological response of the GFS to brain stimulation remains, suggests that the *Pas* mutation dominantly disrupts the pathway between the eye and the GF.

Deficiency mapping of *Pas*: After the original mapping of *Pas* to *Df(1)16-3-22* (WYMAN and THOMAS 1983), KOTO (1983) further defined the location to within *Df(1)B57*, indicating a 19E location at the base of the *X* chromosome. We examined the response to brain stimulation of flies heterozygous for *Pas* and for deficiencies spanning the region (Figure 4). All combinations were viable. Two deficiencies bracket the region of interest: $Df(1)mal^{10}$ deletes the distal end while *Df(1)Q539* deletes the proximal end. The phys-

TABLE 1

Modes of induction of mutations in *X* chromosome subdivision 19E

Complementation group	Mutation	Mutagen[a]	Reference
runt[b]	*HM449*	HMS	KRAMERS *et al.* (1983)
	VE726	EMS	LINDSLEY and ZIMM (1990)
	VE751A	EMS	LINDSLEY and ZIMM (1990)
	YE96	EMS	GERGEN and WIESCHAUS (1986)
Passover	*shakB*^{2}	EMS	HOMYK, SZIDONYA and SUZUKI (1980)
	Pas	EMS	THOMAS and WYMAN (1984)
	17-189	Neutrons	LINDSLEY and ZIMM (1990)
	17-360	Neutrons	LINDSLEY and ZIMM (1990)
	E81	EMS	LIFSCHYTZ and FALK (1969)
	EC201	EMS	LINDSLEY and ZIMM (1990)
	EF535	EMS	LINDSLEY and ZIMM (1990)
	HM437	HMS	KRAMERS *et al.* (1983)
	L41	X-ray	LINDSLEY and ZIMM (1990)
	R-9-29	EMS	LIFSCHYTZ and FALK (1969)
R-9-28	*R-9-28*	EMS	LIFSCHYTZ and FALK (1969)
	DA507	EMS	LINDSLEY and ZIMM (1986)
	EC242	EMS	LINDSLEY and ZIMM (1986)
EC235	*EC235*	EMS	LINDSLEY and ZIMM (1986)
	D83	MR-induced	EEKEN *et al.* (1985)
	HM435	HMS	KRAMERS *et al.* (1983)
	VE909	EMS	LINDSLEY and ZIMM (1986)
little fly			SCHALET and LEFEVRE (1976)
varied outspread[c]			SCHALET and LEFEVRE (1976)

[a] HMS, hycathone methanesulfonate; EMS, ethyl methanesulfonate; MR, male recombination mutator system.
[b] *legless* (SCHALET and LEFEVRE 1976) is an earlier name for this complementation group.
[c] No alleles of *varied outspread* are extant. In late pupae and early adults of heterozygotes carrying deficiencies overlapping in bands 19E7-8 the eyes show a varied or mottled distribution of eye pigment. The eye phenotype has been shown to be temperature sensitive (normal when raised at 17–18° but mottled when raised at 24–25°) in *Df(1)B57/Df(1)Q539* (SCHALET and LEFEVRE 1976) and Df(1)A118/Df(1)Q539 (A. P. SCHALET, unpublished).

TABLE 2

Genetic extent and mode of induction for deletions involving *X* chromosomal subdivision 19E

Deficiency	Deficient for loci	Mutagen	Reference
16-3-35	*mal–R-9-29*	Neutron	SCHALET and LEFEVRE (1976); YAMAMOTO and MIKLOS (1987)
16-3-22	*mal–eo*	Neutron	SCHALET and LEFEVRE (1976)
17-489	*EC235–bb*	Neutron	MIKLOS *et al.* (1987); A. SCHALET (unpublished)
A118	*R-9-28–vao*	X-ray	SCHALET and LEFEVRE (1976)
A53	*EC235–eo*	X-ray	G. LEFEVRE (unpublished); PERRIMON, SMOUSE and MIKLOS (1989)
B57	*run–unc*	X-ray	SCHALET and LEFEVRE (1976)
D43L1	*unc–su(f)*	Cs^{137}	SCHALET and LEFEVRE (1976)
HC279	*R-9-28–vao*	X-ray	G. LEFEVRE (unpublished); PERRIMON, SMOUSE and MIKLOS (1989)
LB7	*R-9-28–su(f)*	EMS or Mitomycin C	A. SCHALET (unpublished); PERRIMON, SMOUSE and MIKLOS (1989)
LB23	*vao–bb*	EMS	SCHALET and LEFEVRE (1976)
mal^{10} [a]	*sw–mell*	X-ray	SCHALET and LEFEVRE (1976)
Q539	*vao–A112*	EMS	SCHALET and LEFEVRE (1976)
T2-14A	*lf–vao*	Tritiated deoxycytidine	SCHALET and LEFEVRE (1976)

[a] The *mal*^{10} deficiency was induced on the chromosomal inversion *sc*^{8}.

iological responses from *Pas/mal*^{10}, and *Pas/Q539* flies are statistically indistinguishable from that of *Pas/FM6*. Heterozygotes with five of the deficiencies (*B57*, *16-3-35*, *A118*, *HC279* and *LB7*) produce an extreme, mutant phenotype (Figure 4) like that of *Pas* homozygotes. In these flies the DLMs do not respond to GF stimulation at all. The TTMs respond abnormally. The TTM response is abolished in all *Pas/B57* individuals and in different percentages of the other genotypes (Figure 4). In those muscles that respond at all, the TTM response shows an increase in mean latency of 53–85% over the control (*Pas/FM6*) muscles (Figure 4). These results place *Pas* near the boundary between the *R-9-29* and *R-9-28* complementation groups.

To confirm this placement, we examined the re-

TABLE 3

The escape response of *Pas*/+ and *shakB*^{2}/+ individuals

Genotype	Mean response rate	Standard deviation	No. of flies tested
Pas/+	1%	3	16
shakB^2/+	79%	15	24
+/+	89%	9	43

All genotypes carried the autosomal mutations *brown* and *scarlet* which render the eyes white in color, and enhance the escape response.

sponse to brain stimulation of flies carrying one or two deficiencies of the 19E region (Figure 5). Only two of the thirteen genotypes tested exhibit the extremely abnormal electrophysiological phenotype of homozygous *Pas* flies. In both of these deficiency combinations (*16-3-35/A118* and *16-3-35/HC279*) the DLM response is totally abolished. Both combinations reduce the TTM maximum response rate from the wild-type value which is greater than 100 Hz to below 15 Hz. The TTM still responds in *16-3-35/A118*, but its latency (1.38 ± 0.11 msec SD) is always longer than that of the *16-3-35/FM6* control (0.89 ± 0.12 msec SD). Only 1/3 of the TTMs of *16-3-35/HC279* responded to brain stimulation at all. These results again place *Pas* in the vicinity of the boundary between *R-9-29* and *R-9-28*.

The neural defect lies with the central circuitry rather than with the motor neurons, muscles or neuromuscular synapses since both the TTMs and DLMs respond normally to high frequency (>100 Hz) stimulation of their motor neurons in the thorax. The abnormal response may represent an altered GF-TTM motor neuron connection, or the presence of another descending pathway masked in the wild type by the rapid GF-TTM response. These deficiency genotypes also produced the uncoordinated leg movements seen in *Pas* and *shakB*^{2} mutants under ether anesthesia (see below).

As can be seen in Figure 5, deficiency *16-3-35*, coming into the region from the distal side, uncovers no known lethal loci that are also uncovered by deficiencies *A118*, *HC279* or *LB7* coming in from the proximal side. Although initial cytological studies indicated some overlap of the region deleted between deficiencies *16-3-35* and *A118* (SCHALET and LEFEVRE 1976), we have obtained recombination in crosses between *16-3-35* and each of the other deficiencies. For deficiencies *16-3-35* and *A118*, seven adult male progeny were found among 21,653 female progeny. For *16-3-35* and *HC279* four putative recombinants were found out of 6071 female progeny. For *16-3-35* and *LB7*, four putative recombinants were found among 7324 female progeny. The putative male recombinants were mated to *C(1)A,y* females. Males from each of the three deficiency crosses proved to be fertile and in turn their male progeny exhibited normal GF responses. If the recombination in these events was homologous and in register then there must be DNA between the proximal breakpoint of *16-3-35* and the distal breakpoints of *A118*, *HC279* or *LB7*.

In a second experiment using a chromosome bearing markers closely flanking the deficiencies, there were 9 male progeny (among 14,004 females) which bore the *car*$^+$ and *Dp(1:1)sc*^{V1} markers expected in flies bearing a chromosome resulting from a crossover in the interval between *Df(1)A118* and *Df(1)16-3-35*. All of these males were fertile; the first two males of independent origin were tested and shown to have normal GF responses.

The normal phenotypes produced in males by these recombinant chromosomes also demonstrate that *16-3-35* does not have additional proximal genetic defects that contribute to the mutant phenotype. Similarly, the *A118*, *HC279* and *LB7* chromosomes do not have additional distal genetic defects that disrupt the GF response. Aberrations of these types would be recovered in the recombinants and cause an abnormal GF response.

These results indicate that *Pas* lies, at least partly, in the DNA between the proximal break of *16-3-35* and the distal breaks of *A118*, *HC279* and *LB7* and is impinged upon by these deficiencies from both sides.

Allelism with the *R-9-29* complementation group: When *Pas* is combined with lethal alleles of the four available complementation groups (*runt*, *R-9-29*, *R-9-28* and *EC235*) in 19E between *Df(1)mal*^{10} and *Df(1)Q539*, it is seen that only *R-9-29* alleles cause a phenotype like that of *Pas* homozygotes. When *Pas* is combined with the *R-9-29* alleles (Table 4), all combinations are mutant except for the *Pas/L41* and *Pas/EF535* combinations which produce control values for DLM and TTM latencies. With the noncomplementing alleles none of the individuals have a DLM response to brain stimulation. The TTM response is either abolished in some or all individuals of a genotype or exhibits a large increase in its latency (Table 4). When they do respond, the maximum response rate is reduced to 25 Hz or less.

The *VE726* allele of the *runt* locus produces an increase in TTM latency when combined with the *Pas* allele (Table 4). However, when other *runt* alleles (*VE751*, *HM449* and *YE96*) are combined with either the *Pas* or *shakB* (see below) alleles, the latencies observed are indistinguishable from control values. Thus, the interaction of *VE726* with *Pas* is peculiar to this allele, and does not extend to other alleles of *runt* or *Pas*.

Viable allele: *shaking-B*^{2} (HOMYK, SZIDONYA and SUZUKI 1980) is an independently isolated behavioral mutation that has been placed in the *R-9-29* complementation group (MIKLOS *et al.* 1987). The *shakB*^{2} stock was kindly provided by THEODORE HOMYK, JR. *shakB*^{2} and *Pas* flies are defective in the motor control

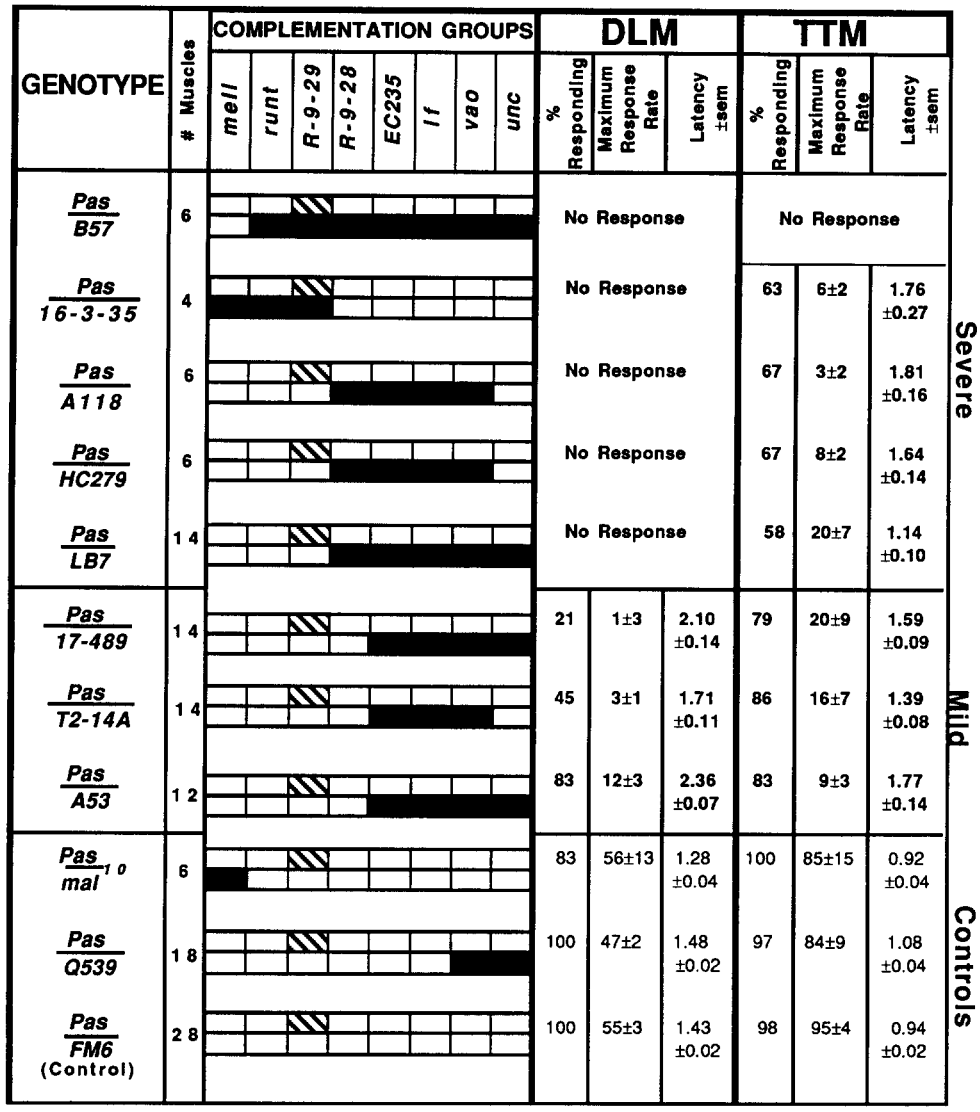


FIGURE 4.—The DLM and TTM responses of flies heterozygous for the *Passover* mutation and for chromosomal deficiencies of the 19E subdivision. The genetic extent of each deficiency relative to the complementation groups listed above is indicated by the black bars. All TTM and DLM latencies in the "severe" and "mild" groups differed significantly (*t*-test: $P < 0.0001$) from those of sibling controls (*Pas/FM6*).

of their legs. The position in which the legs are held during flight is abnormal and there is a slight tremor of the legs during ether anesthesia. The leg tremor phenotype of *Pas* and *shakB*^{2} flies is very different, however, from that of the potassium channel mutant, *Shaker* (SALKOFF and WYMAN 1981). Even the weak alleles of *Shaker* shake much more vigorously and at higher frequency than *Pas* or *shakB*^{2} (E. O. ACEVES-PINA, unpublished results). The shaking effect in *Pas* or *shakB*^{2} flies is better described as an uncoordinated movement rather than the rhythmic movement seen in *Shaker* individuals. The legs of an etherized *Shaker* fly, after being severed from the body, will continue to shake (GANETZKY and Wu 1982), demonstrating a generalized membrane hyperexcitability independent of central circuitry. The severed legs of *Pas* flies do not shake, indicating a central defect, possibly the disconnection of an inhibitory input.

Homozygous *shakB*^{2} do not jump to a light off stimulus. However, unlike *Pas*, *shakB*^{2} does not act as a dominant in elimination of the jump. *shakB*^{2}/+; *bw*; *st* individuals respond nearly as well as +/+; *bw*; *st* individuals (Table 3) indicating that *shakB*^{2} is a recessive allele with respect to the escape response.

We find the electrophysiological phenotype of *shakB*^{2} to be very similar to that of *Pas* (Figure 2, B and C) (BAIRD 1986, 1988). *Pas* and *shakB*^{2} do not complement each other. The heterozygote *Pas/shakB*^{2} has the same mutant phenotype as *Pas* or *shakB*^{2} homozygotes. In all three of these genotypes the DLMs do not respond to brain stimulation, the TTM latency is increased and its maximum response rate decreased (Tables 4 and 5). The defect is central since the muscles respond normally to stimulation of the motor axons.

In a variety of combinations with other alleles and deficiencies *shakB*^{2} behaves in much the same way that *Pas* does (Tables 4 and 5; Figures 4 and 6). When the *shakB*^{2} allele is combined in heterozygotes with our panel of 19E deficiencies, it is seen (Figure 6) that combination with the two deficiencies that bracket the region, *shakB*^{2}/*mal*^{10} and *shakB*^{2}/*Q539*, yield control values for both the DLM and TTM latencies. Four combinations (*shakB*^{2}/*B57*, *shakB*^{2}/*16-3-35*, *shakB*^{2}/

GENOTYPE	# Muscles	COMPLEMENTATION GROUPS								DLM			TTM			
		mell	*runt*	*R-9-29*	*R-9-28*	*EC235*	*lf*	*vao*	*unc*	% Responding	Maximum Response Rate	Latency ±sem	% Responding	Maximum Response Rate	Latency ±sem	
16-3-35 / A118	4									No Response			100	11±2	1.38 ±0.11	Severe
16-3-35 / HC279	6									No Response			33	2±1	1.05 ±0.05	Severe
16-3-35 / LB7	6									92	61±13	1.64 ±0.04	92	25±11	1.50 ±0.06	Mild
16-3-35 / T2-14A	12									100	64±7	1.37 ±0.03	100	17±4	1.39 ±0.05	Mild
16-3-35 / 17-489	8									100	73±12	1.45 ±0.03	100	32±16	1.30 ±0.06	Mild
16-3-35 / A53	8									100	75±6	1.37 ±0.04	100	32±14	1.23 ±0.09	Mild
16-3-35 / FM6	8									100	66±5	1.32 ±0.02	100	>100	0.89 ±0.03	Controls
A118 / FM6	6									100	66±6	1.30 ±0.02	100	>100	0.92 ±0.04	Controls
T2-14A / FM6	8									100	26±4	1.44 ±0.02	100	>100	0.98 ±0.02	Controls
16-3-35 / Q539	16									100	62±3	1.35 ±0.01	100	99±1	0.95 ±0.02	Controls
T2-14A / Q539	14									96	32±5	1.65 ±0.04	100	99±1	1.04 ±0.03	Controls
B57 / FM6	8									100	60±4	1.26 ±0.02	100	>100	0.84 ±0.03	Controls
16-3-22 / FM6	6									100	>100	1.38 ±0.07	100	>100	0.90 ±0.03	Controls
Wild Type (Oregon-R)	12									100	91±2	1.43 ±0.02	100	>100	1.06 ±0.03	Controls

FIGURE 5.—The DLM and TTM responses of flies heterozygous for chromosomal deficiencies involving *X* chromosome subdivision 19E. All TTM latencies in the "severe" and "mild" groups differed significantly ($P < 0.0001$) from those of sibling controls (*16-3-35/FM6*). *t*-test was used in all cases except for *16-3-35/HC279* which, because of the large fraction of nonresponders, was tested with the nonparametric Mann-Whitney *U* test.)

A118 and *shakB²/HC279*) totally abolish the DLM output (Figure 6). In these same four combinations the mean TTM latencies range between 1.6 and 2.5 msec, a substantial increase from the average value of 0.9 msec in *shakB²/FM6* sibling controls. These four deficiencies, when combined with *Pas*, also generate the severely mutant phenotype.

Similarly, when the *shakB²* allele is combined with lethal alleles of the four available complementation groups in bands 19E2 through 19E5, it is seen that alleles of runt, *R-9-28* and *EC235* result in a normal phenotype. Only heterozygotes with *R-9-29* alleles abolish the response of the DLM and abolish the TTM response, or cause a large increase in its latency (Table 5). As for Pas, *shakB²/L41* and *shakB²/EF535* produce latencies indistinguishable from control values whereas *shakB²/R-9-29*, *shakB²/E81* and *shakB²/17–189* produce mutant responses (Table 5). The *R-9-29* and *EC201* alleles also do not complement the leg-shaking phenotype of *shakB²* (MIKLOS *et al.* 1987).

Amorphic alleles: Since the *shakB²*, *R-9-29*, *E81* and *17-189* alleles over *shakB²* all produce the same severe phenotype as the deficiency combination *shakB²/B57*, these alleles are amorphic as far as the neural phenotype is concerned.

Complex interaction with the *R-9-29* complementation group: The *R-9-29* complementation group, with nine extant alleles, demonstrates a pattern of complex complementation with the viable alleles *Passover* and *shakB²*. In establishing the basic genetic properties of the *R-9-29* locus, we first examined seven lethal alleles of this complementation group. These lethal alleles cause inviability of the organism when tested *inter se*, while all *Pas/lethal* and *shakB²*/lethal

TABLE 4

DLM and TTM responses of individuals heterozygous for *Passover* and alleles of the complementation groups of subdivision 19E

Complementation group	Genotype	No. muscles tested	Mean latency msec ± SD (% muscles responding)			
			DLM		TTM	
runt	*Pas/HM449*	4	1.4 ± 0.6	(100)	1.0 ± 0.1	(100)
	Pas/VE726	4	1.7 ± 0.3	(100)	1.4 ± 0.7	(100)
	Pas/YE96	8	1.4 ± 0.7	(88)	1.1 ± 0.2	(81)
R-9-29	*Pas/Pas*	32	**No response**	**(0)**	**1.6 ± 0.8**	**(21)**
	Pas/shakB²	4	**No response**	**(0)**	**2.4 ± 1.3**	**(100)**
	Pas/17-360	8	**No response**	**(0)**	**No response[a]**	**(0)**
	Pas/E81	6	**No response**	**(0)**	**1.6 ± 0.4**	**(100)**
	Pas/EC201	7	**No response**	**(0)**	**1.7 ± 0.5**	**(79)**
	Pas/HM437	6	**No response**	**(0)**	**No response**	**(0)**
	Pas/R-9-29	4	**No response**	**(0)**	**No response[a]**	**(0)**
	Pas/EF535	16	1.6 ± 0.3	(95)	1.1 ± 0.2	(97)
	Pas/L41	20	1.5 ± 0.2	(100)	1.0 ± 0.3	(100)
R-9-28	*Pas/R-9-28*	6	1.2 ± 0.06	(100)	1.0 ± 0.2	(100)
	Pas/DA507	6	1.6 ± 0.3	(100)	1.1 ± 0.3	(100)
EC235	*Pas/EC235*	8	1.7 ± 0.2	(100)	1.0 ± 0.2	(81)
	Pas/D83	6	1.5 ± 0.1	(100)	1.1 ± 0.3	(100)
	Pas/HM435	8	1.4 ± 0.1	(100)	1.0 ± 0.2	(100)
	Pas/VE909	4	1.7 ± 0.2	(100)	1.1 ± 0.2	(100)
(Control)	*Pas/FM6*	28	1.43 ± 0.1	(100)	0.94 ± 0.1	(98)

The TTM latencies in boldface showed a significant ($P < 0.0001$) increase in latency from that of the *Pas/FM6 controls.*
[a] A spike occurred once after a stimulus, it probably was spontaneous rather than a response to the stimulus.

TABLE 5

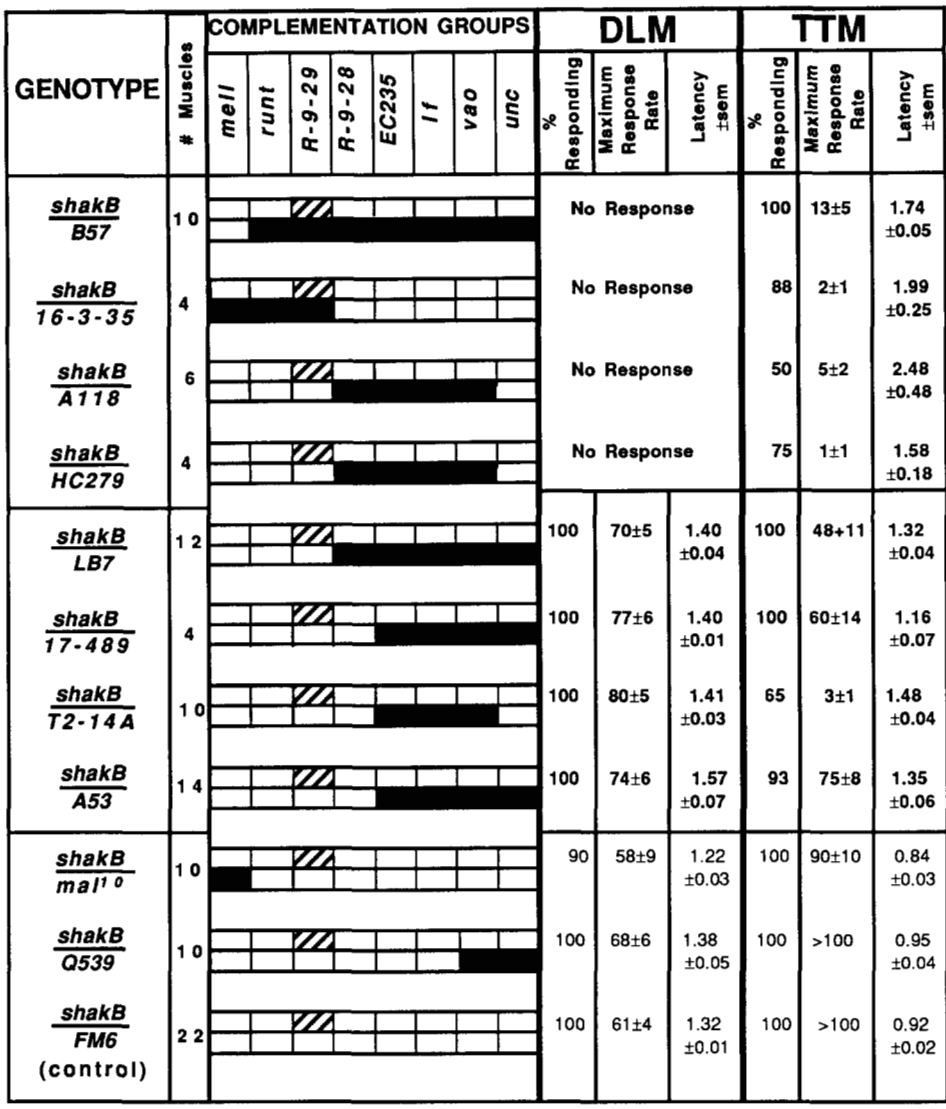
DLM and TTM responses of individuals heterozygous for *shaking-B²* and alleles of the complementation groups of subdivision 19E

Complementation group	Genotype	No. muscles tested	Mean latency msec ± SD (% muscles responding)			
			DLM		TTM	
runt	*shakB²/HM449*	6	1.4 ± 0.05	(100)	1.0 ± 0.07	(100)
	shakB²/VE751A	4	1.2 ± 0.1	(100)	0.9 ± 0.2	(100)
	shakB²/YE96	8	1.2 ± 0.06	(100)	0.9 ± 0.1	(100)
R-9-29	*shakB²/shakB²*	6	**No response**	**(0)**	**1.8 ± 0.2**	**(80)**
	shakB²/17-189	4	**No response**	**(0)**	**No response**	**(0)**
	shakB²/E81	4	**No response[a]**	**(0)**	**1.3 ± 0.2**	**(50)**
	shakB²/R-9-29	4	**No response**	**(0)**	**No response**	**(0)**
	shakB²/EF535	12	1.3 ± 0.1	(100)	0.9 ± 0.1	(100)
	shakB²/L41	8	1.4 ± 0.1	(100)	1.0 ± 0.3	(100)
R-9-28	*shakB²/R-9-28*	18	1.2 ± 0.1	(94)	0.9 ± 0.3	(97)
	shakB²/EC242	4	1.4 ± 0.08	(100)	1.0 ± 0.1	(100)
EC235	*shakB²/D83*	6	1.4 ± 0.1	(100)	0.9 ± 0.08	(100)
	shakB²/HM435	6	1.5 ± 0.07	(100)	1.1 ± 0.1	(100)
(Control)	*shakB²/FM6*	22	1.32 ± 0.12	(100)	0.92 ± 0.08	(100)

The TTM latencies in boldface showed a significant ($P < 0.0001$) increase in latency from that of the *shakB²/FM6* controls.
[a] A spike occurred once after a stimulus, it probably was spontaneous rather than a response to the stimulus.

individuals are viable. While most alleles fail to complement the neural phenotype of *Passover*, two alleles (*EF535* and *L41*) complement the phenotype (Table 4). This suggests that there are two independently mutable functional domains at this locus, a neural and a lethal function.

Summary of severe effect of homozygous 19E3 mutations: Figure 7, M and N, collect the TTM response rates and latencies for the genotypes labeled as "severe" in Figures 4, 5 and 6. These should be compared with the collected data from the control genotypes from Figures 4, 5 and 6 displayed in Figure 7, C and D. While Figures 4, 5 and 6 present the mean values for the responses, Figure 7 presents the full distribution of the responses. It can be seen that the TTM distributions for the severe genotypes have very little overlap with the distributions for the control genotypes. In the analysis of variance (ANOVA) model described in MATERIALS AND METHODS the difference between distributions C and M, as well as D and N was significant at the 99.999% level (using both Fisher PLSD and Scheffe *F*-tests). The DLMs, as



GENOTYPE	# Muscles	COMPLEMENTATION GROUPS: *mell*	*runt*	*R-9-29*	*R-9-28*	*EC235*	*lf*	*vao*	*unc*	DLM: % Responding	DLM: Maximum Response Rate	DLM: Latency ±sem	TTM: % Responding	TTM: Maximum Response Rate	TTM: Latency ±sem	
shakB / B57	10									No Response			100	13±5	1.74 ±0.05	Severe
shakB / 16-3-35	4									No Response			88	2±1	1.99 ±0.25	Severe
shakB / A118	6									No Response			50	5±2	2.48 ±0.48	Severe
shakB / HC279	4									No Response			75	1±1	1.58 ±0.18	Severe
shakB / LB7	12									100	70±5	1.40 ±0.04	100	48+11	1.32 ±0.04	Mild
shakB / 17-489	4									100	77±6	1.40 ±0.01	100	60±14	1.16 ±0.07	Mild
shakB / T2-14A	10									100	80±5	1.41 ±0.03	65	3±1	1.48 ±0.04	Mild
shakB / A53	14									100	74±6	1.57 ±0.07	93	75±8	1.35 ±0.06	Mild
shakB / mal^{10}	10									90	58±9	1.22 ±0.03	100	90±10	0.84 ±0.03	Controls
shakB / Q539	10									100	68±6	1.38 ±0.05	100	>100	0.95 ±0.04	Controls
shakB / FM6 (control)	22									100	61±4	1.32 ±0.01	100	>100	0.92 ±0.02	Controls

FIGURE 6.—The DLM and TTM responses of flies heterozygous for the *shaking-B^2* mutation and for chromosomal deficiencies of the 19E subdivision. All TTM latencies in the "severe" and "mild" groups differed significantly (*t*-test: $P < 0.0001$) from those of sibling controls (*shakB^2/FM6*).

described above, do not respond in any of the "severe" flies, while essentially all of the DLMs in the control genotypes respond (Figure 7, A and B).

The *R-9-29* alleles are larval lethals: The lethal phase of several *R-9-29* alleles was determined. The hatching rate of eggs laid by mothers bearing an *R-9-29* allele is comparable to that of wild type (Table 6), and is greater than that using mothers bearing a known embryonic lethal (deficiency *16-3-22*, deficient for *runt* for example). This was true for both the *L41* allele, which complements the GF defect of the viable *Pas* alleles, as well as for the *R-9-29* and *E81* alleles which do not complement *Pas*. Thus the three *R-9-29* alleles examined do not increase embryonic lethality over the small amount observed in a wild-type control. Male larvae of the genotype *lethal/Y* were isolated from each of the egg collections in which the hatching rate was determined. With rare exception these larvae died after a prolonged first instar stage of two to three days and were never observed to pupariate.

Mild *trans* effect of 19E5-6 deficiencies: When *Pas*, *shakB^2* or *Df(1)16-3-35* is combined in *trans* with proximal deficiencies in the 19E region, eight combinations result in an electrophysiological phenotype similar to, but less severe than, *Pas* (Figures 4, 5 and 6). *16-3-35* or *shakB^2* yield this mild phenotype when made heterozygous with any one of the deficiencies *T2–14A*, *A53*, *LB7* or *17–489*. The mean TTM response latency is increased to 1.3–1.5 msec (Figure 4) and the TTM maximum response rates are severely reduced. The histograms of Figure 7, G and H, display the TTM maximum response rates and latencies of these eight genotypes; they can be compared with the control genotypes of Figure 7, C and D. There is little or no effect on the DLM response as can be seen by comparing the histograms of Figure 7, E and F, with the controls in Figure 7, A and B. A record of this mildly abnormal GF response is shown in Figure 2D.

When *Pas* is combined with these same deficiencies, not only is the TTM response weakened, but the DLM is now affected. All four comparisons A through D with I through L were significant in ANOVA at 99.999% level. The TTM latency is increased 50–75% from controls and their ability to respond to each

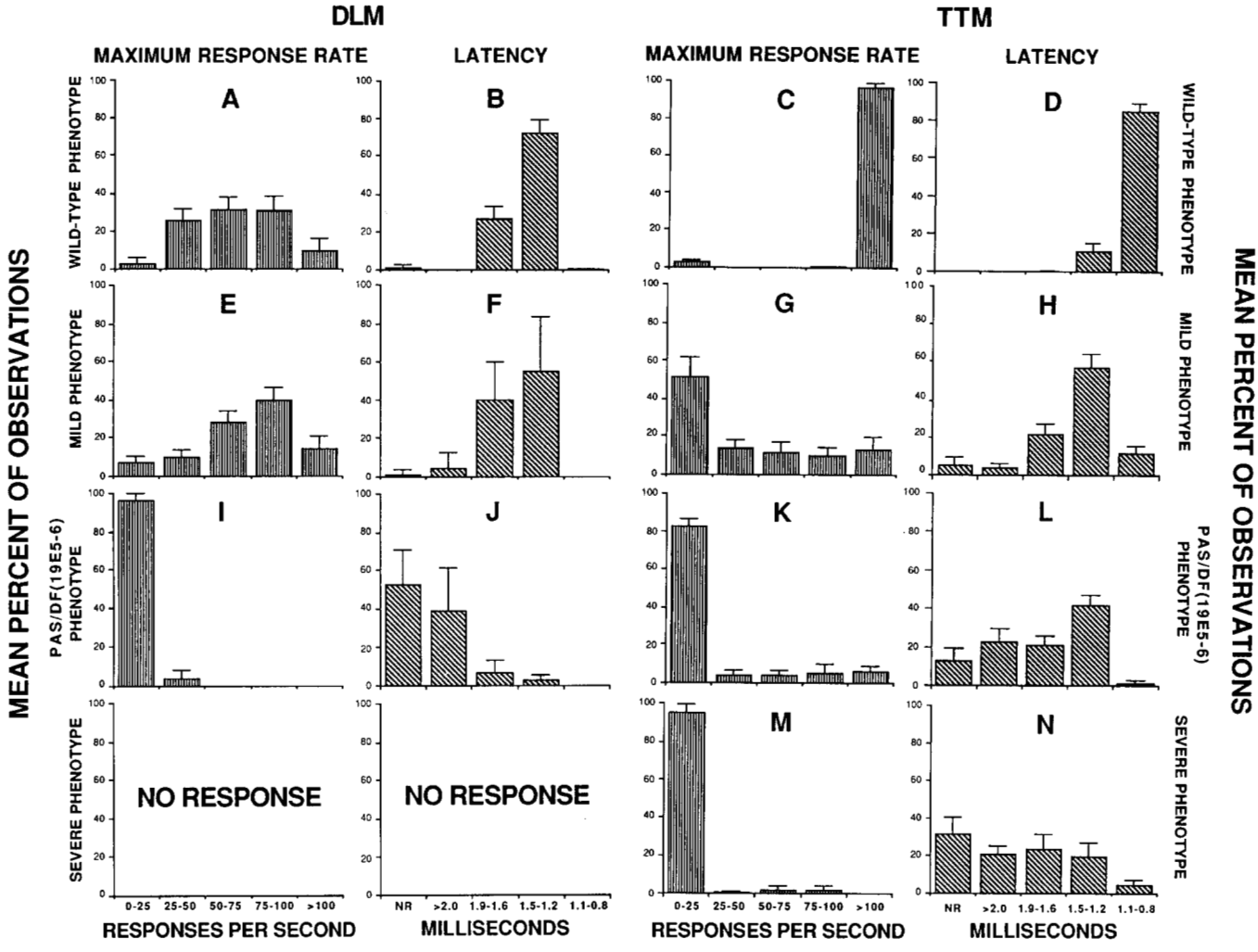


FIGURE 7.—Distribution of response parameters for different classes of genetic lesions. ROWS: Top row (A–D): "Control." Genotypes in which at least one chromosome is normal both 19E3 and 19E5–6 region. Includes all genotypes listed as controls in Figures 4, 5 and 6. Second row (E–H): "Mild." Disruption of 19E3 combined with deficiency for 19E5-6; includes all the genotypes listed as "mild" in Figures 5 and 6. Third row (I–L): Heterozygotes between *Passover* and a deficiency for 19E5–6; includes all genotypes listed as "mild" in Figure 4. Fourth row (M–N: "Severe." Homozygous disruption of 19E3; includes all genotypes listed as "severe" in Figures 4, 5 and 6. In each histogram weak responses are in the bins to the left, while strong responses are in the bins to the right. Rows are arranged so that there is a general degradation of response from top to bottom in all columns. The TTM maximum response rates (C and G) and latencies (D and H) are stronger in controls than in the "mild" genotypes. The DLM response is not much changed. In *Pas/Df(19E5-6)* genotypes, the TTM response (K and L) is further degraded and the DLM response (I and J) is now clearly affected. In the "severe" genotypes, the DLMs do not respond at all and there is very little overlap between the TTM histograms (M and N) and those of the control genotypes. For each genotype, the percentage of responses in each bin was calculated. These percentages, for the relevant genotypes, were averaged to generate the histograms in this figure. Analysis of variance of the raw data (not converted to percentages) shows that 77% of the variance in DLM maximum response rate and 74% of the variance in TTM maximum repsonse rate is accounted for by the separation of the data into the four groups of this figure (calculation of ω^2, WINER 1971). Since latencies could not be measured in the most strongly affected flies (those with nonresponding muscles) the latency statistics do not include the nonresponding class. Nevertheless, even excluding this extremely affected group, analysis of variance shows that 50% of the variance in DLM latencies and 43% of the variance in TTM latencies is accounted for by the separation of the data into the four groups of this figure.

of a rapid train of stimuli is severely reduced. Comparing Figure 7, K and L (*Pas* combinations) with 7, G and H (*shakB*^{2} and *16-3-35* combinations) indicates that *Pas* has a more severe effect on the TTM responses than does *shakB*^{2} or *16-3-35* when combined with deficiencies of 19E5–6. ANOVA comparisons of G with K and H with L were significant at the 99.999% level. The DLM response is abolished in all *Pas/LB7* individuals and in 1/2 to 3/4 of *Pas/17-489* and *Pas/T2-14A* individuals (Figure 4). When DLM responses are present in these genotypes, and in *Pas/A53*, the latency is greater than 1.7 msec and their maximum response rate falls to 12 Hz or less (Figure 4). Compare Figure 7, I and 7J (*Pas* combinations) with Figure 7, E and F (*shakB*^{2} and *16-3-35* combinations) to see the stronger effect of the *Pas* combination genotypes. ANOVA comparisons of E with I and F with J were significant at the 99.999% level.

The heterozygotes that produce the mild phenotype are deficient for two non-adjacent regions. The mild phenotype occurs in the three deficiency/deficiency heterozygotes in which the *R-9-28* lethal complemen-

TABLE 6

Percentage hatching for eggs laid by mothers bearing *R-9-29* mutant or control chromosomes

Genotype of mother	Type of stock	Percent hatched	No. laid
E81/FM6	Neural-lethal	86	86
R-9-29/FM6	Neural-lethal	90	388
L4/FM6	Non-neural-lethal	93	100
Oregon-R/FM6	Wild type (negative control)	88	330
Df(1)16-3-22/FM6[a]	(positive control)	73	51

All fathers were of the genotype *FM6/Y*.
[a] Deletion including *Pas* and *runt*, an embryonic lethal.

tation group is not deleted but lies between the breakpoints of the deficiencies. Alleles of the *R-9-28* complementation groups fully complement the 19E3 mutants and the *16-3-35* deficiency. Thus the two 19E regions which are necessary for a normal GF response are separated at least by the *R-9-28* locus. Alleles of *EC235* also fully complement *16-3-35* and the 19E3 mutants and thus that locus may also lie between the two regions affecting the GFS. We did not have alleles of little fly to test, but given the deficiencies available, the *lf* complementation group may also intervene. The normal phenotype of *16-3-35/Q539* sets a proximal boundary on the location of the interacting region. In accord with this, the *vao* locus seems to be not involved since the three *vao* combinations, *T2-14A/Q539*, *A118/Q539* and *A118/LB23*, have normal phenotype (data not shown).

None of the deficiencies, even those deleting all of 19E, have any measurable electrophysiological effects on either the DLM or TTM output when heterozygous with an intact chromosome. Each of the deficiencies *16-3-35*, *A118*, *T2-14A*, *B57* and *16-3-22* (all over *FM6*) produce wild-type electrophysiological responses (Figure 5). In contrast, the *trans* combinations of *16-3-35* with the nonoverlapping deficiencies *T2-14A*, *17-489* or *A53* do disrupt the GF response. The two different portions of 19E when deleted in *cis* produce a normal phenotype, but when deleted in *trans* affect the GF response. Thus, the wild-type phenotype is observed when both regions are normal on at least one chromosome and the mutant phenotype is observed only when *both* homologs are mutant.

DISCUSSION

Mapping *Passover* and *shaking-B*^{2}: Deficiency mapping of the neural phenotype of these mutations places them between the proximal breakpoint of *mal*^{10} and the distal breakpoint of *Q539*. Alleles of four complementation groups in this region are available. Of these, *Pas* and *shakB*^{2} interact only with the *R-9-29* group. It appears that *Passover*, *shaking-B*^{2} and the *R-9-29* alleles affect a common genetic element. The lethality of *R-9-29* maps cleanly to one genetic position with no ambiguity (SCHALET and LEFEVRE 1973; MIKLOS *et al.* 1987). In the current cytogenetic map (LEFEVRE and WATKINS 1986) *R-9-29* is assigned to 19E2–3. The placement of *Pas* in 19E3 is consistent with the deficiency analysis of Figures 4, 5 and 6. The relationship of the distal break of *LB7* to *Pas* is uncertain; *Pas/LB7* has the severe phenotype, as if LB7 disrupted the locus, while *shakB*^{2}*/LB7* and *16-3-35/LB7* have the mild phenotype, as if *LB7* did not disrupt the 19E3 locus, but had its effect solely through its deletion of the 19E5–6 region.

Complex complementation with the *R-9-29* alleles: Eight alleles of the *R-9-29* locus were found to be lethal when tested in various combinations among themselves. None of these are lethal when heterozygous with *Pas* or *shakB*^{2}. Some of these alleles complement the strong neural phenotype of the *shakB*^{2} and *Pas* mutations whereas most do not. In particular, two alleles, *L41* and *EF535*, which are homozygous lethal, have no noticeable effect on the GF response when heterozygous with either *shakB*^{2} or *Pas*. The two phenotypes can also be separated by the different deficiencies. Deficiency *16-3-35* uncovers both the lethal and neural phenotypes; deficiencies *A118* and *HC279* uncover only the neural phenotypes. Deficiency heterozygotes *16-3-35/A118* and *16-3-35/HC279* produce the extreme neural phenotype yet show no reduced viability. Thus, there are clearly two genetic functions in 19E3, one lethal and one neural, with complex complementation between them. The alleles and deficiencies discussed can be envisaged as forming three groups: (1) the lethal/non-neural group consists of alleles *L41* and *EF535* and is uncovered by *16-3-35*; (2) the viable/neural group consisting of *shaking-B*^{2} and *Passover* and is uncovered by *16-3-35*, *A118* and *HC279*; and (3) the lethal/neural group consists of *HM437*, *R-9-29*, *E81*, *EC201*, *17-189* and *17-360* each of which fails to complement alleles in both of the first two groups. Given that deficiency *16-3-35* uncovers the lethality of *R-9-29* alleles, and is distal to deficiencies *A118* and *HC279*, which uncover only the neural phenotype, we predict that the lethal function resides in a more distal part of 19E3 than the neural function.

Noncomplementation of mutations in the 19E3 and 19E5–6 regions: *trans*-Heterozygotes of deficiencies which both have breakpoints encroaching on the 19E3 area (*16-3-35/A118*, for example) strongly affect the pathways from the GF to *both* the DLM and TTM. *trans*-Heterozygotes of nonadjacent deficiencies of the 19E3 and 19E5–6 regions, such as *16-3-35/T2-14A*, affect the TTM pathway but have little or no effect on the DLM pathway of the GFS. Similarly the extreme mutant phenotype is obtained when the *Pas* or *shakB*^{2} alleles are made heterozygous with most alleles of the *R-9-29* locus, or with deficiencies abutting band 19E3, but only a mild neural phenotype occurs in

complementation tests with deletions removing bands 19E5-6.

The proximal boundary of the interacting region is set at 19E6 by deficiency *Q539* which deletes material proximal to this region. Deficiency heterozygotes *16-3-35/Q539*, *A118/Q539* and *T2-14A/Q539* have a wild-type electrophysiological phenotype, as do heterozygotes of 19E3 mutants with deficiency *Q539*. This lack of effect of the more proximal *X* chromosome regions on the electrophysiological phenotype is also borne out by the fact that both deficiency *17-489* and deficiency *A53* extend much more proximally than *T2-14A*, yet *16-3-35/17-489* or *16-3-35/A53* heterozygotes have a GF response similar to *16-3-35/T2-14A* heterozygotes. Thus, the material deleted by *17-489*, *A53* and *Q539* from the *vao* area in a proximal direction is not involved in the GFS phenotypes in question.

The distal boundary of the interacting region is at 19E5, since neither deficiencies *17-489*, *A53* nor *T2-14A* uncover the *R-9-28* locus in 19E4. Within the 19E5 to E6 region, the only mutations available are those of the *EC235* complementation group, and all the *EC235* lethal alleles we have tested fully complement the 19E3 mutants and deficiencies. Thus, if this interaction between 19E3 and 19E5-6 is due to a particular genetic locus in 19E5-6, it must either be a previously unknown complementation group between *R-9-28* and *EC235* or it is in the *lf-vao* region distal to deficiency *Q539*.

There is at least one complementation group (*R-9-28*) and there may be as many as three complementation groups (*R-9-28*, *EC235* and *lf*) between the two interacting regions.

The two regions interact in *cis*: The possibility that two separate genes in the two regions are involved is made unlikely by the results of our *cis-trans* test. A recessive mutation at either locus alone results in a normal phenotype, but in the double heterozygote recessive mutations at both loci cause a mutant phenotype. If the combined insufficiency of gene products at two independent loci were the underlying cause of the mutant phenotype, then it should not matter whether both loci are mutant on the same chromosome (*ab*/++) or on different chromosomes (*a*+/+*b*). We have used two large deletions, *B57* and *16-3-22*, each of which uncovers both the 19E3 and the 19E5–6 regions. Both *B57*/+ and *16-3-22*/+ individuals (which are of the *ab*/++ class) are electrophysiologically normal. Thus, the wild-type phenotype is observed when both regions are normal on at least one chromosome and the mutant phenotype is observed only when *both* homologs are mutant. The two regions must both be undisturbed in *cis*.

Possible mechanisms of interaction of the 19E3 and 19E5-6 regions: Several possibilities exist for the *cis*-interaction of the two separated regions. *T2-14A*, *17-489* and *A53* might *not* harbor a neural locus, but instead they may disrupt 19E3 expression due to their rearrangement of the chromosome. These proximal deficiencies may exert their influence by bringing new DNA sequences into juxtaposition with the *Pas* locus. The likelihood that this type of position effect is occurring is made less plausible because the four deletions have different proximal breakpoints (19E7-8, 20A1-2 and two different breaks in 20F; SCHALET and LEFEVRE 1976, A. Schalet, unpublished) thus they likely juxtapose different sequences, yet they produce the same electrophysiological effect. On the other hand, the proximal *X* is studded with repetitive sequences (MIKLOS *et al.* 1988) and it is conceivable that several different breaks do bring similar DNA sequences near to the *Pas* locus. If reduction in expression at 19E3 occurs due to a position effect, then a position effect might also be expected on the *R-9-28* locus since it is closer to the break points. However, we find no reduction in viability in at least one of these *R-9-28*/proximal deficiency heterozygotes (*T2-14A/R-9-28*, A. SCHALET, unpublished data).

The *Pas* gene may be split by an intervening gene (or genes) sitting in an intron (or introns) of *Pas*. Several examples of a gene within a gene have been identified in Drosophila, such as the tRNA genes in the *decapentaplegic* gene complex (GELBART *et al.* 1985) and a pupal cuticle protein gene in the *Gart* locus (HENIKOFF *et al.* 1986). With current evidence we can not distinguish between the possibilities of a split coding region and another form of split where there is a coding region at 19E3 and a long range interaction due to an enhancer or other control region in 19E5-6.

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LITERATURE CITED

BAIRD, D. H., 1986 Genetic mapping, alleles and gene dosage effects of *Passover*, a *Drosophila* mutant altering synaptic connections. Soc. Neurosci. Abstr. **12:** 1164.

BAIRD, D. H., 1988 Genetics of *Passover*, a *Drosophila* mutant affecting neuronal connectivity. Ph.D. thesis, Yale University.

EEKEN, J. C. J., F. H. SOBELS, V. HYLAND and A. P. SCHALET, 1985 Distribution of MR-induced sex-linked recessive lethal mutations in *Drosophila melanogaster*. Mutat. Res. **150:** 261–275.

EGGER, M. D., R. S. NOWAKOWSKI, B. PENG and R. J. WYMAN, 1989 Patterns of synaptic interactions within a peripheral nerve in *Drosophila*: in wildtype strains and in *Passover*. Soc. Neurosci. Abstr. **14:** 128.

GANETZKY, B., and C. F. WU, 1982 Indirect suppression involving behavioral mutants with altered nerve excitability in *Drosophila melanogaster*. Genetics **100:** 597–614.

GELBART, W. M., V. F. IRISH, R. D. JOHNSTON, F. M. HOFFMANN, R. K. BLACKMAN, D. SEGAL, L. M. POSAKONY and R. GRIMAILA, 1985 The decapentaplegic gene complex in *Drosophila melanogaster*. Cold Spring Harbor Symp. Quant. Biol. **50:** 119–125.

GERGEN, J. P., and E. WIESCHAUS, 1986 Dosage requirements for *runt* in the segmentation of *Drosophila* embryos. Cell **45:** 289–299.

GREEN, M. M., M. T. YAMAMOTO and G. L. G. MIKLOS, 1987 Genetic instability in *Drosophila melanogaster*: cytogenetic analysis of MR-induced X-chromosome deficiencies. Proc. Natl. Acad. Sci. USA **84:** 4533–4537.

HENIKOFF, S., M. A. KEENE, K. FECHTEL and J. W. FRISTROM, 1986 Gene within a gene: nested *Drosophila* genes encode unrelated proteins on opposite DNA strands. Cell **44:** 33–42.

HOMYK, T., JR., J. SZIDONYA and D. T. SUZUKI, 1980 Behavioral mutants of *Drosophila melanogaster*. I. Isolation and mapping by direct visual observations of behavioral phenotypes. Mol. Gen. Genet. **177:** 553–565.

KEPPEL, G., 1982 *Design and Analysis, A Researcher's Handbook*, Ed. 2. Prentice-Hall, Engelwood Cliffs, N.J.

KING, D. G., and M. A. TANOUYE, 1983 Anatomy of motor axons to direct flight muscles in *Drosphila*. J. Exp. Biol. **105:** 231–239.

KING, D. G., and R. J. WYMAN, 1980 Anatomy of the giant fiber pathway in *Drosophila*. I. Three thoracic components of the pathway. J. Neurocytol. **9:** 753–770.

KOTO, M., 1983 Morphology of giant fiber system neurons in wild-type and mutant *Drosophila melanogaster*. Ph.D. thesis, Yale University.

KOTO, M., M. A. TANOUYE, A. FERRUS, J. B. THOMAS and R. J. WYMAN, 1981 The morphology of the cervical giant fiber neuron of *Drosophila*. Brain Res. **221:** 213–217.

KRAMERS, P. G. N., A. P. SCHALET, E. PARADI and L. HUISER-HOOGTEYLING, 1983 High proportion of multi-locus deletions among hycanthone-induced X-linked recessive lethals in *Drosophila melanogaster*. Mutat. Res. **107:** 187–201.

LEFEVRE, G., and W. WATKINS, 1986 The Question of total gene number in *Drosophila melanogaster*. Genetics **113:** 869–895.

LIFSCHYTZ, E., and R. FALK, 1968 Fine structure analysis of a chromosome segment in *Drosophila melanogaster*: analysis of x-ray induced lethals. Mutat. Res. **6:** 235–244.

LIFSCHYTZ, E., and R. FALK, 1969 Fine structure analysis of a chromosome segment in *Drosophila melanogaster*: analysis of ethyl methane sulphonate-induced lethals. Mutat. Res. **8:** 147–155.

LIFSCHYTZ, E., and N. YAKOBOVITZ, 1978 The role of X-linked lethal and viable male-sterile mutations in male gametogenesis of *Drosophila melanogaster*: genetic analysis. Mol. Gen. Genet. **161:** 275–284.

LINDSLEY, D. L., and G. ZIMM, 1986 The genome of *Drosophila melanogaster*. Part 2: lethals, maps. Drosophila Inform. Serv. **64.**

LINDSLEY, D. L., and G. ZIMM, 1990 The genome of *Drosophila melanogaster*. Part 4: Genes L-Z; balancers, transposable elements. Drosophila Inform. Serv. **68.**

MIKLOS, G. L. G., L. E. KELLY, P. E. COOMBE, C. LEEDS and G. LEFEVRE, 1987 Localization of the genes *shaking-B, small optic lobes, sluggish-A, stoned* and *stress-sensitive-C* to a well-defined region on the X-chromosome of *Drosophila melanogaster*. J. Neurogenet. **4:** 1–19.

MIKLOS, G. L. G., M. T. YAMAMOTO, J. DAVIES and V. PIRROTTA, 1988 Microcloning reveals a high frequency of repetitive sequences characteristic of chromosome 4 and the β-heterochromatin of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **85:** 2051–2055.

PARADI, E., E. W. VOGEL and E. SZILAGYI, 1983 Effect of storage and dose on MMS-induced deletions: complementation analysis of X-chromosomal recessive lethals in the *zeste-white* and *maroon-like* regions of *Drosophila melanogaster*. Mutat. Res. **111:** 145–159.

PERRIMON, N. D., D.SMOUSE and G. L. G. MIKLOS, 1989 Developmental genetics of loci at the base of the *X* chromosome of *Drosophila melanogaster*. Genetics **121:** 313–331.

SALKOFF, L., and R. WYMAN, 1981 Genetic modification of potassium channels in *Drosophila Shaker* mutants. Nature **293:** 228–230.

SCHALET, A. P., 1986 The distribution of and complementation relationship between spontaneous X-linked recessive lethal mutation recovered from crossing long-term laboratory stocks of *Drosophila melanogaster*. Mutat. Res. **163:** 115–144.

SCHALET, A., and G. LEFEVRE, 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 G. LEFEVRE, 1976 The proximal region of the *X* chromosome, pp. 845–902 in *The Genetics and Biology of Drosophila*, Vol. 1b, edited by M. ASHBURNER and E. NOVITSKI. Academic Press, New York.

SWAIN, G. P., R. J. WYMAN and M. D. EGGER, 1990 A Deficiency Chromosome in *Drosophila* alters neuritic projections in an identified motoneuron. Brain Res. (in press).

TANOUYE, M. A., and D. G. KING, 1983 Giant fiber activation of direct flight muscles in *Drosophila*. *J. Exp. Biol.* **105:** 241–251.

TANOUYE, M. A., and R. J. WYMAN, 1980 Motor outputs of the giant nerve fiber in *Drosophila*. *J. Neurophysiol.* **44:** 405–421.

THOMAS, J. B., 1980 Mutations affecting the giant fiber system in *Drosophila*. *Neurosci. Abstr.* **6:** 742.

THOMAS, J. B., and R. J. WYMAN, 1982 A mutation in *Drosophila* alters normal connectivity between two identified neurones. *Nature* **298:** 650–651.

THOMAS, J. B., and R. J. WYMAN, 1984 Mutations altering synaptic connectivity between identified neurons in *Drosophila*. *J. Neurosci.* **4:** 530–538.

WINER, B. J., 1971 *Statistical Principles in Experimental Design*, Ed. 2. McGraw-Hill, New York.

WYMAN, R. J., and J. B. THOMAS, 1983 What genes are necessary to make an identified synapse? *Cold Spring Harbor Symp. Quant. Biol.* **48:** 614–652.

WYMAN, R. J., J. B. THOMAS, L. SALKOFF and D. G. KING, 1984 The *Drosophila* giant fiber system, pp. 133–161 in *Neural Mechanisms of Startle Behavior*, edited by R. EATON. Plenum Press, New York.

WYMAN, R. J., J. B. THOMAS, L. SALKOFF and W. COSTELLO, 1985 The *Drosophila* thorax as a model system for neurogenetics, pp. 513–535 in *Model Neural Networks and Behavior*, edited by A. I. SELVERSTON. Plenum Press, New York.

YAMAMOTO, M.-T., and G. MIKLOS, 1987 Cytological analysis of deficiency *16-3-35* at the base of the *X* chromosome of *Drosophila melanogaster*. Drosophila Inform. Serv. **66:** 154.

ZUSMAN, S., D. COULTER and J. P. GERGEN, 1985 Lethal mutation induced in the proximal X-chromosome of *Drosophila melanogaster* using P-M hybrid dysgenesis. Drosophila Inform. Serv. **61:** 217–218.

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