THE BEHAVIOR OF FOUR NEUROLOGICAL MUTANTS OF DROSOPHILA*

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SINGLE gene changes offer an efficient and attractive way to study the genetic control of behavior. Drosophila, with its numerous technical advantages, would seem to provide a fruitful approach in working out the complexities of neurological control. The four neurological mutants discovered serendipitously and described in this report present just such an opportunity.

The phenotype common to all four is a rapid shaking of the legs following etherization. Because the mutants appeared, among the progeny of four different males of the original thirty treated, they represent four independent mutational events. Subsequent study has disclosed that three separate gene loci are involved, all on the *X*-chromosome.

This shaking phenotype has been described previously in *D. melanogaster* by CATSCH (1944) who localized the dominant gene at 58 on the *X*-chromosome and named the mutant, Shaker, because of its resemblance to the sex-linked dominant in *D. funebris* (LUERS 1936). Since then three other occurrences of the Shaker phenotype in *D. melanogaster* have been reported, (NOVITSKI 1949; FAHMY and FAHMY 1959) all produced by a gene change at the original Shaker locus. To our knowledge, however, these stocks no longer exist nor were the studies carried beyond the description of the phenotype and the localization of the gene.

MATERIALS AND METHODS

Subjects: Drosophila melanogaster stocks were grown on corn meal, sucrose, yeast and agar medium at 25°C, in constant light. The neurological mutants were produced feeding adult Canton-S (C-S) males upon a 0.025 M solution of ethyl methane sulfonate (EMS) in 1% sucrose (LEWIS and BACHER 1968). After treatment males were mated individually to FM6 *w dm*+ (FM6K) females in order to measure the frequency of induced sex-linked recessive lethals (GRELL and LEWIS 1956; KIDD 1966). FM6, a multiply-inverted *X*-chromosome, is recombinationally isolated from its homologue so that, as in this case, the treated homologous *X*-chromosome may be carried in crosses without losing its identity. FM6 carries the recessive marker yellow and the dominant marker for Bar eye. The modification, FM6K, adds the recessive gene for white eye and the replacement of *dm* (diminutive) with its wild-type allele, *dm*+, permits the use of homozygous females which otherwise would be sterile. The particular FM6K stock used in these experiments had previously been Cantonised; the autosomes, except for the fourth chromosome, had been replaced, through a series of controlled crosses, by Canton-S chromosomes. This enabled us to finish the experiment with mutant stocks identical to Canton; except for possible mutations in retained treated chromosomes.

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The method of measuring the frequency of sex-linked recessive lethals is, in principle, the Muller-5 technique (SPENCER and STERN 1948), except that the FM6K chromosome was used in place of Muller's Basc chromosome since FM6K is a more efficient and stable balancer. The frequency of induced sex-linked lethals is scored in the F_2 generation, in which each vial represents a treated Canton-S *X*-chromosome. In order to detect the kind of mutation for which the experiment was originally designed we had to examine the contents of each F_2 vial, at which time the shaker stocks were noted. In some 3000 F_2 vials four separate cultures were shakers. An isogenic stock was derived, subsequently, from a single male in each instance.

The gene loci were mapped using the multiply-marked chromosome *y cv v f car* for two of the mutants, Shaker^5 (*Sh*^{5}) and Hyperkinetic^{2T} (*Hk*^{2T}), and *sc ec cv ct v g f* for the other two, Hyperkinetic^{1P} (*Hk*^{1P}) and Ether à go-go (*Eag*). The ordering of the marker genes is from LINDSLEY and GRELL (1968).

Throughout the text, wherever the term, shaker, with a small "s" is used, it refers to all four mutants.

Etherization: The etherizers used consisted of ½ pint milk bottles containing about 7.5 grams of absorbent cotton packed tightly at the bottom, and a perforated plastic vial at the end of a metal funnel which is fitted tightly to the mouth of the bottle. About 2 ml of diethyl ether were poured on the cotton at the start of the day, with fresh ether added as necessary. Flies were shaken directly from culture bottles into the etherizer and held in the vial for about 20 sec.

Behaviorial assays on unetherized flies:

Activity level based on buzzing events: For each genotype, 12 males and 13 females, whose average age at collection was 6 hrs, were placed in an empty glass vial, 83 × 31 mm, closed with a fine nylon net. The vial was inverted upon a microphone within a sponge rubber-lined glass jar. This, in turn, was placed within a large sponge rubber-lined jar and covered with a box. This arrangement effectively provided, for recording the activity of flies, an environment from which external light and sound were excluded. After a three-minute relaxation period, the number of buzzing events which occurred over a ten-minute period was counted by ear using a simple amplifier and headphone. Activity was scored over a period of seven days at roughly the same time each day, the actual starting time varying from 1:00 to 3:00 PM. The several genotypes were scored separately and in the same sequence each day. In the tests reported here the flies were etherized only once, on the first day after their emergence.

Kinetogenic response: When rapid passes are made by a hand about one inch above a vial containing flies, *Hk*^{1P} and *Hk*^{2T} flies fall over on their backs, rolling about in a completely disorganized fashion, which might be described as a "kinetogenic seizure." To measure the response, flies of known age were placed individually, without etherization, into an empty vial stoppered by an absorbent cotton plug. The vial was placed on its side on the laboratory bench, upon a white background. After a one-minute relaxation period, the number of times the fly responded to the motion of the hand in a total of 50 passes was recorded. Passes were made at the rate of 1 per sec except that, as necessary, time was allowed for the fly to right itself completely following a positive response. At the end of the trial each fly was returned to a fresh food vial to be used the next day.

Fly speck deposition: A second way in which response to motion was measured was by comparing two groups of flies, one beneath a rotating vane, the other below a stationary one, with respect to the number of fly specks produced during a half-hour period. A moving vane elicits the same response in *Hk*^{1P} and *Hk*^{2T} as does the moving hand.

To do this, vials containing 25 males and 25 females were arranged, on their sides, as spokes of a wheel under a vane 15 cm long and 30° arc, rotating once per second 9 cm above the vials. To collect the specks, a strip of aluminum foil 2 × 8 cm was inserted into the vial so that a marked 2 × 4 cm portion, shiny side up, was between the cotton plug and the food, forming the floor of the vial. Two vials of each stock were prepared. One, group A, was subjected to the moving vane for 30 min ("disturbed") after which the foil was changed and specks were collected during a second 30-minute period without movement of the vane ("undisturbed"). For the other vial of each stock, group B, the order was reversed, first being "undisturbed" then

"disturbed." The first day's test was begun at 7:30 PM; on subsequent days at 3:00 PM. The flies were transferred to new food vials after each day's testing.

Phototactic Response: The responses to and from light were measured for Hk^{1P}, Sh^5 and our Canton-S stock. These measurements were made at the California Institute of Technology in the laboratory of Professor S. BENZER and were carried out according to the countercurrent distribution technique devised by him (BENZER 1967).

RESULTS

Localization of the genes: When the contents of the four F_2 vials, in which the mutants were discovered, were examined after etherization under a dissecting microscope, the FM6K males and females lay quite still, whereas the Canton males and heterozygous Canton/FM6K females were shaking. This observation indicated that a dominant mutation had occurred in the Canton *X*-chromosome. Sex-linkage was confirmed by crossing males of each stock to attached-X females. In all cases only the sons were shakers. Table 1 gives the results of mapping the four mutants. Only one of the four is at the Shaker locus, 58.1, and has accordingly been called Shaker^5. The others represent new loci.

Comparison of the phenotypes: Shaker^5 shakes so vigorously that movement among the etherized flies may be observed with the naked eye. The leg shaking is jerky and erratic. In addition, etherized males and females show a strong scissoring of the wings, one of the elements of courtship behavior (MANNING 1959). Their abdomens twitch, but not in a regularly rhythmic pattern.

Hyperkinetic^{1P} and Hyperkinetic^{2T} are vigorous leg shakers, Hk^{1P} being somewhat more vigorous than Hk^{2T}. Their shaking, in contrast with Sh^5, is relatively steady and rhythmic and the activity may be seen with the naked eye. However, they do not show wing scissoring. For reasons given below the Hk^{1P} and Hk^{2T} genes are regarded as alleles.

Ether à go-go is the least vigorous shaker of the four, but it is readily distinguishable from normal flies. It resembles the Hyperkinetic mutants in its

TABLE 1

Localization of mutant genes on X-chromosome

Genotype	Number of offspring counted	Number of crossovers between:		Recombinational fraction between mutant and closer of two markers	Position on *X*-chromosome
Hyperkinetic^{1P}	2431	*ct* and Hk^{1P}	262	2.1	30.9 ± 0.6*
		Hk^{1P} and *v*	51		
Hyperkinetic^{2T}	2027	*cv* and Hk^{2T}	333	2.6	30.4 ± 0.7*
		Hk^{2T} and *v*	52		
Shaker^5	1770	*f* and Sh^5	24	1.4	58.2 ± 0.6*
		Sh^5 and *car*	69		
Ether à go-go	2479	*g* and *Eag*	139	5.6	50.0 ± 1.0*
		Eag and *f*	179		

* Limits of recombinational fraction corresponding to level of significance of one in twenty. Based upon method of STEVENS (1942).

rhythmicity and the lack of wing activity. There is only an occasional twitching of the abdomen.

Analysis of the movement pattern is difficult by ordinary means since the shaking is so rapid. It appears that a rapid shaking of the tarsus is superimposed upon a less rapid movement of femur and tibia.

No morphological changes associated with the shaker phenotype have been detected, nor are there any observable sex differences.

Behavior of hybrids: Female hybrids of all four shakers and Canton-S show that the shaking is dominant, but incompletely so, the leg-shaking being less vigorous than in the respective mutant homozygotes. This applies also to the wing scissoring effect of Sh^5.

Other anesthetizing agents: We have observed the effects of chloroform and ethyl acetate upon shaker flies under the same conditions as those used for etherization. The shaking was not observed. However, for valid comparisons, the partial pressures of the agents to which the flies were exposed must be controlled, since it is known that for a given exposure period a compound can be inert, anesthetic, or lethal, within narrow limits of partial pressure (CHERKIN 1968).

Behavioral assays on unetherized flies: (a) Buzzing activity level. There are obvious differences in behavior between shaker and wild-type flies. When stock bottles are allowed to stand on the laboratory bench, wild type flies settle; some feed on the food surface, others rest on the sides of the bottle or on the cotton stopper. There is little moving about except for the tandem courtship pairs gliding through the population. On the other hand, in a bottle of shaker flies, tremors of activity move through the population starting at one point and spreading as a chain reaction. This kind of activity results in numerous short flights.

When their bottles are pounded upon the laboratory bench, the wild-type flies fly about and then quickly settle, but shaker flies fly first in one direction then another, or fall over on their backs and roll over in attempts to right themselves. They require a longer time to settle to their former level of activity than do the wild-type flies.

These observations led us to investigate in more detail the level of activity of undisturbed, unetherized Canton and shaker flies, as defined by the number of buzzing events recorded over a measured period of time.

Table 2 summarizes the data showing the relation of age to the number of buzzing occurrences per minute of 25 flies. All the shakers are more active than Canton by this test, Hk^{1P} and Sh^5 being the most active genotypes. The activity increases with age of the flies, reaching a plateau near five days.

Since light-adapted Drosophila have been shown to be phototactically neutral to red light (FINGERMAN and BROWN 1953), we were able to observe them in the darkroom to determine the activity which contributed to the recorded buzzes. Although shakers made constant restless movements, we found that only flies in flight made the buzzes recorded in this experiment, and that each separate buzzing occurrence may represent the activity of one or many flies since it appears that the flight of one fly may set off activity in others. Indeed, preliminary experi-

TABLE 2

Buzzes per 25 flies/minute in relation to age of adults (wild type and shakers)

Genotype		Age in days 1	2	3	4	5	7	16	17
Canton	$\bar{x}$	2.3	10.4	12.0	11.4	27.2	13.4	2.9	15.2
	±SE	0.5	1.0	1.1	1.1	1.6	1.2	0.5	1.2
Hk^{1P}	$\bar{x}$	8.1	41.8	71.6	84.7	90.8	100.2	83.6	86.1
	±SE	0.9	2.0	2.7	2.9	3.0	3.1	2.9	2.9
Hk^{2T}	$\bar{x}$	0.6	13.3	30.9	46.8	57.1	41.7	61.0	50.4
	±SE	0.2	1.1	1.7	2.2	2.4	2.0	2.5	2.2
Eag	$\bar{x}$	2.2	11.1	31.7	39.8	47.8	25.0	17.6	27.7
	±SE	0.5	1.1	1.8	2.0	2.2	1.6	1.3	1.7
Sh^{5}	$\bar{x}$	1.6	43.1	74.4	91.6	109.2	67.6	87.8	105.0
	±SE	0.4	2.1	2.7	3.0	3.3	2.6	3.0	3.2

ments (KAPLAN and TROUT 1968) indicate that this test measures both activity and reactivity as defined by EWING (1963).

Note that *Hk^{1P}* and *Hk^{2T}* differ markedly; the differences are statistically significant. (For each day the difference between the two mutants is at least six times greater than its standard error; $P < 0.001$.) *Hk^{2T}* is consistently the lower in activity both in this instance and in other parameters we have measured. The two genes are on the X-chromosome in the region between 30 and 31 and are possibly allelic. The likelihood of their allelism is strengthened by the data derived from measurements of shaker hybrids presented in Tables 3 and 4. (In Table 3 the number of buzzes is based upon 10 flies per min.) *Hk^{1P}/Hk^{2T}* hybrids are more active than the other hybrids with *Hk^{1P}* or *Hk^{2T}*. (In each case the difference between *Hk^{1P}/Hk^{2T}* and the other hybrids is at least 3.6 times greater than its standard error; $P < 0.003$.) Moreover, the activity of *Hk^{P}/Hk^{2T}* lies midway between *Hk^{1P}/Hk^{1P}* and *Hk^{2T}/Hk^{2T}*. (With respect to both homozygotes the differences between them and *Hk^{1P}/Hk^{2T}* are significant on all days at the 0.1% level of significance except in the case of *Hk^{1P}/Hk^{1P}* on day 6 where significance is attained at the 2% level.)

(*b*) *Kinetogenic response.* While working with shaker mutants in the laboratory it was observed that movement of a hand near a bottle containing *Hk^{1P}* flies

TABLE 3

Buzzes per 10 flies/min
(Age 2–3 days) shaker/shaker hybrids

	Genotypes *Hk^{1P}/Hk^{2T}*	*Hk^{1P}/Eag*	*Hk^{1P}/Sh^{5}*	*Hk^{2T}/Eag*	*Hk^{2T}/Sh^{5}*	*Sh^{5}/Eag*
1)*	19.0 ± 1.4	12.4 ± 1.1	6.8 ± 0.8	7.6 ± 0.9	2.6 ± 0.5	10.0 ± 1.0
2)*	23.0 ± 1.5	11.0 ± 1.1	8.4 ± 0.9	9.9 ± 1.0	10.7 ± 1.0	12.4 ± 1.1

* Two measurements made on same day; 1 at 1 PM, 2 at 6 PM.

TABLE 4

Buzzes per 25 flies/min
Comparison of Hk^{1P}/Hk^{2T} *with* Hk^{1P} *and* Hk^{2T} *homozygotes*

Genotype	Age in days 1	2	3	4	6
Hk^{1P}/Hk^{1P}	29.6 ± 1.7	39.4 ± 1.9	88.6 ± 3.0	125.5 ± 3.5	127.0 ± 3.6
Hk^{1P}/Hk^{2T}	16.2 ± 1.3	23.2 ± 1.5	47.8 ± 2.2	81.6 ± 2.9	116.5 ± 3.4
Hk^{2T}/Hk^{2T}	1.2 ± 0.4	13.4 ± 1.2	23.6 ± 1.5	33.6 ± 1.8	70.0 ± 2.6

results in a great deal of activity. Wild-type flies rarely respond in this fashion. To measure this response, flies were tested individually in vials by passing a hand above the vial and recording how often the fly jumped or fell over. Table 5 records the data for all shakers, doubly heterozygous shakers, and shaker/Canton hybrids. Since hybrids are genetically female the table shows the response of females only. Canton, Hk^{1P} and Hk^{2T} males have been tested but no statistically significant differences exist between males and females of the respective genotypes.

Both Hk^{1P} and Hk^{2T} respond markedly, the response increasing with age, the former responding to a greater degree than the latter. (χ^2 determinations by means of 2×2 contingency tables, df = 1, indicate that on the five days when testing corresponded, the differences were significant at the 1% level of significance.) Unlike other aspects of the behavior that have been measured this is a recessive component of the phenotype since it disappears in all hybrid combinations except Hk^{1P}/Hk^{2T}. In this case the response was as great as the more responsive homozygote, Hk^{1P}. In other parameters Hk^{1P}/Hk^{2T} lay between the two homozygotes. Thus, these two mutants appear to be non-complementing.

The mean jumping response of Sh^5 and *Eag* is, on some days, slightly higher than the control level, usually because only one or several flies jumped occasionally up to 23 times in 50 hand-waves. This behavior was not consistent from day to day, suggesting that although Sh^5 and *Eag* are capable of exhibiting an unmistakable kinetogenic response, the penetrance is marginal and irregular under these experimental conditions.

(*c*) *Fly speck deposition:* A possibly more sensitive test of the response to movement is suggested by the work on emotional behavior in rats, namely, frequency of defecation (HALL 1934). The data derived from experiments designed to measure fly speck deposition, indeed indicate that shaker flies show such a response (Table 6).

The ratios of the number of fly specks deposited during the time the flies were disturbed (D) to the number deposited during the time they were undisturbed (U) have been computed for groups A and B, the difference being that the flies in the A vials were first undisturbed, and then disturbed during the second half-hour period, while the flies in group B were first disturbed and then left undisturbed during the second period.

In every instance the D/U ratios for Hk^{1P} and Hk^{2T} are higher than one and, in all cases but one, the differences between these two mutants and Canton are

TABLE 5

Response to hand movement in relation to age
(Mean positive responses in 50 trials ± SE)

Genotype	Number of flies	Day	1	2	3	4	5	6	7	8	9	10	11
Canton ♀	15	$\bar{x}$	0.0	0.5	..	..	..	0.3	..	0.3	..	0.3	..
		±SE	..	0.2	..	..	..	0.1	..	0.2	..	0.2	..
Hk^{1P} ♀	15		30.7	36.6	35.5	43.7	..	44.8	..	43.3	..	40.8	..
			2.2	1.4	2.1	1.4	..	0.8	..	1.5	..	2.1	..
Hk^{2T} ♀	14		1.7	11.3	14.7	..	14.9	24.3	25.0	20.2	..	..	..
			0.7	2.9	2.0	..	3.6	4.6	3.5	4.2	..	..	..
Sh^{5} ♀	15		1.1	0.9	5.7	..	9.2	..	2.3	0.9	..	..	..
			0.5	0.3	1.5	..	1.7	..	0.6	0.3	..	..	..
Eag ♀	15		0.3	0.7	0.7	0.7	0.2	..	0.4	..	2.2	..	0.9
			0.2	0.6	0.6	0.6	0.1	..	0.2	..	0.8	..	0.5
Hk^{1P}/*Hk*^{2T}	15		11.1	20.0	37.9	..	..	42.7	42.9	43.6	44.3	..	..
			1.8	3.0	1.4	..	..	0.9	0.7	0.7	0.8	..	..
Hk^{1P}/C	10		0.0	0.1	0.3	0.6	..	..	0.2	0.6	2.0	0.0	..
			..	0.1	0.1	0.4	..	..	0.2	0.4	1.1	..	..
Hk^{2T}/C	10		0.0	0.1	0.1	..	0.5	0.8	..	0.1	0.2	..	0.8
			..	0.1	0.1	..	0.2	0.7	..	0.1	0.2	..	0.4
Sh^{5}/C	10		0.0	0.2	0.1	0.4	..	..	1.5	0.3	..	1.0	0.8
			..	0.2	0.1	0.2	..	..	0.7	0.2	..	0.6	0.7
Eag/C	10		0.0	0.1	0.2	0.1	..	0.5	..	0.8	..	0.3	0.3
			..	0.1	0.2	0.1	..	0.3	..	0.8	..	0.3	0.3
Hk^{1P}/*Sh*^{5}	10		0.0	0.5	..	2.2	..	..	3.4	1.5	1.3	1.5	..
			..	0.3	..	1.0	..	..	1.0	0.7	0.4	0.6	..
Hk^{2T}/*Sh*^{5}	10		0.3	0.1	..	0.5	..	..	0.8	0.9	0.1	0.4	..
			0.3	0.1	..	0.2	..	..	0.5	0.4	0.1	0.3	..
Hk^{1P}/*Eag*	10		0.0	0.0	..	0.8	..	..	1.6	1.1	0.1	..	0.2
			..	..	..	0.5	..	..	0.3	0.5	0.1	..	0.1
Hk^{2T}/*Eag*	10		0.0	0.7	..	1.8	..	..	0.3	1.4	0.7	..	0.3
			..	0.6	..	0.4	..	..	0.3	0.5	0.3	..	0.1

significant at at least the 5% level. The exceptional case is day 2 of the B vials where, for Canton as well as shaker, the number of specks deposited during the U period is extremely low. Therefore, although the shaker ratios are high, statistical significance is not attained. The conditions responsible for this atypical pattern are unknown.

Sh^{5}, which is a very vigorous shaker, differs significantly from Canton in its fly speck response in only 3 cases out of 7 among the A vials, and 3 cases out of 7 among the B vials. *Eag* is significantly different from Canton on five days in each of the two series. However, in all cases but one, *Eag* on day 1, the differences are in the same direction.

Increased fly speck deposition in response to movement seems to be related to kinetogenic seizures since the same two mutants respond markedly in both ways. In the case of kinetogenic seizures, although *Sh*^{5} and *Eag* showed low levels of response, several individuals responded at a fairly high level. The fly speck response, however, appears to be more penetrant and under appropriate con-

TABLE 6

Fly specks deposited by 50 flies in 30 min when undisturbed (U) and disturbed (D) by rotating vane

	Vial A U then D		Vial B D then U		Vial A Ratio D/U	Vial B Ratio D/U		Vial A U then D		Vial B D then U		Vial A Ratio D/U	Vial B Ratio D/U
Day 0							*Day 4*						
Canton	44	35	27	47	0.8	0.6	Canton	40	52	48	31	1.3	1.5
Hk^{1P}	73	102*	78	29**	1.4	2.7	*Hk^{1P}*	16	71**	121	0**	4.4	. .
Hk^{2T}	64	96*	94	47**	1.5	2.0	*Hk^{2T}*	57	134*	212	7**	2.4	30.3
Sh^{5}	53	47	82	36**	0.9	2.3	*Sh^{5}*	48	75	87	11**	1.6	7.9
Eag	70	89*	93	40**	1.3	2.3	*Eag*	25	84**	94	30*	3.4	3.1
Day 1							*Day 5*						
Canton	56	49	54	25	0.9	2.2	Canton	42	25	22	17	0.6	1.3
Hk^{1P}	51	76*	115	21**	1.5	5.5	*Hk^{1P}*	19	29*	55	1**	1.5	55.0
Hk^{2T}	32	74**	173	29**	2.3	6.0	*Hk^{2T}*	74	74*	201	1**	1.0	201.0
Sh^{5}	27	27	40	18	1.0	2.2	*Sh^{5}*	29	72**	35	21	2.5	1.7
Eag	107	74	94	21*	0.7	4.5	*Eag*	29	85**	42	43	2.9	7.0
Day 2							*Day 6*						
Canton	27	7	34	5	0.3	6.8	Canton	55	26	36	34	0.5	0.7
Hk^{1P}	21	36**	68	11	1.7	6.2	*Hk^{1P}*	19	58**	81	3**	3.1	6.3
Hk^{2T}	17	78**	88	7	4.6	12.6	*Hk^{2T}*	55	110**	87	2**	2.0	43.5
Sh^{5}	6	42**	51	2	7.0	25.5	*Sh^{5}*	30	19	16	18	0.6	0.7
Eag	47	53**	105	7	1.1	15.0	*Eag*	25	19	80	25**	0.8	3.2
Day 3													
Canton	50	31	37	59	0.6	0.6							
Hk^{1P}	34	42*	92	2**	1.2	46.0							
Hk^{2T}	91	97*	173	4**	1.1	43.2							
Sh^{5}	41	61**	51	30**	1.5	1.7							
Eag	34	99**	141	18**	2.9	7.8							

* $P<0.05$.
** $P<0.01$.

Statistical evaluations of differences between shakers and Canton in number of deposited fly specks are based upon χ^2 determinations by means of 2×2 contingency tables with one degree of freedom.

ditions may be shown to be a measurable component of the shaker syndromes.

In a number of cases the shaker D/U ratios for group B are greater than for A, possibly because a disturbed shaker may deposit so much fecal material that there is little available for the following undisturbed period. That this may be so is suggested by the results of a second experiment, summarized in Table 7. In this case only males of *Hk^{1P}* and C-S were tested and were nine days old at the time of testing. The flies were divided into two groups, each group consisting of three vials of 30 C-S males each, and four vials of 30 *Hk^{1P}* males per vial. All the flies in Group I were subjected to a regimen of three consecutive 30 min periods during which they were disturbed by the rotating vane. At the same time, the flies of Group II experienced a U–D–U regimen. Fewer specks were deposited each succeeding time by shaker flies in Group I, but the normal flies remained

TABLE 7

Number of fly specks deposited per 30 min period
(Average of 4 vials (3 for Canton))

Group I	D	D	D
Hk^{1P}	289/4 = 72	157/4 = 39	92/4 = 23
Canton	59/3 = 20	64/3 = 21	66/3 = 22
Group II	U	D	U
Hk^{1P}	40/4 = 10	347/4 = 87	69/4 = 17
Canton	64/3 = 21	72/3 = 24	89/3 = 30

constant, apparently not losing enough specks to affect later trials. The response level of Canton-S flies in Group II remained constant through the three periods also, whereas the response of Hk^{1P} flies during the D period is markedly elevated above the two U periods. The falling off of speck production in the case of shaker flies is clearly, in itself, a measure of the response. Loss of fecal material may not be the only explanation for the fall in production. Responses in optic nerves have been obtained in crabs and crayfish when an object moved with regard to the background. These fibers have been shown to habituate (WIERSMA 1967). Thus, the fall in fly speck production may represent an habituation phenomenon. These are matters for future investigation.

(*d*) *Phototactic response:* Hk^{1P}, Sh^{5} and the C-S stock from which they arose were compared for their phototactic behavior. All behaved as expected of normal, positively phototactic flies as described by BENZER (1967) in both to-light and from-light responses.

DISCUSSION

The phenotype common to the four mutant stocks described is a rapid leg-shaking following etherization. The physiological basis for the shaking is unknown. A much smaller degree of shaking may sometimes be observed in wild-type and mutant stocks of *Drosophila melanogaster*. Thus, possibly, a normally present potentiality has become exaggerated in the mutants. This might be effected by overstimulation of the muscles controlling the shaking or by the removal of a normally present inhibitory influence.

Although all the mutants are similar in phenotype because they shake, there are qualitative and quantitive differences between them in both their conscious and their anaesthetized behavior. For example, only Sh^{5} shows wing scissoring and only Hk^{1P} and Hk^{2T} show a consistent kinetogenic response. Since more than one gene locus is involved among the four mutants, and since the mutants do not form a graded series of responses with respect to all parameters, it does not seem likely that the basic flaw is the same in all cases.

Hk^{1P} and Hk^{2T} represent an alteration of the same gene product. They exhibit a similar spectrum of responses, are possibly at the same genetic locus, and are non-complementing. The differences between them are quantitative and always in the same direction. In this case, the differences may reflect different concentrations of the same gene product, or different alterations of the same gene.

Although the kinetogenic response of Hk^{1P} and Hk^{2T} is recessive, shaking shows incomplete dominance. This situation, in which different aspects of a syndrome may show different dominance relations is well-known in Drosophila, where, for example, the lethality of the Notch series of phenotypes is recessive, whereas the incising of the wings is dominant (LINDSLEY and GRELL 1968). It is analogous also to that of sickle cell anemia in man, where the clinical symptoms of the anemia are recessive, but the presence of an unaltered protein and the *in vitro* sickling of red blood cells can be detected in heterozygotes (NEEL 1949 and PAULING *et al.* 1949). With respect to the kinetogenic response, we are still in the stage of being able to observe only the "clinical symptoms" of the affected homozygote. Identification of a biochemical abnormality associated with the behavior may more accurately define the heterozygote. In this connection, a study of fly speck deposition by shaker hybrids may prove informative since the deposition is a more sensitive measure of the response to movement than the kinetogenic response.

The most comprehensive work on the heritability of visceral reactivity is HALL's on emotional elimination in the rat. HALL (1934) and BROADHURST (1958) both regard this as a valid method of measuring general emotionality in rats but others have questioned its general significance (HUNT and OTIS 1953). However, without making any assumptions concerning the psychological significance of defecation, it was established by HALL's work that visceral reactivity is inherited.

This kind of response has not previously been studied in insects but it is clear that the visceral reactivity of flies may be modified, as in the case of these four mutants, by a single gene change. The D/U ratios for Canton flies are rarely more than one which suggests that wild-type flies have an inherited mechanism for maintaining their coordination when disturbed by sudden movements, a stability which seems to have been lost in shaker flies.

Hopefully, an understanding of the problems outlined will come from further studies of these mutants. We plan to use the shakers to investigate the biochemical and neurophysiological changes that have been brought about in these four instances, each by a single gene change.

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SUMMARY

Four sex-linked mutants have been produced in *Drosophila melanogaster* following the treatment of adult males by ethyl methane sulfonate. The phenotype common to all is a rapid shaking of the legs following etherization, but other aspects of both anesthetized and conscious behavior distinguish them from each other and from wild-type flies. Three independent gene loci are involved.

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