BEHAVIORAL MUTANTS OF *DROSOPHILA MELANOGASTER*. I. ISOLATION AND MAPPING OF MUTATIONS WHICH DECREASE FLIGHT ABILITY[1]

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ABSTRACT

A flight test box was developed and used in the isolation and initial characterization of *Drosophila melanogaster* mutants defective in flight behavior. Forty-eight mutants were isolated from F_1 progeny of ethyl methanesulfonate-treated males. Genetic mapping and complementation tests show that the mutations reside at thirty-four different sites on the *X* chromosome. Different mutants show different degrees of flight ability compared to controls. Forty-six mutations are recessive, while two appear to be semi-dominant with respect to flight behavior. In addition to flight defects, five mutants have visible defects, five behave as temperature-sensitive lethals and three exhibit abnormal electroretinograms. Alleles of each of the previously known behavioral mutations, Hyperkinetic, ether à go-go and Shaker were found. Preliminary studies also suggest that the flight behavioral phenotype of mutations at seven sites is affected by the temperature at which the flies develop.

THE vast array of genetic tools available for the study of *Drosophila melanogaster* has been highly useful for the study of neuromuscular systems involved in normal behavior of the species. Studies using mutations affecting a variety of behavioral phenotypes have already been successful (HOTTA and BENZER 1972; IKEDA and KAPLAN 1970; KONOPKA and BENZER 1971; GRIGLIATTI, SUZUKI and WILLIAMSON 1972; POODRY, HALL and SUZUKI 1973; PAK 1975). Coupled with the development of electrophysiological techniques to probe the mutants, important insights are being obtained (IKEDA and KAPLAN 1970; ALAWI and PAK 1971; LEVINE and WYMAN 1973; IKEDA, OZAWA and HAGIWARA 1976; SIDDIQI and BENZER 1976).

We have selected mutants that have reduced flight behavior with a view to studying the function and development of the elements involved in flight. The present paper describes the initial isolation and genetic characterization of the mutants. Specific behavioral abnormalities determined from simple observation experiments and mosaic analyses of several mutants are presented in the following paper (HOMYK 1977).

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MATERIALS AND METHODS

Mutant strains and nomenclature: Adult Oregon-R males less than forty-eight hours old were fed 0.025 M ethyl methanesulfonate (EMS) by the method of LEWIS and BACHER (1968). The males were crossed to *C(1)DX* females in quarter-pint bottles (20 males and 20 females per bottle). Putative mutant males were separated on the basis of flight behavior as described below. The phenotypes of different classes of mutants retained are described in RESULTS, and in the following paper (HOMYK 1977). New symbols to describe mutants exhibiting different behaviors and visible phenotypes include: *fli*, flightless; *flrd*, flight reduced; *hypo*, hypoactive; *ses*, stress sensitive; *crp*, crippled; *thic*, thickened aristae; and *rde*, reduced eye size. Mutations in different loci but exhibiting similar phenotypes have been given the same designation, but are distinguished from one another by use of a capital letter (*e.g., fli A* and *fli B*). Mutant alleles are further distinguished by numeral superscripts (*e.g.*, $fli\ A^1$ and $fli\ A^3$).

All of the mutations reported here are sex linked and are maintained in two ways: (1) in males by crossing to females carrying a compound *X* chromosome (*fli/Y* ♂ × *C(1)DX, y f/Y* ♀), and (2) in both sexes where females are homozygous or heterozygous with the multiply-inverted *X* chromosome, *FM6*, which is marked with yellow and Bar (*fli/Y* ♂ × *FM6/fli* ♀). Further description of the *C(1)DX* and *FM6* chromosomes is given in LINDSLEY and GRELL (1968). Additional mutants used in complementation and comparative behavioral test are Hk^{1P}, *eag* and Sh^5 (KAPLAN and TROUT 1969), obtained from DR. W. KAPLAN, and tan, (*t*) obtained from the California Institute of Technology Stock Center, Pasadena, California.

Separation of flying from nonflying flies: A plexiglass box (SHEPPARD 1974) was employed for isolating mutant flies, for mapping and complementation studies and for the initial characterization of each mutant strain's flight ability. The box is a large rectangular compartment (59 cm × 15 cm × 21 cm), with a smaller, open-topped cylindrical chamber (15 cm in diameter, 10 cm high) fixed to the floor. The inner wall of the cylinder is lined by a horizontal copper grid, with bars approximately 1 mm apart, which is connected to a power source. When in use, a current with 100V potential applied to the grid, providing nonlethal shocks, prevents flies from climbing over it. Thus, in order to escape from the cylinder, flies must be able to fly the height of its wall. Each chamber is also fitted with a loose lid which can be removed and replaced quickly.

For separation, two to three hundred adult flies were collected and shaken onto the floor of the cylindrical chamber. The lid to the main chamber was replaced to prevent flies from escaping from the box, and the box agitated for three to ten minutes. The lid to the cylindrical chamber was then replaced and the flies in each chamber anesthetized with CO_2, collected and counted. Flies which escape the cylindrical chamber are termed fliers, while the flies which do not are nonfliers.

Putative mutant males were mated individually to *C(1)DX, y f* females, the F_2 male progeny examined for flight ability by visual inspection, and those strains showing flight impairment retained (SHEPPARD 1974).

In the initial mapping experiments, flies were lightly anesthetized with CO_2, introduced into the cylindrical chamber and allowed ten minutes to recover and fly out. It was later observed that such anesthetization has a slightly adverse effect on flight performance. For this reason, in later experiments, including the flight performance and complementation tests, flies were not anesthetized and were allowed only three minutes to escape from the cylinder. Unless otherwise stated, all separation experiments were carried out at 22°.

Mapping experiments: The mutations were mapped relative to the following markers (followed by their symbols and map positions): yellow (*y*) 0.0, crossveinless (*cv*) 13.7, vermilion (*v*) 33.0, forked (*f*) 56.7 and carnation (*car*) 62.5. More detailed descriptions of these mutations can be found in LINDSLEY and GRELL (1968).

Complementation tests: Mutations which appeared to be closely linked genetically were tested for complementation in females heterozygous for two different mutations. The flight ability of mutants and heterozygous mutant combinations were compared using one or more

of the following tests: (1) flies were grown to a density of 200–300 per bottle and tested for flight ability in the flight test box, as described above, (2) females were placed individually in pint-sized milk bottles and their ability to hop and fly measured against a metric scale, (3) flies under CO_2 anesthesia were glued with clear fingernail polish to a small loop of wire on the dorsal thorax. After an hour, each tethered fly was examined for flight behavior.

Examination for temperature sensitivity: To examine the effects of high temperature on viability or subsequent adult behavior, adults were allowed to lay eggs for 48 hr at 29° and discarded; the cultures were kept at 29°. To examine the effect of low temperature, eggs deposited at 22° over a 72 hr period were shifted to 17° until development was completed. Parallel controls were kept at 22°. Where behavior is concerned, mutants raised to adults at the three temperatures were compared as described.

RESULTS

Isolation and initial characterization of flight ability of prospective mutants: The mutant strains obtained by SHEPPARD (1974) were examined for external physical defects and those with visible wing defects discarded. The remaining stocks were maintained as mutant males crossed to *C(1)DX, y f* females and their flight compared, visually, with an Oregon-R control. Strains which demonstrated poorer flight ability than the control were kept for further study and, where possible, were made homozygous. Several mutants could not be maintained as homozygous stocks owing to the reduced fertility of the females.

Each mutant was raised at 17°, 22° and 29° and tested, by visual inspection, for flight ability in half-pint milk bottles at 22° and at 29°. In this test most of the mutants showed no differences in flight behavior, regardless of the temperature at which they were raised or tested. The flight performances of these temperature-insensitive mutants, raised and tested at 22°, are presented as a fraction of the total population that escape the cylindrical chamber of the flight test box (Table 1). The mutants show a wide range of flight ability. Thus, some strains (*e.g.*, $fli\,A^1$, $fli\,F^1$, $fli\,H^1$ and Sh^{101}) never escape the test chamber, while other strains (*e.g.*, $flrd\,A^1$, $flrd\,L^1$, $hypo\,B^s$, Sh^{102} and t^{101}) fly nearly as well as wild type.

The flight behavior of nine mutants was found to be affected by developmental temperature (Table 2). As in previous studies of mutants in which the conditionally expressed phenotype depends on temperature (EDGAR and LIELAUSIS 1964; SUZUKI 1970), the temperature at which the mutant phenotype is expressed is termed the restrictive or nonpermissive temperature, and the temperature at which the phenotype is more normal is termed the permissive temperature. As will be shown in the following section, these temperature-sensitive (ts) mutations fall into seven complementation groups.

The eag^{101} mutant shows reduced flight ability when raised at 22° (Table 2), although all mutants are capable of some flight and carry their wings in normal position. When raised and kept at 29° until three days after eclosion, however, 80% of these mutants carry their wings in an upright position and none can fly, even after a two-day incubation at the permissive temperature (Table 2). Preliminary studies also indicate that eag^{101} mutants raised to eclosion at 22° become increasingly debilitated after prolonged incubation at 29° (unpublished data).

TABLE 1

*Measured flight performance of temperature-insensitive mutants**

Identity	Females % Fliers	Females Total	Males % Fliers	Males Total	Identity	Females % Fliers	Females Total	Males % Fliers	Males Total
fli A^{1}	0	215	0	316	*hypo B*^{1}	46	322	53	249
fli A^{2}	0	171	0	135	*hypo B*^{2}	62	268	44	242
fli A^{3}	0	63	0	55	*hypo B*^{3}	70	234	62	199
fli F^{1}	0	128	0	95	*hypo C*^{1}	31	213	36	204
fli G^{1}	28	165	25	137	*hypo C*^{2}	64	233	57	291
fli G^{2}	4	54	0	69	*hypo D*^{1}	67	280	60	213
fli H^{1}	0	384	0	312					
fli 385	0	23	0	37	*ses A*^{1}	36	389	36	347
					ses B^{1}	14	608	22	345
flrd A^{1}	60	305	63	249	*ses C*^{1}	7	435	1	210
flrd B^{1}	2	132	12	121					
flrd C^{1}	28	223	38	159	*HK*^{101}	27	168	31	191
flrd E^{1}	39	191	19	164	*Sh*^{101}	0	123	0	88
flrd F^{1}	0	372	1	301	*Sh*^{102}	73	289	66	235
flrd G^{1}	62	45	16	51	*eag*^{101}	—	—	30	275
flrd H^{1}	1	75	2	64					
flrd I^{1}	25	548	37	537	*crp A*^{1}	—	—	20	171
flrd J^{1}	3	548	2	237	*rde*	0	135	2	161
flrd K^{1}	12	68	23	98	*t*^{101}	78	316	72	257
flrd L^{1}	74	420	62	299	*thic*	—	—	25	193
flrd 393	—	—	2	136					
flrd 397	14	79	19	94	Ore-R	82	370	73	310
hypo A^{1}	40	296	46	279	*Sh*^{101}/Ore-R	9	526	—	—
					fli N^{1}/Ore-R	0	272	—	—

* % Fliers refers to the fraction of flies which escape the cylindrical chamber in a three minute interval (see MATERIALS AND METHODS). Mutants were raised and tested at 22°.

In each of the remaining eight ts mutants, the temperature at which the adult mutants are incubated does not affect their behavior. Thus, mutants raised through one-day post-eclosion at the permissive temperature exhibit normal behavior when incubated and tested at the restrictive temperature and, conversely, mutants raised to one-day post-eclosion at the restrictive temperature still exhibit the mutant phenotype after a two-day incubation at the permissive temperature. Because the effect of temperature on their flight ability occurs prior to one-day post-eclosion, the flight tests of each ts mutant raised at both permissive and nonpermissive temperatures were performed at 22° (Table 2).

Mutant *flrd D*^{1} is a cold-sensitive mutant for which 22° is a restrictive temperature and 29° a permissive temperature. *fli B*^{1} and *fli E*^{1} are also cold-sensitive mutants for which 22° is the permissive temperature and 17° the restrictive temperature (Table 2).

fli C^{1} and *fli C*^{2} are alleles for which 22° is the permissive and 29° the restrictive temperature. *fli C*^{1} has good viability at 29°. Because *fli C*^{2} shows poor viabil-

TABLE 2

Flight performance of temperature-sensitive mutants raised at different temperatures

Identity	Developmental temperature*	Females % Fliers	Females Total	Males % Fliers	Males Total
fli B^1	17°	0	451	0	290
fli B^1	22°	20	246	42	191
fli C^1	22°	8	87	14	103
fli C^1	29°	—	—	0	401
fli C^2	22°	9	95	23	86
fli D^1	17°	49	297	16	163
fli D^1	22°	0	231	0	77
fli E^1	17°	0	324	0	204
fli E^1	22°	75	212	84	157
fli I^1	17°	18	280	26	238
fli I^1	22°	0	197	0	232
fli I^2	17°	29	291	38	236
fli I^2	22°	0	251	0	250
fli I^3	17°	0	258	0	163
fli I^3	22°	0	466	0	376
flrd D^1	22°	0	1016	1	635
flrd D^1	29°	15	248	38	146
eag^{101}	22°	—	—	30	275
eag^{101}	29°	—	—	0	391
Oregon-R	17°	54	466	60	261
Oregon-R	22°	81	411	87	308
Oregon-R	29°	76	356	82	241

* Temperature at which flies were raised to one day post eclosion. Adults were then incubated on fresh medium for 48 hr at 22° and tested at 22°.

ity when raised at 29°, only the flight behavior of this mutant raised at 22° is reported (Table 2).

fli I^1, *fli I*^{2} and *fli I*^{3} comprise a group of noncomplementing mutations. In the case of mutants *fli I*^{1} and *fli I*^{2}, 22° is the restrictive temperature and 17° is the permissive temperature. The mutant phenotype for *fli I*^{3} is temperature-insensitive at the temperatures reported (Table 2) and at 29° (unpublished data).

In addition to being flightless, *fli D*^{1} has short thin bristles when raised at 22° and is lethal when raised at 29°. When raised at 17°, this mutant has a wild-type phenotype for flight (Table 2) and bristles.

Mapping and complementation. Mapping studies were performed by evaluating the behavior of recombinant male progeny of females heterozygous for a multiply marked *X* chromosome (*y cv v f car*) and an *X* chromosome carrying a particular behavioral mutation. In the case of those mutations designated as *fli*, flightless, or *flrd*, flight-reduced, behavior was judged by scoring the recombinant male progeny for flight ability, using the flight test box. Most of these mutations reduced flight enough to allow unambiguous separation of flier and nonflier mutant recombinants. The positions assigned to the *fli* and *flrd* mutations on the basis of these data are given in Figure 1.

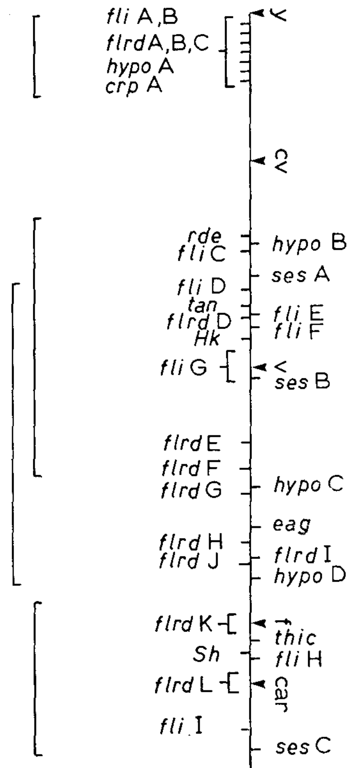

FIGURE 1.—Genetic map of behavioral mutations. The positions indicated are relative to the marker mutations *y*, *cv*, *v*, *f* and *car*. Mutations *fli G*, *flrd K* and *flrd L* mapped close to one of the respective marker mutations, but the data were insufficient to assign the interval. The large braces below enclose mutations which were combined systematically for complementation tests.

Fourteen of the mutants have specific behavioral phenotypes that can be judged more accurately than their simple reduction in flight ability reported in Table 1. These additional phenotypes and their symbols are: (1) crippled (*crp*), walk in crippled or uncoordinated fashion; (2) hypoactive (*hypo*), inactive and difficult to arouse into an excited state necessary for flight; (3) stress-sensitive (*ses*), reversibly paralyzed when exposed to mechanical shock; (4) *eag*, *Hk* and *Sh*, designating alleles of previously described mutations causing hyperactive and ether-shaking phenotypes (KAPLAN and TROUT 1969). One strain, designated *hypo* D^1, has several behavioral abnormalities including hypoactivity, cold-sensitive paralysis and ether-shaking phenotypes. More detailed description of the behavioral phenotypes in these strains is given by HOMYK (1977). Map positions determined for the mutations in the strains responsible for these specific behavioral phenotypes are also given in Figure 1.

Seven of the mutants recovered have additional lethal and/or visible phenotypes (Table 3). Mapping data indicate that the ts lethal phenotypes of *fli* D^1, *flrd* I^1, *ses* A^1 and *ses* C^1 result from the same mutations affecting their flight or stress-sensitivity phenotypes described above. In addition, the *fli* D^1 mutation

TABLE 3

Mutations with lethal and/or visible phenotypes

Identity	Phenotype*
fli D^1	lethal at 29°; small bristles at 22°
flrd I^1	lethal at 29°
ses A^1	lethal at 29°
ses C^1	lethal at 29°
rde	reduced eye size; palpi often missing
thic	lethal at 29°; thickened aristae and sex combs; dark specks in eye
t^{101}	tan body color

* All phenotypes are recessive. Lethal effects of high temperatures occur during development for those strains noted.

is responsible for the small bristle phenotype. Mapping data indicate that the mutations responsible for the visible phenotypes of *rde* and the lethal and visible phenotypes of *thic* (Table 3) are also responsible for the reduced flight ability observed in these strains (Table 1).

Physiological analysis (HOMYK 1977) has shown that three of the mutants have abnormal electroretinograms (ERG). The mutation in one, t^{101}, is responsible for the abnormal ERG and body-color phenotypes (Table 3). Mapping data also show that the abnormal ERG phenotypes of *hypo* D^1 and *ses* C^1 are due to the mutations causing the hypoactive and stress-sensitive phenotypes, respectively (Figure 1).

The mapping studies indicate that the reduced flying ability or abnormal behavioral phenotypes of most of the mutants can be accounted for by single site mutations located near the positions indicated in Figure 1. None of the single recombinants involving strain *fli 385*, however, could fly. Among the double recombinants, eight of twelve *y* + + *f car* recombinants flew, while none of the five + + *v f* + and + *cv v f* + recombinants could fly. This suggests that strain *fli 385* contains two mutations, one near *y* and the other near *car*, each of which

TABLE 4

Flight performance of heterozygotes for noncomplementing mutations

Genotype	% Fliers	Total No. scored
fli A^1/*fli* A^3	0	466
fli C^1/*fli* C^2	12	—
fli I^1/*fli* I^2	0	420
fli I^1/*fli* I^3	0	204
fli I^1/*fli 385**	0	177
fli G^1/*fli* G^2	39	—
hypo B^1/*hypo* B^2	49	279
hypo B^1/*hypo* B^3	44	—
hypo C^1/*hypo* C^2	60	181

* *fli 385* carries two recessive mutations reducing flight. The mutation which fails to complement *fli* I^1 has been designated *fli* I^4.

reduces flight. In addition, the positions of mutations in strains *flrd 393* and *flrd 397* could not be resolved.

Since the genetic maps provide unambiguous assignment of positions relative to the visible markers, complementation tests *inter se* were carried out within the groups shown by brackets in Figure 1. Using flight ability in the flight test box as a criterion for judging complementation (Table 4), the study revealed six loci in which two or more alleles were recovered.

In most cases, females heterozygous for one of the mutations and a normal *X* chromosome fly as well as Oregon-R (data not shown), demonstrating that the mutations are recessive. Females heterozygous for a normal *X* and either of the mutations Sh^{101} or *fli* H^1, however, were found to be poor fliers (Table 1), suggesting that these mutations are partially dominant. That they are not completely dominant is shown in two observations. (1) While homozygous Sh^{101} and *fli* H^1 fail to initiate flight from a horizontal surface, the respective heterozygotes hop and fly a short distance of 5 to 7 cm. (2) Females heterozygous for either mutation oscillate their wings for several minutes in tethered flight. When tethered, females homozygous for either mutation extend and raise their wings as if attempting to initiate flight, but fail to begin wing oscillation.

In complementation tests, females heterozygous for Sh^{101} and any of the mutations mapping proximal to *f* behave like the $Sh^{101}/+$ females described above. Similarly, on this basis, *fli* H^1 complements all of the mutations proximal to *f*.

The phenotypes and map positions (Figure 1) of five mutations suggested that they were possibly alleles of previously described mutations. One, t^{101}, failed to complement an allele of tan for body color (LINDSLEY and GRELL 1968) and defective ERG (HOTTA and BENZER 1969; PAK, GROSSFIELD and WHITE 1969) phenotypes. The recessive ether-shaking phenotype of one mutation failed to complement the recessive shaking behavior of the Hk^{1P*} mutation, while that in another failed to complement the recessive ether-shaking phenotype of *eag* (KAPLAN and TROUT 1969). These new mutations are designated Hk^{101} and eag^{101}, respectively.

The dominant character of the hyperactive and ether-shaking phenotypes of two mutations prohibited evaluation of complementation tests with one another and with Sh^5. Because of their map positions, between *f* and *car*, and dominant shaking phenotype, it has been assumed that these mutations are alleles of the Sh^5 mutation (KAPLAN and TROUT 1969); therefore, they are designated Sh^{101} and Sh^{102}.

DISCUSSION

SHEPPARD (1974) isolated forty-eight mutants of *Drosophila melanogaster* that exhibit a wide range of flight ability (Table 1) and a variety of behavioral phenotypes (HOMYK 1977). Genetic mapping studies and complementation test show that, altogether, these strains contain mutations representing at least thirty-four different loci. The map positions given in Figure 1, however, should be

* We have retained a capital designation for a recessive mutation in agreement with KAPLAN and TROUT (1974).

considered approximate since the mapping data obtained suffer errors for several reasons: (1) The use of a multiply marked *X* chromosome, which often leads to inequality of reciprocal recombinant classes. In the present instance, recombinants inheriting the *cv* marker were under-represented by 20–40% compared to their reciprocal classes. (2) Possible interaction of one of the marker mutations and a behavioral mutation. (3) The retention of a large number of nonmutant flies in the cylindrical chamber (see the Oregon-R control, Table 1) and the apparent "leakiness" of the flight defective phenotype in those mutants for which flight ability is only partially debilitated (Table 1). This represents a source of error for the mapping data concerned the *fli* and *flrd* mutations, since the behavior of recombinants involving these mutations was judged using the flight test box.

A statistical analysis concerning the level of confidence which could be assigned to the map positions in Figure 1 is not given, as such analysis would assume no interaction of the behavioral mutations and the marker mutations regarding judgment of behavior or viability. Should it become useful or necessary, more accurate mapping would best be done using other available marker mutations (LINDSLEY and GRELL 1968) within the same genetic intervals as determined for each of the behavioral mutations in the present study.

Complementation studies have shown that the new mutations, eag^{101}, Hk^{101} and t^{101}, are alleles of previously described mutations affecting behavior. Although complementation tests have not been made, mapping data place *hypo* D^{1}, which has abnormal ERG and ether-shaking phenotypes, close to the ERG defective *non A* mutations (PAK 1975) and Ocd^{ts} (SØNDERGAARD 1975), which has an ether-shaking phenotype.

It is likely that many of the remaining mutations represent new genes and will be useful for studying the genetic control of sensory, neural and muscular elements involved in behavior. In addition, the behavioral phenotypes of mutations in seven genes were found to be affected by temperature. By studying the time dependence of the mutant behavioral phenotype on permissive/restrictive and pulse temperature shifts during development of these mutants (SUZUKI 1970), it should be possible to determine at which time during development particular gene products function.

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