Drosophila Stocks wild :

1. *Drosophila melanogaster*
2. *Drosophila jambulina*
3. *Drosophila kikkawai*
4. *Drosophila malerkotliana*
5. *Drosophila immigrans*
6. *Drosophila nepalensis*
7. *Zaprionus indianus*

Nongthomba, U., and N.B. Ramachandra. Drosophila Stock Centre, Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysore 570 006, India. Induction and isolation of chromosome specific indirect flight muscle mutations in *Drosophila melanogaster*.

The indirect flight muscles (IFMs) of *Drosophila melanogaster* provide a unique model system to genetically dissect muscle structure and function (Sparrow *et al.*, 1991). These are the bulk of thoracic muscles consisting of two groups, namely dorsal longitudinal muscles (DLMs) which are composed of six fibers from dorsal to ventral and dorso-ventral muscles (DVMs), DVM I, DVM II, DVM III which are formed by 3, 2, and 2 fibers, respectively. The wild type IFM development has been studied (Crossley, 1978; Fernades *et al.*, 1991). *Drosophila* is also a suitable system because mutations that affect IFMs development can readily be isolated and these mutations do not affect much on the viability of the flies. Hence, the genetic analysis of mutants has greatly advanced the understanding of muscle development.

A number of mutations affecting the IFMs have been reported (Crossley, 1978; Lindsley and Zimm, 1992). Most of these mutations are quite general in their expression which affect all the flight muscles. However, the genes involved in the development of IFMs have not been identified systematically. So far no other chromosome 2 specific mutation has been reported which affects the IFMs development except *Mhc* gene. In view of this, investigations are made to identify and characterize genes involved in IFM development which reside on chromosome 2 by using ethyl methanesulfonate (EMS) mutagenesis. Here we report the induction and isolation of 16 new viable mutations on the second chromosome in *Drosophila melanogaster* which affect the indirect flight muscles.

Table 1. Scheme for Ethyl Methanesulfonate Mutagenesis

Generation	Cross		
	Females		Males
Parental	CyO / Tft	x	+ / + (EMS treated)
F_1	CyO / Tft	x	* / #
F_2	* / CyO	x	* / CyO
F_3	* / * males and females scored for abnormal wing and IFMs		* / CyO males and females females retained for stocks

CyO / Tft = Chromosome 2 marker; * = mutagenised chromosome # = CyO 0r Tft male.

The Canton-S strain and Curly Oster/Tufted (*CyO/Tft*) mutant strain of *Drosophila melanogaster* were used as wild type and dominant markers of chromosome 2, respectively (Lindsley and Zimm, 1992). All the stocks were cultured on standard wheat cream agar medium at 24 ± 1°C. 25mM of EMS was administered to the Canton-S male flies following the procedure of Grigliatti (1986). The protocol used for induction and detection of mutation on chromosome 2 is presented in Table 1. Control experiments for EMS mutagenesis were made using X^XY stocks. For muscle analysis, thoracic whole mounts were prepared following the procedure described by Fyrberg *et al.* (1995). Complementation analysis was done by crossing the virgins of each of the newly-isolated mutants reciprocally and analyzing the progenies for the defects in wings and IFMs.

The summary of the EMS mutagenesis on chromosome 2 which affect IFMs phenotypes in *D. melanogaster* is given in Table 2. A total of 3283 mutation induced lines were screened. Of these, 70.5%, 2.2% and 27.3% mutations were lethal, sterile and viable, respectively. Out of the 27.3% viable homozygotes scored, 3% were of wing mutants, of

Table 2. Summary of the Ethyl Methanesulfonate mutagenesis

Particulars	1st EMS	2nd EMS	3rd EMS	4th EMS	5th EMS	Total	%
Lines screened	623	686	582	493	900	3283	—
Lethal lines	424	557	442	308	583	2314	70.5
Sterile lines	10	13	19	22	08	72	2.2
Viable homozygotes	189	116	120	163	209	897	27.3
Viable wing mutants	05	05	01	04	14	29	0.9
Viable muscle mutants	05	—	—	03	10	16	0.5

which nearly 60% had shown IFM defects. Not all the wing mutants showed defects in the muscle phenotypes indicating that the wing phenotype is not completely penetrant. However, all the muscle mutants showed defects in wing phenotypes. Similarly, Cripps *et al.* (1994) have reported that a number of flightless mutants display abnormal wing positions but all have not shown the muscle defects. The complementation analysis revealed that, of all the 16 mutants, only one has 9 alleles and all other mutants complement to each other. Thus, these 16 newly-isolated mutations belong to 8 complementation groups. The salient features of these mutations are as follows:

1) all the mutations were viable and recessive except only one which is semi-dominant
2) wings were held up or extended or looping
3) most of these were flightless or weak in flight
4) all these had shown defects in IFMs, two of these had shown more than 80% degeneration in both DLMs and DVMs, another two of them had shown more than 50% of defect in DLMs, the remaining four had shown about 20% degeneration of IFMs
5) five of these mutations were fertile in homozygous condition
6) all these had shown various levels of penetrance and expressivity.

The combination of genetic analysis of mutations and the molecular characterization of the normal and mutant genes and proteins has permitted powerful correlations of function and structure and initiation of experiments testing mechanisms active in complex developmental processes (Epstein and Bernstein, 1992). Several mutants are already known which affect the development of IFMs. Most of these were X chromosome mutants, namely, *erect wing* (*ewg*), *flap wing* (*flw*), *indented thorax* (*int*), *upheld* (*up*), *shibire*^{ts} (*shi*^{ts}), *vertical wing* (*vtw*). All these mutants showed defects in DLMs and DVMs except *shibire*^{ts} where the DVMs were normal. There are two mutations on chromosome 3, namely, *Actin 88F* which showed defect in both DLMs and DVMs and *stripe* (*sr*) showed defects in only DLMs. *Myosin heavy chain* (*Mhc*) is the only gene known on chromosome 2 which is involved in both DLMs and DVMs formation. Many alleles of this gene have been reported which affect IFMs at various degrees (Lindsley and Zimm, 1992). In the present study, in all the 16 newly isolated mutants, both DLMs and DVMs were affected and showed different levels of penetrance and expressivity. However, DLMs were more severely affected than the DVMs. Of the 16 mutants 4 of them showed defects in DVMs at various levels. Investigations in this direction will be interesting to find out the genes involved for DVMs development. Further characterization, mapping and developmental analysis of these mutations are in progress.

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