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Flight ability of the newly isolated three indirect flight muscle mutations in *Drosophila.*

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Abstract: Indirect flight muscle development is not well understood in *Drosophila.* We have generated a number of mutations to understand the genes involved in indirect flight muscle development. Here we report the flight ability of three new mutations. The analysis revealed that the drooping wing flies are either flightless or weak fliers with maximum sterility.

Introduction

The investigation of genes discovered by their homology to *Drosophila* genes is now one of the most successful approaches to understand the genetic control of vertebrate development. However, mechanisms in the fly are in several instances likely to be unique to this specialized organism (Nusslien-Volhard, 1996). In the fruit fly, *D. melanogaster*, muscle development takes place twice – in the embryo during the formation of the muscles of the larva and during pupal development when adult muscles are made (Bate, 1990; Fernandes *et al.*, 1991; Reedy and Beall, 1993; Baylies *et al.*, 1998). Muscle development in the embryo involves specification of the mesoderm (Thisse *et al.*, 1988; Azpiazu *et al.*, 1996; Riechmann *et al.*, 1997), the choice of a muscle founder cell (Bate, 1990; Ruchton *et al.*, 1995), and the consequent fusion of myoblasts to form a fibre which attaches to specific sites on the epidermis and gets correctly innervated (Bate, 1990; Broadie and Bate, 1993). During embryonic myogenesis, signals from the ectoderm and the mesoderm result in the selection and specification of a muscle progenitor cell (Carmena *et al.*, 1998) which divide asymmetrically to give rise to two daughter cells, one of which becomes an adult muscle progenitor (Baylies *et al.*, 1998). In the thoracic and head segments, these progenitor myoblasts associate with imaginal disc cells and proliferate during larval life (Poodry, 1980; Lawrence and Brower, 1982; Fernandes *et al.*, 1991; Fernandes and Vijay Raghavan, 1993; Roy and Vijay Roghavan, 1997, 1998).

The flight of insects such as *Drosophila* is powered by relatively large, striated indirect flight muscles (IFMs) that are similar in structure to vertebrate skeletal muscle (Sparrow, 1995). The IFMs, which are a bulk of muscle mass in the mesothorax, are divisible into sub-sets: the dorsal longitudinal muscle (DLMs) and dorso-ventral muscles (DVMs) which are structurally, physiologically, and biochemically identical. These two groups of IFMs have distinct development histories: the DLMs develop by fusion of disc myoblasts with the larval scaffold which serves as a template during metamorphosis while the DVMs develop *de novo* fusion of the imaginal disc myoblasts (Bate, 1993; Anant *et al.*, 1998).

Ethyl methane sulphonate (EMS) has been the most widely used chemical mutagen for inducing mutations in *Drosophila* (Grigliatti, 1986). An EMS mutagenesis of the second chromosome of *D. melanogaster* was undertaken and a total of 3283 mutagenized chromosomes were generated by Upendra and Ramachandra (1997). Of these, 897 viable recessive lines were recovered in F3. Flies homozygous for the mutagenized second chromosome from each of these 897 lines were analysed for IFM defects (Upendra and Ramachandra, 1997, 1999). In this report, we analyse the flight ability of three IFM mutations and have made an attempt to correlate this with the wing abnormality.

Materials and Methods:

Fly Stocks: The following stocks were used to perform the flight test: 1) RU 536, 2) RU 486, 3) RU 664.

Upendra and Ramachandra (unpublished data) have generated these mutant strains during 1996. In brief, ethyl methane sulphonate (EMS) at 25mM concentration was administered to two-day-old Canton-S adult male flies following the procedure described in Grigliatti (1986). EMS treated males were crossed to Tft/CyO virgin females. F1 male offspring were aged for 2 days. Each of these males which had either Curly or Tufted phenotypes were crossed separately to Tft/CyO virgin females. Progeny from the above cross carrying the CyO balancer chromosome were crossed in the homozygous condition. The IFMs of the viable homozygotes were screened for possible muscle defects using polarized light. All these mutants have shown IFM defects. All the stocks were cultured on standard wheat cream agar medium at 25$\pm$1°C in half pint bottles.

Flight Test: This was performed by following the methodology of Vigoreaux *et al.* (1993) with slight modifications as described by Upendra and Ramachandra (1999). Individual virgin flies were placed in empty milk bottles and observed for wing beat and flight. Flies were scored on a scale from zero to three as flightless, weak fliers, moderate fliers and normal fliers, respectively. Flies that never beat their wings and fell straight to the bottom when the bottle was tapped were termed flightless and were assigned a score of zero. Flies that landed on the wall or bottom with some wing vibration but otherwise did not fly when the bottle was tapped were assigned a score of one (weak flier). Flies that vibrate their wings in the absence of tapping of the bottle, and flew sporadically were assigned a score of two (moderate fliers). Normal fliers with very active wing beat were assigned a score of three.

Fertility Test: After the flight test was performed the homozygotes of each of these three strains were crossed and observed for the progeny. The line of crosses which produces flies are treated as fertile and the one which does not produce any progeny is treated as sterile.

Results and Discussion

Earlier, it was observed by Upendra and Ramachandra (unpublished) that the mutations RU 536, RU 486, and RU 664 show IFM defects at different degrees. The penetrance and expressivity of indirect flight muscle phenotype in these mutants is incomplete. Table 1 provides the different wing and flight phenotypes of the IFM mutations under study. Two primary kinds of wing phenotypes noticed in the homozygotes of these three strains are normal and drooping wings. It is seen that the number of flies with drooping wing phenotype is significantly greater than the number of flies with normal wing phenotype in all these three strains. Figure 1 shows the normal and drooping wing phenotypes of the strains under study. All the three IFM mutants showed the drooping wings. Each one of these mutations is derived from independent lines and complement each other. Therefore, they are not alleles of the same gene.

Table 1. Summary of the wing and flight phenotypes of the three *ifm* mutant strains in *Drosophila melanogaster.*

Strain	Total no. of flies scored	Wing phenotypes			Flight				
		Normal	Drooping	$\chi 2$	Flightless	Weak fliers	Moderate	Normal	$\chi 2$
RU 536	50	17 (34)	33 (66)	4.50*	18 (36)	25 (50)	07 (14)	-	9.88*
RU 486	50	08 (16)	42 (84)	21.78*	23 (46)	26 (52)	01 (02)	-	22.34*
RU 664	50	08 (16)	42 (84)	21.78*	30 (60)	20 (40)	-	-	1.62

* = Significant at 5% level; (Number in bracket represents the percentage)

Table 2. Summary of the fertility of the three *ifm* mutations under study.

Strains	Sterility	Fertility	$\chi 2$
RU 536	10 (40)	15 (60)	1.00
RU 486	21 (84)	04 (16)	10.24*
RU 664	24 (96)	01 (04)	23.04*

* = Significant at 5% level; (Number in bracket represents the percentage)

The flies of the IFM mutations, RU 536 and RU 486 are more likely weak fliers than flightless and a few are moderate fliers. While the RU 664 homozygotes showed more of flightless than flies with weak flight. In all these mutations none have shown normal flight (Table 1). Further analysis revealed that the number of weak fliers (50%) having drooping wing phenotype in the RU 536 strain is greater than the number of moderate fliers (14%) and flightless flies (36%). Similarly, a greater number of flies with drooping wing phenotype (33%) are weak fliers. This holds true for the strain RU 486 but the striking feature of this strain is that the number of weak fliers with drooping wing is significantly greater than the number of weak fliers with normal wing phenotype. This proves to a certain extent that the drooping wings confer weak flight to these flies. In RU 664, the number of flightless flies with drooping wing phenotype (50%) exceeds the number of weak fliers (34%). It is also seen that more flies that are flightless have drooping wing phenotype. This suggests that drooping wing phenotype is responsible for the flightlessness in these flies.

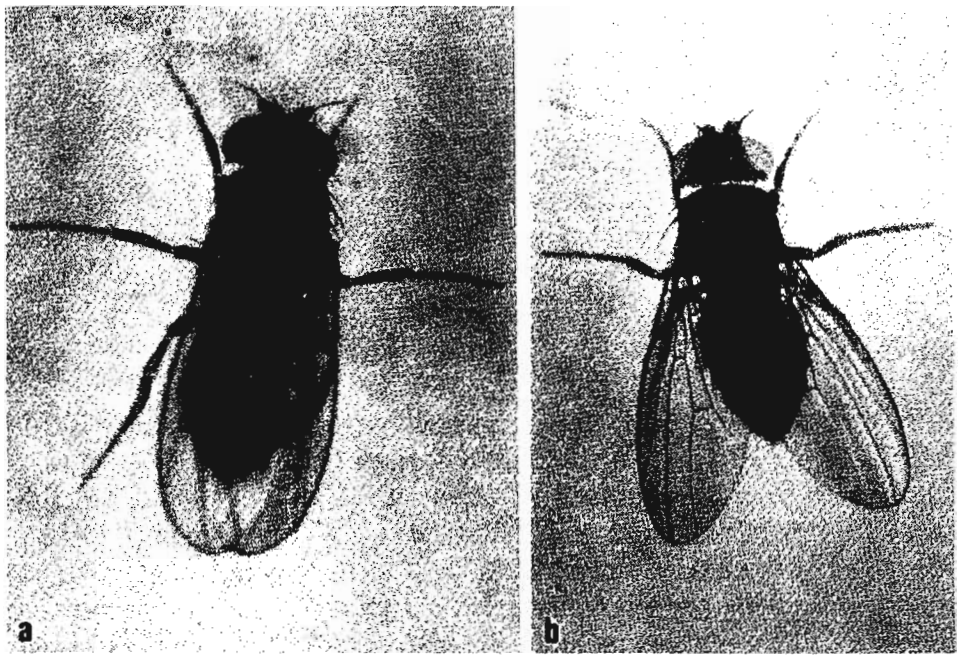

Figure 1: Wing phenotype of the newly isolated IFM mutations in *Drosophila melanogaster*. a, Normal winged: Canton-S; b, Drooping wing phenotypes seen in RU 536, RU 486 and RU 664.

Screens for the adult flight muscle mutants have been few and far between. Many X-linked flightless mutants were isolated during the screening for mutants that showed wing position abnormalities (Homyk and Sheppard, 1977; Deak *et al.*, 1982). Many of these genes have been analyzed during development and the molecular analysis of some of them has also been fruitful (Costello and Wyman, 1986; Homyk and Emerson, 1988; De La pompa *et al.*, 1989; De Couet *et al.*, 1995). The autosomal screens for muscle mutants have concentrated on isolating and studying dominant phenotypes. These studies have largely resulted in identifying mutations in genes that encode muscle structural components. In the present study it can be concluded that drooping wing phenotype may be a deciding factor for the flightless and weak flight in these IFM mutant strains.

Table 2 presents the fertility of the IFM mutants under study. In RU 486 and RU 664, almost all the flightless and the weak fliers are sterile while in RU 536 such a correlation is not possible wherein only 40% of the homozygotes are sterile. It appears that the drooping wing flies are either flightless or weak fliers with maximum sterility. Thus, these studies suggest that the wing phenotypes and flight ability are correlated with sterility of these mutations in homozygous condition.

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