Defect in the reproductive systems of the sterile Indirect Flight Muscle mutants isolated in *Drosophila*.

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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 two sub-sets: the dorsal longitudinal muscle (DLMs) and dorso-vertral 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 by *de novo* fusion of the imaginal disc myoblasts (Bate, 1993; Anant *et al.*, 1998).

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. The dose requirements of these gene products are stringent or because a small perturbation in muscle geometry can result in flight abnormalities (Mogami and

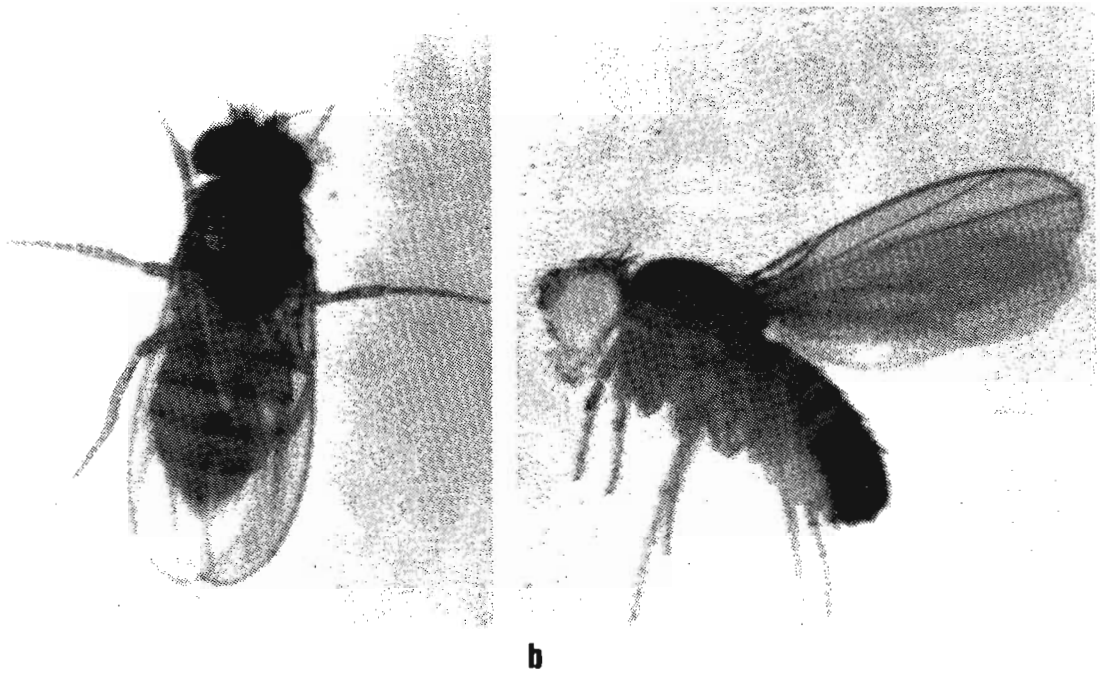

Figure 1. Wing phenotypes of the newly isolated mutations in *Drosophila melanogaster*: a) normal winged fly of Canton-S b) *ifm(2)RU2* homozygote showing raised wing.

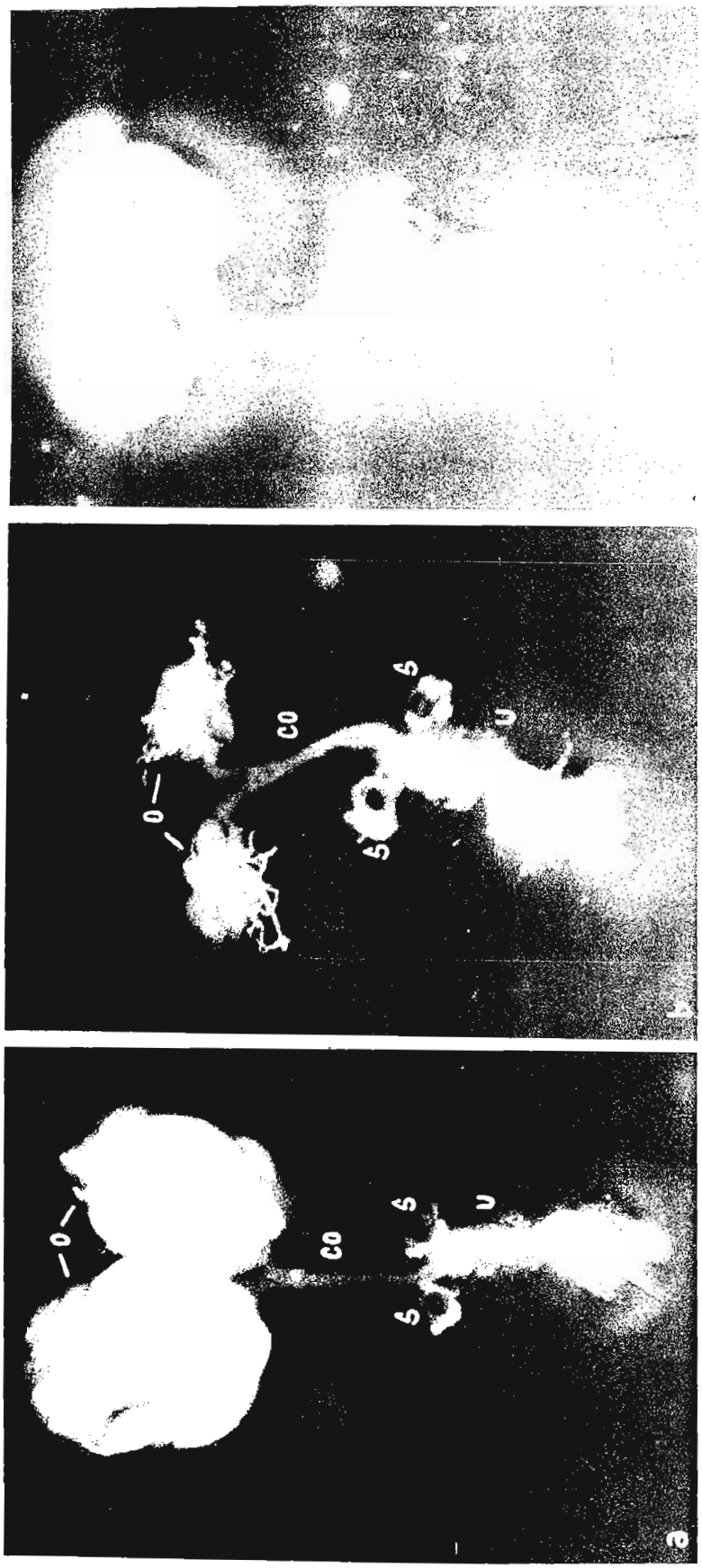

Figure 2. Female reproductive system of: a) Canton-S b) *ifm(2)RU2*[2] c) *ifm(2)RU2*[3]. O-ovaries; CO-common oviduct; S-spermathecae; U-uterus.

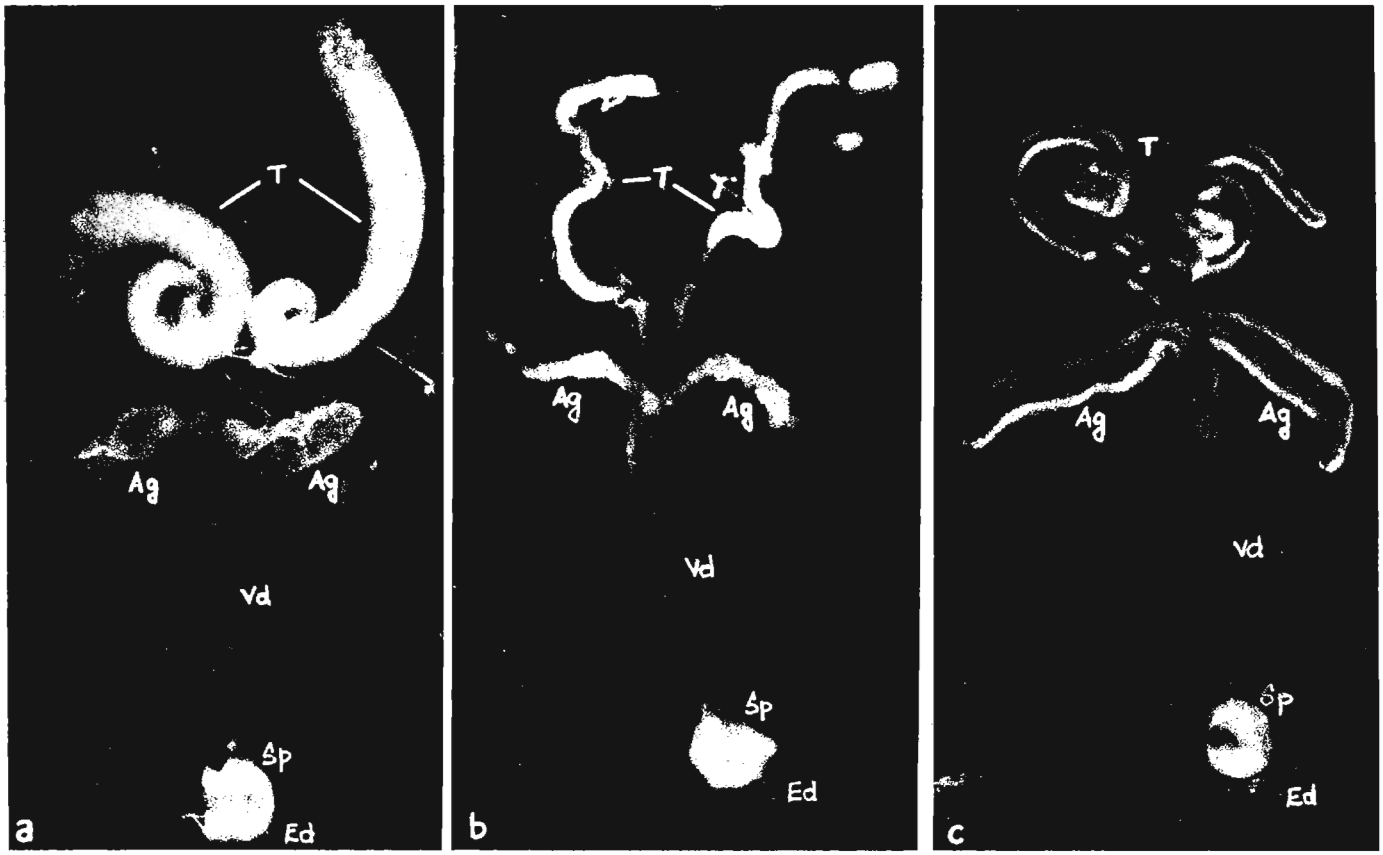

Figure 3. Male reproductive system of: a) Canton-S b) *ifm(2)RU2*^{2} c) *ifm(2)RU2*^{3}. T-testes; Ag-Accessory gland; Vd-vas deferens; Sp-sperm pump; Ed-ejaculatory duct.

Hotta, 1981; Cripps *et al.*, 1994). Many genes like *Myosin heavy chain* (*Mhc*), *Actin* (*Act*), *Myosin light chain 2* (*Mlc-2*), *Tropomyosin* (*Tm*) *Paramyosin* (*Prm*), *Troponin* (*Tn*) have been identified which code for structural proteins of the muscle (Mogami and Hotta, 1981; Bernstein *et al.*, 1983; Fyrberg and Beall, 1990; Cripps *et al.*, 1994) and some of them like *Act* and *Tn-I* are expressed in the early stages of muscle formation (Fernandes *et al.*, 1991; Barthmaier and Fyrberg, 1995). But still their role in muscle patterning and development remains to be unraveled.

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 (1999). 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). Of these some of them are sterile in homozygous condition and yet to be analysed.

Animals homozygous for *ifm(2)RU2* and its alleles have raised wings (Figure 1b). The DVMs of these animals showed thinning in the anterior parts to various degrees and the DVMs are disorganized and degenerated. The muscle fibrils are split, spongy and loosely packed. The homozygotes of this mutation are completely flightless and sterile. This mutation maps in the second chromosome region 36A8-9; 36B-C1. The developmental analysis revealed that *ifm(2)RU2* shows defect in fiber differentiation (Upendra and Ramachandra, 1999).

In the present investigation we have made an attempt to study the reasons for the sterility of the *ifm(2)RU2* alleles by dissecting out their reproductive systems and comparing it with the reproductive systems of the wild type strain Canton-S.

The *ifm(2)RU2*^{2} and *ifm(2)RU2*^{3} were produced by Upendra and Ramachandra (1999) by EMS mutagenesis and are maintained balanced, since they are homozygous sterile. As a standard Canton-S is used. All these stocks are maintained in a standard wheat cream agar medium at 25±1°C. Twenty male and female reproductive systems of the above strains were dissected out in physiological saline and observed under a microscope. The photographs of these were taken using the Leica DMRB microscope.

Drosophila, which is a bisexual organism, has distinct male and female reproductive systems. The male reproductive system in *Drosophila* consists of the external genitalia with the phallic organ, the ejaculatory duct, sperm pump, vas deferens, a pair of accessory glands and a pair of testis arising from the tip of the vas deferens. The testis is the main sperm producing organ and is connected to the vas deferens by a very thin tube-like structure called the seminal vesicle. The paragonial secretions of males stimulate egg laying, even if they are sterile (X0). Male *Drosophila melanogaster* is sexually fully mature about 12 hours after eclosion (Ashburner, 1989).

The female reproductive system consists of a pair of ovaries, which are unequal in size, a common oviduct, vertral receptacle, spermathecae, accessory glands, uterus and external genitalia. *Drosophila* females have two types of sperm storage organs, the spermathecae and the ventral receptacle. These organs can usually maintain large quantities of sperms for a sufficiently long enough period to fertilize many eggs. The spermathecae are a pair of mushroom-shaped organs attached to the upper end of the uterus. The ventral receptacle is a single tube-like structure, usually coiled, which also inserts into the upper end of the uterus (Ashburner, 1989).

Both the male and female reproductive system of Canton-S and *ifm(2)RU2*^{2} and *ifm(2)RU2*^{3} were dissected out. Figure 2a depicts the female reproductive system of the wild type fly Canton-S that is used as a control. It has two ovaries, slightly unequal in size and this is a general characteristic of the ovaries of *D. melanogaster*. The ovaries are completely gravid and roughly oval in shape. The ovaries of *ifm(2)RU2*^{2} are degenerated to a very great extent (Figure 2b). The number of ovarioles is reduced. The ovaries in the case of *ifm(2)RU2*^{3} (Figure 2c) show less degeneration compared to that of *ifm(2)RU2*^{2}. The ovaries are of unequal size and are smaller than the ovaries in C-S. Dissecting out and examining the female reproductive systems of these strains reveal marked variations in the nature of the ovaries where as the other organs in the female reproductive system are not affected.

The male reproductive system of Canton-S (Figure 3a) shows pigmentation with gradation of yellow colour. It has highly coiled testis and translucent paragonia. The testis of the *ifm(2)RU2*^{2} (Figure 3b) are not as highly coiled as compared to C-S. They are also not as broad and thick as in C-S and are whitish in colour. The paragonia is also whitish in colour and very thin. The testis and the paragonia in the case of *ifm(2)RU2*^{3} (Figure 3c) are transparent and the paragonia is longer that in Canton-S. The testis is also highly coiled. It is also seen that both *ifm(2)RU2* alleles produce sperms of various sizes with different head shapes. The sperm count in each of these cases does not appear to be very high.

This strongly confirms that *ifm(2)RU2* gene or its products is involved in fertility. It is also possible that this gene is involved in other processes in the mesoderm for follicle cell development, maturation or in other tissues like ovary and sperm. Therefore, this gene will be a master gene required to regulate the early developmental and differentiation processes of not only muscle, but also other processes. With these observations one can suggest that the sterility of *ifm(2)RU2* alleles is due to the abnormalities in the reproductive system.

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The glossy eye of *lozenge* (*lz*) studied by high power scanning electron microscopy (SEM) of compound eyes and ocelli.

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Mutants of the *lozenge* (*lz*) gene have been known since 1925 (Lindsley and Grell, 1968). Among their widespread defects are grossly abnormal eyes. There is substantial contemporary interest in the molecular and developmental mechanisms in the *lz* eye (Batterham *et al.*, 1996; Crew *et al.*, 1997; Daga *et al.*, 1996; Flores *et al.*, 1998). The compound eyes of *lz* appear shiny, hence the name "glossy" of one allele. A fine-grained roughness of the eye's surface, the "corneal nipple array," serves as an antireflection coating in some insects (Bernhard *et al.*, 1970; Bernhard *et al.*, 1965), and corneal nipples are present in *Drosophila* compound eyes and simple eyes (ocelli) (Stark *et al.*, 1989). The purpose of this study was to examine the surface of *lz* eyes with scanning electron microscopy (SEM) at a high enough magnification to resolve corneal nipples.

Two *lz* alleles were examined: lz^{77a7} (red-eyed) is an eye-specific null (Flores *et al.*, 1998) while lz^{r15} (white-eyed) is a complete null (G. Flores, personal communication). The shininess of eyes illuminated with a Fiber-Lite (Dolan-Jenner 180) was assessed with a dissection microscope (Olympus SZ40), and the light microscopic images were captured with an MTI CCD72 camera and printed with a Sony UP-5200MD video printer. For SEM, heads were dissected, dehydrated in an ethanol series, critical point dried, fixed to stubs, sputter coated and viewed on a Hitachi S570 SEM.

The accompanying plate shows control animals on top (Figures 1-5), red-eyed lz^{77a7} in the middle (Figures 6-10), and white-eyed lz^{r15} on the bottom (Figures 11-14]) The calibration bar for