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PHENOTYPIC AFFECTS OF TROPONIN-T MUTATION *UP[1]* ON *DROSOPHILA MELANOGASTER* BEHAVIOUR

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ABSTARCT

Mutations in the muscle proteins can have variable affects in the body. If the mutation is in the constitutive exon all the muscles will be affected whereas if it is in the non-constitutive one than effects will be localized to the area where the protein from that exon is involved. To observe and analyze the effects of the mutation by statistical methods is one of the most powerful tools to highlight the degree of the effect. Here we are reporting in detail the behavioural effects of a muscle Troponin-T gene mutation known as *up[1]* and its comparison to that of the wild-type flies.

Key-words: *Drosophila*, troponin gene, mutation

INTRODUCTION

Muscle cells express many functionally important components involved in contraction, which are organised in a very precise regular manner important for the contractile function. In striated muscles, like the indirect flight muscles (IFM) of *Drosophila*, contraction occurs as the thick and thin filaments slide pass each other to increase the amount of overlap. Thick filaments are mainly composed of myosin whereas, thin filaments are made up of actin and the regulatory proteins tropomyosin, and troponins T, (tropomyosin binding), I (inhibitory) and C (calcium binding) (reviewed in Gordon *et al*., 2000).

Drosophila melanogaster, commonly known as the fruit fly, has been used for biological research for a very long period. the entire genome of *Drosophila* has been sequenced (Kornberg and Krasnow, 2000), and analysis of the *Drosophila* genome sequence indicated that >60% of the genes implicated in human diseases have *Drosophila* orthologues (Bernards and Hariharan, 2001). The IFM of the *Drosophila* are not necessary for the survival of the flies in the laboratory and many mutants of various proteins including the ones specific for the IFM have been studied in details.

All TnT isoforms of *Drosophila* are encoded by a single gene *upheld* (*up*) comprising of 12 exons of which two are mutually exclusive (Herranz *et al*., 2005, Nongthomba *et al*., 2007). Earlier we have discussed the effects of the *up[1]* mutation, where TAG is changed to TTG at the 3` end of the intron preceeding the wild-type exon 10A, on the muscle development with the summary of the phenotypic effects on the behaviour (Nongthomba *et al*., 2007), here we are reporting the complete data on the phenotypic effects of the *up[1]* mutant and comparison with the wild type flies.

MATERIALS AND METHODS

Fly strains: Flies were maintained at 25° C on a yeast-agar medium. Stock of *ccw[1] sma[1] up[1] mal[F1]/FM7c* was obtained from the Bloomington Stock Center. Other flies were used as described in the FlyBase (http://www.flybase.org). For wild-type controls, Texas and Canton-S flies were used.

Making of the *up[1]* homozygous flies: The *up[1]* stock available from the fly stock centre was maintained heterozygous with the balancer FM7c. In order to characterise and study the mutant, making of homozygous stocks is a prerequisite. As all the males in the stock were FM7c therefore the problem was to get *up[1]* on a non-lethal X-chromosome. The virgin *up[1]*/FM7c females were used to make the *up[1]* stocks.

Walking test: This was performed as described by Naimi *et al*. (2001). Flies were collected from the vials on the day of their eclosion and separated on the basis of their sex after anaesthetising under CO_2. A total of ten males and ten females were selected from each genotype and their wings were cut off using microdissection scissors. The flies were than transferred to separate vials with food and were allowed to recover at 25°C for a day. Next day the walking speed of the flies was recorded by transferring them to a 100 ml measuring cylinder with a mark at 10.5 cm

distance from the base. Flies were tapped down gently by knocking the cylinder on to the rubber pad and immediately the stopwatch was started to note down the time taken by 50% (5) of the flies to cross the 10.5 cm mark. This was repeated 5 times and the average was recorded. In this way the speed was recorded for all the genotypes and the data were recorded until the sixteenth day after eclosion. Statistical analysis was performed on the data to see if there is a difference in the walking abilities among different genotypes.

Jumping test: Jumping test was performed as described earlier (Nongthomba *et al*., 2007). Briefly flies of different genotypes were collected and their wings were removed. They were than placed on a transparent petri plate having a ruled paper under it with lines after every 0.5 cm. The jump response was stimulated by lightly touching the dorsal surface of the thorax with a paintbrush. 8-10 jumps were observed of each of the fly from each genotype. The numbers of lines jumped by the flies were counted and the mean distance jumped for each genotype was calculated. Data were recorded every alternate day for each fly for ten days or till the majority of genotypes were no longer able to jump. Statistical analysis was performed on the data (specified in the results section) to see if there is a difference in the walking abilities among different genotypes.

Flight testing: 3-4 day old flies were separated under CO_2 on the basis of sex into different vials from each genotype to be tested and were given 2-3 hours to recover before testing. Flight test was performed as described by Drummond *et al*. (1991) in a Perspex flight chamber. A light source was set above the chamber and a petri plate at its base to collect the flightless flies. The flies were scored according to the zone in which they landed, up, horizontal, down, or none.

RESULTS

Making of homozygous stocks: Two types of up^1 stocks were made. The first one was the up^1 with the attached X-chromosome (permits all males to inherit X-chromosome), so that there were enough flies to make the second stock, which was the homozygous for the up^1 gene. The scheme for the preparation of the stocks is given in Fig. 1.

Walking ability:. The up^1 gene seems to have no effect on the walking ability of the flies as no significant difference was found between hemi-, homo- and heterozygous up^1 flies compared to the wild type. Also no significant interaction was found between genotypes and days. However, only in the case of up^1 homozygous females highly significant difference was found in the variation due to days as determined statistically by the analysis of variance (ANOVA) shown in Table1 and Fig. 2.

Table 1. Comparison of walking ability between hemi-, hetero-, and homozygous up^1 and wild type flies showing non significant differences between various genotype qalking abilities.

Genotype	Source of variation between	F value	P< 0. 01
up^1 / +	Genotypes	0.1607	non sig.
vs	Days	5.10	high. sig.
wild type	Genotypes X days	1.67	non sig.
up^1 / up^1	Genotypes	1.162	non sig.
vs	Days	4.64	high. sig
wild type	Genotypes X days	1.32	non sig.
up^1 / Y	Genotypes	0.20	non sig.
vs	Days	2.49	non sig.
wild type	Genotypes X days	0.85	non sig.

Table 2. Comparison of jumping ability between *up*^{1} heterozygous and wild type flies showing highly significant difference between the genotypes jumping abilities.

Genotype	Source of variation between	F value	P< 0. 01
up^1 / +	Genotypes	267.88	highly Sig.
vs	Days	0.055	non Sig.
wild type	Genotypes X days	0.127	non Sig.

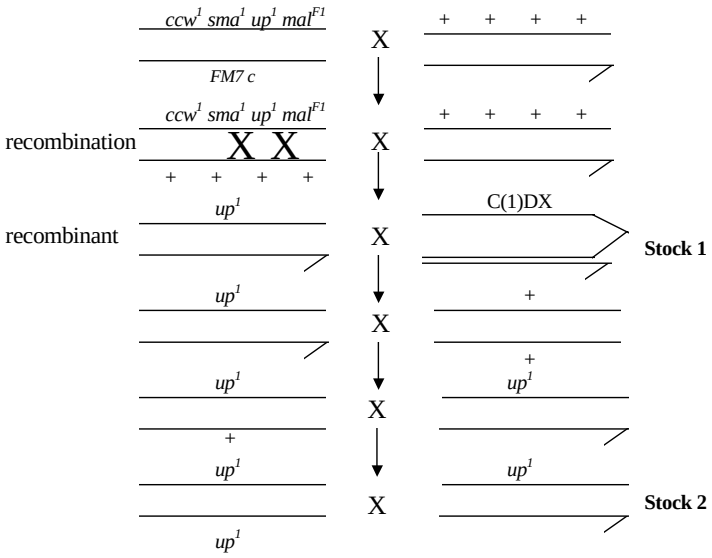

Fig. 1. Scheme for making *up*^{1} homozygous and *up*^{1}/C(1)DX stocks.

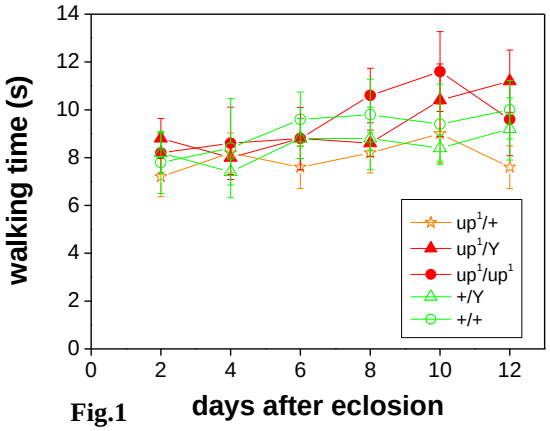

Fig.1

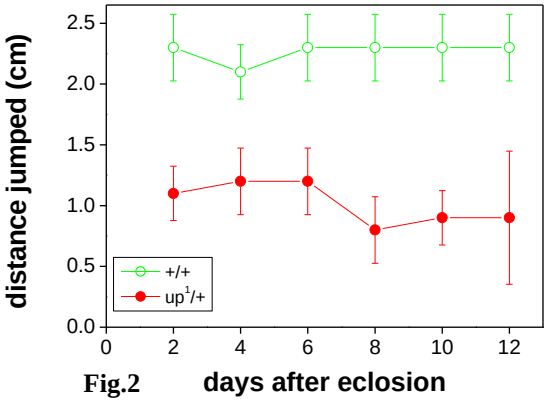

Fig.2

Fig. 2. Graph showing the mean time taken by the different genotypes to cross the 10.5 cms diatance. The bars represent the standard deviations. No significant difference was noted between the various genotypes.

Fig. 3. Graph showing the mean distance jumped by the heterozygous *up*^{1} and wild type flies. The bars represent the standard deviations. Note that there was a significant difference noted between the two genotypes, whereas the *up*^{1} homo and hemizygous flies were barely able to jump.

Jumping ability: The results obtained from the jumping experiment suggested that jumping ability of up^1 flies is poor from the very beginning of adult life and deteriorates with age. Few up^1 flies both hemi- and homozygous were able to jump to a maximum of 0.5 cm on the day of eclosion but after couple of days they lost the jumping ability completely. The heterozygous up^1 flies showed better jumping ability than the homozygous ones although never as good as wild type as determined statistically by the ANOVA shown in Table 2 and Fig. 3.

Flying ability: Although the wings of the majority of the up^1 flies were in the up held position, the flight ability was checked to see if the up^1 flies with the normal wing position and the heterozygous up^1/+ can fly. It was found that none of the up^1 nor the up^1/+ flies was able to fly suggesting that up^1 is a completely penetrant dominant mutation for the flight trait.

DISCUSSION

The up^1 mutation was the first TnT mutation of the up series and apart from up^{101} (Glu 88 Lys) is the only mutation currently available from the stock centres. Although the majority of the flies up^1 flies had their wings in the up position, similar to that of the up^{101} flies, difference was noted in the heterozygotes as up^1/+ were flightless whereas up^{101}/+ were flighted (Fyeberg *et al*., 1990; Naimi *et al*., 2001; Nongthomba *et al*., 2007). Another important difference was seen between the up^1 and the up^{101} mutants. The site and degree of IFM hypercontraction (HC), a condition of muscle damage due to the disturbance in the regulatory mechanism, they cause. In the IFM of the up^{101} HC is mainly observed in the middle with some partial HC also whereas in the mostly partial HC is observed (Naimi *et al*., 2001; Nongthomba *et al*., 2007).

In order to see the affects of the up^1 mutation on various muscles, walking, jumping and flight tests were performed and compared to that of the wild type lies. The walking ability of the up^1 flies seemed to have no effect of the mutation and the flies were able to walk normally as that of the wild type ones. This ability was seen throughout their life indicating that the up^1 mutation is not involved in the TnT protein isoform expressed in the leg muscles. Statistical analysis of the F values also confirmed that there was no significant difference between the two types of flies. The jumping ability of the up^1 flies was found to be absent from the very beginning and the ones that were able to show a very small jump of about 0.5 cm (possibly due to the presence of the foetal TnT isoform) also completely lost it after couple of days. The wild type flies were able to jump throughout their lives. This confirmed that the TnT isoform expressed in the IFM is affected as was shown earlier by Nongthomba *et al*. (2007). Flight was also absent in the up^1 flies and also in the heterozygote up^1 /+ that had their wings in the normal position. This suggested that effected TnT isoform is also involved in the IFM of the flies that is necessary for the flight.

As the up^1 mutation showed a very severe effect on the muscles therefore a detail study of the interactions of various sites among different muscle regulatory proteins in the future may help in understanding the various muscle diseases.

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