



A new climbing assay protocol for *Drosophila melanogaster*.

Deeksha, K.,* Paithankar Jagdish Gopal, and Rajashekhar K. Patil. Department of Applied Zoology, Mangalore University, Mangalore 574 199, Karnataka, India; *e-mail address: deekshakunder@gmail.com

Introduction

Rapid iterative negative geotaxis (RING) in *Drosophila melanogaster* is a frequently employed behavioral assay for addressing questions on motility in relation to ageing, nutrition, and in use of the fly as a disease model. The protocol employs tapping the flies down in a vial and observing the number of flies that climb up to certain level to arrive at a climbing index (Todd *et al.*, 2004; Gargona *et al.*, 2005; Nicole *et al.*, 2009; Charles *et al.*, 2012; Rakshith *et al.*, 2013). The modified RING assay design described in the present study is a variant, wherein a 25 cm tube containing flies is rotated by a motor at fixed programmable intervals to elicit negative geotaxis. This rotation repeatedly places the flies at the bottom of the tube and elicits climbing. Similar studies have been carried out by Sean *et al.*, 2016 to measure genetic variation in response to gently induced exercise.

Keywords: Fly, climbing index, negative geotaxis.

A.

B.

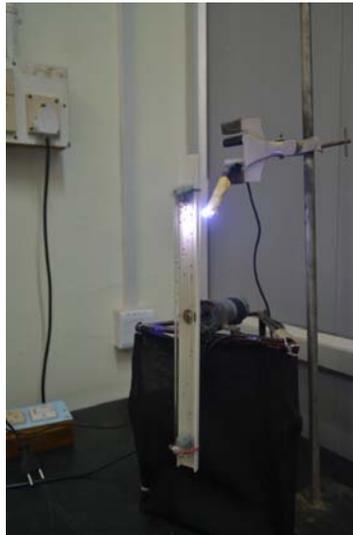
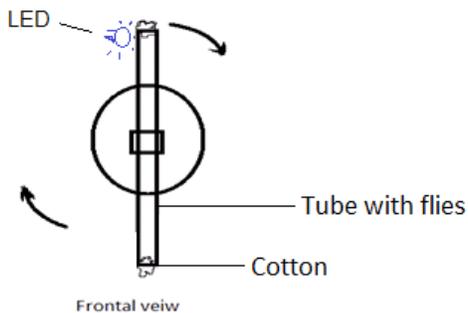


Figure 1. (A) Rotor-RING assay. A schematic representation of the apparatus is provided in A. The rotor rotates clockwise at intervals of 2 minutes. (B) Photograph of the apparatus.

Material and Methods

Fly stocks (Canton-S) obtained from *Drosophila* stock centre, University of Mysore, was maintained in the laboratory as an inbred stock. The stock was maintained at 22°C. Two-day-old flies were used for studies. A motor-operated apparatus with a 25 cm tall tube, fixed in the middle, was made to rotate a half turn of 180° (Figure 1). Two-day-old *Drosophila melanogaster* (Canton-S) flies were used. The interval between successive rotations is programmable in the apparatus. The flies which are at the top of the tube will be at the bottom when the tube makes a half rotation. This induces their innate instinct of negative geotaxis and the flies start climbing up. The tube is designed for the flies to climb a distance of 25 cm. The tube was divided into 4 equal parts using a marker. Time taken for a half rotation is 2 sec. The imparted centrifugal force is negligible and the time between two successive turns was 2 minutes. Climbing index was calculated by

counting the flies which were present in the upper most quadrant. The experiment was conducted for 30 min which thus completes 15 cycles. For each experiment 50 flies were taken. The experiments were carried out before noon. All the experiments were carried out in three sets. Males and females were considered separately for the experiment.

Table 1. Showing number of flies in different divisions of the tube.

Number of rotations	Division 4	Division 3	Division 2	Division 1 Uppermost
1		1	2	47
2				50
3	4		2	44
4	3		2	45
5			1	49
6	2		2	46
7		1		49
8	1		1	48
9		1		49
10	1		1	48
11		1		49
12	1	1	2	46
13	3		2	45
14	2	2		46
15	2	1		47

Observations

Flies that are placed at the bottom of the tube tend to move up due to negative geotaxis. Repetitive stimulation of 15 cycles placed the flies in the bottom of the tube and evoked the flies to climb repeatedly. The time taken for one session of training was 30 minutes following which the flies rested. Climbing index was calculated by counting the number of flies manually (Table 1).

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Antioxidant properties of fruit/vegetable substrates suitable for culturing *Drosophila melanogaster*.

Pavković-Lučić, S.¹, J. Trajković¹, T. Savić², L. Lučić¹, D. Miličić¹, and S. Gorjanović³.

¹University of Belgrade, Faculty of Biology, Studentski trg 16, 11000 Belgrade, Serbia;

²University of Belgrade, Institute for Biological Research „Siniša Stanković“, Despota Stefana Blvd. 142, 11000 Belgrade, Serbia; ³Institute of General and Physical Chemistry, Studentski trg 12/V, 11000 Belgrade, Serbia.

In year 2000, we established four *Drosophila melanogaster* strains reared on substrates cooked with fruits and vegetables (banana - B strain; apple - A strain; T - tomato strain; and C- carrot strain), which represent resources used for feeding in natural conditions (Shorrock, 1972). After culturing flies for more than 60 generations, we have published those recipes as suitable for maintaining *D. melanogaster*, as well as species with similar nutritional requirements (Kekić and Pavković-Lučić, 2003). Later, we have used those strains in experiments devoted to the influence of nutrition on morphological characteristics, cuticular