A rapid method for measuring olfactory responses of *Drosophila* larva*

The olfactory responses of *Drosophila* larvae are measured by following its chemotactic movements on a petri dish. Several variants of this test have been employed. In the commonly used version of the experiment[1–3], diluted odorant is placed near the edge of the dish and a spot of the diluent diametrically opposite. After 5 min, the larvae on the two halves of the dish, the odour side (O) and the control side (C) are counted, and the response index is computed as $O - C/O + C$. This method of measuring olfactory response suffers from certain disadvantages. The test is slow; since the odour spreads on the dish by diffusion, it takes a few minutes for the larvae on the farther side of the odour source to respond. On the other hand, the larvae near the source respond early and, if the concentration of the odorant is high, they become desensitized and wander-off. We describe here a modification of the larval test, which enables us to assess the attraction response rapidly by measuring the initial rate of entry in a zone around the odour source.

CsBZ flies were grown on standard cornmeal yeast medium at 24°C and

*Dedicated to Prof. S. Ramaseshan on his 80th birthday.

maintained on a 12 h day/night cycle. Standard procedures were used in handling cultures[4,5]. Larvae at a prescribed stage of development were separated from the cornmeal culture medium by floating on 30% polyethylene glycol and washed free of debris in tap water. A fixed number of larvae, between 30 and 50, were placed at the centre of a petri dish filled with 1% agar and allowed to spread out. As they reach an outer 3 cm ring, 10 µl of diluted odorant ethyl acetate (EA) was placed in a small well at the centre (Figure 1). As the larvae sense the odour, they turn around and move towards the centre. The response is measured by the initial rate at which the larvae cross the inner 1 cm ring, marked on the dish. The response index may be defined as per cent of larvae entering the ring per minute.

The petri dish is photographed with a digital camera at short intervals of 15 s or less, and the larvae outside the ring are counted, either from a printout of time-lapse photographs or using a computer program written by one of the authors (V.A.). The automation of the experiment eliminates the errors in timing as well as counting, and greatly reduces experimenter's bias.

The response of third instar larvae to varying dilutions of EA in the range 10^{-7} to 10^{-4} is shown in Figure 2. It may be seen that attraction is proportional to concentration of the odorant over a hundred-fold change, but falls at concentrations greater than 10^{-5}, as repulsion sets in.

Tracks of individual larva can be reconstructed from the photographic record taken at intervals of 5 s. The larva turns towards the odour source gradually (Figure 1). The usual track is circular or spiral, although an occasional larva might make a sharp 180° turn. The curvature of the track shows considerable variation. Analysis of larval tracks provides valuable information about the mechanism of orientation[6]. The crawling speed increases with age and is correlated with an increase in body size (Figure 3). The average length of the first instar larva was 0.88 mm and its speed 1.71 cm/s. The third instar larva had a length of 3.58 mm and a crawling speed of 5.88 cm/s. The average speed in body-lengths thus remained unchanged. The larval speed also increased with the concentration of the odorant. For a tenfold increase in concentration, the speed in our experiment went up by 3.3 mm/min.

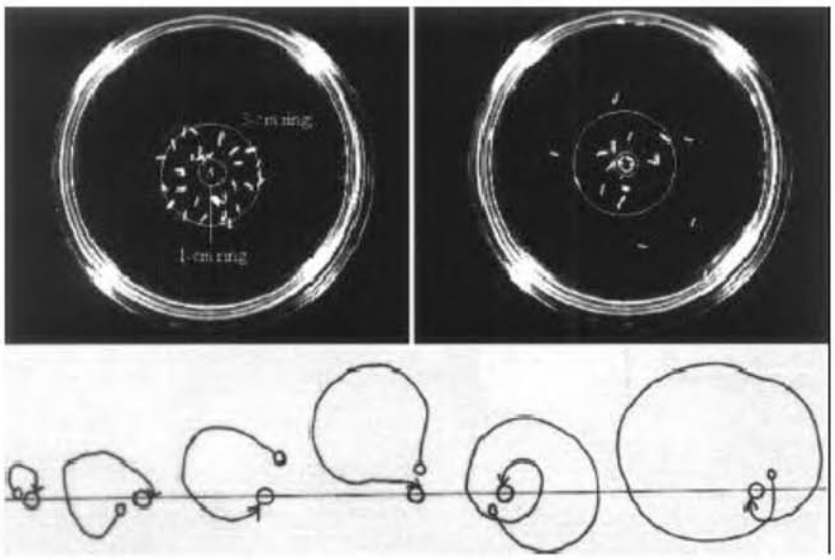

Figure 1. (Top) Test plate at 15 s (left) and 180 s (right). Tracks show larvae spiralling into the odour well. Response is measured by the rate at which they enter the inner 1 cm ring.

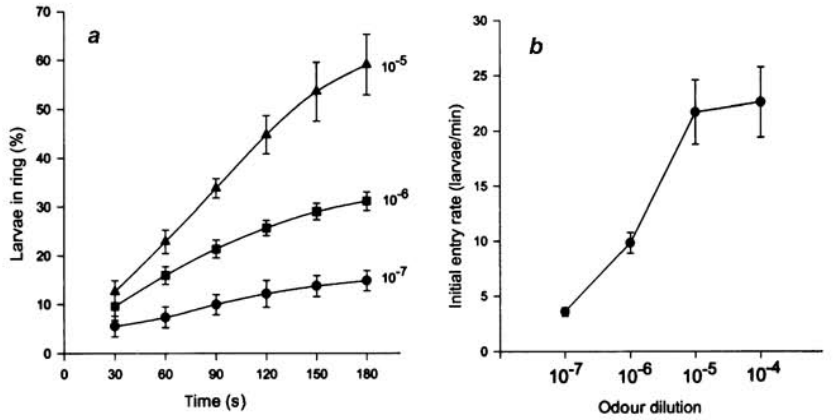

Figure 2. Entry curves showing response of larvae to increasing concentration of ethyl acetate (*a*). Initial rate of entry is proportional to odorant concentration in the range 10^{-7} and 10^{-5}. Attraction declines above 10^{-5} (*b*).

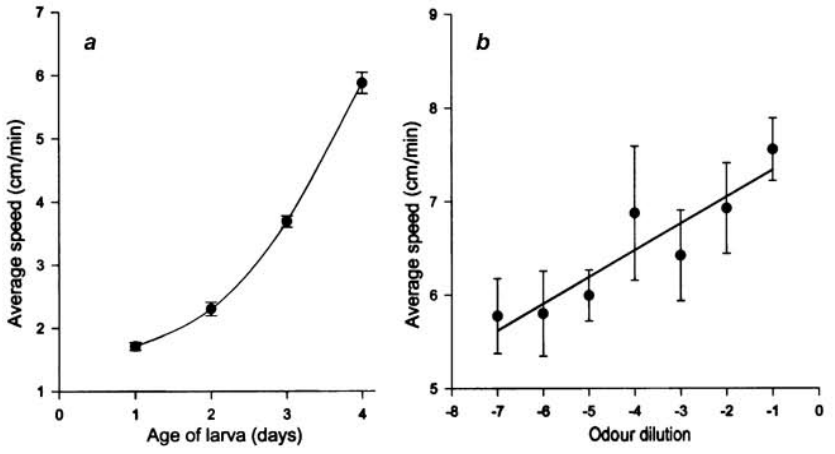

Figure 3. Crawling speed of larvae increases with age (*a*) and odorant concentration (*b*).

The chief merits of the method described above are its rapidity and accuracy. The initial rate of entry can be determined in less than 2 min and the standard error of the measurement can be easily brought down to less than 10%. The paradigm can be used for analysing the tracks (Figure 1) of individual larvae, and is specially suitable for studying olfactory learning and memory.

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