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A novel assay to screen for mutants with strong olfactory defects.

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A novel OLFACTORY TRAP ASSAY was developed to facilitate large scale screening of flies with olfactory defects. This was necessitated because the conventional olfactometers did not permit large scale screening of flies. For example, Carlson's trap (Woodard, 1989) and the Y-maze (Ayyub, 1990) allowed screening of only about 10 flies and 100 flies per run, respectively. In contrast, the new olfactory trap assay reported here, facilitates screening of at least 500 flies per run. In order to determine the efficiency of this assay, experiments were carried out with the wild type strain *CS* and the mutant- *olfD*^{x9} (Ayyub, 1990). *olfD*^{x9} is the strongest known olfactory mutant and is anosmic to multiple odours. Normal fly medium (cornmeal agar medium) and well fermented yeast were used as attractants in the assay. The results show a strong response to the attractants by both the wild type and the anosmic mutant, *olfD*^{x9}, opening up a new and simpler way to obtain olfactory mutants having stronger olfactory phenotypes.

Design of olfactometer: The olfactometer consisted of a perspex cubical box of internal dimensions 24cm × 24cm × 24cm. A shutter provided on one side of the box facilitated opening and closing of the box. Several orifices on two side walls of the box provided ventilation. A hole on the top facilitated introduction of flies into the box. Each box contained four traps placed at the corners of the box. A moist filter paper on the floor of the box provided humidity. The trap consisted of either normal *Drosophila* food (Cornmeal Agar traps) or fermented yeast (Yeast Traps) in a split bottle (joined together by sticky tape) as attractant. In case of cornmeal agar traps, about ten grams of yeast granules were sprinkled on the surface of the medium. In case of the yeast traps, yeast paste was allowed to ferment for at least 18 hours at 37 °C. The consistency of the yeast paste was readjusted by adding additional yeast granules. This was then applied to the wall of the bottle containing 1.0 % sucrose agar medium. The open end of the bottle was covered with a glass funnel attached to a 1 ml plastic micropipette tip severed at the pointed end. The funnel allowed the free entry of flies into the trap but restricted their exit (Figure 1 a and b).

Assay: Three- to five-day-old flies obtained from uncrowded bottles were used in all the experiments. Flies were starved in bottles containing moist filter paper for about 18 hours, prior to the test. Starved flies were then introduced into the box containing traps. The experimental run was for 24 hours in dark and at the end of each run, the persplex boxes with flies were placed at 4°C to knock out the flies. The flies were then collected from both the box and the traps and screened under the microscope. The percentage of flies in the traps was calculated as given below:

$$\% \text{ Flies trapped} = \frac{\text{Number of flies in traps}}{\text{Total number of flies in the box}} \times 100$$

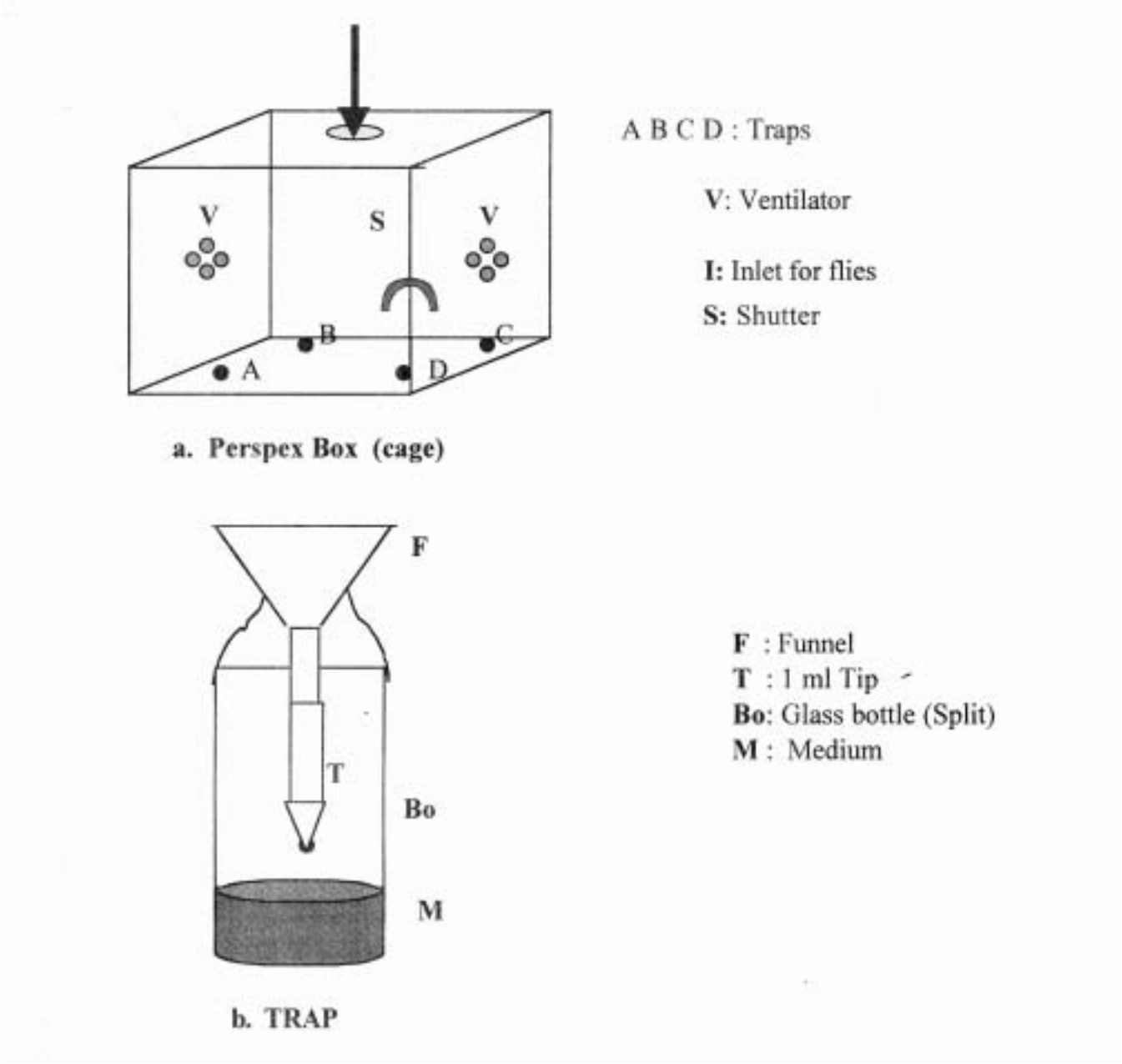

Figure 1 a and b. The experimental set up.

Results:

Cornmeal agar trap assay: Experiments with *Canton-S* showed that 96.0% of the flies entered the cornmeal agar traps. About 7.0% of the total flies died during the experiment. About 86.0% of homozygous $olfD^{x9}$ and 90.0% of the $olfD^{x9}$ / FM7C were in the traps (Figure 2 a).

Yeast trap assay: Experiments carried out with *Canton-S* flies and $olfD^{x9}$ (Ayyub, 1990) showed that both the strains responded to the well fermented yeast. Nearly 96.0% of *CS* flies were found in the traps. About 6% of the total flies were found dead during the experiment. Surprisingly,

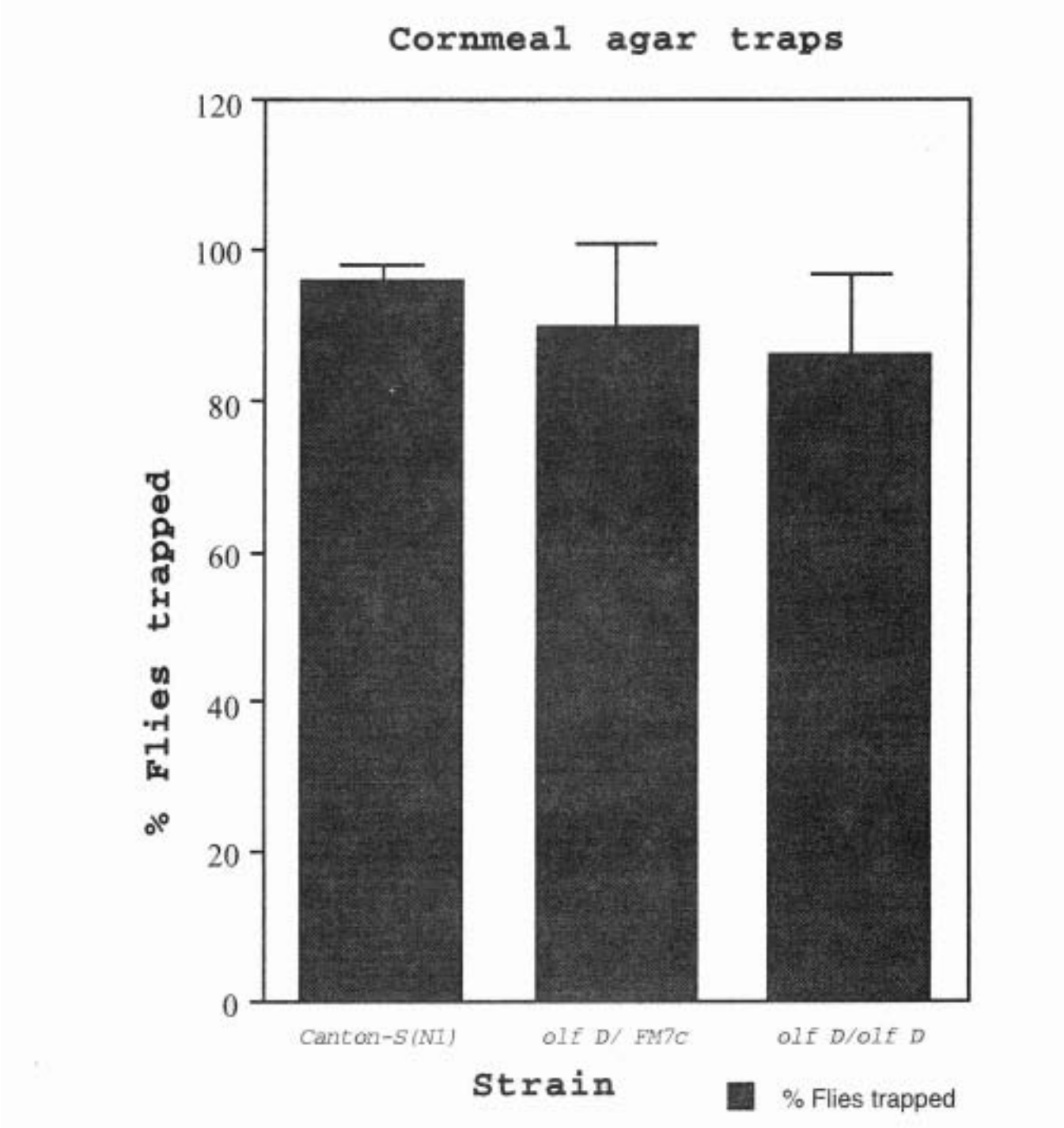

Figure 2a. Cornmeal agar traps. Each reading represents an average of the percentage of flies trapped in cornmeal agar traps. Error bars represent standard deviation.

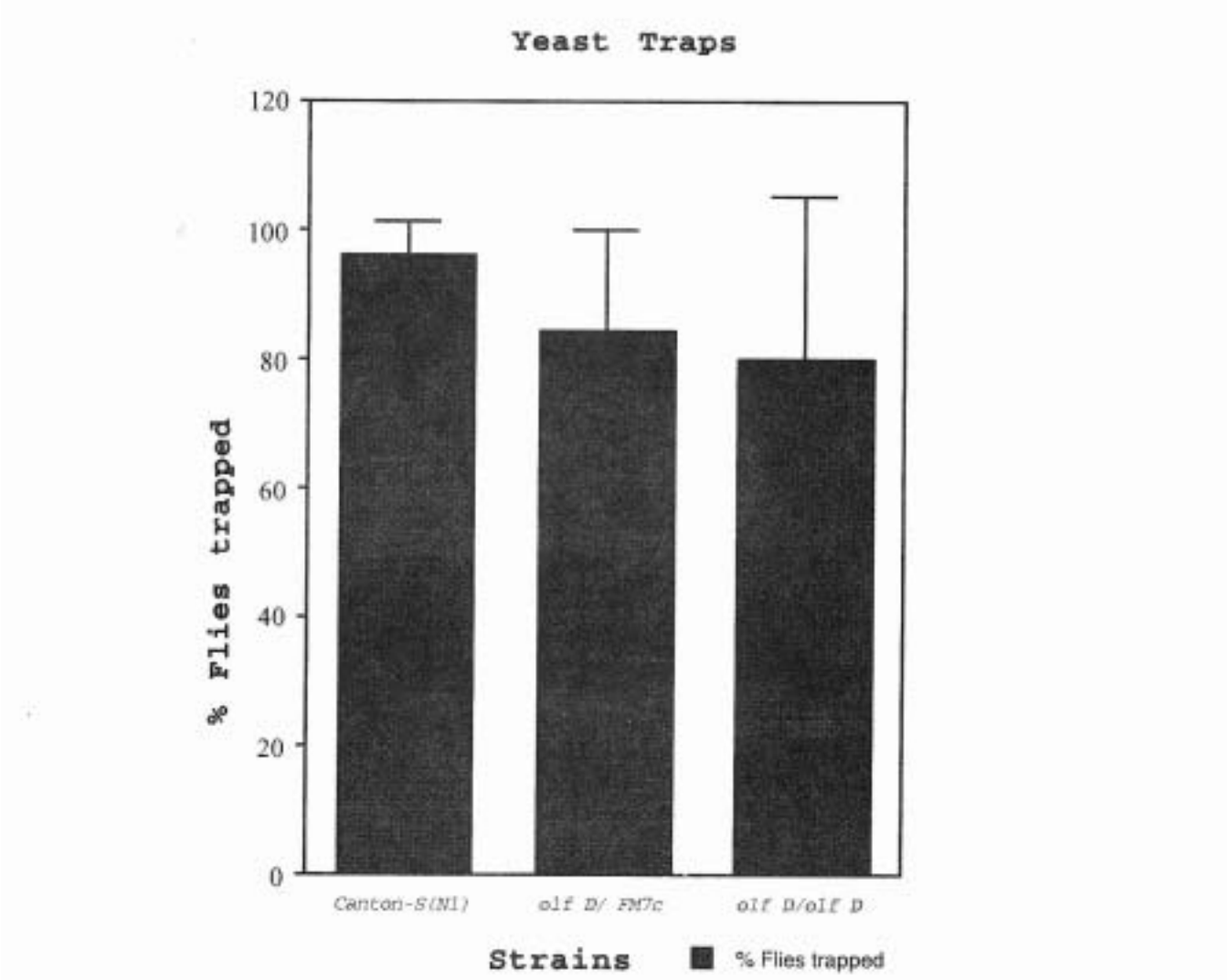

Figure 2b. Yeast traps. Each reading represents an average of the percentage of flies trapped in yeast traps. Error bars represent standard deviation.

about 80.0% of the homozygous *olfD*^{x9} mutant were also found in the traps. Whereas, 84.0% of the *olfD*^{x9}/ FM7C flies responded to the yeast traps (Figure 2 b).

These results suggest that this assay could serve as a valuable tool in isolating mutants with strong olfactory defects, hitherto not permitted by the conventional olfactory paradigms.

References: Ayyub, C., J. Paranjape, V. Rodrigues, and O. Siddiqi 1990, J. Neurogenet. 6(4): 243-262; Woodard, C., T. Huang, H. Sun, S.L. Helfand, and J. Carlson 1989, Genetics 123(2): 315-326.