This finding confirms previous observations that *D. nebulosa* is at its best in the savanna environments where dry seasons alternate with rainy ones (Dobzhansky, 1950), and that it occurs in dry habitats where no sibling species is found (Ayala *et al*., 1974). The finding here reported extends the putative region of ocurrence for this species in about 230,000 square kilometers and into the semi-arid regions of the Andean mountain range.

Finally, we would like to encourage further attempts to summarize and compile the distributional data of other Neotropical species of *Drosophila*, a task dearly needed for the better assessment of the biodiversity and too infrequently performed.

Acknowledgments: To M. Polihronakis who independently confirmed the species identification. This study was supported by CONICET, ANPCyT and Universidad of Buenos Aires. JP is recipient of a postgraduate scholarship of CONICET. IMS is a member of Carrera del Investigador Científico (CONICET).

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Behavioral characterization of P-element insertion lines of *Drosophila melanogaster*.

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Abstract

As continuation of our previously published results (Ahsan *et al.*, 2008) we further characterized the *Drosophila* P-element insertion lines behaviorally in our standardized adult and larval olfactory behavioral paradigms for their olfactory response phenotype. We tested these lines with three chemicals - Ethyl acetate, Isoamyl acetate, and 1-Hexanol at concentrations varying from 10^{-1} to 10^{-9}. We also compared the conditioned and unconditioned olfactory responses. We found insertion lines with varying degree of olfactory responses to the tested chemicals.

Introduction

The fruit fly, *Drosophila melanogaster*, can smell and discriminate a wide variety of odors with remarkable sensitivity and specificity. Its olfactory system is somewhat similar to vertebrates, but indeed a lot simpler structurally, and may help unravel fundamental principles of chemosensation and contribute to understanding of the more complex process of olfaction in higher organisms. Because of its anatomical simplicity, the availability of genetic information, the well-established physiological and behavioral analysis techniques, and its ability to learn in simple olfactory-based associative learning paradigms, *Drosophila* has become an ideal model organism for studying olfaction (de Bruyne *et al.*, 2001).

Recent advances in genomics and molecular neurobiology have provided an unprecedented level of detail into how the adult *Drosophila* olfactory system is organized. Volatile odorants are sensed by two bilaterally symmetric olfactory sensory appendages, the third segment of the antenna and the maxillary palps, which, respectively, contain approximately 1200 and 120 olfactory receptor or sensory neurons (ORNs or OSNs) each. These ORNs express a divergent family of seven transmembrane domain odorant receptors (ORs) with no homology to vertebrate ORs, which determine the odor specificity of a given ORN.

Adults and larvae are anatomically and behaviorally much different, reflecting their different lifestyles. For example, adult *Drosophila* flies need to find food (as well as mates, egg-laying sites, etc.), which requires sophisticated odor-driven behavior. Fly larvae, in contrast, live on their food source and hence do not need long-range odor detection to find food. Although larvae respond to a variety of chemicals (Rodrigues *et al.*, 1980; Cobb *et al.*, 1999; Heimbeck *et al.*, 1999; Cobb and Domain 2000), one may expect the chemosensory systems of both developmental stages to display significant differences in terms of cell number, organization, and behavioral function. The cephalic chemosensory apparatus of the larva includes three external sense organs, dorsal organ (DO), terminal organ (TO), and ventral organ (VO), as well as three internal, pharyngeal organs (Singh and Singh, 1984; Singh, 1997; Python and Stocker, 2002a; Gendre *et al.*, 2004). Each of them consists of several sensilla, a sensillum comprising one to several sensory neurons and three accessory cells, all housed below a common cuticular structure or terminal pore. The DO is composed of the central "dome" and six peripheral sensilla. The dome, whose wall is perforated by thousands of pore tubules, is innervated by the profuse dendritic arbors of 21 olfactory receptor neurons (ORNs). The odorant receptor family in *Drosophila* consists of 62 members (Clyne, 1999; Vosshall, 1999; Robertson, 2003) compared to more than 1000 in rodents. At least 25 of the 62 receptors are expressed in the fly larva. Of these 25, 14 are larval-specific, while the rest are expressed in both adult and larval olfactory systems. As in the adult, the large majority of the 21 larval olfactory sensory neurons express one conventional odorant receptor, along with an atypical receptor, OR83b.

The evolution of studies on olfaction in *Drosophila* has come a long way, starting from the first odor attraction studies by Barrows (1907), establishment of role of antenna in odor response using genetic approaches (mutant antennaless) by Begg and Hogben (1946), further use of neurogenetics to study various neurological phenomenon by Benzer (1971, 1973), isolation of first single-gene olfactory mutations by Rodrigues and Siddiqi (1978, 1981), and since then many more single-gene mutations are isolated and are being studied in great detail by various researchers. A majority of these mutations have been identified using adult olfactory screens, but in several cases larvae of the mutants show abnormal responses as well. Rodrigues (1978) and Ayyub (1990) isolated olfA, olfB, olfC, olfD, and olfE mutants. olfD shows reduced response to all the odors while olfA, olfB, and olfE show reduction in response to benzaldehyde. olfC shows reduced response to acetate

esters. olfD was found to be allelic with smellblind (sbl, Aceves Pina and Quinn, 1979) and was found to be localed in the para, a class of Na+ channel gene (Lilly *et al.*, 1994). The mutant olf413 larvae show reduced response to only Ethyl acetate and it is normal for other chemicals tested (Tickoo, 2001). chsB is a chemosensory mutant (Trivedi, 2003). Defects in the olfactory behavior reflect lesions at any step of the pathway from odor detection, to transduction, processing, and motor response.

Isolation of *Drosophila* olfactory mutants is important in identifying the genes mediating the sense of smell. First single-gene olfactory mutations were isolated by Rodrigues and Siddiqi (1978, 1981). The P-element has been the workhorse of *Drosophila* genetics since it was developed as a tool for transgenesis in 1982; the subsequent development of a variety of systems based on the transposon have provided a range of powerful and flexible tools for genetics and genomics applications. P-element insertions are frequently used as starting-points for generating chromosomal deletions to remove flanking genes, either by screening for imprecise excision events or by selecting for male recombination events. One of the early uses for P-elements was in large-scale mutagenesis screens, the major advantage over traditional chemical or radiation methods being that mutants were molecularly tagged by virtue of the P-element sequence (Russell *et al.*, 2003). In addition to phenotypic screening, P-elements also can be used to study the pattern and timing of gene expression by enhancer trapping (Figure 3.1) (O'Kane *et al.*, 1987; Wilson *et al.*, 1989).

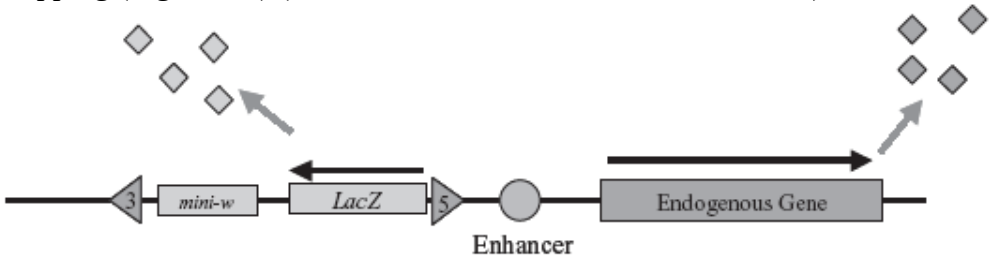


Figure 1. Enhancer trapping. A P-element construct containing a transformation marker, in this case a functional copy of the white gene (mini-w), and a LacZ reporter gene driven by a weak basal promoter inserts near a gene. An endogenous enhancer (grey circle) may then control the expression of the LacZ reporter in a similar pattern to the endogenous gene (black arrows). P-element ends are shown as triangles (5 and 3), and gene products are shown as squares.

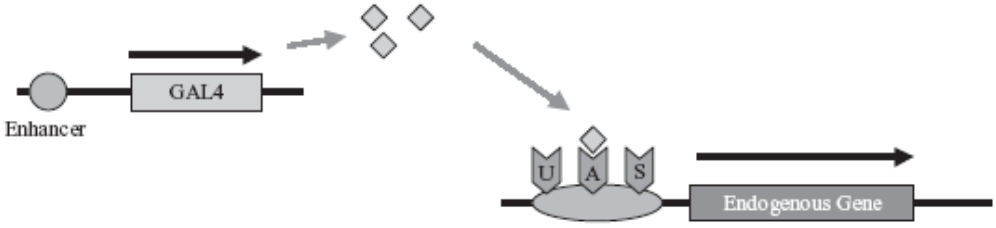


Figure 2. GAL4-activated gene expression. In the GAL4-UAS system, a construct containing the GAL4 gene is inserted randomly in the genome. As with the enhancer trap strategy shown in Figure 2, it may come under the influence of a genomic enhancer and express GAL4 in a pattern dictated by the enhancer. The GAL4 protein can then act at any UAS sites in the genome to activate expression of a gene of interest. Two scenarios are possible. In the first, a gene of interest is introduced into the genome in a P-element construct carrying UAS sites. In the second, a set of UAS sites in a P-element (an EP-element) are mobilised at random in the genome; if they insert in the vicinity of an endogenous gene, GAL4 can be used to activate the expression of that gene.

One widely-used variant of the enhancer trap strategy is the GAL4-UAS system developed by Brand and Perrimon (1993). This binary system utilizes enhancer trapping with a construct carrying the *Saccharomyces cerevisiae* transcriptional activator, GAL4, as a reporter gene and the activity of the GAL4 protein as a transcription factor can be detected by monitoring the expression of a second reporter gene under the control of a GAL4 responsive promoter, or upstream activation sequence (UAS) (Figure 3.2). On the one hand, reporter genes such as LacZ or GFP can be used to visualize the expression pattern of the enhancer. On the other hand, and far more importantly, any gene placed downstream of the UAS sequences in a construct can be activated by the GAL4 protein.

A set of P-element insertions containing P[lArB] (Figure 3.3) and P[GawB] (Figure 3.4) were screened in our lab on the basis of their LacZ and GFP reporter expression patterns in olfactory organs, their adult and larval behavior phenotypes were characterized by me, Nixon (2001), Sukant (2003), Bilal (2004), Shamsudeen (2005), Hisham (2005), Satyajit (2006), Amulya (2007), Deepitha (2007), Shobhana (2007), Shwetha (2007), and others.

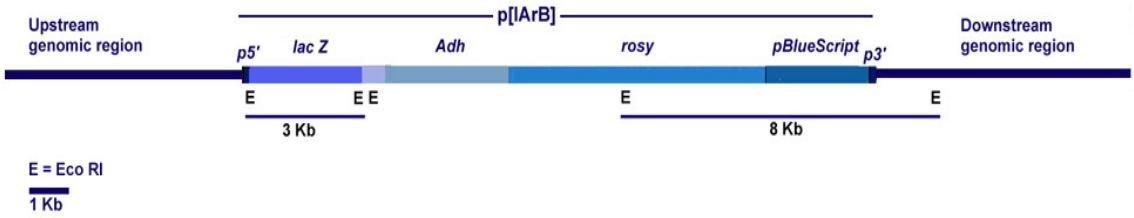


Figure 3. Schematic of the molecular structure of P[lArB]. It consists of enhancer trap *lacZ* and selectable markers *rosy* and *Adh*.

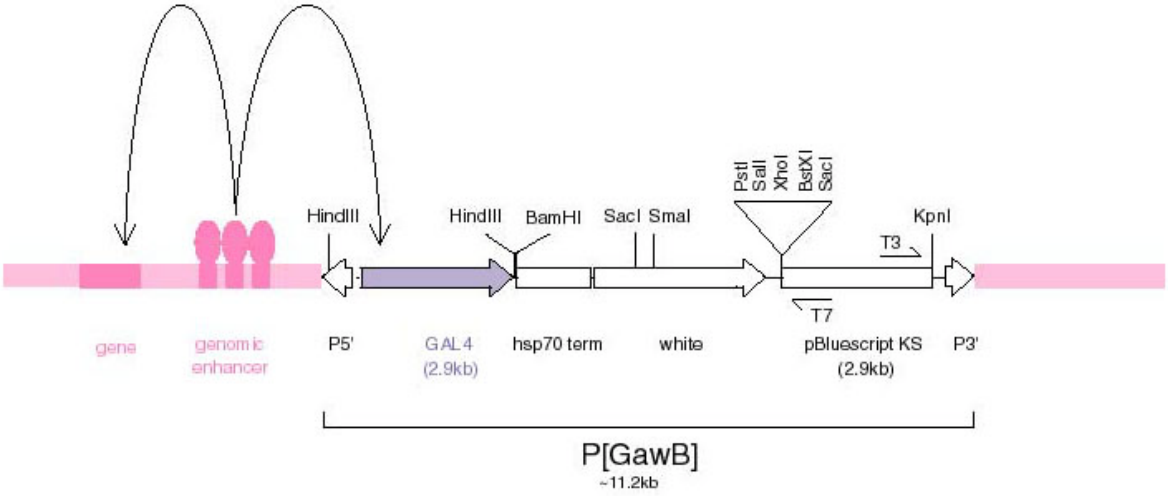


Figure 4. Schematic of the molecular structure of P[GawB]. It consists of enhancer trap *GAL4* (binary system) and selectable marker *white*.

Materials and Methods

Drosophila stocks and maintenance

Drosophila melanogaster imagoes (all the strains tabulated below) were maintained at 24ºC in standard cornmeal agar medium with 14-hour light and 10-hour dark cycle. Standard procedures were used for handling the cultures (Roberts, 1986). Flies were allowed to lay eggs and transferred

every 24 hours for expansion. For larval work, flies were allowed to lay eggs in media bottles for 12 hours.

List of fly strains

(1) Canton Special Benzer (CSBz), Wild type stock, TIFR Stock Centre, Mumbai; (2) 003, 030, 191, 1110, OK66, OK140, OK284, OK294, OK301, OK309, Cahir O'Kane, University of Cambridge, Cambridge, UK; (3) 238Y, Josh Dubnau, CSHL.

Results and Discussion

Adult olfactory responses of P[GawB] insertion lines

Adult flies of eight P[GawB] insertion lines were characterized behaviorally (after four days of conditioning) using the T-trap assay (Chakraborty *et al.*, 2011, 2009). There are different classes of insertion lines, which show varying degree of conditioned and unconditioned responses for the three chemicals tested (Ethyl acetate, Isoamyl acetate, and 1-Hexanol) when compared to the wild type response (Table 1). In the line 238Y, the unconditioned response is increased for both Ethyl acetate and Isoamyl acetate when compared to the wild type (the conditioned response is normal).

Table 1. Adult behavioral phenotype of P[GawB] insertion lines compared to wild type.

Strains	Ethyl acetate		Isoamyl acetate		1-Hexanol	
	CR	UCR	CR	UCR	CR	UCR
003	↓	↓	↓	N	↓	↓
030	↓	N	↓	↓	↓	↓
191	N	↓	↑	N	N	↓
OK66	N	↑	↑	N	↓	↓
OK284	N	N	↓	N	↓	↓
OK301	↓	↓	↑	↑	↓	N
OK309	↓	N	↓	↓	↓	↓
238Y	N	↑	N	↑	↓	↓

CR : Conditioned Response; UCR : Unconditioned Response; N : Normal;
↑ : Increased; ↓ : Decreased

Larval olfactory responses of P[GawB] insertion lines

The early third instar larval behavioral phenotype for the same eight insertion lines was measured using the larval plate test (Khurana *et al.*, 2009). The lines were tested for a range of log dilution (10^{-1} to 10^{-9}) of three chemicals (Ethyl acetate, Isoamyl acetate, and 1-Hexanol). There are again different classes of insertion lines showing varying responses across different chemicals tested. Interestingly, when compared to wild type, the responses of few insertion lines are different at certain log dilutions but normal at other log dilutions of the same chemical (Tables 2, 3 and 4) (Figures 5 to 12). The line 238Y shows increased response to all the three chemicals tested at lower dilutions.

The unconditioned response of 238Y line adult flies is increased for both Ethyl acetate and Isoamyl acetate when compared to the wild type and the conditioned response is normal. The third instar larvae show increased response to all the three chemicals tested at lower dilutions.

Isolation of *Drosophila* olfactory mutants is important in identifying the genes mediating the sense of smell. The P-element insertion (P[lArB] and P[GawB]) lines described in this thesis were screened in our lab on the basis of their LacZ and GFP reporter expression patterns in olfactory organs. Eight P[GawB] insertion lines were tested both in adult and larval stages using the T-trap

Table 2. Larval behavioral phenotype of wild type and P[GawB] insertion lines for Ethyl acetate.

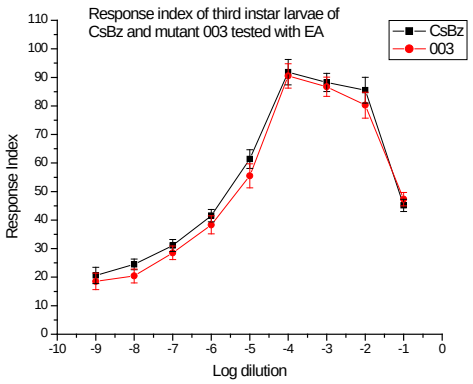
Log dilution	CsBz	003	030	191	OK66	OK284	OK301	OK309	238Y
10^{-1}	45.15 ± 2.12	47.32 ± 2.36	43.54 ± 3.28	43.19 ± 2.59	46.65 ± 3.19	46.88 ± 3.12	41.57 ± 3.21	46.17 ± 2.56	43.21 ± 3.24
10^{-2}	85.53 ± 4.53	80.26 ± 4.54	86.89 ± 2.76	82.45 ± 4.23	88.74 ± 4.38	82.49 ± 4.23	80.92 ± 4.28	82.76 ± 3.42	80.12 ± 4.31
10^{-3}	88.23 ± 3.17	86.72 ± 3.39	86.72 ± 4.23	90.73 ± 3.01	90.87 ± 4.52	85.31 ± 3.82	85.37 ± 3.54	87.39 ± 3.41	83.61 ± 4.64
10^{-4}	91.83 ± 4.45	90.53 ± 4.27	92.1 ± 4.43	92.56 ± 3.29	91.95 ± 3.29	90.99 ± 4.21	80.53 ± 4.52	92.14 ± 4.21	85.9 ± 2.28
10^{-5}	61.38 ± 3.27	55.54 ± 4.21	52.55 ± 3.21	65.01 ± 4.52	57.52 ± 2.49	65.32 ± 2.38	51.76 ± 3.16	55.26 ± 2.41	62.15 ± 3.5
10^{-6}	41.56 ± 2.18	38.32 ± 3.15	45.02 ± 2.76	50.56 ± 2.35	44.67 ± 3.25	50.92 ± 2.73	32.47 ± 2.57	35.19 ± 2.53	56.43 ± 3.45
10^{-7}	31.11 ± 2.1	28.52 ± 2.38	35.67 ± 2.91	38.42 ± 2.53	32.8 ± 3.2	40.42 ± 2.45	24.19 ± 2.45	24.42 ± 2.15	51.8 ± 2.76
10^{-8}	24.5 ± 1.86	20.43 ± 2.43	25.32 ± 2.19	30.23 ± 2.19	25.98 ± 2.43	30.31 ± 1.99	20.32 ± 2.42	19.29 ± 2.1	49.1 ± 3.85
10^{-9}	20.62 ± 2.85	18.52 ± 2.87	22.74 ± 2.84	25.65 ± 2.85	21.84 ± 2.79	24.67 ± 2.42	16.12 ± 1.67	17.15 ± 1.98	36.5 ± 2.19

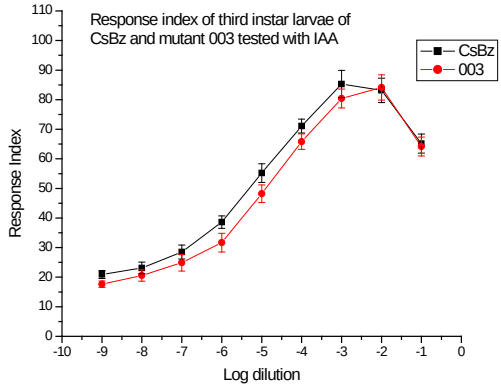
Table 3. Larval behavioral phenotype of wild type and P[GawB] insertion lines for Isoamyl acetate.

Log dilution	CsBz	003	030	191	OK66	OK284	OK301	OK309	238Y
10^{-1}	65.16 ± 3.21	64.18 ± 3.18	63.17 ± 2.43	69.79 ± 3.21	69.72 ± 3.19	69.72 ± 3.81	66.83 ± 2.45	67.91 ± 2.84	62.16 ± 3.16
10^{-2}	83.15 ± 4.12	84.13 ± 4.28	77.32 ± 3.12	80.41 ± 2.56	81.83 ± 2.62	80.19 ± 4.12	82.35 ± 3.41	78.31 ± 2.87	85.35 ± 2.45
10^{-3}	85.29 ± 4.6	80.42 ± 3.19	75.79 ± 2.51	82.94 ± 3.63	83.52 ± 4.17	84.39 ± 2.15	84.19 ± 3.67	80.74 ± 3.74	90.15 ± 4.23
10^{-4}	71.09 ± 2.36	65.81 ± 2.63	63.61 ± 2.72	75.23 ± 2.69	70.94 ± 2.35	72.74 ± 3.51	75.78 ± 2.49	66.73 ± 2.69	75.32 ± 3.27
10^{-5}	55.19 ± 3.14	48.23 ± 2.98	48.36 ± 3.17	60.35 ± 3.52	57.29 ± 3.15	60.86 ± 2.31	63.71 ± 2.68	55.81 ± 4.12	70.45 ± 2.48
10^{-6}	38.61 ± 2.12	31.68 ± 3.15	29.16 ± 2.1	50.52 ± 3.19	41.82 ± 3.67	49.92 ± 3.27	50.18 ± 3.72	32.93 ± 2.73	47.85 ± 3.19
10^{-7}	28.51 ± 2.34	24.92 ± 2.83	24.62 ± 2.19	38.1 ± 2.15	36.52 ± 2.13	36.61 ± 2.54	38.21 ± 2.81	24.76 ± 1.89	35.74 ± 2.31
10^{-8}	23.12 ± 1.97	20.56 ± 1.92	20.14 ± 1.92	30.46 ± 1.98	27.42 ± 1.38	28.85 ± 1.96	32.98 ± 1.93	20.75 ± 1.67	30.98 ± 2.18
10^{-9}	20.9 ± 1.35	17.63 ± 1.1	16.28 ± 1.31	24.91 ± 2.17	24.91 ± 1.42	24.96 ± 2.01	26.99 ± 2.14	17.82 ± 1.2	27.16 ± 1.97

Table 4. Larval behavioral phenotype of wild type and P[GawB] insertion lines for 1-Hexanol.

Log dilution	CsBz	003	030	191	OK66	OK284	OK301	OK309	238Y
10^{-1}	68.15 ± 3.21	70.28 ± 4.14	64.91 ± 3.16	70.8 ± 4.13	70.45 ± 2.91	67.82 ± 4.15	65.97 ± 2.31	66.93 ± 2.19	66.32 ± 2.13
10^{-2}	79.12 ± 3.53	77.81 ± 2.21	78.45 ± 3.52	76.29 ± 3.61	81.19 ± 2.76	80.19 ± 4.21	76.12 ± 4.37	76.19 ± 4.27	78.94 ± 3.61
10^{-3}	61.81 ± 2.47	62.94 ± 3.18	63.21 ± 4.81	60.57 ± 3.53	59.39 ± 3.27	62.76 ± 2.84	59.52 ± 3.19	60.65 ± 3.17	72.01 ± 3.72
10^{-4}	57.5 ± 3.76	51.85 ± 2.74	50.81 ± 2.36	58.98 ± 4.01	51.98 ± 4.1	54.86 ± 3.91	54.87 ± 3.26	51.87 ± 3.32	70.61 ± 2.78
10^{-5}	43.61 ± 2.14	36.82 ± 2.85	34.17 ± 3.19	47.78 ± 2.91	45.83 ± 2.98	44.29 ± 2.38	37.19 ± 2.16	35.62 ± 2.91	53.82 ± 2.19
10^{-6}	31.18 ± 2.41	25.91 ± 1.47	24.31 ± 2.64	36.47 ± 2.1	30.98 ± 2.13	36.19 ± 2.13	21.91 ± 1.31	25.78 ± 2.45	44.87 ± 3.52
10^{-7}	24.52 ± 1.91	20.1 ± 2.15	20.67 ± 1.69	29.32 ± 1.48	23.16 ± 1.38	30.28 ± 2.87	16.35 ± 1.2	18.32 ± 1.25	40.62 ± 1.98
10^{-8}	17.19 ± 1.01	14.13 ± 1.24	13.16 ± 1.03	22.91 ± 1.31	13.31 ± 1.12	20.91 ± 1.25	12.81 ± 1.91	11.53 ± 1.73	30.84 ± 2.01
10^{-9}	10.12 ± 1.23	8.27 ± 1.09	8.23 ± 1.14	17.78 ± 1.3	10.19 ± 1.41	14.21 ± 1.01	7.06 ± 1.41	7.32 ± 1.05	20.41 ± 1.57





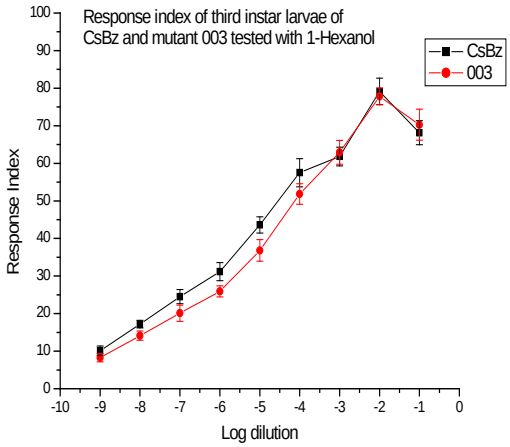


Figure 5 (previous page). Olfactory response of third instar larvae of wild type and 003 line tested for varying log dilutions of Ethyl acetate, Isoamyl acetate, and 1-Hexanol. Each response index (RI) represents the mean of six experiments, each experiment consisting six petri-dishes. The standard error was calculated as the standard error of mean.

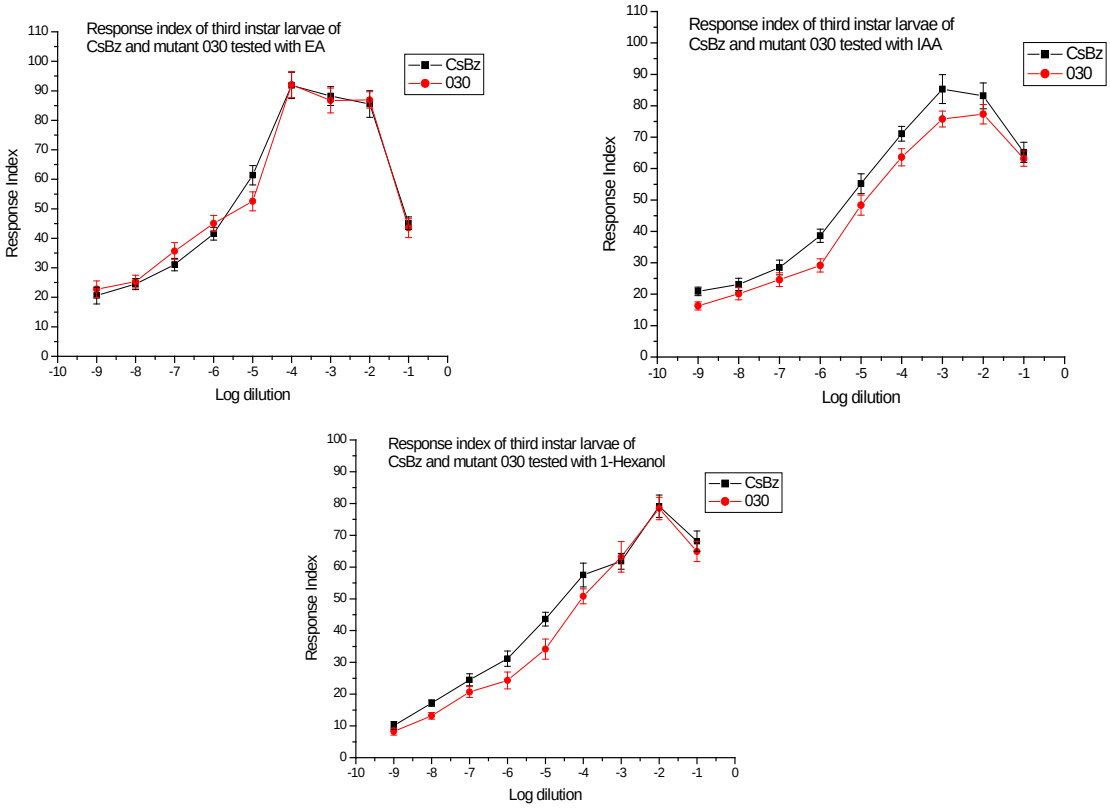


Figure 6. Olfactory response of third instar larvae of wild type and 030 line tested for varying log dilutions of Ethyl acetate, Isoamyl acetate, and 1-Hexanol. Each response index (RI) represents the mean of six experiments, each experiment consisting six petri-dishes. The standard error was calculated as the standard error of mean.

and larval plate assay, respectively, for three chemicals (Ethyl acetate, Isoamyl acetate and 1-Hexanol). In adults, both conditioned and unconditioned responses were measured and compared with that of wild type. The conditioning was done in Thorpe's media in presence of one of the three odors at standardized concentration. For Ethyl acetate, conditioning at 10^{-4} dilution and testing at 10^{-5} dilution gives maximum difference between conditioned and unconditioned response, means maximum amount of conditioning. These concentrations were used to screen mutants. Different classes of insertion lines were found with varying responses to the chemicals at different concentrations both in adult and larval stages. In larval stages, the difference with wild type was mainly at lower concentrations of the odor. When the larval response was compared with that of adult unconditioned response (the innate response), larvae of most of the lines show no carryover of the response to adults, meaning the specific response at larval stage does not go to adult necessarily. This indicates toward the brain re-arrangement during metamorphosis (Stocker, 2008). Adult flies need to find food (as well as mates, egg-laying sites, etc.), which requires sophisticated odor-driven

behavior. Fly larvae live on their food source and hence do not need long-range odor detection to find food. Although larvae respond to a variety of chemicals (Rodrigues *et al.*, 1980; Cobb *et al.*, 1999; Heimbeck *et al.*, 1999; Cobb and Domain, 2000), one may expect the chemosensory systems of both developmental stages to display significant differences in terms of cell number, organization, and behavioral function. There are few odorant receptors that are specific to adult and larvae and few are common. For example, larvae of the *003* line show normal response to all the concentrations of the three odors tested, but adults show decreased response to Ethyl acetate and 1-Hexanol and normal response to only Isoamyl acetate. The larvae of *191* line show decreased response to Ethyl acetate and Isoamyl acetate and normal response to 1-Hexanol, but adults show decreased response to Ethyl acetate and 1-Hexanol and increased response to Isoamyl acetate. Thus, the two systems, larvae and adults, behave differently for most of the lines tested. The pleiotropic mutations like these affecting responses to so many chemicals probably exemplify genes whose products are used downstream. In contrast, ligand specific mutations are expected to be in the genes whose products are closer to the receptor end of the pathway. Thus, these P-element insertion lines are important reagents to study the mechanism of olfactory response at the molecular level. And the first crucial step would be to localize the P-element insertions in these lines at base pair level.

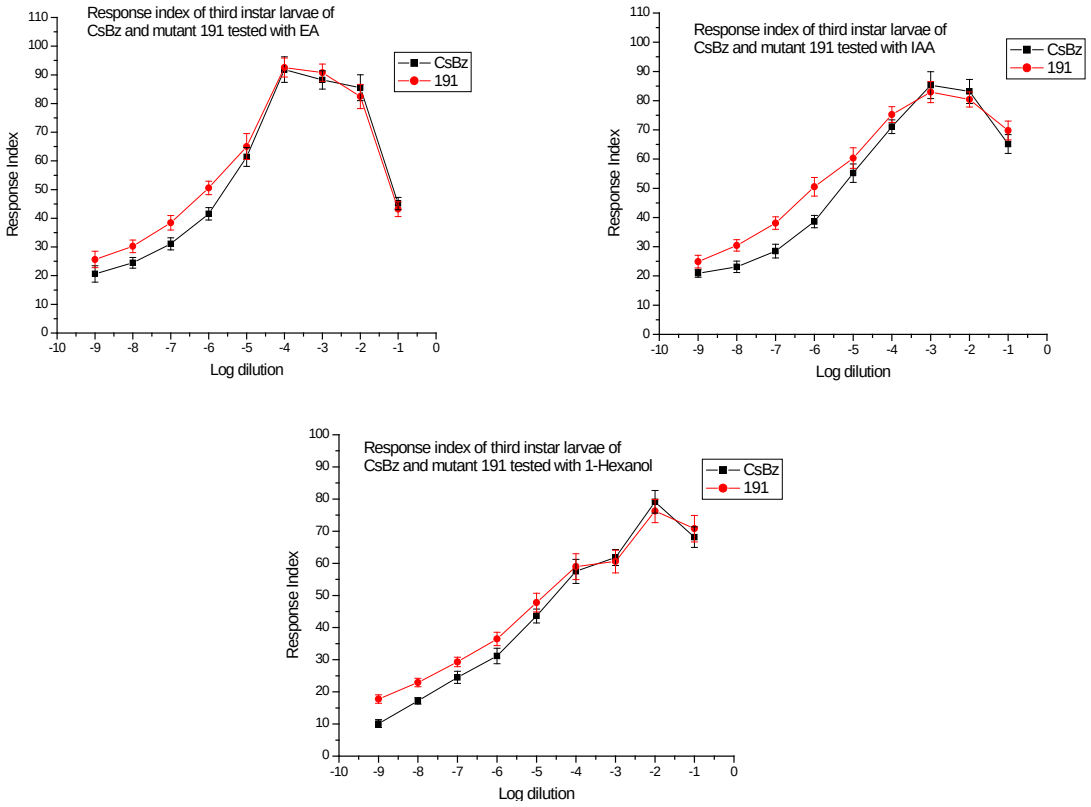


Figure 7. Olfactory response of third instar larvae of wild type and 191 line tested for varying log dilutions of Ethyl acetate, Isoamyl acetate, and 1-Hexanol. Each response index (RI) represents the mean of six experiments, each experiment consisting six petri-dishes. The standard error was calculated as the standard error of mean.

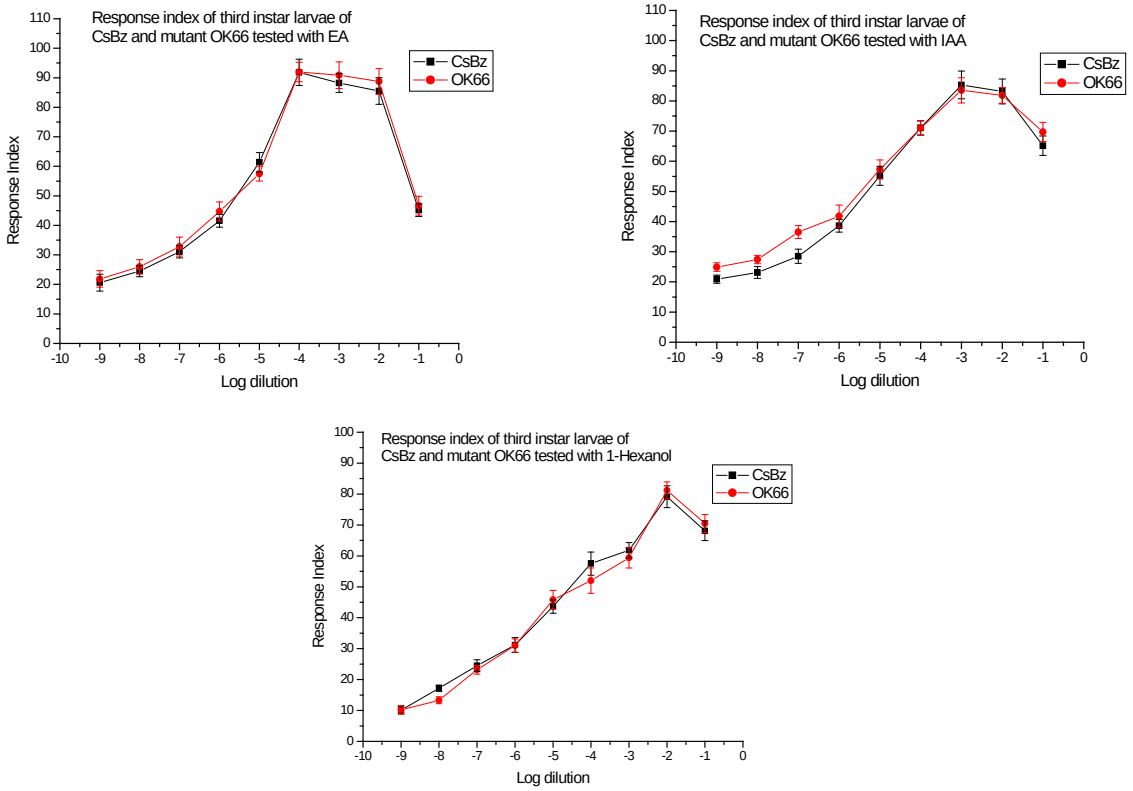


Figure 8. Olfactory response of third instar larvae of wild type and OK66 line tested for varying log dilutions of Ethyl acetate, Isoamyl acetate, and 1-Hexanol. Each response index (RI) represents the mean of six experiments, each experiment consisting six petri-dishes. The standard error was calculated as the standard error of mean.

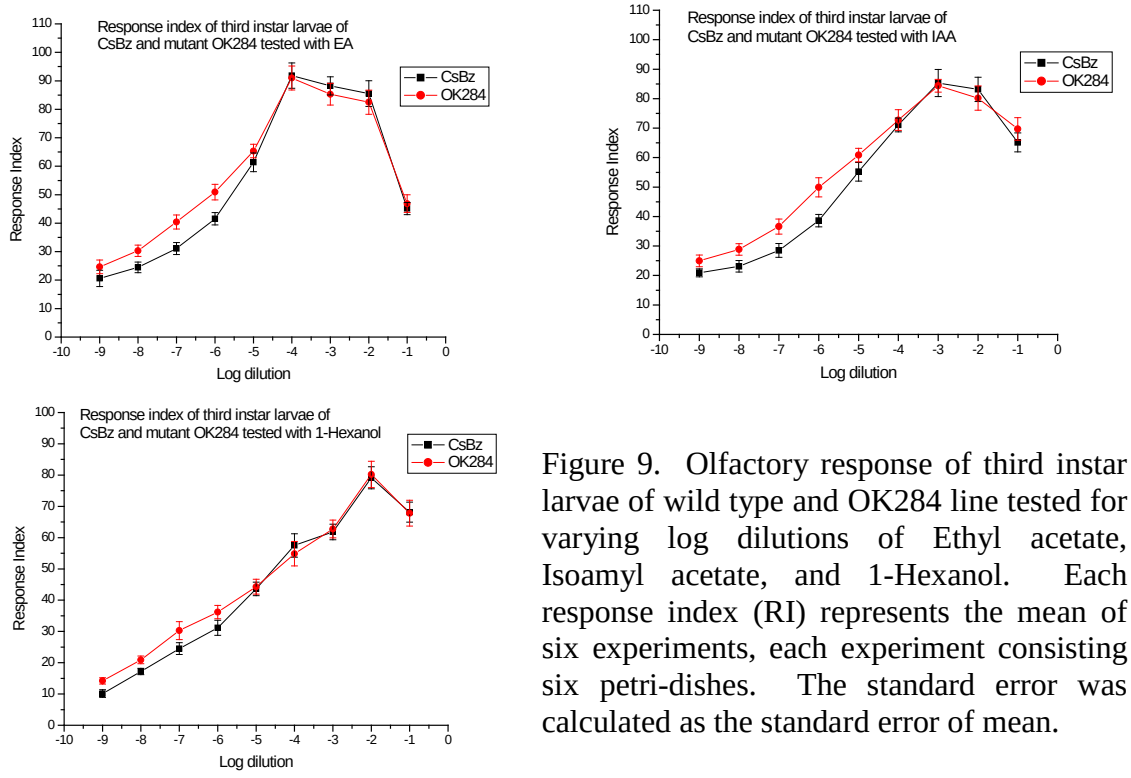


Figure 9. Olfactory response of third instar larvae of wild type and OK284 line tested for varying log dilutions of Ethyl acetate, Isoamyl acetate, and 1-Hexanol. Each response index (RI) represents the mean of six experiments, each experiment consisting six petri-dishes. The standard error was calculated as the standard error of mean.

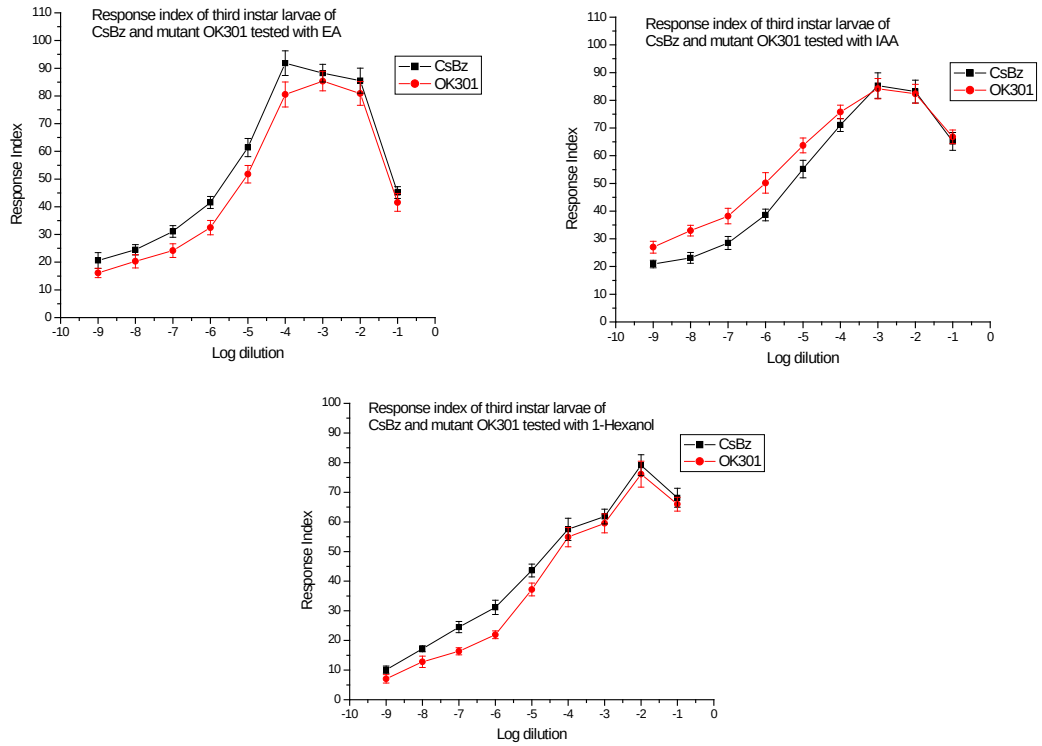


Figure 10. Olfactory response of third instar larvae of wild type and OK301 line tested for varying log dilutions of Ethyl acetate, Isoamyl acetate, and 1-Hexanol. Each response index (RI) represents the mean of six experiments, each experiment consisting six petri-dishes. The standard error was calculated as the standard error of mean.

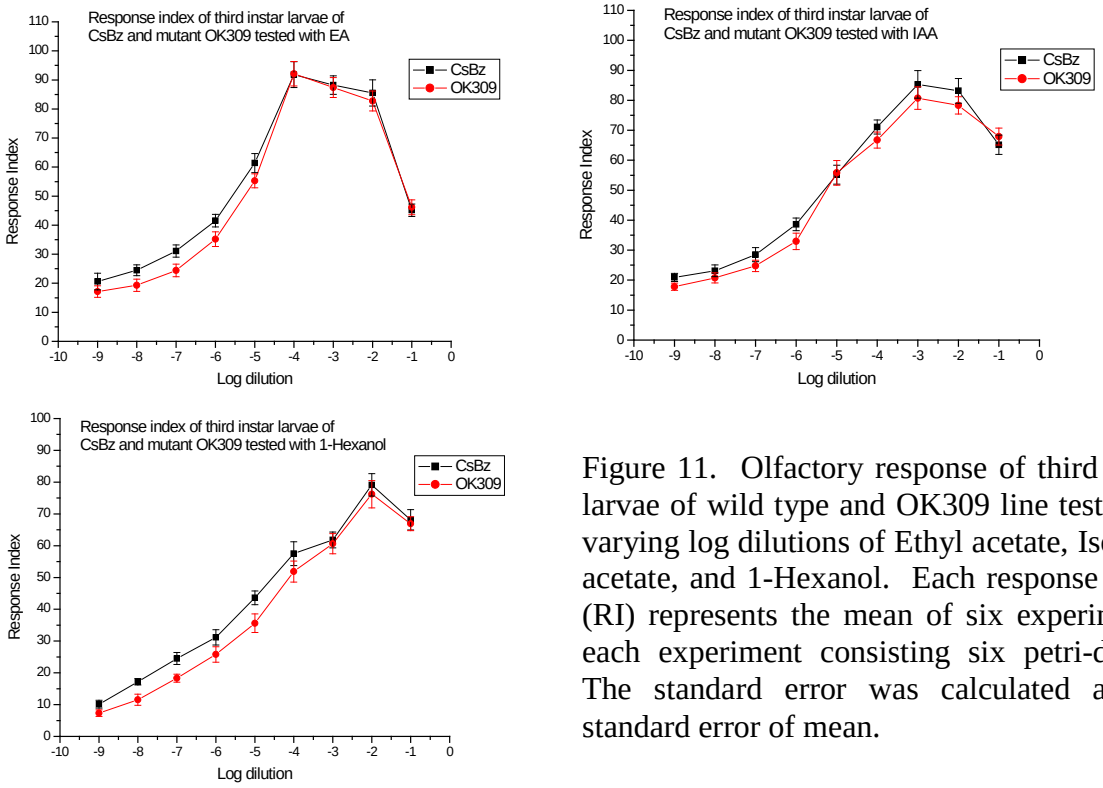


Figure 11. Olfactory response of third instar larvae of wild type and OK309 line tested for varying log dilutions of Ethyl acetate, Isoamyl acetate, and 1-Hexanol. Each response index (RI) represents the mean of six experiments, each experiment consisting six petri-dishes. The standard error was calculated as the standard error of mean.

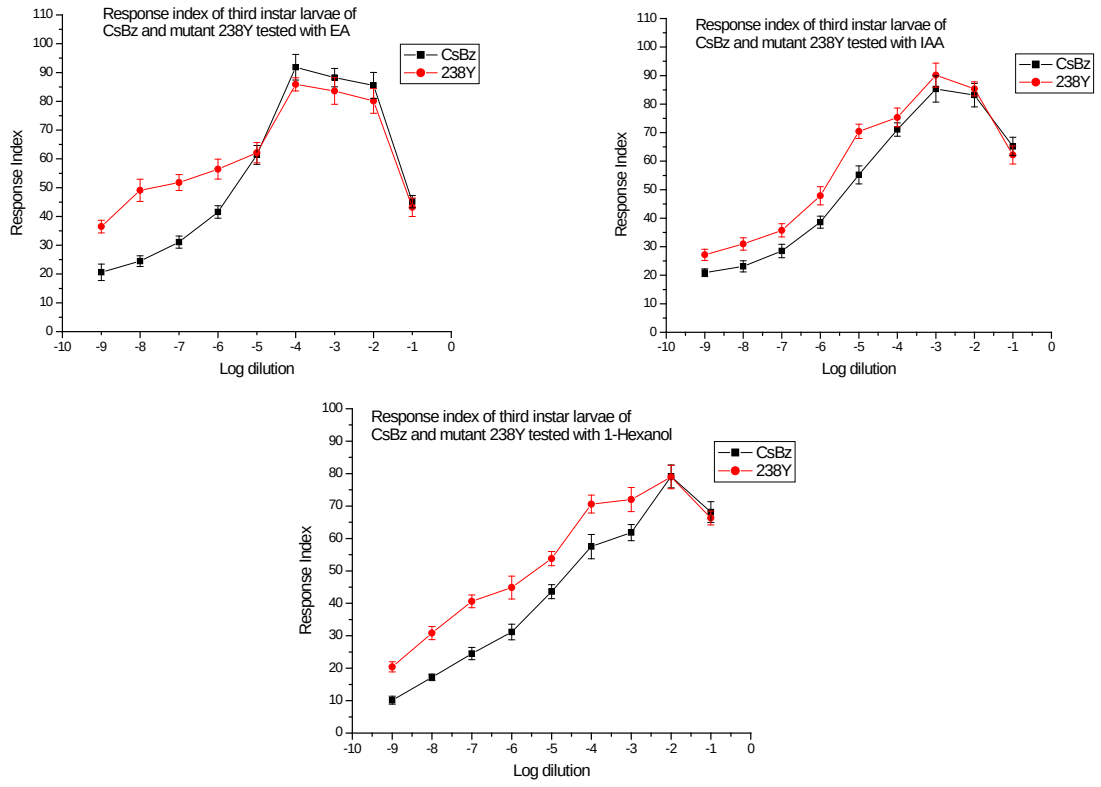


Figure 12. Olfactory response of third instar larvae of wild type and 238Y line tested for varying log dilutions of Ethyl acetate, Isoamyl acetate, and 1-Hexanol. Each response index (RI) represents the mean of six experiments, each experiment consisting six petri-dishes. The standard error was calculated as the standard error of mean.

In our previous and this paper, nine P-element insertion lines have been localized at base pair level. We have olfactory behavioral data for many of these lines at both larval and adult stages, and few of them show interesting phenotypes and few show interesting GFP expression patterns in olfactory organs. The molecular localization of P-element in these lines gave us a list of candidate genes, and the roles of these genes could be established in mediating those interesting olfactory behavioral phenotypes. Few insertions are in intron regions of genes, and they show mutant phenotype and would be really worth investigating. Electrophysiological recordings (EAG and single unit) would tell more about these lines and the mechanism of sense of smell at peripheral level. The plasmid rescue and inverse PCR protocol have been standardized, and using these methods more P-element insertions could be localized and studied if required.

The line *238Y* shows increased olfactory response to certain chemicals at larval and adult stages, and also GFP is expressed specifically in mushroom bodies. It is established that this altered behavioral phenotype in this line is indeed due to the presence of P-element insertion. The molecular and behavioral characterization of this line show that this altered phenotype is not due to *frizzled* gene. So, there could be other possibilities like this insertion might be affecting some gene in trans or enhancer elements of some gene. These possibilities need to be investigated further to understand the molecular mechanism of this altered olfactory behavior in *238Y* line. The larvae of this line show increased initial learning in electroshock paradigm, which is due to the presence of P-element in the

genome. Further characterization of this line at the molecular level and knowing which gene products (if not *frizzled*) are affected in this line should give answers to all these questions.

Acknowledgments: Financial assistance in the form of Research Fellowship from Indian Council of Medical Research (ICMR), New Delhi to J.A. is gratefully acknowledged. This project was funded by a grant of Defense Research and Development Organization (DRDO).

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P-element disruption of the *Drosophila melanogaster* homolog of human cancer susceptibility gene does not increase fertility of female heterozygotes.

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Abstract

BRCA1 and BRCA2 function in homologous meiotic recombination, and mutations in these genes cause human breast and ovarian cancer. Nevertheless, disruptive mutations in these genes are present at an unexpectedly high frequency in human populations. A recent study suggested that BRCA1/2 mutations are maintained because they increase the early-life fertility of their carriers (Smith *et al*., 2011). However, further study of this unexpected fitness advantage in humans is impeded by family planning strategies. Just like its human counterpart, *Drosophila* BRCA2 (*dmbrca2*) interacts with RAD51 and functions in DNA repair by homologous recombination (Klovstad *et al*., 2008) and thus may experience similar selective pressures. We produced flies heterozygous for a BRCA2 knockout and compared fecundity to flies not bearing the mutation in the first two days postcopulation to test for a fitness advantage similar to that observed in humans. In contrast to humans, *Drosophila* heterozygous for a disruption of BRCA2 do not show increased