Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 2908–2912, April 1989
Neurobiology

Isolation and characterization of an olfactory mutant in *Drosophila* with a chemically specific defect

(specific anosmia/chemosensation behavior/pigmentation/fertility)

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Communicated by Melvin J. Cohen, December 16, 1988 (received for review September 29, 1988)

ABSTRACT **A *Drosophila* mutant was isolated and shown to exhibit defective response to the chemical odorant benzaldehyde in two distinctly different behavioral assays. The defect exhibited chemical specificity: response to three other chemicals was normal. The mutant also showed abnormalities in pigmentation and fertility. Genetic mapping and complementation analysis provide evidence that the olfactory, pigmentation, and fertility defects arise as a result of a lesion at the pentagon locus. The specificity of the olfactory defect suggests the possibility that the mutation may define a molecule required in reception, transduction, or processing of a specific subset of chemical information in the olfactory system.**

The olfactory system is an exquisitely sensitive system for the detection and discrimination of airborne chemicals. Despite its ubiquitous presence throughout the animal kingdom and its importance as a primary sensory modality, little is understood about the molecular mechanisms underlying its action in any higher organism. In recent years, several powerful approaches have been used to identify molecular components associated with the olfactory systems of selected animals (e.g., refs. 1–5). Some of the most effective approaches have begun with the identification of molecules by virtue of tissue localization, structural properties, or binding properties *in vitro*, followed by efforts to define their roles *in vivo*.

The experimental plan of this article is based on an alternative strategy. The approach used here is to identify olfactory system components by virtue of their function *in vivo* and then to characterize their properties in detail. This approach is particularly feasible in organisms amenable to genetic analysis, such as *Drosophila*, the organism used in this study: mutants defective in olfactory function due to a single gene mutation may be isolated, and the affected genes and their products may then be characterized at the genetic and molecular levels. This approach has proven to be highly effective in dissecting the *Drosophila* visual system (6).

Behavior offers a useful means of isolating olfactory mutants (7). Since behavioral assays require that an animal identify and respond to a stimulus, they may identify mutants with defects in stimulus reception, transduction, processing, transmission, or motor output. Mutants with lesions at different levels are expected to exhibit different characteristics. One particularly interesting type of mutant is that exhibiting a defective response only to a subset of chemical odorants. This class of mutants, displaying what are known as specific anosmias, may include those with defects in olfactory receptor molecules or other molecules specific to individual functional pathways. In this paper, we describe the isolation, behavioral characterization, and chromosomal localization of a mutant in this class.

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MATERIALS AND METHODS

Drosophila melanogaster. Canton-S-5 (CS-5) is homozygous for an X chromosome derived from a Canton-S strain obtained from O. Siddiqi (Tata Institute, Bombay). It was constructed from this Canton-S strain and from an *FM7* balancer stock (S. Benzer, California Institute of Technology) whose autosomes were of Canton-S origin. *C(1)A y* and *FM6/FM7c*, both in a Canton-S background, and *Dp(1;2)-FN107* (8) were from D. Kankel (Yale University); *y cv v f* was from L. Tompkins (Temple University); and ct^+ y^+ *Y* (9) was from A. Schalet (Yale University). *Df(1)KA14* (8) was from A. Spradling (Carnegie Institute of Washington); $ptg^{fs(1)M71}$ was from D. Mohler (University of Iowa); and ptg^2 and ptg^4 were from the Mid-America *Drosophila* Stock Center (Bowling Green State University). Flies were grown as per Monte *et al.* (10), at 25°C except where indicated otherwise.

Chemicals. Benzaldehyde, ethyl acetate, propionic acid, and paraffin oil were from Fluka, and 3-octanol and ethylene glycol were from Aldrich; all were of the highest purity available. Ethyl methanesulfonate was from Sigma.

Mutagenesis. Two-day-old CS-5 male flies were fed ethyl methanesulfonate (11) and then mated to *C(1)A y* female virgins. Males were removed 72 hr after the onset of the mating period. Single F_1 males were mated to virgin *C(1)A y* females to establish lines. One percent of the F_1 males had obvious visible phenotypes, 20% produced no offspring, and 2% produced only female offspring.

Olfactory T Maze. The olfactory T maze (see Fig. 1) was based on the olfactory-driven learning T maze of Dudai *et al.* (12) as modified by Tully and Quinn (13). Benzaldehyde and 3-octanol acted as strong repellents, in a concentration-dependent manner. The assays were performed in dim red light, to reduce the potential role of external visual cues. No appreciable response was obtained in the absence of chemical odorant; at the beginning and end of each testing session, flies were tested with diluent alone as stimulus to verify the absence of any bias or external cues. Control experiments in which flies were tested after surgical removal of antennae provided evidence that response is dependent on input from these organs, which are the primary olfactory organs of the fly (14, 15).

Olfactory Jump Assay. A detailed description of the jump assay will be published elsewhere. Briefly, a fly is placed in a vertically oriented plastic tube and allowed to crawl halfway up the side. An airstream is pulled through the tube, which has a small hole at the top, at a rate of 1 liter/min. The introduction of a chemical odorant into the airstream induces the fly to jump. A positive response is scored if the fly jumps off the wall of the tube and lands at the bottom of the tube within 3 sec after introduction of the odorant. Flies were tested once and then discarded.

Abbreviation: CS-5, Canton-S-5.

All experiments using the 3D18 mutant line were performed with matched control flies, which were tested in parallel. In most experiments in which the jump response of 3D18 flies was measured, the animals were scored in a blind fashion; i.e., the investigator scoring the jump response was unaware of the genotype being tested. This was possible because of the highly penetrant hyperpigmentation phenotype characteristic of 3D18 (see *Results*), which, although difficult to see with the naked eye, could easily be scored under the microscope after testing jump response. Controls, moreover, were internal: 3D18 and control flies were mixed into a single vial, tested individually, divided into jump and nonjump categories, and then scored for pigmentation. In this manner, the proportion of positive responses (% jumping) could be calculated for both 3D18 and control groups.

In the experiment shown in Fig. 3, males and females were tested separately for response to benzaldehyde. No significant differences were found between sexes at any concentration tested, for either CS-5 or 3D18. The data for males and females were therefore pooled in this experiment, and testing in other experiments was performed without regard to sex. Another experiment showed that 3D18 males exhibited a defective jump response whether their mothers were homozygous or heterozygous for the mutation. Benzaldehyde was used at a 3×10^{-2} dilution in paraffin oil, and ethyl acetate was used at a 1×10^{-1} dilution in water except where indicated otherwise. Tests of statistical significance were performed with the Student *t* test following arcsine transformation of the jumping percentages.

Other Tests. Flight was tested by dropping flies into a large unobstructed space and observing their behavior, and electroretinograms were as per Pak *et al.* (16). Larval olfactory assays were as in Monte *et al.* (10). Briefly, larvae were placed at the center of an agarose Petri dish containing two diametrically opposed filter discs, one holding ethyl acetate and the other a control. Larvae were allowed to migrate for a period of 5 min, after which response was measured by counting the number of larvae on the odorant and control halves of the plate. This procedure provided consistent results when ethyl acetate, but not benzaldehyde, was used as odorant. Courtship was tested by L. Napolitano and L. Tompkins as described in ref. 17. Antennae were examined under a Zeiss dissecting microscope at a magnification of × 128. Flies were fixed, embedded, sectioned, silver stained (18), and examined with a Leitz compound microscope. Embryos were examined by staining with diamidinophenylindole, which allowed evaluation of the number of nuclei (19).

RESULTS

Isolation of a Mutant That Demonstrates a Specific Chemical Odorant Defect in the Olfactory T Maze. Using the repellent benzaldehyde as a stimulus in an olfactory-driven T maze (Fig. 1), we screened males of 1150 lines, each line carrying a unique X chromosome mutagenized with ethyl methanesulfonate. Among several lines identified on the basis of low response index, one was chosen for detailed analysis. Fig. 2*A* shows that the response index of line 3D18 for benzaldehyde is significantly lower than that of the matched wild-type CS-5 control ($P < 0.001$; Student's two-tailed *t* test) but is normal for a second repellent, 3-octanol.

3D18 Demonstrates a Specific Chemical Odorant Defect in the Olfactory Jump Assay. When tested in the olfactory jump assay, 3D18 again demonstrated a selective defect in response to benzaldehyde. Fig. 2*B* shows that 3D18 was significantly defective in its response to benzaldehyde ($P < 0.001$) but was normal in its response to odorants of two other chemical classes, ethyl acetate (an acetate ester) and propionic acid (an organic acid). (3-Octanol does not elicit a strong response in the wild type in the jump assay.)

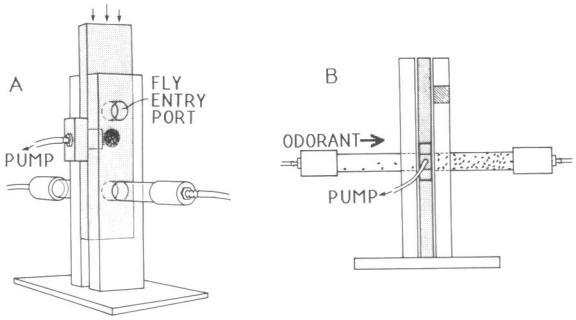

FIG. 1. Olfactory T maze. (*A*) Fifty to 100 flies are entered through the entry port into a chamber in the central, vertical sliding plate. (*B*) The plate is slid down into the bottom position, from which flies can escape either to the left or the right. The collecting tube on the left contains chemical vapors; the collecting tube on the right contains control air. Air is continuously drawn through the system by a pump (Cole-Parmer KNF 7056-25) at a rate of 1 liter/min. Air entering the system from the left is drawn over the surface of a chemical odorant solution in a sidearm flask; control air entering from the right passes over diluent. Air leaves the system through a set of holes too small for a fly to pass through. After a period of 1 min, the numbers of flies in the two tubes are counted. A response index is computed by subtracting the number of animals on the odorant side from the number on the control side and dividing by the total. Flies are tested only once and are then discarded.

Dose–response curves for benzaldehyde and ethyl acetate showed that the selectivity of the defect persisted over a range of stimulant concentrations, including nonsaturating concentrations. Fig. 3 shows that for concentrations of chemical odorants spanning two orders of magnitude, giving wild-type responses ranging from ≈25% to ≈80%, 3D18's response was significantly lower than wild type ($P < 0.05$) at all tested concentrations of benzaldehyde, but it was not significantly different from wild type at any concentration of ethyl acetate. Although in this experiment mean responses to ethyl acetate were greater for the mutant than for CS-5 at all concentrations, at no concentration is the difference statistically significant. This identity of response to ethyl acetate receives further support from the experiments shown in Fig. 2*B*, in which mean CS-5 response was not significantly greater than that of the mutant, and in Table 3, in which mean responses were identical.

3D18 Has a Pigmentation Phenotype and a Fertility Defect. In addition to the olfactory defect, 3D18 flies have a visible

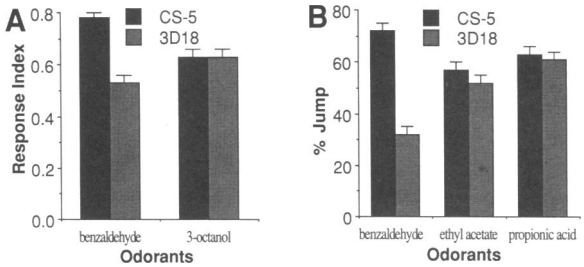

FIG. 2. 3D18 shows a selective defect in both the olfactory T maze and the jump assay. (*A*) T maze. Benzaldehyde was diluted 1×10^{-3} in water; 3-octanol was diluted 1×10^{-2} in ethylene glycol. Each test was of a population of ≈50 male flies. $n = 42$ tests for benzaldehyde, and $n = 26$ tests for 3-octanol. 3D18 flies and the matched controls were tested in alternating fashion. Flies used in this experiment were grown at 29°C; flies raised at other temperatures ranging between 18°C and 29°C also showed statistically significant differences in response to benzaldehyde. Error bars indicate SEM. (*B*) Jump assay. Benzaldehyde was used at a 3×10^{-2} dilution in paraffin oil; ethyl acetate and propionic acid were used undiluted. $n = 10$ tests of 30–60 flies each for each of the three chemical odorants. Tests were scored blind. Error bars indicate SEM.

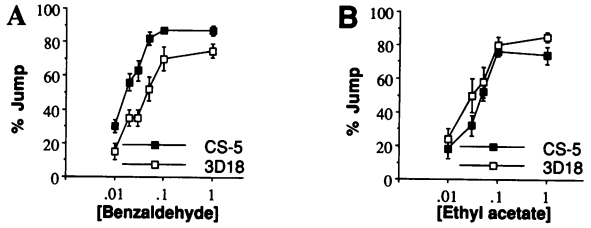

FIG. 3. The selective defect of 3D18 in the jump assay is preserved throughout the entire spectrum of the dose–response curve. 3D18 and CS-5 flies were tested at each concentration of the indicated chemicals on each of 5 days. Error bars represent SEM. (*A*) Benzaldehyde. Sixty flies, 30 of each sex, were used in each test. Dilutions were in paraffin oil. (*B*) Ethyl acetate. Dilutions were in water.

cuticular pigmentation phenotype, which is more severe in females. The cuticle is marked by a discrete patch of hyperpigmentation on the mesonotum of the dorsal thorax, which appears as a pentagonal spot from which three narrow stripes of pigmentation extend anteriorly, resembling a trident. The scutellum, the mesopleura, and a small region of the dorsal aspect of the head, adjacent to the eyes, are also darkened. Although a pigmentation pattern similar in type to 3D18 has been observed in a number of wild-type strains, the hyperpigmentation of 3D18 is intense, and its penetrance is very high. All 3D18 females and virtually all 3D18 males (>95%) are easily distinguished from the parental wild-type CS-5.

Homozygous stocks of 3D18 exhibit low fertility. Preliminary examination suggested that this infertility was due to a defect in fertilization. Further analysis (Table 1) documented this effect and showed that the infertility is primarily a consequence of the maternal genotype. Wild-type females mated to mutant males show normal fertility. Homozygous 3D18 females, on the other hand, lay normal numbers of eggs, but they produce few progeny, whether mated to 3D18 or wild-type males. We have observed no external defects in the eggs, but only 10%–20% of them undergo fertilization and normal embryonic development. Staining with the nucleophilic dye diamidinophenylindole, followed by fluorescence microscopy, revealed that the eggs either fail to become fertilized or are blocked before multiple nuclei become apparent. Those eggs that do undergo embryonic development continue to develop to adulthood with normal viability (data not shown).

The Mutant Appears Normal in Other Tests. Tests of other behaviors revealed no defects. 3D18 animals are capable of negative geotactic and positive phototactic responses. Larvae have a normal olfactory response to ethyl acetate. Tests of courtship behavior and flight ability have shown no abnormalities. Recordings of visual system physiology—electroretinograms—were normal. No gross anatomical defects have been observed, either in the external appearance of the antennae and the maxillary palps or in sections through the central nervous system, which included the antennal lobes, mushroom bodies, and central brain.

Meiotic Recombination Mapping of 3D18. As a first step in determining whether the olfactory, pigmentation, and infertility defects all result from a single mutation on the X chromosome, the visible hyperpigmentation defect of 3D18 was mapped by meiotic recombination. By using an X chromosome marked with the visible mutations, yellow, crossveinless, vermilion, and forked (*y cv v f*), 1690 flies were scored for recombination events. The calculated intervals between the markers agreed well with the conventional map distances between them: the *y* to *cv*, *cv* to *v*, and *v* to *f* intervals were approximately 10, 18, and 21 map units as compared to the accepted map distances of 13.7, 19.3, and 23.7 (20). The hyperpigmentation phenotype mapped to the *cv* to *v* interval. Scoring of 330 crossovers in this interval allowed the hyperpigmentation phenotype to be localized to approximately map position 23.

Four recombinants produced in this cross were chosen for an initial test of the hypothesis that the olfactory phenotype is a consequence of the same mutation at map position 23. The recombinants, of which the first two listed are double recombinants, are as follows: (*i*) *y cv* 3D18-hyperpigmentation + *f*, (*ii*) *y* + 3D18-hyperpigmentation *v f*, (*iii*) *y cv* 3D18-hyperpigmentation + +, and (*iv*) + + 3D18-hyperpigmentation *v f*. Limited data suggested that all four recombinant chromosomes failed to complement the olfactory defect of the parental 3D18 chromosome when tested in the olfactory jump assay (data not shown). These results tentatively localized the olfactory defect, like the pigmentation defect, to the *cv* to *v* interval, a localization that was then tested further by mapping with duplications and deficiencies covering parts of this interval.

Duplication and Deficiency Mapping of 3D18. *Dp(1;2)FN107*, a duplication that spans the region of the X chromosome extending from 7A8 to 8A4-5, rescues the olfactory, pigmentation, and infertility phenotypes of 3D18 (Table 2). While control 3D18 flies had a jump response of 44±3, 3D18 flies carrying the *Dp(1;2)FN107* duplication chromosome had a jump response of 75±3, which is close to the wild-type control value of 73±3. Evidence that this restoration of normal jumping behavior is an effect of the duplication and not an effect of genetic background comes from the fact that sibling 3D18 flies that did not inherit the duplication chromosome (they inherited a marked bw^D chromosome instead) had a jump response of 47±3, which is close to the 3D18 value. Moreover, CS-5; bw^D *and CS-5; Dp(1;2)FN107* flies

Table 1. Fertility defect of 3D18

Genotype				% hatched	% fertilized	
Male	Female	Progeny*	*n*†	eggs‡	eggs§	*n*¶
3D18	3D18	6 ± 3	20	19 ± 4	10	391
CS5	3D18	13 ± 4	22	20 ± 3	19	469
3D18	CS5	52 ± 5	24	>90	95	565
CS5	CS5	67 ± 8	21	>90	90	203

*Single pair mates were brooded for 96 hr. The number of adult progeny was counted 18 days after beginning of mating period. The values given are the means ± SEM.
†Number of single pair mates.
‡Eight- to 15-hr egg collections, from 3- to 5-day-old females, were incubated an additional 36 hr and then were examined for the percentage of hatched eggs. The values given are the means ± SEM.
§Twelve-hour egg collections, from 3- to 5-day-old females, were incubated an additional 2 hr, stained with diamidinophenylindole, and then examined for the presence of multiple nuclei.
¶Number of eggs scored.

Table 2. Duplication mapping of 3D18 by using *Dp(1;2)FN107*

Genotype	% jump: benzaldehyde (mean ± SEM)	*n**	Hyperpigmentation†	Female fertility‡
CS-5	73 ± 3	12	+	+
CS-5;bw^D§	70 ± 6	4	+	+
CS-5;*FN107*	70 ± 5	4	+	+
3D18	44 ± 3	6	↑↑	−
3D18;bw^D	47 ± 3	12	↑↑	−
3D18;*FN107*	75 ± 3	12	+	+

*Number of tests; 30–50 flies per test.
†+, normal wild-type pigmentation; ↑, a small dark pentagonal patch with trident on the dorsal thorax; ↑↑, a larger pentagonal patch and patches of pigmentation on the head and mesopleura.
‡+, Normal female fertility; −, female infertility.
§bw^D is the marked second chromosome of sibling flies that did not receive the *Dp(1;2)FN107* chromosome.

have jump responses indistinguishable from those of their CS-5 parents. We note, however, that from this experiment we can not exclude the possibility that restoration of wild-type jump response is due to a genetic factor on the duplication chromosome other than the duplication.

The deficiency *Df(1)KA14*, which deletes the chromosomal material between cytogenetic bands 7F1 and 8C6, uncovers the olfactory, pigmentation, and fertility defects of 3D18 (Table 3). The fact that 3D18/*Df(1)KA14* but not control CS5/*Df(1)KA14* flies are mutant with respect to all three phenotypes argues that the mutant phenotypes of 3D18/*Df(1)KA14* are due to the uncovering of the 3D18 chromosome by the deficiency and not merely to the influence of genetic background; the 3D18/CS-5 control demonstrates that all three phenotypes are recessive.

Table 3 also shows that 3D18 satisfies a critical part of the formal definition of an amorphic allele: there is no significant difference in the olfactory, pigmentation, or fertility defect between 3D18 flies and 3D18/*Df(1)KA14* flies. The chemical specificity of the 3D18 olfactory defect is also preserved in 3D18/*Df(1)KA14* heterozygotes.

Consistent with the results obtained with *Dp(1;2)FN107* and *Df(1)KA14*, the duplication $ct^+ y^+ Y$, which spans the region 6E to 7C4, did not cover the 3D18 hyperpigmentation phenotype. Its effect on the olfactory phenotype was not tested.

3D18 Is Allelic to pentagon. Taken together, the cytogenetic mapping localizes the olfactory and pigmentation phenotypes to the 7F1 to 8A4-5 interval of the X chromosome. There are no reports of olfactory genes in this interval, but the interval does include a pigmentation gene, pentagon (*ptg*), discovered by Bridges in 1922 (20). Like 3D18, pentagon has also been shown to be covered by *Dp(1;2)FN107* and uncovered by *Df(1)KA14* (8). Its recombinational map position, 23.2 (20), agrees well with that determined for the 3D18 pigmentation phenotype: 23.

pentagon alleles have pigmentation phenotypes similar in pattern to that of 3D18, although less extreme in extent and intensity. Like 3D18, $ptg^{fs(1)M71}$ has a female fertility defect, although more extreme than that of 3D18. Furthermore, Table 4 shows that of two *ptg* alleles tested, ptg^2 and ptg^4, both are severely defective in their response to benzaldehyde in the jump assay.

Complementation analysis shown in Table 4 indicates that neither ptg^2 nor ptg^4 complements 3D18 in the olfactory jump assay. Testing of ptg^2/CS-5 and ptg^4/CS-5 indicates that both of these *ptg* alleles are recessive, as was shown for 3D18 in Table 3. $ptg^{fs(1)M71}$ does not complement 3D18's fertility defect (data not shown). The simplest interpretation of these results is that 3D18 is an allele of the pentagon locus.

Examination of the pigmentation patterns indicated in Table 4, however, reveals an unexpected result. Although ptg^4 and 3D18 each fail to complement ptg^2 with respect to pigmentation, ptg^4/3D18 heterozygotes appear to show interallelic complementation: their pigmentation is wild type.

Table 3. Deficiency mapping of 3D18 by using *Df(1)KA14*

Genotype	% jump* (mean ± SEM) Benzaldehyde	Ethyl acetate	Hyperpigmentation†	Female fertility‡
CS-5	81 ± 3 (5)	67 ± 6 (5)	+	+
3D18	49 ± 4 (6)	67 ± 4 (20)	↑↑	−
3D18/*KA14*	43 ± 4 (10)	72 ± 6 (5)	↑↑	−
CS-5/*KA14*	82 ± 4 (5)	NT	+	+
3D18/CS-5	81 ± 4 (5)	NT	+	+

NT, not tested.
*The numbers in parentheses are the number of tests (30–50 flies per test).
†Hyperpigmentation patterns are designated in Table 2.
‡+, Normal female fertility; −, female infertility.

Table 4. 3D18 and pentagon complementation

Genotype	% jump:benzaldehyde (mean ± SEM)	*n**	Hyperpigmentation†
CS-5	81 ± 3	5	+
3D18	49 ± 4	6	↑↑
ptg^2	33 ± 4	3	↑
ptg^2/3D18	37 ± 4	6	↑↑
ptg^2/CS-5	77 ± 1	5	+
ptg^4	26 ± 2	3	↑
ptg^4/3D18	40 ± 2	3	+
ptg^4/CS-5	85 ± 1	5	+
ptg^2/ptg^4	48 ± 6	5	↑

*Number of tests; 30–50 flies per test.
†Hyperpigmentation patterns are designated in Table 2.

This result is of particular interest in light of the fact that ptg^4/3D18 heterozygotes have a mutant phenotype in the olfactory assay. Not only does this uncoupling of the two phenotypes argue that the olfactory defect is not a simple consequence of abnormal pigment deposition, but the complementation pattern suggests the possibility that the pentagon locus may be genetically complex.

DISCUSSION

This paper illustrates the use of behavioral genetics to identify genes required for olfactory response. We have isolated a *Drosophila* olfactory mutant that exhibits defective response to benzaldehyde in two distinct olfactory assays. The mutant responds normally to three other chemicals, indicating a specific chemical anosmia. The chemical specificity of the defect argues against the possibility that the mutant has a motor abnormality or a generalized neural defect; rather, it suggests the possibility that the mutant may define a molecule required in transduction or processing of a specific subset of chemical information.

Genetic mapping of this mutant has provided evidence that the origins of the olfactory, pigmentation, and fertility phenotypes lie in a small region of the X chromosome near the pentagon locus. The finding that independently derived pentagon alleles also exhibit olfactory, pigmentation, and fertility defects suggests a common origin for all three phenotypes. These results, in conjunction with the failure of 3D18 to complement the olfactory phenotype of either ptg^2 or ptg^4, provide strong evidence that the product of the pentagon locus is required for normal response to benzaldehyde as well as for normal pigmentation and fertility.

The coincidence of olfactory and pigmentation phenotypes was unexpected. Although it can be rationalized—e.g., melanin and catecholamine neurotransmitters are both synthesized through a common metabolic pathway—and the association of albinism with neural connectivity defects is well documented in mammalian sensory systems (21), reasonable speculation as to its basis must await further genetic and molecular data. Both phenotypes, like fertility, may be sensitive to a variety of genetic perturbations; both, for example, have been found to be influenced by genetic background (ref. 20; unpublished results). Pleiotropy, moreover, has often been observed among *Drosophila* mutants exhibiting neural defects (see ref. 22 for a recent discussion) and, in fact, the mutants tan and ebony exhibit defects in both pigmentation and visual system physiology (16, 23).

What is perhaps most striking about the mutant described here is the specificity of its olfactory defect: although severely affected in response to benzaldehyde, its response to 3-octanol, ethyl acetate, and propionic acid appears unaffected. Chemical specificity would be expected of mutants with lesions in receptor, transduction, or processing molecules specific to one subset of chemicals. It is more

difficult to explain such specificity in terms of a lesion in a more general neuronal or motor function. One might postulate that the 3D18 product plays a role in response to all chemicals but that the 3D18 mutation produces only a partial reduction in gene activity and that benzaldehyde response is more sensitive to this decrement than are other responses. Arguing against this possibility, however, is the finding that the phenotype of 3D18/*Df(1)KA14* flies is no more severe than that of 3D18 flies, indicating that the 3D18 allele encodes little if any activity.

In considering the possibility that benzaldehyde acts through a functional pathway distinct from that of several other chemicals, we note that reported precedent for an aldehyde-specific *Drosophila* mutant has come from work using other olfactory paradigms (7). There is evidence that different pheromones elicit different behaviors in *Drosophila* (24, 25), and experiments in moths with pheromones (26) and in rat pups with odor cues that stimulate suckling (27) have provided evidence for subsystems stimulated by particular odorants. The specific anosmia demonstrated by the mutant described here may reflect a basic underlying design of the olfactory system according to which subsets of chemical odorants are segregated into specific functional pathways.

Extension of the genetic analysis described here to the molecular level should provide molecular probes useful in examining patterns of expression of the pentagon locus. If pentagon is expressed in a subset of olfactory system neurons, then detailed characterization of its distribution of expression may be useful in testing models of functional organization in the *Drosophila* olfactory system.

We thank T. Tully, M. McKenna, and M. Lilly for their help in developing the T maze assay; R. Ayer, H. Sun, D. Kankel, P. Lewis, G. Fitzgerald, and A. Schalet for advice and helpful discussion; C.-Ting Wu, N. Scottgale, S. K. Inouye, and B. K. I. Helfand for help with the manuscript; and P. Monte for technical help. This work was supported by a National Institutes of Health Physician Scientist Award HD00736 to S.L.H. and in part by National Institutes of Health grant GM36862 (to J.R.C.). J.R.C. is an Alfred P. Sloan Research Fellow.

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