GENETIC ALTERATION OF OLFACTORY FUNCTIONS IN *DROSOPHILA MELANOGASTER*

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Received December 21, 1972

An olfactory function has been assigned to a wide variety of systems such as narsal receptors in vertebrates and frequently to the antennal receptors in insects. A general problem in olfaction is to ascertain the basis for odor specificity and the nature of its transducing systems. Theories attempting to explain odor perception in chemical constitution of odorants have revolved about vibrational energy levels, molecular shapes and functional groups (for a review see Moulton, and Beidler 1967). Single cell recordings from a receptor indicate that even a cell is sensitive to a variety of odorants (Shibuya, and Tucker 1967; Boeckh, Kaissling, and Schneider 1965). Interactions of odorants at the receptor cell can be complex; although an opposite geometrical isomer of the sex pheromone masks or blocks its activity (Roelofs, and Comeau 1968; Jacobson 1969), certain pairs of odorants show indeed a marked synergistic action (Silverstein, Rodin, and Wood 1966). It has been assumed that the complex interplay of odors was caused by the action of odors upon different sites of the receptor cell membrane or upon different receptor cells. It would be desirable, therefore, to begin with a simple system. A promising approach is the use of the genetic alterations (Benzer 1971). Studies in insects and in man suggested that various aspects of olfaction are genetically determined (Dethier, and Yost 1952; Kay, Eichner, and Gelvin 1967; Brown, and Robinette 1967). *Drosophila* offers a certain advantage in such studies. This paper describes the first result of such analysis.

MATERIALS AND METHODS

Isolation of the mutant: A line of flies attracted to acetic acid was selected from the Oregon R strain of *Drosophila melanogaster*. The resulting strain AA75-1 was reared on a yeast medium (5% yeast extract, 10% sucrose, 0.8% propionic acid) at 25°C. Males of the strain AA75-1 aged 1 to 2 days were fed on 0.5% ethyl methane sulfonate (EMS) with 1.5% sucrose for 18 hr, then on a fly medium for 24 hr. They were then mated singly with untreated virgin females of the same strain four days and transferred to mate with new females another four days (Fig. 1). Each population coming from respective pair might involve flies having a variety of genotypes as +/+; +/+, +'/+; +/+ and +'/+'; +/+. To detect populations in which mutants involved, changes in behavioral responses to several odorants were examined in descendants (F₃ or F₄) by an olfactometry: acetic acid (AA) balanced with odor mixture (OM) (see Olfactometry).

Fig. 1. Crossing scheme employed to isolate olfactory mutants.

The suspect populations, showing abnormal behaviors in the present olfactometry, were fractionated repeatedly by either of the stimulants. Flies thus fractionated may involve mutants, both homozygotes and heterozygotes, and normal flies mingled with by chance. To establish an isogenic line of the mutant, males of suspects were mated singly with virgin females of the 'mutant isolator strain' T4 having genotype of *Cy/Pm; H/Sb* with an attached X chromosome (also with marker *y w^a su(w^a) bb*). The female hybrids, *Cy/+; H/+* or *Cy/+; Sb/+*, were backcrossed singly to the T4 males. Thus homozygotes, of which the 2nd chromosomes came from one side of the chromosomal pair of each suspects, were isolated. Responses of these homozygous flies were examined again in the same manner as in F₃ and F₄ to detect mutants.

Olfactometry: (1) *Apparatus:* Responses were assayed with an olfactometer devised to evaluate attractancy or repellency for odorants as well as to fractionate mutants (Fig.

Fig. 2. Olfactometer and its delivery system. A: Diagram of apparatus for stimulus control. B: Side view of olfactometer.

2). The compressed air that flows into glass tubes (8 mm inside diameter) is cleaned with silica gel (Sg) and active charcoal (Ch). The flow rate is controlled with needle valve (N), glass teflon stopcocks (Sc) and flowmeter (F, Matheson Co. Inc.). The air current thus cleaned is bubbled in 500 ml gas washing bottles (G) kept in a thermostat (Th) at 20°C. When the streams reach the olfactometers through flowmeter (F) and glass tubes, they would attain the ambient temperature of 25°C. To avoid adsorbance of odors, the connection between lines of glass tubing is made with spherical ground joints (J) or with teflon tubes. Olfactometer is constructed with five parts (Fig. 2, B). (1) A starting bulb (S): A 100 ml round flask with a branch (8 mm inside diameter). (2) A choice bulb (C): A 500 ml reaction flask with three necks, (3) Two trapping bulbs (T): Bulbs with the same make-up as the starting bulb (S), (4) An adaptor (A_{s-c}) connecting the S with the middle neck of C-bulb (15 mm inside diameter). (5) Two adaptors (A_{c-t}) bent right angle (about 20 cm in length and 15 mm inside diameter) to connect the T-bulbs with the outer necks of the C-bulb. A funnel shaped open end of each adaptor A_{c-t} (5 mm inside diameter) is stuck down into trapping bulb (T). By this device, the trapped flies are not allowed to go out of the bulb (T), while they can go into the bulb (T) from the bulb (C) through the open end of the adaptor without mechanical obstruction. Branches of both starting (S) and trapping bulb (T) are plugged with cotton filaments to keep flies from going out. The odor streams, flowing into the bulbs (T) from their branches, pass through adaptors A_{c-t} and thereby flow together into C-bulb. The odor currents thus mixed pour into the bulb (S) and flow out from

its branch. The flies transferred to the startining bulb (S) move towards the choice bulb (C), in which they are allowed to choose either of the two entrances of the adaptors (A_{c-i}) for odorant. The perpendicular part of the adaptor (A_{c-i}) is a device to sort out the flies attracted by the odor from flies that happen to enter it, because the later hesitates to move further towards odor source against their geotaxis.

(2) *Behavioral assay*: For isolation of the mutant, several hundred flies reared on the yeast medium (aged 0–36 hr) where starved at 25°C for 17 hr. Freshly prepared solution (250 ml each) of odorants were kept in gas washing bottles at 20°C. Testing vapors (420 ml each: almost equal to the capacity of each olfactometer channel) were sent at the rate of 50 ml/min for 8 min, and subsequently at the rate of 1 ml/min for 20 min. Tests were done at the flow rate of 1 ml/min for 1 hr at 25°C. For comparison of the responses between the mutant and parent strain to a variety of odorants, a large number of flies were needed. The flies were therefore reared on the corn medium (7% yeast extract, 15% sucrose, 15% corn powder, 0.4% propionic acid) and starved for 28 hr before testing. Olfactometry was also slightly modified as follows; testing vapors were sent at the rate of 100 ml/min for 4 min, and subsequently at the rate of 2 ml/min for 10 min. Tests were done at a flow rate of 2 ml/min for 1 hr. When additional tests were needed to fractionate mutants or to define the homogeneity of population, trapped flies (reared on yeast medium) were cooled for 1 hr to permit weighing or counting, allowed to take the fly medium for 8 hr, starved 14 hr and tested again as described above. Responses of flies to odorants were evaluated by the ratio of flies trapped in odor-side to total number trapped: Response value for odor (OD) vs. water (W) was represented as $K_{OD/W}$. A more carefully controlled evaluation has been performed by exchanging two stimulants between two sides of the olfactometer to neutralize any right-left bias. The response was profoundly affected by changes in flow rate through 1 hr-testing, so that the flow rate was adjusted every 20 minutes and measurement for the mutant flies was carried out simultaneously with the parent, employing paired system of two olfactometers connected to the same odor source. The olfactometer and gas washing bottles were cleaned with detergent, rinsed with organic solvents, and dried in an oven at 140°C for 1 hr to avoid any hazard caused by contaminative odor in previous experiments. The air delivery lines were cleaned by passing compressed air for 10 or more minutes.

Screening of the mutants: The behavioral alteration of a suspect population, in which mutants may be involved, was examined by olfactometry, employing a balanced system with acetic acid vs. a mixture of several chemicals, so that the resulting unbalance with changes in response to any of the odorants was revealed at once. The 'odor mixture' was made up with a variety of chemicals: 2×10^{-3} M 2,3-butanedione, 2×10^{-5} M trans-4-phenyl-3-buten-2-one (trans-PBO), 2×10^{-5} M 4-(o-hydroxyphenyl)-3-buten-2-one (HPBO), 2×10^{-4} M 3-heptanone, 2×10^{-4} M 5-nonanone and 2M ethanol. Concentrations of acetic acid, 3-heptanone, ethanol and trans-PBO were determined so as to be approximately intermediate between attractancy and repellency of the strain AA75-1 and for the repellents, 5-nonanone, 2,3-butanedione and HPBO, the concentration employed were approximately threshold values: an average value for acetic acid over water ($K_{AA/W}$) being 0.50 ± 0.01 , for ethanol 0.36 ± 0.02 , for 3-heptanone 0.50 ± 0.04 , for 5-nonanone

0.45 ± 0.04 , for 2,3-butanedione 0.48 ± 0.02 , for HPBO 0.49 ± 0.02 and for trans-PBO 0.62 ± 0.03 .

Odors and diluent: Nearly all of the chemicals used as stimulants were of reagent grade purchased from Baker, Eastman Organic Chemicals, Tokyo Kasei Co. and Wako Pure Chemicals Ins., or chemicals purified in the laboratory by repeated distillation or crystalization. Trans-4-phenyl-3-buten-2-one and trans-4-(o-hydroxyphenyl)-3-buten-2-one were synthesized from acetone and aldehydes. Highly purified geraniol, nerol, farnesol and pellilaldehyde were given by Dr. T. Nishino, Tohoku university, and tropolone was generously given by Dr. Y. Kitahara of the same university. Several chemicals still retained impurities according to thin layer or gas chromatography: morin, 5-nonanone (impurity about 6%), 3-heptanone (1%) and 4-heptanone (1%). The compressed air as the diluent or carrier gas for the odorants was cleaned with silica gel and charcoal.

RESULTS

Isolation of a mutant strain: The responses of the Oregon R wild strain to the odor of 10^{-4} M acetic acid (AA) varied with samples from different vials from extreme attraction to extreme repulsion: Average value of the responses to acetic acid vs. water ($K_{AA/W}$) was 0.39 ± 0.065 . For further studies using mutagenesis it was essential to isolate a line isogenic in odor responses. The flies of the Oregon R stock were then fractionated repeatedly by 10^{-4} M AA vs. water to isolate a line attracted to AA (see Method). The resulting line in F_7 (AA75-1) showed high level of attraction to 10^{-4} M AA, giving an average value ($K_{AA/W}$) of 0.699 ± 0.02 . From 80 pairs with 40 EMS-treated males of the strain AA75-1, about 1,000 hybrid progenies from 30 pairs were obtained (50 were lethal or sterile owing to potent action of EMS). Of 30 populations thus obtained, seven turned out to be suspects showing behavioral alterations, five being more attracted to acetic acid (AA) vs. odor mixture (OM) as E211 in F_3 and E205 in F_4 and two being attracted to OM as E118 and E125 (Fig. 3). The average value of attraction for AA vs. OM ($K_{AA/OM}$) in the EMS-treated was, however, similar to that in the untreated: 0.608 ± 0.022 and 0.615 ± 0.021 for F_3 and F_4 treated hybrids, respectively, and 0.590 ± 0.015 for the untreated. Fractionations of the suspects by 10^{-4} M AA vs. OM gave profiles indicating that two AA-attractive lines, E205 and E211, were behaviorally heterogeneous, suggesting that mutants may be involved (Fig. 4). Two of the OM-attracted lines, E118 and E125, were also heterogeneous (Fig. 4), while others gave a profile similar to the control. Six AA-attracted males, four from E211 and two from E205, were isolated by four trials of the fractionation; Fractionation of E118 and E125 gave no suspect males. The AA-attracted males thus obtained were mated singly with virgin females of the mutant isolator T4 having the genotype of *Cy/Pm;H/Sb* with an attached-X chromosome (see Method). Although all of E205 and two of E211 were sterile, female hybrids, *Cy/+;H/+ Cy/+;Sb/+*, coming from two males of the E211 (male a and b) were backcrossed singly to the T4 males. The homozygotes, of which the 2nd chromosomes came from one side of the chromosomal pair of each suspects, were isolated in descendants. Of six lines

Fig. 3. Isolation of mutants induced by EMS (solid circle), comparing to parent strain (open). Each value represents the ratio of flies attracted by acetic acid (AA) vs. odor mixture (OM). P: Parent strain (AA75-1); F₃ and F₄: Flies treated by EMS (solid), comparing to the untreated (open in F₃); F₅: Flies fractionated by AA vs. OM; F₈₋₁₀: Female homozygotes coming from line E211 and from parent strain with attached X chromosome (AA75-2).

examined so far, five from male b (line b-2, b-3, b-4, b-6) and male a (line a-1) turned out to be true mutants showing a heritable alteration in response: $K_{AA/OM}$ was 0.89 to 1.00 (Fig. 3, F₈₋₁₀). These homozygotes have received their sex chromosomes (the attached-X and the X) from T4 flies, so that the behavioral alteration is almost certainly due to a mutation on the autosomes. The line b-1 was not different from the control. The line b-2 was therefore used as the mutant stock (HPB-1).

Figure 5 shows the responses of the mutant strain to acetic acid (AA) and the chemicals in the odor mixture, compared to the parent strain. The mutant was more attracted to $10^{-3}M$ acetic acid, whereas for the parent strain grown on corn media this odor was repellent. $0.5M$ ethanol was attractive to both strains. The greatest difference between the mutant and the parent strain was for 4-(o-hydroxyphenyl)-3-buten-2-one (HPBO, Eastman Organic Chemicals), at the concentration of both 2×10^{-4} and

Fig. 4. Fractionations for line E205 and E211 by 10^{-4} M acetic acid (AA) vs. odor mixture (OM), and for E118 and E125 by OM vs. AA, comparing to parent flies (AA75-1). Each value represents the ratio of flies isolated through n trials of fractionation.

Fig. 5. Responses of parent (solid bar) and mutant flies (open), genotype $+/+;+/+$, to each odorant of acetic acid, 4-(o-hydroxyphenyl)-3-buten-2-one, its trans isomer and ethanol vs. water ($K_{OD/w}$). Flies were reared on corn media except those reared on yeast media for 2×10^{-5} M HPBO. F: Female, M: Male.

$2 \times 10^{-5} \text{M}$, giving a $K_{\text{HPBO}/\text{W}}$ value of 0.73 for the mutant, and 0.36 for the parent. However, the highly purified trans isomer of HPBO isolated from the purchased or the synthesized chemical, turned out to be inactive for both strains, which implies that there remains an unknown impurity. The response of females to these odorants was identical with males of the same strain (Fig. 5).

Factors affecting behavioral alteration of the mutant strain: (1) Larval medium and starvation-period: The behavioral characteristics of the mutant strain are dependent on the combination of the larval medium and the length of the starvation-period before testing. As mentioned earlier, the odor of commercial $2 \times 10^{-5} \text{M}$ 4-(o-hydroxyphenyl)-3-buten-2-one (HPBO) produces a behavioral alteration in the mutant strain reared on the yeast medium with 17 hr starvation (Fig. 5). For flies grown on corn medium with 17 hr or 28 hr starvation this odor is inactive; these flies show no oriented movement towards the odor source. The mutant strain reared on the corn medium showed, however, some behavioral alteration for HPBO at tenfold greater concentration ($2 \times 10^{-4} \text{M}$) with 28 hr starvation (results with a 17 hr-starvation time were inconclusive). The mutant strain supplied water through 28 hr starvation-period became less attracted to HPBO, resulting in masking an apparent strain difference. (2) Odor concentration: Figure 6 shows the behavioral responses of the mutant and parent strains to different concentration of salicylaldehyde. These results indicate that the behavioral alteration of the mutant strain is apparent at the concentration of 3×10^{-4} to $5 \times 10^{-3} \text{M}$. Further increase in concentration masks the strain difference: both the parent and mutant flies

Fig. 6. Responses of parent and mutant flies reared on corn media to odor of salicylaldehyde (SA) vs. water (W) ($K_{\text{SA}/\text{W}}$).

repell. A similar result has been obtained with potent repellents for the parent flies such as 3-heptanone and 5-nonanone; this will be described later.

Specific odors for mutant strain: Odors which were repellent to the parent strain but attractive to the mutant are defined as "specific". To detect features common these, responses of the mutant and parent strain to 97 chemicals were compared. Figure 7 shows the responses of the flies for ketones, aldehydes and esters (N1 to N27),

Fig. 7. Responses of parent (open circle) and mutant flies (solid), including genotype $+/+;+/+$ and $Cy+/+;+/+$, to each odor of aldehydes, ketones and esters vs. water (A) and of alcohols and phenols vs. water (B). Broken line shows difference between two strains is statistically significant (reliability 90%). No. of tests: 156; No. of flies trapped: about 45,000. N1: 5×10^{-4} M *o*-phthalaldehyde, N2: 10^{-3} M 1-phenyl-2-butanone, N3: 10^{-3} M ethylfumarate, N4: 2×10^{-4} M 3-methyl-2-heptanone, N5: 10^{-2} M 2,5-hexanedione, N6: 10^{-2} M dimethylsuccinate, N7: 10^{-4} M 2-methyl-1,4-naphthoquinone, N8: 10^{-3} M 4-phenyl-2-butanone, N9: 10^{-3} M 4-(*p*-methoxyphenyl)-2-butanone, N10: 10^{-3} M 3-methyl-2-heptanone, N11: 2×10^{-4} M *trans*-4-phenyl-3-buten-2-one, N12: 10^{-3} M *iso*-phthalaldehyde, N13: 5×10^{-5} M perillaldehyde, N14: 10^{-3} M ethylmalate, N15: 2×10^{-4} M *p*-(3-oxobutyl) phenyl acetate, N16: 4×10^{-1} M 2-propanone, N17: 10^{-3} M coumarin, N18: 10^{-3} M terephthalaldehyde, N19: 10^{-3} M acetophenone, N20: 10^{-3} M 4-heptanone, N21: 10^{-6} M benzil, N22: 10^{-2} M furfural, N23: 10^{-4} M 3-heptanone, N24: 2×10^{-3} M 4-methylacetophenone, N25: 10^{-4} M 5-nonanone, N26: 10^{-3} M 5-nonanone, N27: 10^{-3} M 3-heptanone, P1: 5×10^{-1} M ethanol, P2: 10^{-2} M pyrocatechol, P3: 10^{-2} M phenol, P4: 10^{-3} M 2,5-di-*tert*-butylhydroquinone, P5: 10^{-1} M ethylene glycol, P6: 10^{-2} M *o*-hydroxybenzyl alcohol, P7: 10^{-2} M piperonyl alcohol, P8: 10^{-3} M 1,1,1-tris (hydroxymethyl) propane, P9: 10^{-2} M furfuryl alcohol, P10: 10^{-3} M hydroquinone, P11: 5×10^{-5} M citronellol, P12: 10^{-2} M 2-methyl-2,4-pentanediol, P13: 10^{-2} M 1,4-butanediol, P14: 5×10^{-5} M geraniol, P15: 5×10^{-5} M farnesol, P16: 10^{-2} M 1,3-butanediol, P17: 10^{-2} M 2,3-butanediol, P18: 10^{-2} M 4-methyl-2-pentanol, P19: 5×10^{-5} M nerol, P20: 10^{-2} M *o*-cresol, P21: 10^{-3} M *n*-octanol.

Fig. 8. Response of parent and mutant flies, including genotype $+/+;+/+$ and $Cy/+;+/+$, to chemicals possessing $=O$ and $-OH$. Molecular feature of each odorant is represented as minimal distance between $=O$ oxygen atom and $-OH$ proton, estimated by a chemical model. For tropolone the infra-red spectrometric data was referred (Koch 1951). Each symbol is same as in Fig. 7. No. of tests: 288; No. of flies trapped: about 82,000. B1: $10^{-1}M$ diacetone alcohol, B2: $2 \times 10^{-3}M$ *o*-hydroxyacetophenone, B3: $10^{-3}M$ ethyl benzoylacetate (enol), B4: $5 \times 10^{-3}M$ *o*-vanillin, B5: $4 \times 10^{-3}M$ methyl salicylate, B6: $10^{-3}M$ *o*-vanillin, B7: $10^{-4}M$ 5-hydroxy-1,4-naphthoquinone (juglone), B8: $10^{-3}M$ benzoylacetone (enol), B9: $10^{-1}M$ methyl acetoacetate (enol), B10: $2 \times 10^{-3}M$ *o*-hydroxypropiophenone, B11: $2 \times 10^{-4}M$ morin, B12: $10^{-3}M$ *o*-hydroxybenzaldehyde, B13: $10^{-1}M$ 2,4-pentanedione (enol), B14: $5 \times 10^{-3}M$ *o*-hydroxybenzaldehyde, C15: $2 \times 10^{-4}M$ purpurogallin, C16: $10^{-1}M$ tropolone, D17: $10^{-2}M$ acetoin, D18: $2 \times 10^{-2}M$ hydroxyacetaldehyde, E19: $10^{-4}M$ phthicol, E20: $10^{-5}M$ 3-hydroxyflavon, E21: $10^{-3}M$ maltol, E22: $10^{-2}M$ 2,3-butanedione (enol, Eastman Organic Chemicals), E23: $10^{-3}M$ kojic acid, E24: $10^{-2}M$ 2,3-butanedione (enol, Tokyo Kasei Co.), F25: $10^{-2}M$ malonic acid, F26: $10^{-2}M$ maleic acid, F27: $5 \times 10^{-3}M$ fumaric acid, F28: $10^{-3}M$ *p*-toluic acid, F29: $10^{-3}M$ iso-valeric acid, F30: $5 \times 10^{-2}M$ succinic acid, F31: $10^{-3}M$ benzoic acid, F32: $10^{-2}M$ succinic acid, F33: $10^{-2}M$ *n*-butyric acid, F34: $10^{-2}M$ *p*-hydroxybenzoic acid, F35: $10^{-2}M$ *m*-hydroxybenzoic acid, F36: $10^{-2}M$ iso-butyric acid, F37: $10^{-1}M$ propionic acid, F38: $10^{-3}M$ *n*-valeric acid, F39: $10^{-3}M$ *o*-toluic acid, F40: $10^{-3}M$ *m*-toluic acid, F41: $10^{-3}M$ acetic acid, F42: $10^{-5}M$ benzoin, F43: $10^{-2}M$ ascorbic acid, G44: $2 \times 10^{-4}M$ *trans*-4-(*o*-hydroxyphenyl)-3-buten-2-one, H45: $10^{-3}M$ 7-hydroxycoumarin, I46: $2 \times 10^{-3}M$ *m*-hydroxyacetophenone, I47: $10^{-3}M$ *m*-hydroxybenzaldehyde, I48: $10^{-3}M$ iso-vanillin, J49: $10^{-4}M$ dimedone (enol), J50: $10^{-3}M$ 4-hydroxycoumarin, K51: $10^{-2}M$ 5-hydroxymethylfurfural, L52: $5 \times 10^{-4}M$ 4-(*p*-hydroxyphenyl)-2-butanone, M53: $10^{-3}M$ *p*-hydroxybenzaldehyde, M54: $10^{-3}M$ vanillin, M55: $2 \times 10^{-3}M$ *p*-hydroxyacetophenone, M56: $2 \times 10^{-3}M$ *p*-hydroxypropiophenone.

and for alcohols and phenols (P1 to P21). From 27 aldehydes, ketones and esters examined so far, four aliphatic monoketones, 2-propanone (N16), 3-heptanone (N23), 4-heptanone (N20) and 5-nonanone (N25), turned out to be specifically attractive for the mutant strain. Of 21 alcohols and phenols, none was specific for the mutant. For compounds giving an average value of about 0.5 (P8 to P17), more measurements are needed to define whether or not given concentrations exceed their threshold values.

Figure 8 shows the responses of flies to 56 odorants possessing both a =O oxygen (carbonyl, aldehyde or carboxylic group) and -OH proton (hydroxyl, phenol or carboxylic group). Certain diketones (2,4-pentanedione, benzoylacetone, dimedone etc.) and an ester (methyl acetoacetate) are included in this figure because of their tendency to take enol form in gas phase. Behavioral alteration of the mutant strain was caused by the following kinds of chemicals: (I) diketones: 2,4-pentanedione (B13), benzoylacetone (B8) and 2,3-butanedione (E22 and E24); (II) an ester: methyl acetoacetate (B9); (III) γ -pyrones: maltol (E21) and kojic acid (E23); (IV) a flavon: morin (E11); (V) monocarboxylic acids: acetic acid (F41), propionic acid (F37), n-butyric acid (F33), iso-butyric acid (F36) and n-valeric acid (F30). All of these odors was repellent to the parent strain but attractive to the mutant.

As demonstrated earlier for acetic acid, ethanol and 4-(o-hydroxyphenyl)-3-buten-2-one (HPBO) (Fig. 5), responses of females were similar to those males. The result was confirmed for additional compounds. This is not always true however; there are cases in which the behavioral alteration in males seems to be dubious (*e.g.* maltol). The heterozygote *Cy/+* showed approximately the same phenotype for almost all of the odorants as the homozygotes *+/+*.

Molecular characteristics of specific odors: Molecular characteristics of odorants are displayed in Figure 8. From the results obtained so far, certain functional groups such as methyl, methoxy and phenyl, do not seem to play any role in producing odor specificity; nor does the size or shape of the odorant molecules seem to be relevant. Hydroxyl, carbonyl, aldehyde and carboxylic groups, on the other hand, appear to effect the specificity. The 10 specific odors, salicylaldehyde, morin, maltol, kojic acid and six carboxylic acids, possess a characteristic structure, consisting of a proton acceptor (=O) and a proton donor (-OH proton), of which minimal distances are 1.65 to 2.5 Å. Furthermore, in gas phase, 2,4-pentanedione and methyl acetoacetate apparently take enol forms (Conant and Thompson 1932), forming structures similar to salicylaldehyde and morin. The specificity of benzoylacetone is very probably related to the formation of the enol form: close to 99 per cent enolization in the absence of solvent (Gould 1959). The α -diketone, 2,3-butanedione, contains about 0.6 per cent of enol form in the absence of solvent, forming a structure similar to maltol and kojic acid (Hammond 1956). Since intermolecular hydrogen bonding occurring in the enol form results in lowering the polarity, it appears that the enol is in practically more volatile than the keto form in the polar solvent as water employed in the present olfactometry. As a result, these 14 specific odors seem to contain either of the following structures: (1) the 2,4-pentanedione type (B in Fig. 8) including salicylaldehyde, benzoylacetone, methyl acetoacetate and morin, in which the average distance between =O atom and -OH proton (average A-D distance (A-D)) is 3.2 Å, varying from 1.65 to 5.0 Å for different con-

formers; (2) the 2,3-butanedione type (E in Fig. 8) including kojic acid and maltol whose A-D are about 3.0 to 3.3Å, varying from 2.3 to 3.7Å for kojic acid and maltol and from 2.3 to 4.3Å for 2,3-butanedione; and (3) the acetic acid type (F in Fig. 8) including propionic acid, n-butyric acid, iso-butyric acid, n-valeric acid and succinic acid whose A-D is about 2.8Å, varying from 2.5 to 3.1Å. It is assumed, therefore, that the odor specificity is produced by a 'bifunctional unit', consisting of a proton-acceptor (A) and proton-donor (D), of which the average A-D distance is about 3Å. This role of the 'bifunctional unit' in specificity is supported further by the following facts: (1) None of 21 alcohols and phenols shown in Fig. 7 revealed specificity. Since these include 10 polyhydric alcohols and diphenols such as 1,4-butanediol, pyrocatechol and o-hydroxybenzyl alcohol, possessing two or more hydroxy groups, the odor specificity cannot be dependent on the proton-donor alone. (2) Of the 13 chemicals whose minimal A-D distances are greater than 3.4Å such as trans-4-phenyl-3-buten-2-one (3.4 to 7.3Å), 7-hydroxycoumarin (4.1 to 5.9Å), dimedone (4.8 to 5.8Å for enol; 96 per cent enolization in water (Schwarzenwach and Felder 1944)) and 4-(p-hydroxyphenyl)-2-butanone (5.8 to 7.8Å) (G to M in Fig. 8), all turned out to be entirely nonspecific; (3) Of the 10 chemicals possessing two =O atoms, all of which are in keto form in gas phase and whose minimal distances between two oxygen atoms range from 1.1Å (2,5-hexanedione and dimethyl succinate) to 6.6Å ((p-oxobutyl)phenyl acetate), none was found to be specific for the mutant.

There are, however, exceptional cases: although four aliphatic ketones, 2-propanone, 3-heptanone, 4-heptanone and 5-nonanone, have not the 'bifunctional unit', these are specific odors. Many chemicals are non-specific in spite of possessing the 'bifunctional unit' (see B, E and F in Fig. 8): the 2,4-pentanedione type: o-hydroxyacetophenone, o-hydroxypropiophenone, o-hydroxybenzoic acid, methyl salicylate and juglone; the 2,3-butanedione type: 3-hydroxyflavon and phthiocol; the acetic acid type: benzoic acid, malonic acid, fumaric acid, maleic acid, o-toluic acid, m-toluic acid, p-toluic acid, m-hydroxybenzoic acid, p-hydroxybenzoic acid, iso-valeric acid. Among those non-specific odors, there are both attractants and repellents, implying that given concentrations as stimulants exceed threshold values. It appears, therefore, that there remains unknown features affecting interactions between odor molecules and receptor sites.

DISCUSSION

Behavioral responses for odorants may involve a complex pathway in nervous system. A defect in any one of the system could lead to a modification or elimination of the olfactory response. The behavior of the mutant described might be explained if the mutant contains missense mutation producing a modification or defect in a receptor site that interacts with the repellent or its nervous system involved. The fact that the mutant is strongly attracted by chemicals repellent to the parent strain implies that there is at least a different receptor site, and that the neural excitation thus produced leads after integration to an oriented movement towards the odor source. Thus, whatever mechanisms involved in the nervous system, an odorant causes actions upon dif-

ferent receptor sites rather than a particular one. This is the reason why theories that attempt to explain the basis for odor qualities have been far satisfactory.

No matter what sort of interaction may be involved between an odor molecule and the receptor site, studies of a system with two binding sites might provide insight into the interaction of more than one. Pheromone masking by geometrical isomers might be explained if it is ascertained that a functional group of the opposite isomer interact with a binding site to obstruct the approach of pheromone molecules.

Since no anomalies in external morphology have been detected in this mutant, the location of the receptor site is not clear. The determination of the region using electrodes will lead to isolation and identification of the missing substance in the mutant. The isolation of additional mutants on the autosomes as well as on the X chromosomes would offer a great opportunity to dissect a complicated network of olfaction.

SUMMARY

An olfactory mutant of *Drosophila melanogaster* has been isolated. In 97 chemicals examined, the mutant is strongly attracted by 18 chemicals repellent to parent strain: These are salicylaldehyde, morin, maltol, kojic acid, methyl acetoacetate, 2,4-pentanedione, benzoylacetone, 2,3-butanedione, six carboxylic acids and four aliphatic monoketones. These chemicals except four monoketones contain a molecular feature as the 'bifunctional unit' consisting of a proton-acceptor (A) and a proton-donor (D) in gas phase, showing an average A-D distance of about 3Å.

ACKNOWLEDGMENTS

Isolation of the mutant has been done at Florida State University. I wish to thank Drs. L. M. Beidler, D. Tucker, and A. G. Debusk for support and helpful suggestion and Dr. S. Benzer for reading and commenting on the manuscript. This work was aided in part by the Scientific Research Fund of the Ministry of Education (Nos. 8411 and 4050).

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