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Multiple Chemosensory Defects in *daf-11* and *daf-21* Mutants of *Caenorhabditis elegans*

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ABSTRACT

Phenotypic analysis of the *daf-11* and *daf-21* mutants of *Caenorhabditis elegans* suggests that they have defects in components shared by processes analogous to vertebrate taste and olfaction. *daf-11* and *daf-21* mutations were previously shown to cause inappropriate response to the dauer-inducing pheromone. By mutational analysis and by disabling specific chemosensory sensilla with a laser, we show that neurons in the amphid sensilla are required for this pheromone response. Using behavioral assays, we find that *daf-11* and *daf-21* mutants are not defective in avoidance of certain non-volatile repellents, but are defective in taxis to non-volatile attractants. In addition, both mutants are defective in taxis to volatile attractants detected primarily by the amphid neuron AWC, but respond normally to volatile attractants detected primarily by AWA. We propose that *daf-11* and *daf-21* mediate sensory transduction for both volatile and non-volatile compounds in specific amphid neurons.

IN vertebrates, the chemosensory processes of taste and olfaction are mediated by distinct populations of sensory neurons that appear, at least in part, to use related transduction mechanisms (LANCET *et al.* 1988; SHEPHERD 1991). Detection of odorant and sweet taste stimuli is thought to be initiated by the binding of a ligand to a seven-transmembrane receptor that is coupled to a second messenger by a trimeric G-protein. The second messenger molecules act directly or indirectly on ion channels to affect their conductance. The recent isolation and analysis of transduction components from vertebrate taste or olfactory tissue has focused mainly on those components that are specific to the tissue of origin. However, given the similarity of some taste and olfaction transduction mechanisms, an intriguing possibility is that a single molecule or set of molecules might function in both processes.

The nematode *Caenorhabditis elegans* has sensory mechanisms analogous to vertebrate taste and olfaction that mediate response to a dauer pheromone (GOLDEN and RIDDLE 1982, 1984a,b), avoidance of volatile and non-volatile repellents (CULOTTI and RUSSELL 1978; BARGMANN *et al.* 1990, 1993), and taxis toward volatile and non-volatile attractants (WARD 1973; DUSENBERY 1974; BARGMANN *et al.* 1993). A simple nervous system of 302 neurons in the adult hermaphrodite and genetic tractability make *C. elegans* an excellent model organism in which to functionally identify and analyze components involved in taste and olfactory transduction.

As in vertebrates (KINNAMON 1987; GETCHELL and GETCHELL 1987), the sensory responses of *C. elegans* to volatile and non-volatile chemicals are mediated by distinct populations of sensory neurons. There are three sets of chemosensory sensilla in *C. elegans*. The cells in each sensillum have ciliated endings that are exposed to the environment through a pore that is formed by a socket cell. The cilia are surrounded by a sheath cell that provides a supportive environment for sensory-cilium function. The bilaterally symmetric amphid sensilla in the head each contain 12 ciliated neurons. Eight of these amphid neurons are directly exposed to the environment, while four other morphologically distinct amphid neurons are less directly exposed (WARD *et al.* 1975; WARE *et al.* 1975; PERKINS *et al.* 1986; WHITE *et al.* 1986). The bilaterally symmetric phasmid sensilla in the tail each contain two exposed neurons (SULSTON *et al.* 1980; HALL and RUSSELL 1986; WHITE *et al.* 1986). A final set of putative chemosensory sensilla are the sixfold symmetric inner-labial sensilla surrounding the mouth, each with a single exposed neuron (WARD *et al.* 1975; WARE *et al.* 1975; WHITE *et al.* 1986). Screens for mutations affecting *d*auer larva *f*ormation (*daf*; ALBERT *et al.* 1981), avoidance of high *osm*olarity (*osm*; CULOTTI and RUSSELL 1978), and *che*motaxis (*che*; DUSENBERY *et al.* 1975; LEWIS and HODGKIN 1977) identified mutations in nine genes, all of which cause structurally abnormal sensory endings in one or more of these chemosensory sensilla (LEWIS and HODGKIN 1977; ALBERT *et al.* 1981; PERKINS *et al.* 1986). All of these mutants were subsequently found to be defective in several chemosensory responses (PERKINS *et al.* 1986). These are referred to as the cilium-structure mutants.

C. elegans displays stereotyped responses to various chemicals in the environment. The remainder of this introduction provides a brief description of each response, as well as the genes and chemosensory neurons implicated in each response.

Dauer pheromone response: In an uncrowded environment with abundant food, the *C. elegans* life cycle consists of four larval stages and the egg-laying adult. However, the presence during early larval stages of high levels of a constitutively secreted pheromone induces a developmentally and morphologically distinct third-stage larva, the dauer larva (CASSADA and RUSSELL 1975). This pheromone is necessary and sufficient to induce dauer formation, although food and temperature modulate response to the pheromone (GOLDEN and RIDDLE 1982, 1984a, 1985). Recovery from the dauer state is also determined by environmental conditions; low pheromone, high food and low temperature encourage a dauer to molt into an L4 larva (GOLDEN and RIDDLE 1982, 1984b). Most dauer formation mutants fall into one of two broad classes: mutants that form dauers in the absence of pheromone are *da*uer-*f*ormation *c*onstitutive (Daf-c) while mutants that fail to form dauers in response to pheromone are *da*uer-*f*ormation *d*efective (Daf-d). The cilium-structure mutants comprise a subclass of the Daf-d mutants. Based on studies of genetic interactions between the Daf-c and Daf-d mutants, the *daf* genes have been ordered into a formal genetic pathway for induction of dauer formation (RIDDLE *et al.* 1981; VOWELS and THOMAS 1992; THOMAS *et al.* 1993). Sensory neurons that regulate dauer formation have also been identified. Animals in which the amphid neurons ADF, ASI and ASG were killed with a laser microbeam formed a dauer larva without pheromone induction. When a fourth exposed neuron, ASJ, was also killed, those dauers were unable to recover to form an L4 larva (BARGMANN and HORVITZ 1991a). Because of their effects on dauer formation, ADF, ASI, and ASG are good candidates for pheromone-responding cells. However, all tests of the effects of the cell kills were performed in the absence of pheromone (BARGMANN and HORVITZ 1991a). Therefore, we have assayed dauer formation in the presence of pheromone to determine whether amphid chemosensory neurons, or neurons in other sensilla, are required for the pheromone response.

Avoidance response: Wild-type *C. elegans* is repelled by solutions of high osmotic strength (CULOTTI and RUSSELL 1978) and other noxious volatile and non-volatile stimuli (BARGMANN *et al.* 1990, 1993). Mutants with defects specific to avoidance of non-volatile repellents have been isolated, but await further characterization (our unpublished data). Cell-kill experiments have shown that the exposed amphid neurons ASH and ADL are required for consistent avoidance of non-volatile repellents (BARGMANN *et al.* 1990). Genes and cells that mediate avoidance of volatile repellents are currently under study (C. BARGMANN, personal communication).

Chemotaxis response: *C. elegans* is attracted to a number of small non-volatile molecules including cyclic nucleotides, biotin, cations, and anions (WARD 1973; DUSENBERY 1974; BARGMANN and HORVITZ 1991b), and to a wide variety of volatile compounds (BARGMANN *et al.* 1993). In a gradient of attractant, worms move toward and accumulate at the peak of the gradient (WARD 1973). Screens for mutants defective in chemotaxis to non-volatile attractants (*che*; DUSENBERY *et al.* 1975; LEWIS and HODGKIN 1977) or to volatile attractants (*odr*; BARGMANN *et al.* 1993) have identified several genes required for normal responses. The results of cell-kill experiments indicate that chemotaxis to the non-volatile attractants Na^+, Cl^-, cAMP and biotin requires the exposed amphid cell ASE; three other exposed neurons, ADF, ASG and ASI, appear to play lesser roles in these responses (BARGMANN and HORVITZ 1991b). Chemotaxis toward volatile attractants requires primarily the indirectly exposed amphid neurons AWA and AWC, each of which mediates responses to a variety of odorants (BARGMANN *et al.* 1993).

In this study, we report the analysis of two genes, *daf-11* and *daf-21*, whose products are good candidates for components involved in both volatile and non-volatile chemosensory transduction processes. Mutations in *daf-11* and *daf-21* cause a Daf-c phenotype; however, preliminary evidence suggests that, unlike the *daf-11* mutations, the Daf-c phenotype of *daf-21(p673)* may not be due to a simple loss of function mutation (E. A. MALONE, personal communication). Previous results showed that *daf-11* and *daf-21* are the only two mutants whose Daf-c phenotype is suppressed by mutations in the Daf-d cilium-structure genes (VOWELS and THOMAS 1992; THOMAS *et al.* 1993), leading to the hypothesis that *daf-11* and *daf-21* are directly or indirectly involved in pheromone sensory transduction. Here we report results suggesting that transduction of the pheromone signal requires only amphid chemosensory neurons. We also report the analysis of the avoidance response of *daf-11* and *daf-21* mutants to non-volatile repellents, and their taxis responses to non-volatile and volatile attractants. These results suggest that *daf-11* and *daf-21* are involved in several amphid-mediated chemosensory responses.

MATERIALS AND METHODS

General genetic methods: General methods of *C. elegans* strain maintenance were as described by BRENNER (1974). The *C. elegans* Bristol strain N2 was used as the standard wild-type strain. Many of the *daf-11* alleles and the *daf-21* allele were generously provided by D. RIDDLE, University of Missouri. This paper follows the standard *C. elegans* nomenclature (HORVITZ *et al.* 1979).

Marker mutations used were:

Linkage group (LG) V: *dpy-11(e224)*, *unc-42(e270)*, *sma-1(e30)*, *egl-1(n986dm)*, *unc-76(e911)*.

Pheromone assays: Preparation of dauer pheromone was modified from GOLDEN and RIDDLE (1984a). *Escherichia coli* strain OP50 was grown to saturation in two volumes of vigorously agitated superbroth [modified from DAVIS *et al.* (1980): 12 g Bacto-tryptone, 24 g Bacto-yeast extract, 8 ml 50% glycerol, 2 ml 10 M NaOH and 33 ml 1.5 M potassium phosphate, pH 6.0, per liter]. *E. coli* was harvested by centrifugation, and

added to wild-type worms in one volume of S-basal medium (SULSTON and HODGKIN 1988). The culture was grown to high density with vigorous agitation at 20°. After all *E. coli* were consumed, worms and other debris were removed by centrifugation. The supernatant was reduced by boiling to a thick brown residue that was then extracted with 95% ethanol repeatedly until the ethanol phase became colorless. The ethanol was evaporated in a vacuum. The resulting residue was resuspended in approximately 10 ml of water per liter of worm culture, and filter sterilized using a 0.2-μm filter. The pheromone preparation was stored at −20°.

We define one unit of pheromone as the amount required to induce 33% dauer formation in a wild-type population at 25°. Pheromone assays were performed at 25° as described in GOLDEN and RIDDLE (1984a) except that 50 μg/ml streptomycin was added to the agar in the assay plates. Dauers (including those that had crawled onto walls and lid of the assay plate) and non-dauer worms were counted in order to calculate the percent dauer formation on each plate. The weighted mean (Σ dauers from all plates/Σ dauers + non-dauers from all plates) and standard error of the mean of all tests at each pheromone concentration were determined.

Cell kills: Cells were killed with a Laser Sciences, Inc. VSL-337 nitrogen laser-pumped dye laser as described by AVERY and HORVITZ (1989), except that kills were confirmed 3–5 hr after the operation. Control animals were treated identically to operated animals, but no cells were killed (mock kills). Pheromone-response assays on operated and unoperated worms were done in parallel at 25°.

Avoidance assays: Assays for avoidance of high osmolarity were slightly modified from CULOTTI and RUSSELL (1978). A 15-mm diameter ring, formed with approximately 10 μl of 8 M glycerol + 0.25 mg/ml crystal violet, was applied to an agar surface. Well fed adult worms were transferred to an agar plate without food and allowed to swim freely for a brief period of time to remove excess bacteria. The worms were then picked to the center of the ring of repellent. During the assay, worms were directly observed to determine that avoidance responses were present and normal. In addition, after 10 min the number of worms that had crossed the ring was recorded. The *daf-11* alleles and numbers of worms tested were: *m47* ($n = 20$), *m87* ($n = 22$), *m84* ($n = 29$), *m124* ($n = 17$) and *sa195* ($n = 25$). *daf-21(p673)* was also tested ($n = 22$).

Assays for chemotaxis to non-volatile and volatile attractants: Population chemotaxis assays were performed as described in BARGMANN *et al.* (1993) with minor modifications. Gradients of non-volatile attractants were established by adding 100 μl of molten agar + attractant to an 8-mm hole 0.5 cm from the perimeter of a 10-cm Petri dish. As a control, the same amount of agar without attractant was added to a similar hole on the opposite side. Following established precedent (BARGMANN and HORVITZ 1991b), the concentration of non-volatile attractant that maximized the chemotactic response of the wild type was tested. The initial attractant concentration at the gradient peak was 0.1 M NH_4Cl, 0.05 M cAMP-NH_4 or 0.1 M biotin-NH_4. Solutions were adjusted to pH 7.0 with NH_4OH (BARGMANN and HORVITZ 1991b). Cl^- gradients were established for 16–18 hr, and cAMP and biotin gradients were established for 24 hr. To enhance the response of wild type to cAMP and biotin, 5 μl of 0.2 M cAMP or biotin was spotted onto the attractant point source once every hour for 3 hr before the assay. Similarly, 5-μl pulses of water were added to the control point source. Response to a cationic attractant (Na^+ or K^+) or to lysine (WARD 1973) could not be adequately addressed because our wild-type strain repeatedly showed a weak or variable response to these attractants. We also assayed response to the best characterized attractants from five of the six classes of volatile odorants studied by BARGMANN *et al.* (1993): isoamyl alcohol, benzaldehyde, pyrazine, diacetyl and 2,4,5-trimethylthiazole. Response of our wild-type strain to the sixth attractant, butanone, was too variable for effective analysis.

To obtain synchronous broods for the chemotaxis assays, 7–15 adult worms were allowed to lay eggs for 7–9 hr. All strains were then grown to the adult stage, and assays were performed, at 20°. However, many Daf-c strains form mostly dauers at this temperature. Therefore, *daf-12(m20)* was used to suppress the Daf-c phenotype of the *daf-11* and *daf-21* mutants (RIDDLE *et al.* 1981; VOWELS and THOMAS 1992; THOMAS *et al.* 1993). *daf-12* is the only strong suppressor of *daf-11* and *daf-21* that does not itself affect chemotactic responses. Although *daf-12* suppressed the Daf-c phenotype of the *daf-1(m40)*, *daf-4(e1364)*, *daf-7(e1372)*, *daf-8(e1393)* and *daf-14(m77)* mutants (RIDDLE *et al.* 1981; VOWELS and THOMAS 1992), it did not suppress their egg-laying (Egl) defects (TRENT *et al.* 1983; THOMAS *et al.* 1993), which limited the size of the synchronous broods to be tested, nor did it suppress their clumpy (Cpy) phenotype (THOMAS *et al.* 1993), which inhibited dispersal of the worms during the chemotaxis assays. Since a mutation in *daf-3* could suppress most of these phenotypes, *daf-1*, *daf-4*, *daf-7*, *daf-8* and *daf-14* were tested in a *daf-3(e1376)* background. In addition, response of each of these single mutants (grown at 15°) to 10^{-2} isoamyl alcohol was tested in a limited number of assays, and the results were similar to those observed for the double mutants containing *daf-13*. A *daf-12* or *daf-3* single mutant control was included with each chemotaxis assay, and in no case was the response significantly different from the wild type at $P \leq 0.01$. To remove residual bacteria that might affect chemotaxis, well fed worms to be tested for response to non-volatile attractants were washed three times with 20° chemotaxis assay buffer [1 mM $CaCl_2$, 1 mM $MgSO_4$, 5 mM potassium phosphate (pH 6.0)]; those to be tested for response to volatile attractants were washed three times with S basal medium and once with water as described in BARGMANN *et al.* (1993). All strains were run in several assays, and a weighted mean chemotaxis index was calculated for each strain as [Σ(worms from all assays at attractant) − Σ(worms from all assays at control]/Σ(worms from all assays). The standard error of the mean was calculated from the chemotaxis indices (BARGMANN *et al.* 1993) of each individual assay of a given strain with a given attractant. Responses of the control and mutant strains were compared statistically using the two-tailed *t*-test.

Dauer formation and recovery assays: To assess percent dauer formation at 15°, 5–10 adult animals were picked to plates and allowed to lay eggs at 23° for 7–9 hr; plates were then transferred to 15°. After 5 days, dauer and non-dauer progeny were counted, including those animals that had crawled onto the walls of the plate (see below). The percent dauer formation at 15° for some alleles of *daf-11* is lower than reported in MALONE and THOMAS (1994). This inconsistency was shown to be due to differences in the strain of OP50 used as a food source (data not shown). Counts reported in VOWELS and THOMAS (1992), THOMAS *et al.* (1993) and this work were all done with the same OP50 strain.

To assess dauer recovery, Daf-c mutants were allowed to form dauers at 25° and were then shifted to 15°. However, the dauers tended to crawl onto the walls of the Petri dish and die, reducing the number of scorable individuals. A discussion of our attempts to solve this problem is found in THOMAS *et al.* (1993). To minimize the problem in this assay, dauers were picked to 10-cm Petri plates which have a larger surface area/circumference ratio than the 5-cm plates standardly used. Dauers, recovered dauers (defined as those worms with a light intestine and obvious pharyngeal pumping) and dauers

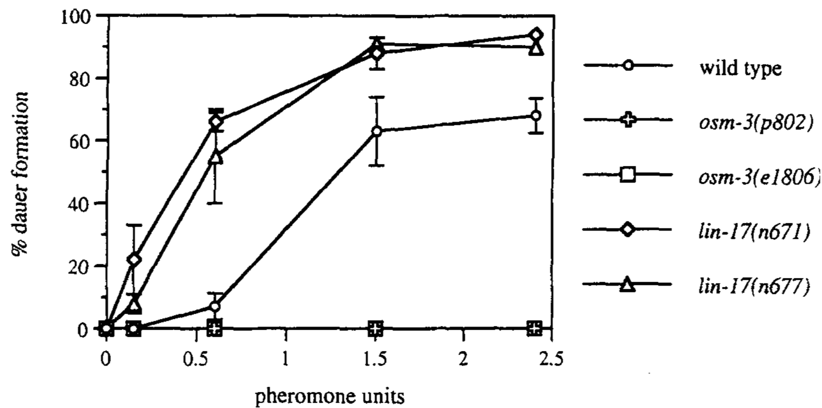

FIGURE 1.—Dauer pheromone response of amphid and phasmid-defective mutants. Dauer formation in response to exogenously added dauer pheromone was assessed at 25°. Each data point represents combined data from three test plates. Error bars represent standard error of the mean among the individual test plates. The modest hypersensitivity of *lin-17* mutants to pheromone is difficult to interpret because first, cells other than the phasmid socket cell are affected in *lin-17* mutants (STERNBERG and HORVITZ 1988), and second, the same *lin-17* mutants were not hypersensitive to pheromone at 20° (data not shown).

trapped on the walls of the plate were counted after 3 days at 15°. Calculation of the percent recovery included only those worms remaining on the agar at the time of counting.

Tests for dominance of Daf-c phenotype: Wild-type males were mated to marked *daf-11(m84, m87, m124* or *sa195)* or *daf-21(p673)* hermaphrodites. The *daf-11* crosses were set at 25°; the *daf-21* cross was set at 20° for 1 day to increase fertility (*daf-21* has a small brood size at high temperatures) and was then transferred to 25°. Parents were transferred to fresh plates daily to obtain synchronous cohorts and approximately 600 cross progeny of each mating were analyzed by counting dauers and non-dauers. In the one case in which dominance was observed, the reciprocal cross was also made: *him-8(e1489); daf-11(m84)* males were crossed to marked hermaphrodites and cross progeny were analyzed as above.

Strain constructions and testing: The *daf-11 daf-21* double mutants were constructed as described in THOMAS *et al.* (1993). To construct the *daf-11 daf-21; daf-12* triple mutants, hemizygous *daf-12/0* males were mated to *daf-11 daf-21* hermaphrodites. Heterozygous progeny were picked to 20°. From the resulting brood, *daf-11 daf-21* dauers were transferred to 15° to recover from the dauer state, and their long progeny were picked to 20° (the *daf-12* mutation confers both Daf-d and Long phenotypes). The presence of both Daf-c mutations was confirmed by complementation testing, and the presence of *daf-12* was inferred from the complete suppression of the Daf-c phenotype.

To generate *daf-11 trans*-heterozygotes, *unc-42(e270) daf-11(sa195)/++* males were mated to either *daf-11(m84) sma-1(e30)* or *daf-11(m124) unc-76(e911)* hermaphrodites at 15°. In a separate cross, *daf-11(m87) unc-76(e911)/++* males were mated to *daf-11(m84) sma-1(e30)* hermaphrodites. Cross progeny hermaphrodites (many of which passed through the dauer stage) were allowed to lay eggs for 7–9 hr and then transferred to 15°. From plates on which unmarked and both types of marked progeny were segregating, percent dauer formation of unmarked progeny at 15° was determined. Dauers that crawled up on the sides of the Petri dish were counted but not included in the calculation because it was difficult to check for the presence of the marker mutation. To quantify dauer recovery, heterozygous parents were allowed to lay eggs at 25° for 16–24 hr. After 3 days, unmarked dauer progeny were picked to plates at 15° and recovery assays were performed as described above. To test odorant response, the *daf-11 trans*-heterozygotes (which did not contain *daf-12* to suppress their Daf-c phenotype) were grown at 15° to prevent the majority of the population from forming dauers. Unmarked L4 *trans*-heterozygotes were transferred to 20° for 16–24 hr and then tested in odorant assays as described above. As controls, wild-type and *daf-11* strains were subjected to a similar temperature-shift regime.

Maternal effect of *daf-21*: To quantify the maternal rescue of the Daf-c phenotype of *daf-21(p673)*, *dpy-11 daf-21/++* and *daf-21/+* individuals were allowed to lay eggs for 16–24 hr at 25°. After 3 days, progeny were analyzed for the presence of dauers. To quantify zygotic rescue, wild-type males were mated to *dpy-11 daf-21* hermaphrodites at 20° for 16–24 hr and then shifted to 25°. After 3 days, cross progeny dauers and non-dauers were counted. The maternal rescue of the *daf-21* odorant response defect was determined by assaying only the unmarked progeny (*daf-21/daf-21*) of individuals carrying *daf-21* in *trans* to a dominant linked marker [*egl-1(n986dm)*]. The strains were grown at 15°, and chemotaxis assays were performed with the temperature-shift regime as described above. Worms were assayed for odorant response to 1% isoamyl alcohol.

RESULTS

Amphid chemosensory neurons are required for dauer pheromone response: Three amphid neurons, ADF, ASI and ASG, are required to prevent dauer formation in the absence of pheromone (BARGMANN and HORVITZ 1991a), but tests for neurons required for dauer formation in response to pheromone have not been reported. It has been previously noted that under conditions of starvation and high population density the cilium-structure mutants form few to no dauers (ALBERT *et al.* 1981; PERKINS *et al.* 1986; SHAKIR *et al.* 1993). This result led to the suggestion that dauer formation is regulated by the only neurons known to be commonly affected in all the cilium-structure mutants, the amphid and phasmid sensory neurons. To more directly address which sensilla mediate the specific response to dauer pheromone, we used a pheromone bioassay (GOLDEN and RIDDLE 1984a) to test both cilium-structure mutants and animals in which specific sensilla were disabled with a laser microbeam. Briefly, dauer formation of a limited population of animals grown in the presence of food and a given level of pheromone was analyzed. We tested *osm-3* mutants, in which the exposed amphid and phasmid cilia are structurally abnormal but the indirectly exposed amphid neurons (AWA and AWC) and the inner labial (IL) neurons are structurally normal (PERKINS *et al.* 1986). Two *osm-3* mutants failed to form dauers in response to levels of pheromone that strongly induced dauer formation in the wild type (Figure 1). Similar re-

TABLE 1

Effect on dauer pheromone response of disabling amphid or phasmid sensilla

Cells killed	Percent dauer formation (*n*)	
	Mock kill[a]	Kill
Amphid sheath[b]	69 (16)	0 (16)
Amphid sheath	48 (40)	0 (22)
PHA, PHB	79 (14)	47 (17)
Phasmid sheath	73 (37)	65 (20)

The number of animals tested is shown in parentheses.

[a] Control worms that were treated identically to operated worms except that no cells were killed.

[b] This experiment was done with approximately 4 units of pheromone per plate. All other experiments were done with approximately 3 units of pheromone per plate.

sults were observed for two other cilium-structure mutants, *daf-10* and *che-11* (data not shown), in which all amphid and phasmid neurons are defective but the IL neurons are normal (ALBERT *et al.* 1981; PERKINS *et al.* 1986). These results suggest that the indirectly exposed amphid neurons and the IL neurons are not sufficient to mediate pheromone response, and that exposed amphid or phasmid neurons are required for this response. Since none of the cilium-structure mutations specifically affect only amphid or only phasmid neurons, they could not be used to separate the requirements for these cells in pheromone response. In *lin-17* mutants, however, the phasmid cilia are unexposed to the environment because the phasmid socket cells that form the sensillar pore are lacking (STERNBERG and HORVITZ 1988). *lin-17* mutants show a strong pheromone response (Figure 1), suggesting that exposure of the phasmid neurons is not required. The differential response of *osm-3* and *lin-17* mutants to pheromone suggests that the exposed amphid neurons are necessary for mediating the pheromone response.

Because the *osm-3* and *lin-17* mutations may have unidentified pleiotropic effects, we did cell-kill studies in the wild type to corroborate the mutant analysis. The role of the amphids in the pheromone response was tested by killing both amphid sheath cells with a laser in early L1 animals. The preferred approach of killing all the amphid neurons was not possible because this causes a Daf-c phenotype (BARGMANN and HORVITZ 1991a). The amphid sheath cells surround the sensory cilia and killing these cells causes defects in three amphid-mediated processes: osmotic avoidance, chemotaxis, and the ability of the amphid neurons to take up a fluorescent dye, fluorescein 5-isothiocyanate (BARGMANN *et al.* 1990; C. BARGMANN, personal communication; our unpublished observations). When the amphid sheath cells were killed, animals did not form dauers when exposed to high concentrations of pheromone, while greater than 50% of the wild-type controls formed dauers under the same conditions (Table 1). In contrast, when the two phasmid sensory neurons, PHA and PHB, or the phasmid sheath cells were killed, animals showed a normal response to pheromone (Table 1). Animals with each type of kill were also grown in the absence of pheromone and were not dauer constitutive (data not shown). In agreement with the mutant analysis, these results suggest that the amphids but not the phasmids are necessary for response to pheromone.

Responses of *daf-11* and *daf-21* mutants to repulsive and attractive non-volatile compounds: The Daf-d cilium-structure mutations suppress the Daf-c phenotype caused by *daf-11* and *daf-21* mutations suggesting that the function of these two gene products requires intact amphid or phasmid cilia (VOWELS and THOMAS 1992; THOMAS *et al.* 1993). Since only amphid neurons are required for response to dauer pheromone, it seemed likely that the *daf-11* and *daf-21* products function in the ciliated endings of amphid sensory cells to mediate the response. To determine whether mutations in these two genes affect other amphid-mediated chemosensory behaviors, we assayed responses to non-volatile repellents and attractants.

To assay the osmotic avoidance response, we measured escape from a ring of repellent (see MATERIALS AND METHODS). Five different *daf-11* mutants and the *daf-21* mutant showed normal avoidance of a standard high osmolarity solution. In contrast, we have found that both *daf-11* and *daf-21* mutants are defective in taxis to a variety of non-volatile attractants. *daf-11* mutants had previously been reported to be chemotaxis defective, but the extent of the defect was not reported (RIDDLE 1988). A strain carrying *daf-21(p673)* was shown to be defective in response to sodium chloride (DUSENBERY 1976). To test more fully the chemotaxis defects caused by *daf-11* or *daf-21* mutations, we tested response of two *daf-11* mutants and the *daf-21* mutant to three non-volatile attractants: Cl^- and cAMP, which are representative of the anion and cyclic nucleotide classes of attractants (WARD 1973), and a recently identified attractant, biotin (BARGMANN and HORVITZ 1991b). The chemotaxis assays were modified slightly from the method of BARGMANN *et al.* (1993) (see MATERIALS AND METHODS). In brief, a population of animals was exposed to a gradient of attractant on an agar-filled Petri plate. Anesthetic was spotted at the peak of the gradient and at a control spot to immobilize animals that reached these points. Response to each chemical was expressed as a chemotaxis index (C.I.) which was calculated from the number of animals at the end of the test period at the attractant, at the control, and at neither [C.I. = (no. of worms at attractant − no. of worms at control)/total no. of worms]. A chemotaxis index of 1 indicates perfect accumulation at the attractant, and 0 indicates no preference for the attractant (BARGMANN *et al.* 1993).

Response of the wild type and the *daf-11* and *daf-21* mutants to each attractant is shown graphically in Figure

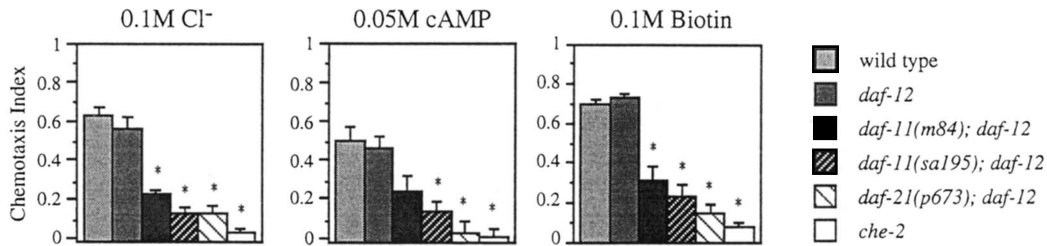

FIGURE 2.—Response of *daf-11* and *daf-21* mutants to non-volatile attractants. Error bars represent the standard error of the mean of the chemotaxis indices calculated from all assays with a given attractant and strain. At least four independent assays were performed for each mutant at each concentration of attractant. *daf-12(m20)* was tested as a positive control; *che-2(e1033)* was tested as a negative control. Asterisks (*) indicate significant difference from wild type and *daf-12* at $P \leq 0.01$ using the two-tailed *t*-test.

2. A *daf-12* mutation was used to suppress the Daf-c phenotype of *daf-11* and *daf-21*. Animals carrying the *daf-12* single mutation were tested as a positive control and responded like wild type to all attractants. The *che-2(e1033)* mutant, which is severely defective in chemotaxis, was tested as a negative control (LEWIS and HODGKIN 1977; BARGMANN and HORVITZ 1991b) (Figure 2). The response of each tested *daf-11* and *daf-21* strain was significantly defective when compared to *daf-12* or wild type with one possible exception (the response of the *daf-11(m84)* mutant to cAMP was significantly different from *daf-12* only at $P = 0.05$). We conclude that *daf-11* and *daf-21* mutants have a general defect in the taxis response to non-volatile attractants.

Chemotaxis responses of *daf-11* and *daf-21* mutants to volatile attractants: To determine whether *daf-11* and *daf-21* are required for response to volatile attractants, we assayed response of *daf-11* and *daf-21* mutants to a representative compound from each of five classes of volatile odorants (BARGMANN *et al.* 1993): an alcohol (isoamyl alcohol; IAA), an aromatic compound (benzaldehyde; BENZ), a pyrazine (PYR), a ketone (diacetyl; DIA), and a thiazole (2,4,5-trimethylthiazole; TMT). Responses to representative attractants from three other classes (esters, aldehydes and diethyl ether) have been less well characterized (BARGMANN *et al.* 1993), so these were not included in our assays. Odorant assays and calculation of population responses were similar to those described above for non-volatile attractants except that the odorant gradient was established immediately prior to the assay (see MATERIALS AND METHODS). Wild-type, *daf-11(sa195)*, *daf-11(m84)* and *daf-21(p673)* strains were assayed for response to at least four concentrations of each odorant. Because *daf-11(m84)* and *daf-11(sa195)* differed substantially in their response to some odorants (see below), we performed a less extensive analysis of four additional *daf-11* alleles, *m597*, *m47*, *m87* and *m124*. The results of these assays are summarized in Figure 3; a detailed presentation of the results for the wild type, *daf-11(sa195)*, *daf-11(m84)* and *daf-21(p673)* is shown in Figure 4, A–E. Since a *daf-12* mutation was used to suppress the Daf-c phenotype of the *daf-11* and *daf-21* mutants, the *daf-12* mutant was assayed as a control.

***daf-11* and *daf-21* mutants show reduced sensitivity to IAA and BENZ:** Responses of *daf-11* mutants to IAA ranged from nearly wild type (*m84*) to severely defective (*m597*, *sa195*) (Figures 3 and 4A). Yet even the most severely defective mutants responded to some extent to high concentrations of IAA. The *daf-21(p673)* mutant had a moderately defective response profile resembling that of *daf-11(m87)*. These results suggest that most *daf-11* mutants and the *daf-21* mutant have decreased sensitivity to IAA.

The attractive response of wild type and *daf-12* to BENZ peaks at around 10^{-2} BENZ, and drops sharply at higher and lower concentrations (BARGMANN *et al.* 1993; Figures 3 and 4B). At the most attractive concentration of BENZ (10^{-2}), response of both *daf-11* and *daf-21* mutants was significantly reduced from wild-type and *daf-12* levels but was not eliminated. In response to undiluted BENZ, wild-type, *daf-12*, *daf-11* and *daf-21* animals moved toward the attractant, but many stopped before reaching the peak of the gradient. This behavior is consistent with previous observations suggesting that high concentrations of BENZ are repulsive to *C. elegans* (BARGMANN *et al.* 1993). Wild-type and *daf-12* animals also appeared to be moderately repelled by 10^{-1} BENZ, while many *daf-11* mutants appeared to be significantly less repelled. One possible explanation for the altered responses of the *daf-11* and *daf-21* mutants is that they have a reduced sensitivity to both the attractive and repulsive aspects of BENZ, causing their response curves to shift toward higher concentrations.

***daf-11* and *daf-21* mutants respond strongly to PYR and DIA:** In contrast to their defective response to IAA and BENZ, all *daf-11* and *daf-21* mutants had strong responses to each concentration of the attractant PYR (Figures 3 and 4C). Similarly, three *daf-11* mutants and the *daf-21* mutant responded normally to concentrations of DIA ranging over three orders of magnitude. However, the strongest *daf-11* alleles, *m597*, *sa195* and *m47* caused a slight but statistically significant decrease in response to 10^{-2} DIA. In our assays, undiluted DIA appeared to have a strong repulsive effect on wild-type and *daf-12* animals. The repulsion was consistently more pronounced than previously reported (BARGMANN *et al.* 1993), suggesting that different wild-type strains

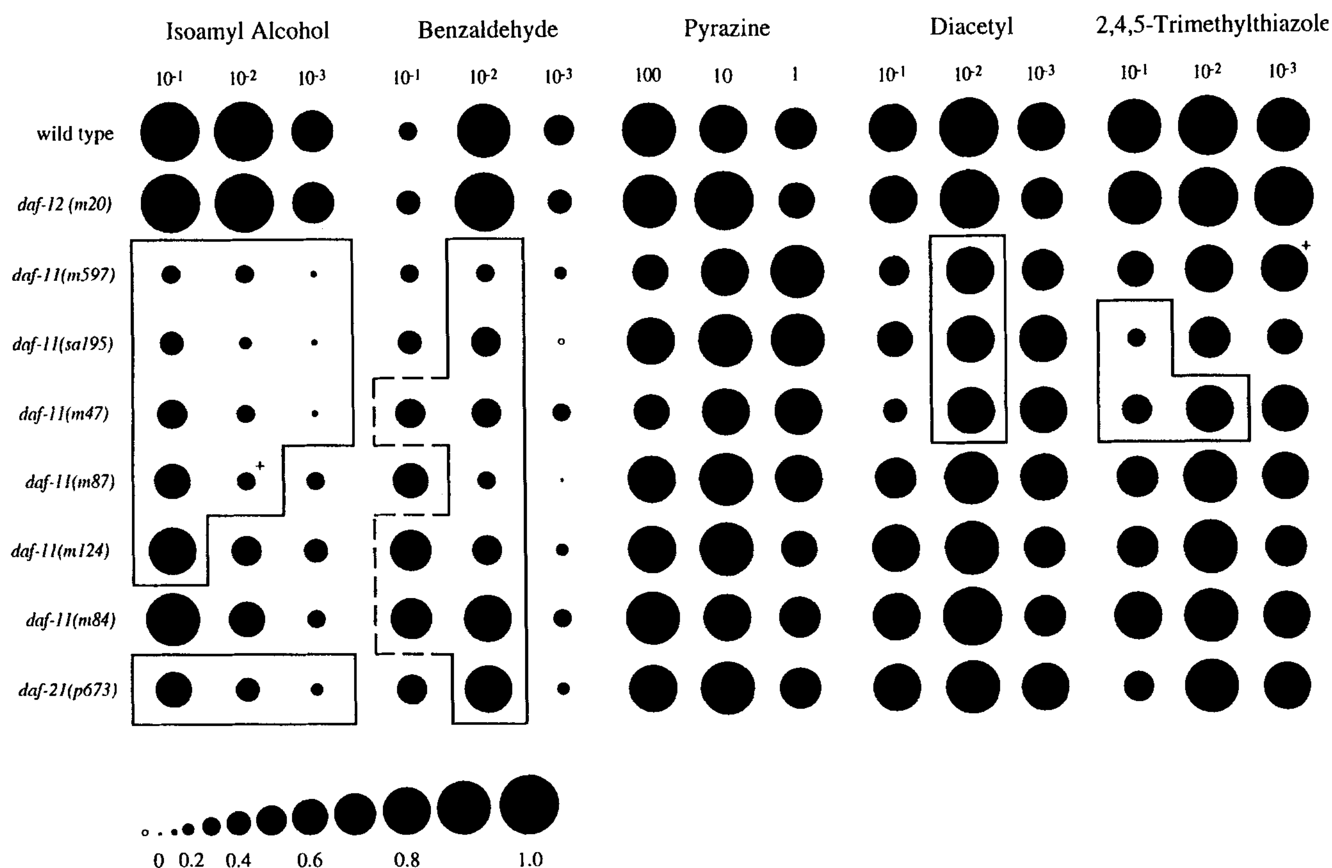

FIGURE 3.—Summary of response to volatile attractants of different *daf-11* mutants and the *daf-21* mutant. C.I. were divided into 12 equally sized bins (*e.g.*, 1.0–0.91, 0.9–0.81, etc.) and are represented by proportionally sized circles. Filled circles are positive indices and open circles are negative indices. C.I. that are significantly decreased from that of wild type and *daf-12* at $P \leq 0.01$ are surrounded by solid lines; those that are significantly increased from that of wild type and *daf-12* at $P \leq 0.01$ are surrounded by dashed lines. All strains contain *daf-12(m20)*. At least three independent assays were performed for each mutant at each concentration of attractant. Relative concentrations are based on volume:volume dilutions of pure liquid attractant in ethanol; concentration of pyrazine (a solid) is in milligrams/ml of ethanol (as in BARGMANN *et al.* 1993). + indicates that one assay was not included because the individual C.I. was more than four standard deviations from the mean C.I.

display some heterogeneity in response to undiluted DIA. Therefore, it is difficult to interpret the fact that *daf-11* and *daf-21* mutants were more attracted than wild type to undiluted DIA.

***daf-11* and *daf-21* mutants show slightly reduced response to TMT:** The odorant TMT is attractive to wild-type and *daf-12* worms over a wide range of concentrations (BARGMANN *et al.* 1993) (Figure 4E), but is repulsive when undiluted. Like wild type, both *daf-11* and *daf-21* mutants avoided the highest concentration of TMT, and their response to all other concentrations of TMT was moderately reduced (Figures 3 and 4E). Only the *daf-11* mutations, *sa195* and *m47*, caused a TMT response that was significantly different from both *daf-12* and wild type. These results suggest that *daf-11* has a small effect on the response to TMT and that the *daf-21* mutation has little or no effect on this response.

Defective odorant response is specific to the group 1 Daf-c genes: The dauer-constitutive mutants have been divided into two groups (group 1: *daf-11* and *daf-21*; group 2: *daf-1*, *daf-4*, *daf-7*, *daf-8* and *daf-14*) on the basis of distinct phenotypes and genetic interactions (THOMAS *et al.* 1993). To determine whether group 1 and 2 Daf-c mutants differ with respect to odorant response, all group 2 Daf-c mutants were assayed for response to three concentrations of each volatile attractant. Group 2 mutants exhibit a population behavior called clumping, which interferes with chemotaxis. Since the clumpy phenotype is suppressed by *daf-3*, but not *daf-12* (THOMAS *et al.* 1993), all group 2 mutants were assayed in a *daf-3* background. As a control, the *daf-3* single mutant was assayed, and in no case was odorant response significantly different from the wild type. Group 2 *daf-c; daf-3* double mutants responded to each attractant like the *daf-3* single mutant (Figure 5). To be sure that the *daf-3* mutation did not suppress any chemotaxis defects caused by group 2 mutations, we assayed response of each group 2 single mutant to 10^{-2} IAA, and response of group 2 *daf-c; daf-12* double mutants to one concentration of each of the five attractants (data not shown). In some of the single and *daf-12* double mutant assays, a sizable fraction of the population remained

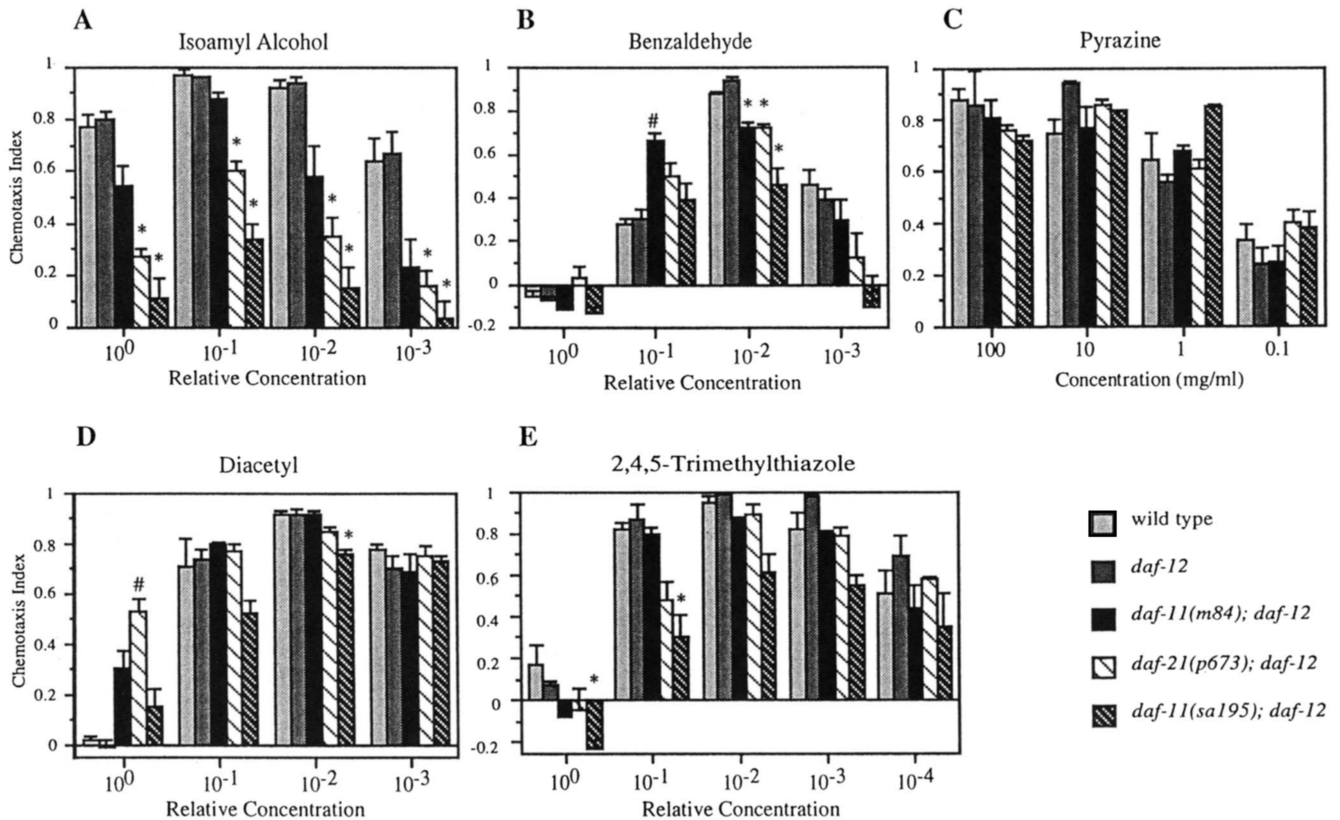

FIGURE 4.—Response of selected *daf-11* mutants and the *daf-21* mutant to volatile attractants. (A) Isoamyl alcohol; (B) benzaldehyde; (C) pyrazine; (D) diacetyl; (E) 2,4,5-trimethylthiazole. Error bars are as in Figure 2. Relative concentrations of IAA, BENZ, DIA and TMT and concentrations of PYR are as in Figure 3. *, Significantly different (decreased) from wild type and *daf-12* at $P \leq 0.01$; #, significantly different (increased) from wild type and *daf-12* at $P \leq 0.01$.

clustered at the origin throughout the duration of the assay despite attempts to disperse them. In these cases, the clumping phenomenon produced an apparent decrease in the chemotaxis index when compared to the *daf-c; daf-3* double mutant response. However, two observations indicate that the decreased indices were solely due to clumping, not to a defect in the ability to discriminate the attractant from the control. First, those animals that dispersed from the clump showed normal preference for the attractants. Second, in those assays in which the animals dispersed normally, the chemotaxis index was not significantly different from the wild-type controls. As an additional control, we determined that *daf-3* did not suppress or enhance the response of two *daf-11* mutants to 10^{-1} IAA (*sa195* and *m84*; data not shown). These findings suggest that *daf-3* has no effect on the chemosensory responses of the Daf-c mutants. The wild-type odorant responses of the group 2 Daf-c mutants reveal an additional difference from the group 1 Daf-c mutants, further supporting the hypothesis that these genes function in distinct pathways (THOMAS *et al.* 1993).

Odorant response of *daf-11 daf-21* double mutants: The *daf-11* and *daf-21* single mutants showed reduced sensitivity to IAA and BENZ, but their responses to these attractants were not completely eliminated. To determine whether mutations in both genes would cause a more severe odorant response defect, two *daf-11 daf-21* double mutants were assayed as described above. In many cases, the response of a double mutant to IAA and BENZ appeared to be slightly more defective, but was not statistically significantly different at $P \leq 0.01$, when compared to both single mutant responses (Figure 6, A and B). Like the single mutants, the double mutants still retained some response to the higher concentrations of these attractants. These results suggest that the remaining response of each single mutant to IAA and BENZ is not completely due to the wild-type function of the other gene. Interestingly, responses of the double mutants to some concentrations of PYR and DIA, although quite strong, were significantly reduced when compared to both single mutant responses (Figure 6, C and D). The defects appear to be more pronounced at the higher concentrations of each attractant. Responses of the single and double mutants to TMT were not significantly different (Figure 6E).

Further phenotypic characterization of *daf-11* alleles: The IAA and BENZ response profiles suggested that the *daf-11* mutations constitute an allelic series. For strength of IAA response defect, the series is *m597* ≈ *sa195* > *m47* > *m87* > *m124* ≈ *m84* (Figure 4). For recovery from the dauer stage to resume the normal life

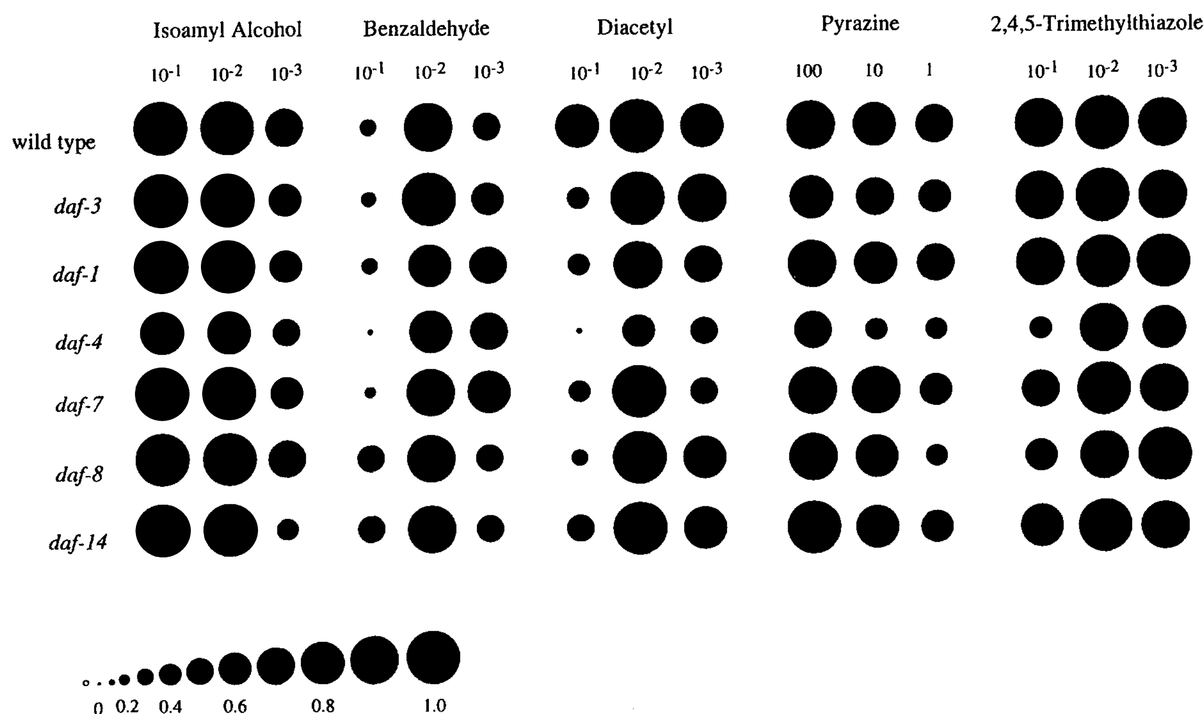

FIGURE 5.—Summary of response to volatile attractants of Group 2 Daf-c mutants. Chemotaxis indices and significant differences are represented as in Figure 3. However, since all strains contain *daf-3(e1376)*, the C.I. are determined to be significantly different from wild type and *daf-3* at $P \leq 0.01$. At least two independent assays were performed for each concentration. Relative concentrations of IAA, BENZ, DIA and TMT and concentrations of PYR are as in Figure 3.

cycle, a related allelic series was observed: few or no *m597*, *sa195*, *m47* and *m87* dauers recovered, an intermediate number of *m124* dauers recovered, and almost all *m84* dauers recovered (Table 2). However, upon analysis of a third phenotype, penetrance of dauer formation at 15°, a nearly opposite allelic series was found (Table 2). The *m84* mutant was strongly Daf-c, the *m124* mutant was intermediate, and *m597*, *sa195*, *m47* and *m87* mutants were only weakly Daf-c (Table 2). These nearly inverted series of phenotypic strengths are intriguing, but are inconsistent with a simple allelic series.

An additional phenotype of *daf-11(m84)* complicates fitting it into a simple allelic series. When *daf-11* heterozygotes (*m84* in *trans* to *sa195* or *m87*) were assayed for odorant response to IAA, dauer recovery, and dauer formation at 15°, it was found that *m84* was strongly dominant to *m87* and *sa195* for all phenotypes (Table 2). We also found that *m124* was dominant to *sa195* for dauer recovery, but not for dauer formation at 15° or odorant response. The dominance of the *m124* allele to *sa195* was less convincing, perhaps because the differences between the single mutants were smaller. The *daf-11(m84)* mutation was also unique in that its Daf-c phenotype was not completely recessive to the wild type. 0.4% dauers were observed among *m84*/+ animals (n = 1127) regardless of parental origin of the *m84* allele, ruling out maternal effect as an explanation. Because *m84* and possibly *m124* retain some wild-type function for dauer recovery and response to volatile odorants, and because *m84* displays weak dominance to the wild type, these two alleles are unlikely to represent the null phenotype. However, in the absence of genetic deficiencies of the *daf-11* region, it is not possible to further test which alleles represent complete loss of *daf-11* function.

daf-21 phenotypes are maternally rescued: *daf-21(p673)* causes a Daf-c phenotype in the larva and a defect in taxis to some odorants in the adult. However, we found that *daf-21*/+ hermaphrodites had no dauer progeny (n = 1,659) indicating that the Daf-c phenotype of the *daf-21*/*daf-21* progeny was rescued by the maternal *daf-21*(+) gene. From a cross between marked *daf-21* hermaphrodites and wild-type males, all cross progeny were non-dauer (n = 608), indicating that the Daf-c phenotype of *daf-21* is fully recessive and is zygotically rescued by *daf-21*(+). In addition, *daf-21*/*daf-21* progeny of *daf-21*/+ hermaphrodites responded normally to 10^{-2} IAA (Figure 7), indicating maternal rescue of even this adult defect. Maternal rescue of late phenotypes is unusual but not unique: both the Daf-c and egg-laying defective phenotypes of some *daf-1* alleles are also maternally rescued (SWANSON and RIDDLE 1981; our unpublished observations). Rescue of the *daf-21* defects may be due to persistence of maternally supplied *daf-21*(+) gene product through the adult stage. A similar phenomenon has been observed in Drosophila; two maternally supplied gene products have been detected in third instar larvae (O'BRIEN and

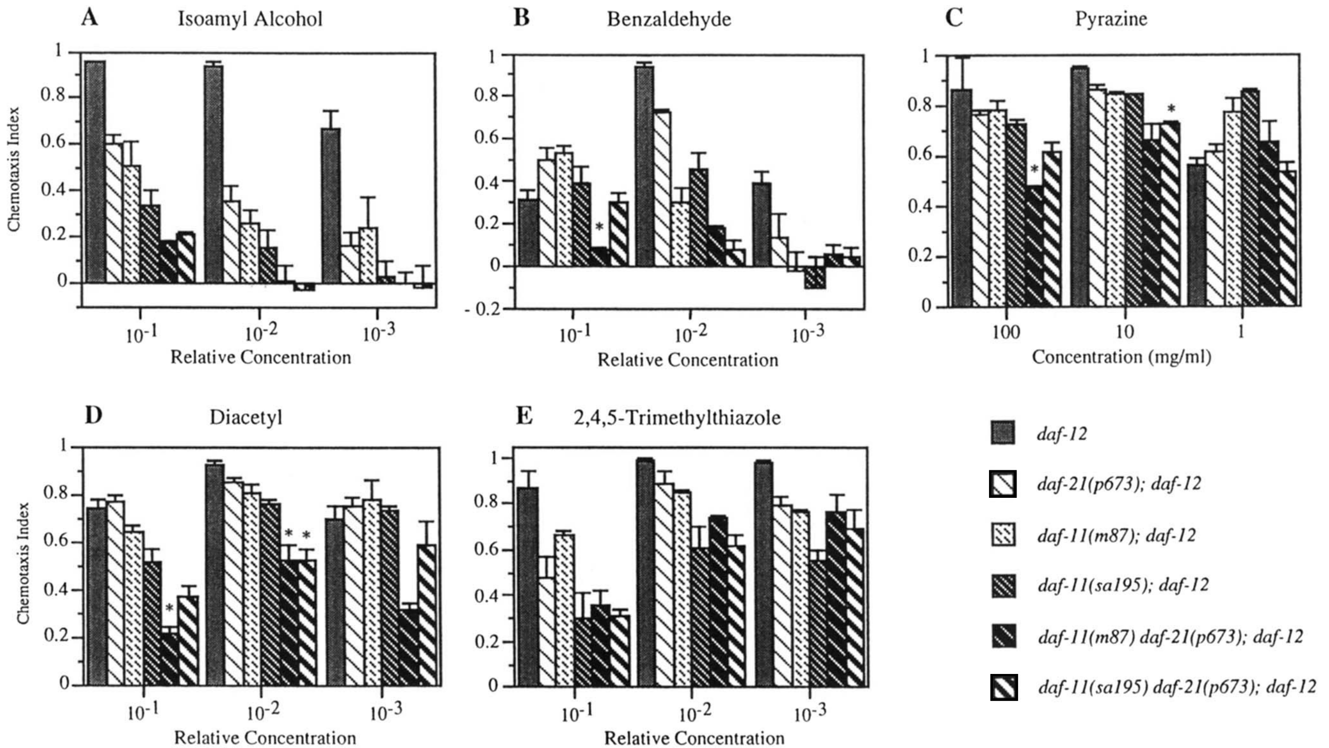

FIGURE 6.—Response of selected *daf-11 daf-21* double mutants to odorants. (A) Isoamyl alcohol; (B) benzaldehyde; (C) pyrazine; (D) diacetyl; (E) 2,4,5-trimethylthiazole. Error bars are as in Figure 2. At least two independent assays on *daf-11(m87) daf-21(p673)* and three assays on *daf-11(sa195) daf-21(p673)* were performed at each concentration. Relative concentrations of IAA, BENZ, DIA and TMT and concentrations of PYR are as in Figure 3. *, Significantly different from response of both parental *daf-11* and *daf-21* mutant strains at $P \leq 0.01$.

TABLE 2

Phenotypes of single *daf-11* mutants and *daf-11* trans-heterozygotes

Strain	Chemotaxis index (SEM) [a]	Percent dauer recovery at 15° (n) [b]	Percent Daf-c at 15° (n) [b]
Single *daf-11* mutants			
daf-11(m597)	ND	2(216)	0.4(1308)
daf-11(m47)	ND	7 (86)	2 (1220)
daf-11(m87)	0.09 (0.05)	0 (97)	3 (319)
daf-11(sa195)	0.06 (0.04)	0(127)	17 (1564)
daf-11(m124)	0.57 (0.09)	25(179)	48 (1238)
daf-11(m84)	0.52 (0.08)	99(496)	97 (188)
daf-11 trans-heterozygotes			
daf-11(sa195) / *daf-11(m84)*	0.47 (0.04)	92(232)	95 (148)
daf-11(m87) / *daf-11(m84)*	0.49 (0.04)	88(347)	81 (221)
daf-11(sa195) / *daf-11(m124)*	0.34 (0.07)	30(101)	12 (330)

[a] Odorant response to 10^{-2} isoamyl alcohol. Both *daf-11* single mutants and *trans*-heterozygotes were temperature-shifted from 15° to 20° as L4 larvae (see MATERIALS AND METHODS). SEM = standard error of the mean.
[b] n = total counted.

MACINTYRE 1978). Alternatively, *daf-21* may be involved in an early developmental event that affects later sensory function. However, at least at a gross level, *daf-21* mutants have structurally normal amphid chemosensory cells and retain normal chemosensory responses to non-volatile repellents and to some volatile attractants.

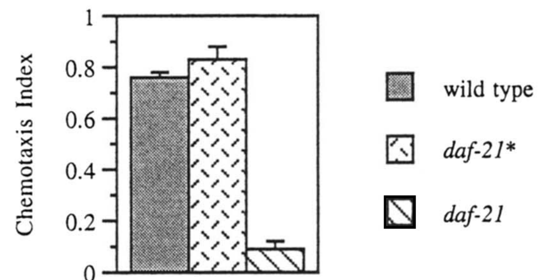

FIGURE 7.—Response of maternally rescued *daf-21* progeny to 10^{-2} isoamyl alcohol. "*daf-21**" indicates homozygous *daf-21* progeny of a *daf-21/+* mother. Error bars are as in Figure 2.

DISCUSSION

***daf-11* and *daf-21* probably act in the sensory endings of amphid neurons to mediate chemosensory responses:** We have shown that normal amphid, but not phasmid, sensory endings are required for response to the dauer pheromone. First, mutations that cause both amphid and phasmid cilium-structure defects also cause a pheromone response defect, while mutations that block exposure of the phasmid cilia to the environment have no effect on pheromone response. Second, killing the

amphid sheath cells, but not phasmid sheath cells or phasmid neurons, eliminates response to dauer pheromone. Previous studies have shown that *daf-11* and *daf-21* mutations require structurally normal sensory cilia for the expression of their Daf-c phenotype (VOWELS and THOMAS 1992; THOMAS *et al.* 1993). This expression does not depend on normal phasmids since *daf-11; lin-17* and *daf-21; lin-17* double mutants are still strongly Daf-c (our unpublished observations). Since dauer formation induced by pheromone or by mutation in either *daf-11* or *daf-21* depends on normal amphid cilia, our current model is that these two gene products function in the pheromone signal transduction pathway in the amphid cilia. Though not directly shown to be responsible for pheromone response, the amphid neurons ADF, ASI, ASG and ASJ regulate dauer formation and recovery (BARGMANN and HORVITZ 1991a) and are thus good candidates for sites of *daf-11* and *daf-21* function.

We have shown that *daf-11* or *daf-21* mutations cause defective response to the non-volatile attractants, Cl^-, cAMP and biotin. Killing the exposed amphid neuron ASE also reduces chemotaxis to these attractants (BARGMANN and HORVITZ 1991b). Although the results of the single-animal assays used to assess the effect of the cell kills are not directly comparable to the population assays used here, it is likely that *daf-11* and *daf-21* mutations cause defects that are comparable to or more severe than the defects caused by killing ASE alone. In contrast, mutations in *daf-11* or *daf-21* have no effect on avoidance of non-volatile repellents, a response that is mediated primarily by the amphid neuron ASH (BARGMANN *et al.* 1990). The simplest explanation of these results is that *daf-11* and *daf-21* are required for the function of ASE, but are not required for the function of ASH. An alternative explanation for *daf-11* and *daf-21* in chemotaxis is that they are required in non-amphid cells to amplify or integrate signals from ASE. Since previous results suggest that *daf-11* and *daf-21* function in the amphid sensory endings to control dauer formation (VOWELS and THOMAS 1992; THOMAS *et al.* 1993), we favor the former explanation. It might be possible to distinguish between these interpretations by killing ASE in a *daf-11* or *daf-21* mutant, and testing for an enhanced chemotaxis defect. However, the mutant responses are already sufficiently weak (Figure 2) that further decreases in the chemotaxis index would be difficult to detect.

A comparison of the odorant response defects caused by killing specific amphid cells and those caused by a mutation in either *daf-11* or *daf-21* suggests that these genes function in specific neurons to mediate chemotaxis to volatile attractants. In *daf-11* mutants, *daf-21* mutants, and individual animals in which the amphid neuron AWC has been killed, response to the volatile attractants IAA and BENZ was strongly reduced, but there was little or no effect on response to PYR, DIA and TMT (BARGMANN *et al.* 1993). The cell kill results suggest that IAA and BENZ are sensed primarily by AWC, that PYR and DIA are sensed primarily by another amphid neuron, AWA, and that either cell can function for response to TMT (BARGMANN *et al.* 1993). Therefore, a simple model to explain the mutant results is that *daf-11* and *daf-21* are required for normal function of AWC, but not AWA. However, since the *daf-11* and *daf-21* mutations may not cause complete loss of gene function, we cannot rule out a requirement for the gene products in AWA.

When compared to both *daf-11* and *daf-21* single mutants responses, the *daf-11 daf-21* double mutant responses to IAA and BENZ were slightly, but not significantly, reduced. In addition, the double mutants showed small, but significant, reductions in response to some concentrations of PYR and DIA. In no case was response of the double mutant to an attractant eliminated at all concentrations. The small decreases in the odorant responses may be explained if *daf-11* and *daf-21* act in a single transduction pathway, and both of the tested *daf-11* and *daf-21* mutations fail to fully block the transduction. At present it is not possible to test this hypothesis because the null phenotype of each gene is unknown. However, if *daf-11* and *daf-21* mutations affect only the function of AWC, this model does not explain why the double mutant responses to PYR and DIA, sensed primarily by AWA, are reduced. Perhaps the double mutant responses reveal a small requirement for *daf-11* and *daf-21* in AWA. Alternatively, these results can be explained if the function of more than one cell is required for response to the full range of concentrations of an attractant. Observations in salamander olfactory neurons provide a physiological basis for this model: a single cell responds to a narrow range of odorant concentrations suggesting that responding to a broader range of concentrations requires neurons with different sensitivities (FIRESTEIN *et al.* 1990). Support for this model also comes from observations in *C. elegans*, in which killing AWA results in defective response to low concentrations of DIA (10^{-3}) (BARGMANN *et al.* 1993), while killing AWC results in slightly defective response only to higher concentrations of DIA (P. SENGUPTA and C. BARGMANN, personal communication). Interestingly, the response of *daf-11* and *daf-21* mutants differs from wild type most clearly at an intermediate concentration of DIA. These findings are consistent with a minor role of AWC, *daf-11* and *daf-21* in the DIA response. A similar secondary role for AWA in the responses to IAA and BENZ might explain the residual response of *daf-11 daf-21* double mutants to high concentrations of these attractants.

In summary, the results of the volatile and non-volatile chemotaxis assays suggest that *daf-11* and *daf-21* function in the amphid neurons AWC and ASE. Is it possible that *daf-11* and *daf-21* function in these cells to mediate

response to dauer pheromone? A role for AWC in pheromone response seems unlikely. Structurally and functionally, these cells are implicated only in response to volatile compounds (BARGMANN *et al.* 1993), and no volatile component of the dauer pheromone is required to induce dauer formation (GOLDEN and RIDDLE 1984a). In addition, *osm-3* mutants have structurally and functionally normal AWC cells (PERKINS *et al.* 1986; BARGMANN *et al.* 1993), but do not respond to dauer pheromone (Figure 1). These observations suggest that AWC is not involved in the response to dauer pheromone. However, it is more plausible that *daf-11* and *daf-21* function in ASE (or other exposed amphid cells) to mediate response to pheromone. Previous studies have suggested that overlapping sets of amphid sensory neurons regulate dauer formation and chemotaxis to non-volatile attractants (BARGMANN and HORVITZ 1991a,b).

***daf-11* and *daf-21* are candidates for transduction components involved in both taste and olfaction:** If *daf-11* and *daf-21* act in cells that control both dauer formation and chemotaxis, the reduced response of these mutants to volatile and non-volatile attractants can be explained in various ways. One hypothesis is that constitutive activation of the pheromone-response pathway in these Daf-c mutants interferes with chemosensory transduction. While such an interference model is possible, it must explain the following points. First, the activated pheromone-response pathway must selectively affect some chemotaxis responses but not others, since *daf-11* and *daf-21* mutants respond normally to PYR and DIA. Second, the reduced response of *daf-11* and *daf-21* mutants to IAA and BENZ is unlikely to be directly due to the activation of the pheromone transduction pathway in AWC (see above). Third, five other mutations that constitutively activate the pheromone-response pathway (*daf-1, daf-4, daf-7, daf-8* and *daf-14*) do not cause chemotaxis defects to volatile attractants (Figure 5) and have not been reported to cause non-volatile chemotaxis defects (RIDDLE 1988). Finally, suppression of the Daf-c phenotype of *daf-11* and *daf-21* does not rescue the odorant response defects.

A simple alternative hypothesis is that *daf-11* and *daf-21* mutations affect shared components of the transduction pathways for response to dauer pheromone and chemotaxis to volatile and non-volatile attractants. Several observations are consistent with this model. First, the only obvious feature common to the processes of dauer formation and chemotaxis is that they involve chemosensory transduction. Second, *daf-11* and *daf-21* mutations cause defects in only a subset of odorant responses, suggesting that they function specifically in chemosensation. Third, since the Daf-c phenotype of *daf-11* is suppressed by a mutation in any one of the nine Daf-d cilium-structure genes (VOWELS and THOMAS 1992), it is likely that *daf-11* functions in the amphid cilia as a component of the pheromone transduction process. By analogy, *daf-11* is likely to perform similar transduction functions in the responses to volatile and non-volatile attractants. This model is consistent with the finding that transduction components in vertebrate olfactory neurons are localized to the cilia (JONES and REED 1989; BAKALYAR and REED 1990). The *daf-21(p673)* mutation causes phenotypes that parallel those of *daf-11* in nearly every detail, suggesting that the two genes are involved in the same transduction pathways (THOMAS *et al.* 1993; this work). However, the partial sterility of *daf-21* at high temperatures, and the maternal rescue of the Daf-c and odorant defect phenotypes complicate the interpretation of this gene. In addition, preliminary results suggest that *daf-21(p673)* is not a simple loss of function mutation (E. A. MALONE, personal communication). Therefore, further studies are required to elucidate the role of *daf-21*(+) in chemosensory transduction.

In contrast to other *C. elegans* genes known to be required for chemotaxis to both volatile and non-volatile attractants, *daf-11*, and perhaps *daf-21*, stand out as members that appear to be specific to chemosensory transduction. The eight Daf-d mutants with defects in taxis to volatile and non-volatile compounds (*che-2, che-3, che-11, che-13, daf-10, osm-1, osm-5* and *osm-6*; BARGMANN *et al.* 1993) have been shown to have grossly abnormal amphid and phasmid sensory cilia (PERKINS *et al.* 1986). Mutations in *unc-31, unc-86* and *lin-32*, which have been reported to cause defective chemotaxis to both volatile and non-volatile attractants (BARGMANN *et al.* 1993), all cause more general neuronal defects. A mutation in *unc-31* causes defects in movement, response to touch, egg-laying, pharyngeal pumping and dauer recovery (BRENNER 1974; AVERY *et al.* 1993), suggesting that it functions in many different neuron classes. Both *unc-86* and *lin-32* regulate developmental processes that specify neuronal cell fates (FINNEY and RUVKUN 1990; CHALFIE and AU 1989). Preliminary characterization of three mutants, *che-7, tax-4* and *tax-6* suggests that these genes are required for taxis to volatile and non-volatile attractants (BARGMANN *et al.* 1993), but further characterization is needed to determine whether there are more general neuronal abnormalities. In *Drosophila,* a mutation in *smellblind* (*sbl* or *olf D*) causes larval defects in volatile and contact chemotaxis (LILLY and CARLSON 1990) but this gene, like many *C. elegans* genes, is probably not specific for chemosensory processes as there are several lethal alleles of this gene. In fact, recent analysis of *sbl* mutations suggests that they are alleles of the *paralytic* (*para*) gene (LILLY *et al.* 1994), which encodes a voltage-gated sodium channel (LOUGHNEY *et al.* 1989; RAMASWAMI and TANOUYE 1989).

If the pheromone and chemotaxis responses in *C. elegans* are analogous to taste and olfaction in vertebrates, *daf-11* and *daf-21* might be members of a small group

of genes active in both of these processes. The model that gustatory and olfactory chemosensory processes share gene products is plausible given the molecular similarities between these processes (SHEPHERD 1991). For example, receptors (BUCK and AXEL 1991; ABE *et al.* 1993), G-proteins (MCLAUGHLIN *et al.* 1992; PACE *et al.*, 1985; PACE and LANCET 1986; JONES and REED 1989) and adenylate cyclases (KURIHARA and KOYAMA 1972; STRIEM *et al.* 1989; PACE *et al.* 1985; SKLAR *et al.* 1986; LOWE *et al.* 1989; PFEUFFER *et al.* 1989; BAKALYAR and REED 1990) have been identified from each type of tissue. In some cases, proteins identified from gustatory or olfactory tissue were shown to be specific to those tissues when compared to non-sensory tissues. However, in no case was taste tissue tested for the presence of the signal transduction components isolated from olfactory tissue, and in only one case was olfactory tissue tested for the presence of a signal transduction component isolated from taste tissue (MCLAUGHLIN *et al.* 1992). In that case, the gene product, gustducin, was shown to be taste-cell specific. However, it is possible that other components are shared between the two systems in vertebrates. Alternatively, although analogous transduction cascades occur in the two tissues, each signal transduction element may be subtly adapted for function in one tissue or the other, and thus be specific to that cell type.

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