# Evidence for Parallel Processing of Sensory Information Controlling Dauer Formation in *Caenorhabditis elegans*

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ABSTRACT

Dauer formation in *Caenorhabditis elegans* is induced by chemosensation of high levels of a constitutively secreted pheromone. Seven genes defined by mutations that confer a dauer-formation constitutive phenotype (Daf-c) can be congruently divided into two groups by any of three criteria. Group 1 genes (*daf-11* and *daf-21*) are (1) strongly synergistic with group 2 genes for their Daf-c phenotype, (2) incompletely suppressed by dauer-formation defective (Daf-d) mutations in the genes *daf-3* and *daf-5* and (3) strongly suppressed by Daf-d mutations in nine genes that affect the structure of chemosensory endings. Group 2 genes (*daf-1*, *daf-4*, *daf-7*, *daf-8* and *daf-14*) are (1) strongly synergistic with group 1 genes for their Daf-c phenotype, (2) fully suppressed by Daf-d mutations in *daf-3* and *daf-5* and (3) not suppressed by Daf-d mutations in the nine genes that affect chemosensory ending structure. Mutations in each group of genes also cause distinct additional behavioral defects. We propose that these two groups of Daf-c genes act in parallel pathways that process sensory information. The two pathways are partially redundant with each other and normally act in concert to control dauer formation.

UNDER unfavorable environmental conditions, *Caenorhabditis elegans* larvae enter an alternative third-larval stage called the dauer larva. The dauer larva is developmentally arrested and is adapted for long term survival under harsh conditions (CASSADA and RUSSELL 1975). When environmental conditions improve, the dauer larva will recover, molt and resume the normal life cycle at the fourth-larval (L4) stage (CASSADA and RUSSELL 1975). The decision whether or not to enter the dauer state is made during the L1 and L2 stages primarily by assessing the concentration in the environment of a constitutively secreted dauer pheromone (GOLDEN and RIDDLE 1982, 1984a). The efficiency of induction of dauer formation by dauer pheromone is modulated by temperature and the abundance of food, such that higher temperatures and lower levels of food encourage dauer formation (GOLDEN and RIDDLE 1984a). Indirect evidence suggests that sensory control of dauer formation is mediated by chemosensory endings that are exposed to the external environment as part of the amphid sensillum (ALBERT, BROWN and RIDDLE 1981; PERKINS *et al.* 1986; BARGMANN and HORVITZ 1991; VOWELS and THOMAS 1992). The amphid neurons ADF and ASI are required to repress dauer formation (or promote the L3 stage) in the absence of pheromone (BARGMANN and HORVITZ 1991). Presumably dauer-inducing environmental conditions, primarily high levels of dauer pheromone, act negatively on these neurons to derepress dauer formation.

A large set of genes that mediate dauer-larva formation have previously been identified (*e.g.*, RIDDLE, SWANSON and ALBERT 1981). Mutations in most of these genes cause one of two types of abnormal development. Dauer-constitutive (Daf-c) mutations cause dauers to form even when environmental conditions are noninducing for dauer formation (no pheromone, abundant food). Nearly all such Daf-c mutations are incompletely penetrant and form more dauers at high temperature due to the intrinsic temperature sensitivity of dauer formation (GOLDEN and RIDDLE 1984b). Dauer-defective (Daf-d) mutations block dauer formation, even when conditions are dauer-inducing (high levels of pheromone, high temperature, low food). Using epistasis analysis, these genes have been ordered into a formal pathway controlling dauer formation (VOWELS and THOMAS 1992; RIDDLE, SWANSON and ALBERT 1981).

Until recently, the large majority of available information about Daf genes that act upstream of the Daf-d gene *daf-12* could be explained by a simple linear pathway of gene action (VOWELS and THOMAS 1992). Several genes that are not clearly upstream of *daf-12* have been proposed to interact in more complex nonlinear ways, most notably *daf-2* (RIDDLE, SWANSON and ALBERT 1981; VOWELS and THOMAS 1992). *daf-2* was not analyzed in this study, but its role is considered in Discussion. However, even for genes that act upstream of *daf-12*, certain observations have been difficult to reconcile with a simple linear model. First, some epistatic interactions involving the dauer-defective genes *daf-3* and *daf-5* were difficult to explain with a linear pathway (VOWELS and THOMAS 1992). Second, the incomplete penetrance of all identified

Daf-c mutations in the genes *daf-1*, *daf-4*, *daf-7*, *daf-8*, *daf-11* and *daf-14* suggested that loss of function of none of these genes fully activates dauer formation (GOLDEN and RIDDLE 1984b). Finally, mutants for all of these six Daf-c genes remain responsive to dauer pheromone (GOLDEN and RIDDLE 1984b), which suggested that a functional pheromone-response pathway remained active in each mutant.

Here we present new observations that greatly extend the body of results that conflict with a simple linear model of gene action. Based on our findings, we suggest that the early steps of the dauer pathway comprise two distinct parallel chemosensory pathways that act in concert to control induction of the dauer larva.

## MATERIALS AND METHODS

**General genetic methods:** General methods of maintenance of *C. elegans* were as described by BRENNER (1974). Growth medium was NG agar (BRENNER 1974), except that agar was 2.0%. This paper follows the standard *C. elegans* nomenclature (HORVITZ *et al.* 1979).

**Marker mutations used:** The linkage groups (LGs) are as follows.

*LGI: unc-13(e1091), lin-11(n566)*
*LGII: unc-52(e444), lin-7(e1413)*
*LGIV: dpy-9(e12), unc-44(e362)*
*LGV: unc-42(e270), sma-1(e30), unc-76(e911)*
*LGX: unc-1(e719), dpy-3(e27), unc-2(e55), egl-17(e1313), lon-2(e1442), unc-58(e665).*

**Origin and mapping of *daf-21(p673)*:** *p673* is a newly identified Daf-c mutation that was recovered on outcross from the strain PR673, originally isolated as chemotaxis defective (R. RUSSELL, personal communication; DUSENBERRY 1976). Following its Daf-c phenotype, we mapped *p673* to chromosome *V* on the basis of 2-factor linkage to *dpy-11*. The only previously identified Daf-c gene on chromosome *V* was *daf-11*. The following data demonstrate that *p673* defines a new locus, distinct from *daf-11*. First, *p673* fully complements the *daf-11* alleles *m47*, *m51*, *m84*, *m87*, *m124* and *sa195* for their Daf-c phenotype. Second, *p673* was mapped between *sqt-3* and *unc-76*, clearly distinct from *daf-11*: from a strain of genotype *daf-21/sqt-3 unc-76*, 5 of 14 Unc non-Sqt recombinants segregated *p673*, and 1 of 1 Sqt non-Unc recombinant segregated *p673*. Finally, *p673* double mutants with *daf-11* were easily constructed by picking Daf-11 non-Unc recombinants from animals of genotype *daf-11 unc-76/p673* (see details below). Since no other genes with related phenotypes lie in this region, *p673* appears to define a new gene and was assigned the gene name *daf-21* (D. RIDDLE, personal communication).

**Isolation of new *daf-3*, *daf-5* and *daf-12* mutations:** L4 to young adult CB1372 *daf-7(e1372)* hermaphrodites were mutagenized with EMS (BRENNER 1974) and were picked to 10-cm plates at 15°, about five $P_0$ animals per plate. Parents were removed after about 200–250 eggs were laid, and the plates were left at 15° until the $F_1$ progeny had reached the L4 to young adult stage. The plates were then shifted to 25° and 3–4 days later were screened for nondauer $F_2$ animals. Nondauer animals were picked individually to plates at 25° and their brood examined for the production of nondauers. Only one suppressor candidate was ultimately retained from each original screening plate to ensure independence of the suppressor mutations. Recessiveness, approximate map position and complementation of existing *daf-3*, *daf-5* and *daf-12* mutations were all assessed by analyzing suppression of the Daf-c phenotype of *daf-7(e1372)*. All newly isolated alleles were outcrossed twice to the wild-type and reisolated as single mutants before use in construction of double mutants with Daf-c mutations, including *daf-7(e1372)*. From this screen three new *daf-5* alleles, seven new *daf-3* alleles and one new *daf-12* allele were identified. From a similar screen for suppressors of *daf-11(m87)* one new *daf-12* allele was identified (W. SCHACKWITZ, personal communication). Both new *daf-12* mutations confer a slightly long (Lon) phenotype similar to that of the original *daf-12(m20)* mutation, indicating that single gene mutations cause both the Lon and Daf-d phenotypes. All alleles of *daf-3* and *daf-5* appear wild-type except for their incompletely penetrant Daf-d phenotype.

**Construction of *daf-c;daf-c* double mutants:** Various approaches were required for the construction of these double mutants. In general, one mutation was tracked in crosses on the basis of its Daf-c phenotype, while the other mutation was tracked by balancing it in *trans* with a tightly linked visible marker. An example is the construction of *daf-7;daf-21/unc-76*. *daf-7* is on chromosome *III*, while *daf-21* and *unc-76* are tightly linked on chromosome *V*. *unc-76/+* males were mated to *daf-7* hermaphrodites and the resulting male progeny were mated to *daf-21* hermaphrodites at 25°. Many cross progeny (nondauer) L4 hermaphrodites were picked singly to plates at 25°. Plates that segregated both Daf-c and Unc progeny were inferred to be from parents of genotype *daf-7/+;daf-21/unc-76* (the Daf-c phenotype of *daf-21* is maternally rescued). *daf-7* homozygotes were obtained by picking recovered dauer progeny singly to plates at 15°. Plates that were homozygous for the *daf-7* egg-laying defective (Egl) phenotype and heterozygous for *unc-76* were inferred to be the desired strain. The presence of *daf-21* in heterozygous state was apparent from the fact that among many progeny of the *daf-7;daf-21/unc-76* parents, about 1/3 produced 100% dauer non-Unc progeny at 15°, while the remaining 2/3 produced many nondauers and segregated *unc-76*. In most cases the identity and presence of both Daf-c mutations was also directly confirmed by complementation testing. Other constructions followed similar methods, although of course the balancing marker varied and not all constructions could take advantage of the maternal rescue. The genotypes of the double mutants were confirmed by complementation testing in all cases in which the presence of a mutation could not be directly observed on the basis of a distinctive phenotype (for example *daf-4* mutations cause a distinctive small phenotype).

**Construction of *daf-c;daf-d* double mutants:** In all cases in which the *daf-d* mutation displayed strong suppression of the *daf-c* mutation, constructions were performed and genotypes confirmed as previously described (VOWELS and THOMAS 1992). *daf-11* double mutants with *daf-3* and *daf-5* alleles were constructed in a similar fashion, but in some allelic combinations the Daf-c phenotype was so poorly suppressed that the presence of the *daf-3* or *daf-5* allele was in some doubt. In such cases (all cases in which suppression was less than 10%, see Tables 3 and 4), the presence of the *daf-3* or *daf-5* mutation was confirmed by a test cross. For *daf-3*, the putative double mutant was crossed to wild-type males and the *daf-3* hemizygous male progeny were crossed to *unc-24 daf-14* hermaphrodites. Several cross progeny were picked singly to plates at 25° and the broods were observed for the presence of the expected fraction of Unc non-Daf non-Egl progeny (*daf-3* suppresses both the Daf-c and Egl phenotypes of *daf-14*; VOWELS and THOMAS 1992). Several such progeny were picked and their broods observed at 25° to confirm the presence of *daf-3*. The presence of *daf-5* alleles was tested similarly, except that *unc-24 daf-14/*

+ males were crossed to the putative double mutant. In every case the *daf-3* or *daf-5* allele was found to be present, indicating that the weak suppression of *daf-11* and *daf-21* displayed by these alleles is significant. These findings also validated the use of partial suppression of *daf-11* mutations as indicators of the presence of a *daf-3* or *daf-5* mutation.

Due to its maternal effect, *daf-21* double mutants were constructed slightly differently. *daf-21* double mutants with unlinked cilium-structure mutations were constructed by crossing *daf-21/+* males to Daf-d hermaphrodites. Presumptive $F_1$ double heterozygotes were picked individually to 10-cm plates, allowed to lay approximately 30 eggs and removed. After growth for two generations at 25°, one of two methods was used to isolated the double mutants. In the first method, dauers were picked in a group to a plate at 15°. When these dauers recovered they were picked individually to plates at 25°. From the progeny of these animals, cilium-structure defective segregants were picked on the basis of suppressing the Daf-c phenotype of *daf-21*. In the alternative method, the cilium-structure mutation was homozygosed first by picking osmotic-avoidance defective (Osm) progeny (CULOTTI and RUSSELL 1978) singly to plates at 25°. Some such parents produced rare Daf-c progeny. Since *daf-21* has strong maternal rescue, these were provisionally identified as double homozygotes. For both construction methods, the presence of the cilium-structure mutation was confirmed by fluorescein isothiocyanate (FITC) filling (PERKINS *et al.* 1986), and the *daf-21* mutation was confirmed by complementation testing for the Daf-c phenotype. Two of the cilium-structure genes, *che-11* and *osm-6*, are linked to *daf-21*. In these cases, double mutants were constructed in a manner similar to that previously described for *daf-11* (VOWELS and THOMAS 1992). Briefly, either *daf-21* or the cilium-structure mutation was linked to an appropriate marker and Daf-c nonmarker or Osm nonmarker recombinants were picked from appropriate heterozygotes to isolate putative double mutants. FITC filling and complementation testing for *daf-21* were then used to confirm the genotype of the double mutants.

Construction of *daf-21* double mutants with *daf-3*, *daf-5* and *daf-12* were performed using recessive *trans* markers linked to the Daf-d mutation. Animals of genotype *daf-21/+;daf-d/marker* were constructed by appropriate crosses. Random unmarked progeny were picked singly to plates at 25° and *daf-21* homozygotes were recognized by the segregation of a high proportion of dauer progeny. Nondauer progeny of the *daf-21* homozygotes were picked singly and screened for failure to segregate the marker. The presence of *daf-21* was subsequently confirmed by complementation testing, and the presence of the Daf-d mutation was confirmed on the basis of suppression of *daf-21*. Since suppression by *daf-3(sa206)* and *daf-5(sa205)* was marginal, their presence was confirmed by test crosses as described above for *daf-11*.

**Construction of triple mutants:** Strains carrying mutations in two *daf-c* genes and a *daf-12* mutation were constructed as follows. *daf-c1* males were mated to *daf-c1;daf-12* hermaphrodites and the resulting *daf-c1;daf-12/0* male progeny were mated to *daf-c2* hermaphrodites at 25°. Cross progeny (nondauer) L4s were picked individually to plates at 25°. Dauer progeny of these parents were allowed to recover at 15° and homozygotes for *daf-c1* were picked on the basis of being non-Egl (in each cross the *daf-c2* mutation causes an Egl phenotype while the *daf-c1* mutation does not). Egl non-Daf-c progeny of such *daf-c1* homozygotes were picked as putative triple mutants. The presence of each *daf-c* mutation was confirmed by complementation testing, as described above. The presence of the *daf-12* mutation was inferred from its suppression of the Daf-c phenotype and from its own Lon phenotype.

**Phenotype counts:** The frequency of dauer formation was assessed under noninducing conditions. Noninducing conditions were uncrowded animals (no more than about 400 larvae) on well seeded 6-cm NG agar plates. The fraction of dauers for each strain was determined using partially synchronized populations. Between 4 and 12 adult hermaphrodites were placed on a plate at the test temperature. After allowing egg laying for a limited time (less than 24 hr at 15° and less than 12 hr at 25°) the parents were removed. This degree of synchrony is generally sufficient to distinguish among dauers, transient dauers, and animals that never formed dauers (VOWELS and THOMAS 1992). As the progeny matured, the plates were checked frequently and L4 nondauers were counted and removed to prevent a new generation of eggs. For many strains it was found to be important to check the sides of the petri dish for trapped dauers. For a few strains, the dauers formed at 15° recovered so quickly that counts of less synchronized populations of animals resulted in dramatic underestimates of the frequency of dauer formation. This was particularly true of *daf-7(e1372)* and to a lesser extent *daf-14(m77)* strains. This fact presumably accounts for major discrepancies between our results and a prior report in which it appears little or no synchrony was employed (SWANSON and RIDDLE 1981). With these strains, as test temperatures increase, dauer recovery becomes increasingly inhibited (data not shown), presumably accounting for the appearance of dramatic increases in dauer formation at higher temperatures (SWANSON and RIDDLE 1981). We too find that dauer formation is higher at increased temperatures but to a quantitatively smaller degree. Even with 24-hr egg lays at 15° the counts on mutants that recover very quickly from the dauer state may include a few animals that formed a dauer but recovered almost immediately. Thus, actual dauer formation rates at 15° may be slightly higher than reported. All counts in Table 2 resulted from egg lays of the same duration, except for the *daf-21* double mutants with group 2 Daf-c mutations, which were made from unsynchronized broods of maternally rescued *daf-21* homozygotes (see below). These *daf-21* broods all formed 100% nonrecovering dauers, so recovery did not confound the counts.

A number of *daf-c;daf-c* double mutants proved impossible to maintain as homozygotes for both *daf-c* mutations. These counts were made in one of two ways. For the *daf-21* double mutants with group 2 Daf-c mutations, counts were made as follows. Progeny of parents homozygous for the group 2 Daf-c mutation and heterozygous for *daf-21* (maintained in *trans* to the tightly linked *unc-76*) were picked individually to plates at 15°. As expected, about one-third of these broods did not segregate Uncs, and all of these broods formed all or nearly all dauers. The counts in Table 2 represent all the progeny from several such parents.

Counts for *daf-11* with the group 2 *daf-c* genes were more difficult because neither *daf-c* mutation showed maternal rescue. *daf-1(m40)* and *daf-1(e1287)* normally display strong maternal rescue, and we anticipated that we could use this rescue to make *daf-11;daf-1* counts in a manner analogous to that described above for *daf-21*. Interestingly, however, the maternal rescue of *daf-1* is affected by the presence of a *daf-11* mutation. Specifically, 51/51 progeny of *daf-11(m47);daf-1(m40)/dpy-9* parents raised at 15° segregated nondauers and Dpys, and 44/44 progeny of *daf-11(m87);daf-1(m40)/dpy-9* raised at 15° segregated nondauers and Dpys. If maternal rescue of *daf-1* were complete, as it is in a wild-type background, we would have expected on the average 31.7 plates (⅓ of 44 + 51) to segregate no Dpys. If *daf-1* had only 9% maternal rescue in a *daf-11* mutant back-

ground, the probability of these results is about 5% ($(1-\frac{1}{3} \times 0.09)^{95}$). We conclude that *daf-11* mutations eliminate or sharply reduce the maternal rescue of *daf-1(m40)*. Similar observations were made for *daf-1(e1287)*. Interestingly, we found that *daf-21* also eliminates or reduces the maternal rescue of *daf-1(m40)* ($P < 0.005$ that maternal rescue was complete), but that none of the group 2 *daf-c* genes, including *daf-1*, affect the maternal rescue of *daf-21*.

The synthetic Daf-c interaction between *daf-11* and the group 2 *daf-c* genes was inferred from two sorts of counts. First, a strain of genotype *daf-c;daf-11/unc-42 sma-1* was constructed. Broods from such heterozygotes were grown at 15° and large numbers of non-Unc non-Sma progeny (over 30 for each of 10 such strains) were progeny tested to determine whether or not *daf-11* was homozygous (no Unc Sma progeny). In each case *daf-11* was never homozygous, suggesting a strong synthetic Daf-c interaction between *daf-11* and the other *daf-c* gene. These tests were performed for both *daf-11(m84)* and *daf-11(m47)* in combination with every group 2 Daf-c gene. Such tests are desirable because they avoid possible marker effects, however it was not possible to directly observe the fate of the *daf-11* homozygous progeny, nor was it possible to accurately quantitate the Daf-c synergy. To solve these problems a second approach was taken. Strains of genotype *daf-c;unc-42(e270) daf-11/sma-1(e30)* were constructed. The recessive *unc-42* mutation permits direct tracking of the fate of *daf-11* homozygotes and permits large numbers of such animals to be analyzed. Groups of such heterozygous parental animals were allowed to lay eggs synchronously at 15° (as above) and the parents were removed. Five and six days later the frequencies of the various progeny classes were determined. This method presented a difficulty: a sizable fraction of the dauers formed crawled up the sides of the dish and desiccated (this is normal for both wild-type dauers and the dauers formed by most Daf-c strains). It was impossible to determine the marker phenotype of the desiccated dauers. Instead, we did three things. First, the number of Unc dauers that had not crawled up the side of the plate was counted and found to be very close to 1/4 of the total progeny (Unc dauers do not move well and presumably are systematically underrepresented among the desiccated population). This finding directly demonstrated that the typical fate of the *daf-c;unc-42 daf-11* animals was indeed dauer formation. Second, the plates were scrutinized for any classes of progeny other than dauers, nondauer L4s, and young adults (eg. dead eggs or arrested larvae). No such classes were found at significant frequencies, ruling out lethal interactions. Third, the nondauers (Unc, Sma, and neither) and total progeny were counted (although the marker phenotype of desiccated dauers cannot be determined, they can be counted). For most of these counts, rare Unc nondauers were found (see Table 2) and each was picked for progeny testing. Every one segregated Sma progeny, indicating that they were recombinants. For *daf-7* and *daf-14* control counts were performed both from parents of genotype *daf-c;unc-42(e270)/sma-1(e30)* and from homozygous strains of genotype *daf-c;unc-42(e270)*. As expected, the frequency of dauer formation among Unc progeny was the same in the two cases. For *daf-4* and *daf-8*, a *daf-c;unc-42* double mutant was counted as the control. Taken together these data indicate the degree to which a homozygous *daf-11* mutation enhances the second *daf-c* mutation, as shown in Table 2.

**Recovery from the dauer state:** Qualitative assessment of the capacity of mutant dauers to recover from the dauer state and continue normal development was made for all single and double mutants. Some attempts were made to quantitate recovery of dauers at 15°, but they met with technical problems. On agar-filled petri plates, wild-type and most Daf-c mutant dauers soon crawl up the plastic sides of the plate and desiccate there. Recovery of dauers on plates thus represents a complex interaction between the kinetics of crawling up the plastic (this varies some from strain to strain) and recovery kinetics. This problem has conventionally been solved (*e.g.*, BARGMANN and HORVITZ 1991) by placing the dauer larvae in liquid medium in microtiter wells with food present. Some Daf-c mutant dauer larvae will recover under these conditions, though all appear to recover more poorly than when left on Petri plates (our data, not shown). Some Daf-c mutant dauers, notably those carrying the *daf-21* allele and most alleles of *daf-11*, recover very poorly under these conditions, despite recovering to some extent on plates. This phenomenon may be due to the availability of much higher concentrations of food on plates. Providing high levels of food in microtiter wells results in the eventual death of the dauer larvae (not shown), presumably from anoxia. Some unsuccessful attempts were made to reduce these problems: use of glass, polycarbonate or polymethylpentene plates did not substantially reduce the frequency of dauers crawling up the sides. These problems made adequate quantitation of recovery difficult for most strains. However, few *daf-21* dauers crawl up the sides of plates, probably because they are very lethargic and tend to lie in a curled posture (*daf-21* nondauers are not lethargic or Unc). This phenotype was also characteristic of all *daf-21* double-mutant dauers, and thus permitted effective qualitative scoring of dauer recovery even with long-term incubations.

**Pheromone tests:** Pheromone tests were performed using pheromone that was crudely purified as described (GOLDEN and RIDDLE 1984b), except that the assay plates contained peptone and the bacteria were not streptomycin treated. These two modifications were made to minimize the tendency of the strains to form dauers, since many of the mutants tested form about 90% dauers even on NG plates. Egg lays were set for up to three hours at room temperature, then the plates were incubated at 15° and were scored for dauer formation 5 or 6 days later. A unit of pheromone was defined as the amount of pheromone per plate required to induce 33% dauer formation in the wild type at 25° using the assay conditions described by GOLDEN and RIDDLE 1984b.

**Map data:** Prior to this work *daf-3* was mapped to an approximate position at the left end of chromosome *X*. We determined a more accurate map position for *daf-3* as a suppressor of the Daf-c and Egl phenotypes of *daf-7*. Animals of genotype *daf-7(e1372);daf-3(e1376)/dpy-3 unc-2* were constructed and 104 suppressed progeny were picked to individual plates at 25°. Of these, three segregated Dpy Unc progeny, and three others segregated Unc progeny, indicating that *daf-3* maps a short distance to the left of *dpy-3*. Similarly from *daf-7;daf-3/unc-1 dpy-3* heterozygotes, 99 suppressed animals were picked singly to plates at 25°. Of these, six segregated Dpy animals, one segregated Unc animals, and one segregated Dpy Unc animals (one of these last two classes must have arisen from a double recombination event). These data map *daf-3* to the left of *dpy-3* and close to *unc-1*.

## RESULTS

**The new Daf-c gene *daf-21* is similar to *daf-11* in its genetic interactions with Daf-d genes:** Identification of the Daf-c mutation *p673* is described in MATERIALS AND METHODS. Although *p673* maps to the same chromosome as *daf-11*, it was shown to define a new gene, which we have named *daf-21*. In order to

TABLE 1

**Percent dauer formation at 25° of *daf-21; daf-d* and *daf-11; daf-d* double mutants**

| Daf-d gene | Daf-c gene: *daf-21(p673)* | | *daf-11(m84)*[a] | |
|---|---|---|---|---|
| + | 70 | (472) | 99 | (1771) |
| *daf-6(e1377)* | 89 | (303)[b] | 96 | (247) |
| *osm-1(p808)* | 16 | (226) | 3 | (525) |
| *osm-3(p802)* | 49 | (294)[c] | 47 | (153)[c] |
| *osm-5(p813)* | 0.5 | (1410) | 0 | (199) |
| *osm-6(p811)* | 1 | (576) | 0 | (356) |
| *che-2(e1033)* | 1 | (599) | 0 | (456) |
| *che-3(e1124)* | 4 | (297) | 0 | (93) |
| *che-11(e1810)* | 0.6 | (467) | 0 | (288) |
| *che-13(e1805)* | 4 | (196) | 0 | (323) |
| *daf-10(e1387)* | 9 | (288) | 0.8 | (265) |

In Tables 1 through 5, the percentage of animals forming dauers is given, with the total number of animals counted given in parentheses.

[a] For comparison with *daf-21*, *daf-11* data are reproduced from VOWELS and THOMAS (1992).

[b] *daf-21* has also been tested with *daf-6(m186)*: 83%, $N = 321$. Other *daf-11; daf-6* allele combinations are found in VOWELS and THOMAS (1992).

[c] Other *osm-3* alleles are stronger suppressors of *daf-11* but have not been tested with *daf-21*.

assess gene interactions, we constructed a set of double mutants between *daf-21* and various Daf-d mutations. The dauer formation phenotypes of these double mutants are shown in Tables 1 and 3. The interaction of *daf-21* with all tested Daf-d mutations was very similar to that previously reported for *daf-11*. Particularly significant is the high degree of suppression of *daf-21* by mutations affecting the structure of the chemosensory cilia (Table 1). This pattern of suppression sets *daf-11* and *daf-21* apart from all other Daf-c mutations (VOWELS and THOMAS 1992).

**Certain *daf-c;daf-c* double mutants display strong synthetic enhancement of their Daf-c phenotype:** All identified mutations in the genes *daf-1*, *daf-4*, *daf-7*, *daf-8*, *daf-11*, *daf-14* and *daf-21* cause an incompletely penetrant Daf-c phenotype at 15° and a more completely penetrant Daf-c phenotype at 25°. The difference between high and low temperatures in these mutants is apparently due to the intrinsic temperature sensitivity of dauer formation in the wild type (GOLDEN and RIDDLE 1984b). The strongest Daf-c mutations cause formation of 80–90% dauers at 15° (Table 2), and upon continued incubation at 15° these dauers recover readily from the dauer state and grow to maturity (not shown). We constructed *daf-c;daf-c* double mutants in order to test whether any pair of *daf-c* mutations would display a novel phenotype when compared to each single mutant. All combinations of double mutants among these seven genes were constructed. The degree of dauer constitutivity for all of these double mutants is shown in Table 2. Some double mutants were readily propagated at 15° because they remained incompletely penetrant for the Daf-c phenotype. Counts for these mutants were made from homozygous double mutant strains. Certain gene combinations were so strongly Daf-c even at 15° that they had to be maintained with one of the two genes in the heterozygous state. Counts for these double mutants were performed in several ways as described in MATERIALS AND METHODS. The *daf-11* counts required the inclusion of a *cis* marker (*unc-42*) for recognizing the *daf-11* homozygous segregants. The appropriate single mutant controls with *unc-42* are also shown in Table 2.

A simple pattern is apparent in these data. All double mutants between mutations in *daf-11* and mutations in *daf-1*, *daf-4*, *daf-7*, *daf-8* or *daf-14* formed 100% dauers at 15°. Similarly, *daf-21* mutations caused nearly 100% dauer formation at 15° in combination with *daf-1*, *daf-4*, *daf-7*, *daf-8* and *daf-14* mutations. For convenient description we will refer to *daf-11* and *daf-21* as group 1 Daf-c genes, and *daf-1*, *daf-4*, *daf-7*, *daf-8* and *daf-14* as group 2 Daf-c genes. In contrast to all double mutants between groups 1 and 2, all double mutants among genes within a group remained incompletely penetrant for the Daf-c phenotype. Some group 2 Daf-c double mutants displayed some degree of synergy, but none raised the level of dauer formation above the approximately 90% characteristic of the strongest Daf-c single mutants. All double mutants of both types were strongly Daf-c at the restrictive temperature of 25° (not shown). Among the total of 6,008 animals doubly mutant for *daf-11* or *daf-21* and a group 2 Daf-c mutation, only one nondauer was found, a *daf-8;daf-21* double mutant. This nondauer was fertile and produced a brood of all dauers at 15°, indicating that it had not accumulated a suppressor mutation. Even this exception to the otherwise absolute synergy was not surprising, since *daf-8(e1393)* is the weakest allele of the group 2 Daf-c genes tested (see Table 2, control counts). Unlike all other group 2 Daf-c alleles, *daf-8(e1393)* may encode a true temperature-sensitive gene product, since it is unique in that all of its 25° phenotypes are reduced or absent at 15°, including the egg-laying defective, clumpy and dark intestine phenotypes (see below; VOWELS and THOMAS 1992).

In addition to the strong synergy for dauer formation between groups 1 and 2, similar observations were made about recovery from the dauer state. For reasons described in MATERIALS AND METHODS it proved difficult to quantitate the dauer recovery phenotype, but qualitative observations were made. All single Daf-c mutants (RIDDLE, SWANSON and ALBERT 1981) and *daf-c;daf-c* double mutants within each group (our data, not shown) can recover from the dauer state at 15°, although the efficiency and time required for recovery varies. In all cases tested, the Daf-c double mutants between group 1 and group 2 genes were deficient for this dauer recovery. This

TABLE 2

**Percent dauer formation of *daf-c; daf-c* double mutants at 15°**

| | + | *daf-4(e1364)* | *daf-7(e1372)* | *daf-8(e1393)* | *daf-14(m77)* | *daf-21(p673)* | *daf-11(m47)* | *unc-42*[a] | *unc-42 daf-11(m47)*[a] |
|---|---|---|---|---|---|---|---|---|---|
| *daf-1(m40)* | 9 (334) | 71 (289) | 82 (158) | 37 (188) | 80 (394) | 100 (432) | NA | 0.7 (417) | 100 (220) |
| *daf-4(e1364)* | 86 (180) | | 46 (176) | 88 (338) | 85 (330) | 100 (388) | NA | 82 (160) | 100 (270) |
| *daf-7(e1372)* | 90 (344) | | | 77 (115) | 90 (399) | 100 (957) | NA | 75 (212) | 100 (193) |
| *daf-8(e1393)* | 3 (322) | | | | 67 (192) | 99.8 (412) | NA | 11 (293) | 100 (264) |
| *daf-14(m77)* | 82 (349) | | | | | 100 (646) | NA | 47 (172) | 100 (164) |
| *daf-21(p673)* | 17 (343) | | | | | | 10 (518) | NA | NA |
| *daf-11(m47)* | 1 (567) | | | | | | | 1 (289) | NA |

| | + | *daf-11(m47)* | *daf-21(p673)* |
|---|---|---|---|
| + | 0 | 1 (567) | 17 (343) |
| *daf-1(e1287)* | 2.5 (248)[b] | 100 (277)[a] | 100 (1,091) |
| *daf-7(m62)* | 73 (175) | ND | 100 (694) |
| *daf-11(m47)* | 1 (567) | NA | 10 (518) |
| *daf-11(m87)* | 3 (187) | NA | 0.7 (272) |
| *daf-11(m124)* | 80 (243) | NA | 0.5 (194) |

[a] These counts were made on the *unc-42 daf-11* progeny of heterozygous parents. The *unc-42* column is the appropriate control for comparison with the *unc-42 daf-11* column. The percent dauer formation was determined as described in MATERIALS AND METHODS. For the *daf-1(m40)* count, seven Unc nondauers were found: six were recombinants and the seventh could not be assessed because it was sterile. For the *daf-1(e1287)* count, two Unc nondauers were found, and both were recombinants. For the *daf-4* count, three Unc nondauers were found, and all three were recombinants. For the *daf-8* count, six Unc nondauers were found, and all six were recombinants. For the *daf-14* counts, two Unc nondauers were found, and both were recombinants. See MATERIALS AND METHODS for description of how recombinant classes were detected.

[b] This count was made of the double mutant *daf-1(e1287); unc-42(e270)*. A similar count with unmarked *daf-1(e1287)* was very similar.

effect was clearest in the case of *daf-21* double mutants, because its maternal rescue permitted the collection of large broods of *daf-c;daf-c* homozygous dauers. In each case the entire complement of dauers shown in Table 2 was incubated at 15° for at least 2 weeks and none ever recovered.

The strong synergy between group 1 and group 2 mutations cannot be explained on the basis of strength of mutant phenotype caused by the single mutations. The *daf-11(m47)* mutation used for much of the analysis was intentionally selected as being one of the weaker *daf-11* alleles (see Tables 2 and 3). Likewise, the *daf-21* mutation causes a relatively weak Daf-c phenotype (Tables 2 and 3). Similarly, among group 2 genes, the *daf-8(e1393)* allele and the *daf-1(m40)* allele cause relatively weak Daf-c phenotypes at 15° (Table 2), and yet they are fully synergistic with the *daf-11* and *daf-21* mutations. In contrast, strongly Daf-c alleles of *daf-4*, *daf-7* and *daf-14* display little or no synergy with each other, but do display strong synergy with the weaker *daf-11* and *daf-21* mutations.

Most of these observations were initially made with single alleles of each gene. Only one allele exists for the *daf-8*, *daf-14* and *daf-21* genes, but several alleles of each other gene have been identified. Additional alleles of *daf-1*, *daf-7* and *daf-11* were tested as shown in Table 2, with results identical to those with the canonical alleles. In addition, less quantitative information was determined for a second *daf-11* allele, *m84*, in double mutants with each group 2 Daf-c gene, with similar results (data not shown, see MATERIALS AND METHODS). These results indicate that the Daf-c group interactions are not allele specific. One group 1 double mutant, *daf-11(m124) daf-21*, actually appeared to be less Daf-c than either single mutant (Table 2), a result we cannot currently explain. Interpretation of these data is considered in the DISCUSSION.

***daf-3* and *daf-5* mutations completely suppress some *daf-c* mutations but suppress others incompletely:** Daf-d mutations in the genes *daf-3*, *daf-5* and *daf-12* have been identified primarily as suppressors of the Daf-c phenotype of *daf-4* and *daf-7* mutants (RIDDLE, SWANSON and ALBERT 1981). The previously isolated *daf-3(e1376)* and *daf-5(e1385)* mutations completely suppress the Daf-c phenotype of mutations in *daf-1*, *daf-4*, *daf-7*, *daf-8* and *daf-14* (Table 3; VOWELS and THOMAS 1992). This strong suppression is not specific for the *daf-c* allele tested. In contrast, the suppression by the same *daf-3* and *daf-5* mutations is incomplete for all seven *daf-11* alleles tested and for *daf-21* (Table 3; VOWELS and THOMAS 1992). Thus, the Daf-c genes can be divided into groups based on the degree of suppression displayed by *daf-3* and *daf-5*. Strikingly, this grouping corresponds exactly to the grouping of genes on the basis of synthetic Daf-c phenotype.

The inability of *daf-3* and *daf-5* mutations to fully suppress *daf-11* and *daf-21* mutations is not due to a stronger Daf-c phenotype caused by the *daf-11* and *daf-21* mutations tested. First, the *daf-11(m47)* and the *daf-21* allele are among the phenotypically weakest Daf-c mutations (Tables 2 and 3), and yet are incompletely suppressed by *daf-3* and *daf-5*. Second, *daf-12* mutations contrast with *daf-3* and *daf-5* mutations in

**TABLE 3**

**Percent dauer formation at 25° for *daf-c; daf-d* double mutants for some *daf-3*, *daf-5*, and *daf-12* mutations**

| | | Daf-d gene | | | | | |
|---|---|---|---|---|---|---|---|
| Daf-c gene | + | *daf-3(e1376)* | *daf-3(sa206)* | *daf-5(e1385)* | *daf-5(sa205)* | *daf-12(m20)* | *daf-12(sa156)* |
| *daf-1(m40)* | 99 (312)[a] | 0 (323)[a] | 0.2 (593) | 0 (419)[a] | 0 (241) | 0 (238)[a] | 0 (510) |
| *daf-1(e1287)* | 99 (167)[a] | 0 (425)[a] | ND | ND | ND | 0 (116)[a] | ND |
| *daf-4(e1364)* | 100 (177)[a] | 0 (274)[a] | 0 (282) | 0 (241)[a] | 0 (176) | 0 (425) | 0 (344) |
| *daf-4(m72)* | 100 (203)[a] | 0 (295)[a] | ND | 0 (629) | ND | 0 (152)[a] | ND |
| *daf-7(e1372)* | 100 (316)[a] | 0 (322)[a] | 1 (783) | 0 (308)[a] | 1.2 (508) | 0 (218)[a] | 0 (547) |
| *daf-7(m62)* | 100 (281)[a] | 0 (264)[a] | ND | 0 (588) | ND | 0 (176)[a] | ND |
| *daf-8(e1393)* | 97 (343)[a] | 0 (309)[a] | 0.2 (508) | 0 (181)[a] | 0 (438) | 0 (281) | 0 (223) |
| *daf-14(m77)* | 99 (409)[a] | 1 (361)[a] | 0.3 (776) | 0 (431) | 0 (485) | 0 (488) | 0 (335) |
| *daf-21(p673)* | 71 (265) | 9 (478) | 82 (320) | 31 (382) | 78 (455) | 0 (434) | 0 (258) |
| *daf-11(m51)* | 100 (746) | 16 (610) | 96.9 (349) | 12 (607) | 84 (851) | 0 (468) | 0 (808) |
| *daf-11(m87)* | 97 (1003)[a] | 32 (281)[a] | 95 (480) | 25 (336)[a] | 84 (267) | 0 (181)[a] | 0 (262) |
| *daf-11(m124)* | 100 (366) | 32 (316) | 98.6 (432) | 44 (537) | 94 (210) | 0 (234) | 0 (514) |
| *daf-11(sa195)* | 100 (209) | 11 (298) | 97 (280) | 33 (367) | 72 (221) | 0 (599) | 0 (327) |
| *daf-11(m47)* | 74 (1375)[a] | 13 (293)[a] | ND | 3 (188) | ND | 0 (307)[a] | ND |
| *daf-11(m84)* | 99 (1771)[a] | 7 (551) | ND | 23 (264) | ND | 0 (325) | 0 (931) |
| *daf-11(m597)* | 99 (195) | 16 (270) | ND | 18 (129) | ND | 0 (323) | ND |

[a] To facilitate comparison with new data, these counts are reproduced from VOWELS and THOMAS (1992).

their complete suppression of all group 1 and group 2 Daf-c mutations (Tables 3 and 4), indicating that full suppression is possible in both cases. Finally, it has been shown that mutations in the group of nine cilium-structure genes strongly suppress the Daf-c phenotype of *daf-11* (VOWELS and THOMAS 1992) and *daf-21* mutations (Table 1), but cause no suppression of *daf-1*, *daf-4*, *daf-7*, *daf-8* or *daf-14* mutations (VOWELS and THOMAS 1992).

We were concerned, however, that the specificity of suppression by *daf-3(e1376)* and *daf-5(e1385)* might not be characteristic of the genes, but rather reflect some peculiarity of these particular mutations. Apart from the weaker *daf-5(e1386)* allele (which also displays a similar specificity for suppression of Daf-c genes; VOWELS and THOMAS 1992) no other *daf-3* or *daf-5* alleles were available to us. To determine whether or not these findings were characteristic of *daf-3* and *daf-5*, we isolated more *daf-3* and *daf-5* mutations by screening for suppressors of the Daf-c phenotype of *daf-7(e1372)*. We chose *daf-7(e1372)* for this screen because it had been shown that *daf-3*, *daf-5*, and *daf-12* mutations are the major classes of suppressors that are isolated (RIDDLE, SWANSON and ALBERT 1981). Approximately 9,000 EMS- mutagenized genomes were screened and 7 *daf-3* alleles, 3 *daf-5* alleles, and 1 *daf-12* allele were isolated (see MATERIALS AND METHODS). In addition, the *daf-12(sa156)* allele had been previously isolated in a screen for suppressors of *daf-11(m87)* (W. SCHACKWITZ, personal communication). The suppressor properties of one randomly selected new allele of each of these three genes was characterized in detail. The additional alleles selected for detailed analysis were *daf-3(sa206)*, *daf-5(sa205)* and *daf-12(sa156)*. The results of this analysis are shown in Table 3. *daf-12(sa156)* was identical to the canonical *daf-12(m20)* allele in its capacity to completely suppress all of the Daf-c genes analyzed. *daf-3(sa206)* and *daf-5(sa205)* displayed the same gene specificity in their suppression that was found for the previously analyzed *daf-3* and *daf-5* mutations. Indeed, the contrast between their incomplete suppression of group 1 genes and strong suppression of group 2 genes was even more dramatic. *daf-3(sa206)* and *daf-5(sa205)* may be slightly weaker alleles of *daf-3* and *daf-5*, since they suppress several of the Daf-c mutations more weakly than *daf-3(e1376)* and *daf-5(e1385)*. All other alleles of *daf-3*, *daf-5* and *daf-12* were subjected to a more limited analysis, by construction as double mutants with one representative mutation from each group of Daf-c genes (Table 4). In every case the *daf-3* or *daf-5* allele displayed the same disparity of suppression, while the single additional *daf-12* allele suppressed fully. Though all the new *daf-3* and *daf-5* mutations were isolated as *daf-7* suppressors,

**TABLE 4**

**Percent dauer formation at 25° of *daf-7* and *daf-11* double mutants with all other *daf-3*, *daf-5*, and *daf-12* alleles**

| | Daf-c gene | |
|---|---|---|
| Daf-d gene | *daf-7(e1372)* | *daf-11(sa195)* |
| + | 100 (316) | 100 (209) |
| *daf-3(sa207)* | 1.3 (307) | 15 (549) |
| *daf-3(sa208)* | 0 (385) | 90 (248) |
| *daf-3(sa209)* | 0.3 (337) | 82 (303) |
| *daf-3(sa213)* | 0.9 (464) | 87 (189) |
| *daf-3(sa214)* | 0 (378) | 50 (146) |
| *daf-3(sa216)* | 5.8 (417) | 99 (327) |
| *daf-5(sa211)* | 0 (500) | 92 (208) |
| *daf-5(sa215)* | 0.6 (534) | 98 (257) |
| *daf-12(sa204)* | 0 (647) | 0 (275) |

they need not have fully suppressed *daf-7*, nor was weak suppression of *daf-11* and *daf-21* necessitated. These results show that the distinct interactions of *daf-3* and *daf-5* with the group 1 and group 2 Daf-c genes are not allele specific. Despite this distinction, most *daf-3* and *daf-5* mutations do significantly suppress *daf-11* and *daf-21*. Interpretation of these findings is considered in the DISCUSSION.

In order to test whether *daf-12* mutations could block the simultaneous activation of dauer formation by both a group 1 and a group 2 Daf-c mutation, we constructed triple mutants between two Daf-c mutations and a *daf-12* mutation (see MATERIALS AND METHODS). *daf-4*, *daf-7* and *daf-14* mutations were combined with *daf-12(m20)* and either a *daf-11* or *daf-21* mutation. In all cases the resulting strain was fully viable and completely dauer defective under both dauer-inducing and noninducing conditions (data not shown), indicating that *daf-12* mutations block concerted activation by both groups of Daf-c genes.

**Pheromone responsiveness of single and double mutants:** The capacity of several Daf-c mutants to respond to dauer-inducing pheromone at 15° has been reported (GOLDEN and RIDDLE 1984b). We have repeated these results and extended the analysis to several other alleles and genes, as shown in Table 5. We have also tested pheromone responsiveness for some selected *daf-c;daf-c* double mutants within each group of Daf-c genes. For group 1, we show data for double mutants between the single existing *daf-21* allele and three different *daf-11* alleles. For group 2, we show data for double mutants between *daf-7* and each of the other four group 2 genes. All single and double mutants tested display a highly significant enhancement of dauer formation in response to pheromone. These results indicate that in each case some part of a functional pheromone-response pathway remains intact.

**The *daf-c* genes can be divided into phenotypic categories that are related to their genetic interactions:** We have noticed that certain of these Daf-c mutants display several other phenotypes in addition to their shared Daf-c phenotype. As has been previously reported, *daf-1*, *daf-7*, *daf-8* and *daf-14* mutations cause an egg-laying (Egl) defect (TRENT, TSUNG and HORVITZ 1983), and this defect is fully suppressed by mutations in *daf-3* and *daf-5* (TRENT, TSUNG and HORVITZ 1983; VOWELS and THOMAS 1992). *daf-4* mutants are also Egl, but their egg laying has a pharmacological response profile that is distinct from the other group 2 Daf-c genes (TRENT, TSUNG and HORVITZ 1983), and their Egl defect is poorly suppressed by *daf-3* and *daf-5* mutations (VOWELS and THOMAS 1992). We have noticed two other phenotypes shared by mutants for *daf-1*, *daf-7*, *daf-8* and *daf-14* (and not by *daf-4*). We call these the *c*lum*p*y (Cpy) and *d*ark *in*testine (Din) phenotypes. The Cpy phenotype can be readily observed during the growth of the brood produced by a single *daf-1*, *daf-7*, *daf-8* or *daf-14* parent (*daf-7* is shown in Figure 1). Most of the progeny at all stages of growth spontaneously group into a few distinct clusters near the edge of the lawn of bacteria. A similar phenotype has been previously noted in *bor-1* mutants (R. CASSADA, personal communication; L. AVERY, personal communication). The Din phenotype consists of abnormally dark intestinal cells, and is most easily scored in the mid adult, although it appears to be expressed in the intermolt periods throughout larval growth as well. At 25° the Egl, Cpy and Din phenotypes of *daf-1*, *daf-7*, *daf-8* and *daf-14* mutants are very similar. All of these mutants also have similar phenotypes at 15°, with the exception of *daf-8(e1393)* which is much weaker for all three phenotypes. Neither *daf-11* nor *daf-21* mutants display the Egl, Cpy or Din phenotypes at any

**TABLE 5**

**Percent dauer formation of single and double Daf-c mutants in response to dauer pheromone at 15°**

| Genotype | Pheromone: None | Pheromone: 2 units[a] |
|---|---|---|
| *wild-type* | 0 (1,469) | 79.1 (1,235) |
| *daf-1(m40)* | 1.2 (328) | 97.5 (122) |
| *daf-1(e1287)* | 0.8 (628) | 97.8 (492) |
| *daf-4(e1364)* | 73.0 (293) | 100 (310) |
| *daf-4(m72)* | 94.8 (310) | 100 (225) |
| *daf-7(e1372)* | 61.4 (606) | 99.7 (303) |
| *daf-7(m62)* | 65.0 (551) | 99.7 (391) |
| *daf-8(e1393)* | 11.7 (643) | 98.8 (518) |
| *daf-14(m77)* | 73.2 (605) | 100 (297) |
| *daf-7(e1372); daf-1(m40)* | 77.7 (256) | 100 (177) |
| *daf-7(e1372) daf-4(e1364)* | 77.3 (366) | 100 (353) |
| *daf-8(e1393); daf-7(e1372)* | 94.6 (427) | 99.6 (252) |
| *daf-7(e1372); daf-14(m77)* | 69.5 (272) | 100 (158) |
| *daf-11(m47)* | 9.2 (437) | 79.9 (278) |
| *daf-11(m51)* | 10.3 (380) | 96.8 (526) |
| *daf-11(m87)* | 16.4 (293) | 94.8 (326) |
| *daf-11(m124)* | 68.2 (277) | 99.6 (243) |
| *daf-11(sa195)* | 36.3 (361) | 100 (257) |
| *daf-21(p673)* | 48.6 (181) | 100 (117) |
| *daf-11(m47) daf-21(p673)* | 26.5 (245) | 85.0 (180) |
| *daf-11(m87) daf-21(p673)* | 1.7 (422) | 65.0 (340) |
| *daf-11(m124) daf-21(p673)* | 6.7 (373) | 85.6 (348) |

Assays were performed as described in MATERIALS AND METHODS. Percent dauer formation without pheromone cannot be directly compared with Tables 1 and 2 since assay conditions were different (see MATERIALS AND METHODS). All data shown are the mean of at least two independent tests, and usually three or more. For each data pair the probability $P$ that the two frequencies were not significantly different was calculated using a normal theory test of proportions for two independent samples with continuity correction, by calculating $z = ((p1 - p2) - 1/2\,(1/n1 + 1/n2))/\text{SE}\,(p1 - p2)$, and determining $P$ from a normal distribution table. In all cases $P < 0.001$. For all cases, the same probabilities were also estimated by a chi-square test (2 by 2 contingency table method) with similar results. For the *daf-4(m72)* and the *daf-8; daf-7* cases, the Chi-square test was less appropriate since some expected values in the contingency table are small. Statistical methods are from ALTMAN (1991).

[a] We have defined a unit of pheromone as that amount required to induce 33% dauer formation in the wild-type under standard assay conditions (GOLDEN and RIDDLE 1984b) at 25°.

temperature. On the other hand, both *daf-11* and *daf-21* mutants are defective in chemotaxis (RIDDLE 1988; DUSENBERRY 1976), a phenotype not shared by Daf-c mutants in the group 2 genes. A summary of these phenotypes is given in Table 6.

The germane point of these observations is that these Daf-c genes fall into distinct functional groups. *daf-11* and *daf-21* are important for chemotaxis, while *daf-1*, *daf-7*, *daf-8* and *daf-14* are involved in regulation of egg laying, dispersal of animals across a plate, and intestinal constitution. *daf-4* mutations uniquely cause small size and a distinct kind of egg-laying defect. Each of these groups of genes seems related to the others only by their common involvement in regulation of dauer formation. With the exception of *daf-4*, these phenotypic groups correspond to the groups defined by synergy and by epistasis analysis.

**Differential effects of *daf-3*, *daf-5* and *daf-12* on the additional phenotypes of the Daf-c mutations:** In addition to differential suppression of the Daf-c phenotype, *daf-3* and *daf-5* show another qualitatively different interaction with group 1 and group 2 Daf-c genes. The Egl, Cpy and Din phenotypes of *daf-1*, *daf-7*, *daf-8* and *daf-14* mutants are all fully suppressed by *daf-3(e1376)* and *daf-5(e1385)*. In contrast, *daf-12(m20)* does not suppress any of these phenotypes, despite fully suppressing the Daf-c phenotype. The effects of *daf-3* and *daf-12* on the Cpy phenotype of *daf-7* are shown in Figure 1. These interactions are also not allele specific, since every allele of *daf-3* and *daf-5* suppresses the Egl, Cpy and Din phenotypes of *daf-7(e1372)*, while *daf-12(sa156)* and *daf-12(sa204)* do not. The failure of *daf-12* to suppress the Egl, Cpy and Din phenotypes indicates that they do not depend on dauer formation *per se*. We interpret these results to mean that *daf-1*, *daf-7*, *daf-8* and *daf-14* function together to control one or more processes in addition to dauer formation. The *daf-3* and *daf-5* genes must also function in these distinct processes, and presumably they play similar roles with respect to the group 2 Daf-c genes, since they display the same epistatic relationship. *daf-12* has no discernible function in these distinct processes, but plays a crucial downstream role in dauer formation.

## DISCUSSION

Four sets of data favor the existence of two parallel pathways for interpreting the sensory stimuli that control dauer formation. First, no mutation in any of the genes analyzed here causes a fully penetrant Daf-c phenotype. Second, the group 1 Daf-c genes are partially redundant with the group 2 Daf-c genes for repressing dauer formation. This fact presumably explains the incomplete penetrance of all single mutants. Third, Daf-d mutations in *daf-3* and *daf-5* strongly suppress the group 2 Daf-c mutations but incompletely suppress the group 1 Daf-c mutations, while the cilium-structure Daf-d mutations in nine genes strongly suppress group 1 Daf-c mutations but do not suppress group 2 Daf-c mutations (VOWELS and THOMAS 1992). Fourth, all of these Daf-c single mutants and all tested Daf-c double mutants remain responsive to dauer pheromone. We consider each of these sets of data in more detail in the following sections.

**Redundancy among the *daf-c* genes:** The strong synergy in Daf-c phenotype in certain *daf-c;daf-c* double mutants is liable to two simple explanations. The *daf-c* genes might lie in a simple linear pathway as previously suggested (VOWELS and THOMAS 1992), and the synergy might result from additive defects of two mutations, each of which incompletely activates dauer formation. Alternatively, the synergy might result from *daf-c* genes that lie on partially redundant parallel pathways. We favor the latter interpretation for several reasons. First, the strongest synergy is restricted only to double mutants between groups 1 and 2, and is not seen in double mutants within either group. Second, the groups of genes as defined by strongest synergy correspond exactly to the groups defined by epistatic interactions with *daf-d* mutations (VOWELS and THOMAS 1992; our unpublished data). Specifically, the Daf-c phenotype of *daf-11* and *daf-21* mutations is strongly suppressed by mutations in the nine cilium-structure genes, while the Daf-c phenotype of *daf-1*, *daf-4*, *daf-7*, *daf-8* and *daf-14* is unaffected. The third reason to favor the parallel pathway model arises from the suppression pattern of *daf-3* and *daf-5* mutations and is discussed in the next section.

**Suppression by *daf-3* and *daf-5*:** We have previously noted that *daf-3* and *daf-5* are difficult to interpret as part of a simple linear dauer-formation pathway (see DISCUSSION, VOWELS and THOMAS 1992). However, this analysis was limited to a single strong allele of each of *daf-3* and *daf-5*, and only one or two alleles of each of the Daf-c genes. We have now extended this analysis by identifying more alleles of both *daf-3* and *daf-5* and testing their suppression of Daf-c genes more completely. A clear pattern emerged: mutations in both *daf-3* and *daf-5* strongly block the Daf-c phenotype of mutations in group 2 genes, but are incomplete in their effect on mutations in group 1 genes. Conversely, it was previously found that Daf-d mutations in nine cilium-structure genes strongly suppress the Daf-c phenotype of group 1 genes but have no effect on group 2 genes (VOWELS and THOMAS 1992). Together, these findings are not easily reconciled with a linear epistasis pathway. Instead, they suggest that *daf-3* and *daf-5* define a branch of the pheromone response pathway in which only the group 2 Daf-c genes function.

**Responsiveness to dauer pheromone:** A parallel processing model predicts that all Daf-c single mutants

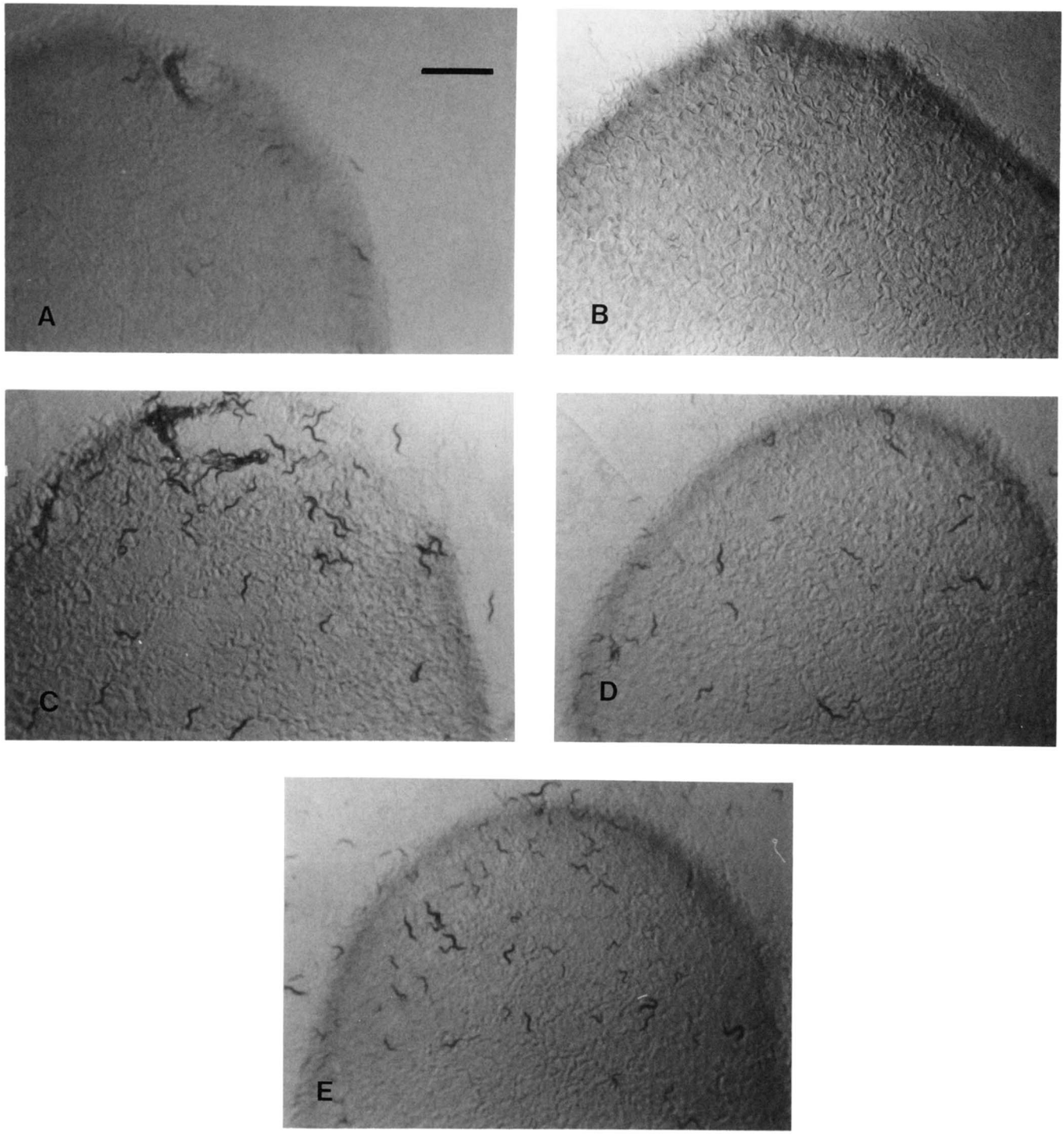


FIGURE 1.—The Cpy phenotype of various single and double mutants. The photographs were made at low magnification in order to emphasize the distribution of animals on the plate at the expense of visibility of individual animals. (A) *daf-7(e1372)* dauers grown at 25°. In this low magnification view, the individual dauers are difficult to see, but one large clump of about 50 dauers is obvious. (B) *daf-11(sa195)* dauers grown at 25°. No clump of dauers is visible. This field contained about 50 evenly dispersed dauers that are difficult to see in the photograph. (C) *daf-7(e1372);daf-12(m20)* nondauers of mixed age, grown at 20°. One large clump of animals is visible. (D) *daf-7(e1372);daf-3(e1376)* nondauers grown at 20°. (E) Wild-type nondauers of mixed age, grown at 20°. For panels C to E, the individual animals are more easily seen because dauer formation is suppressed and the animals have grown larger (the largest animals are late L4s to young adults). All photographs are at the same magnification and show representative sectors of the bacterial lawn on their respective plates. Scale bar approximately 2 mm.

**TABLE 6**

**Phenotypic classes among Daf-c genes**

| Gene | Phenotype | | | | | |
|---|---|---|---|---|---|---|
| | Daf-c | Egl | Cpy | Din | Che | Sma |
| *daf-4* | − | − | + | + | + | − |
| *daf-1* | − | − | − | − | + | + |
| *daf-7* | − | − | − | − | + | + |
| *daf-8* | − | − | − | − | + | + |
| *daf-14* | − | − | − | − | + | + |
| *daf-11* | − | + | + | + | − | + |
| *daf-21* | − | + | + | + | − | + |

Sources: Daf-c phenotype: RIDDLE, SWANSON and ALBERT (1981); this work. Egl phenotype: TRENT, TSUNG and HORVITZ (1983); this work. Cpy and Din phenotypes: this work. Che phenotypes: *daf-11*, RIDDLE (1988); *daf-21*, DUSENBERRY (1976) (where *p673* was called DD73); our unpublished data. Sma: RIDDLE, SWANSON and ALBERT (1981); this work.

and all Daf-c double mutants between genes in the same group will retain a pheromone response. GOLDEN and RIDDLE (1984b) reported pheromone responsiveness of several Daf-c mutants, and we have tested this response in additional Daf-c single mutants and a set of *daf-c;daf-c* double mutants (Table 5). All mutants retained clear pheromone responsiveness, failing to contradict a parallel processing model. An alternative explanation of these results is that each single mutation causes an incomplete block of a linear pathway, leaving some partial pheromone response intact. However, this explanation is not very satisfying since every mutant and double mutant tested must be supposed *ad hoc* to have an incomplete pathway block. In fact, a substantial body of evidence suggests that the null phenotype caused by at least *daf-1*, *daf-4* and *daf-7* mutations is incompletely penetrant dauer formation with strong pheromone responsiveness at low temperatures (GEORGI, ALBERT, and RIDDLE 1990; GOLDEN and RIDDLE 1984b).

**Parallel processing:** All of these data can be explained by the formal model shown in Figure 2. The group 1 and group 2 Daf-c genes act in parallel to control response to environmental conditions. The outcome of each parallel branch of the pathway is integrated at some point downstream to give rise to a single decision about dauer formation. *daf-3* and *daf-5* act only in the group 2 branch of the pathway, downstream of *daf-1*, *daf-4*, *daf-7*, *daf-8* and *daf-14*. The nine cilium-structure genes act in the group 1 branch of the pathway, downstream of *daf-11* and *daf-21*. We note that the *daf-3* and *daf-5* genes and the cilium-structure genes could also act upstream of the Daf-c genes on either branch of the pathway (indeed it seems likely that the cilium-structure genes function upstream of the group 2 Daf-c genes, since they pleiotropically disrupt all the amphid sensory endings). This model accounts for the several observations reported here more adequately than a linear pathway model. The incomplete penetrance of all alleles of the seven Daf-c genes analyzed is explained by the fact that the remaining branch of the pathway remains functional in each single mutant. The synergy between the two groups of Daf-c genes is explained as the result of activation of both branches of the pathway. The strong suppression by *daf-3* and *daf-5* of only the group 2 Daf-c genes is explained by their action only in that branch of the pathway. The capacity to respond to pheromone, which is characteristic of all group 1 and group 2 Daf-c single mutants and Daf-c double mutants within a group, is explained by the function of the remaining branch of the pathway. Finally, the pleiotropic phenotype classes observed among the Daf-c genes (Che for group 1 and Egl, Cpy and Din for group 2) suggest that these genes act as functional groups to control other distinct behavioral processes. The two parallel processes that control dauer formation appear to be the one case in which these functional groups of genes act together to control the same process. The additional phenotypes associated with each group might result from disruption of a single process that results in diverse phenotypic consequences. Alternatively, each group of genes might function together in distinct processes that separately control different phenotypes.

We offer two possible explanations of the fact that *daf-3* and *daf-5* partially suppress *daf-11* and *daf-21*. First, *daf-3* and *daf-5* might each act only in the group 2 branch of the pathway, as shown in Figure 2. If so, inactivating the group 2 branch by a mutation in *daf-3* or *daf-5* must reduce the capacity of the group 1 branch to promote dauer formation. This explanation is plausible in a parallel processing model, since the activities of both branches of the pathway are integrated to control dauer formation. Second, it is possible that *daf-3* and *daf-5* have a second role in the group 1 Daf-c pathway, although to a lesser extent than in the group 2 pathway.

The hypothesized parallel actions of the group 1 and group 2 Daf-c genes might occur in the same or separate cells. Cell-killing studies have shown that the amphid neurons ADF and ASI are required to repress dauer formation in the absence of dauer pheromone (BARGMANN and HORVITZ, 1991; BARGMANN, THOMAS and HORVITZ 1990). Because *daf-3* and *daf-5* mutations completely block dauer formation in ADF/ASI kills (BARGMANN and HORVITZ 1991), these sensory cells are implicated in the group 2 branch of the pathway. The process of dauer repression mediated by ADF and ASI thus remains the most attractive candidate for requiring the function of *daf-1*, *daf-4*, *daf-7*, *daf-8* and *daf-14* (VOWELS and THOMAS 1992; BARGMANN and HORVITZ 1991). In this connection it is interesting that *daf-1* encodes a putative transmembrane receptor with a cytoplasmic serine-threonine kinase domain (GEORGI, ALBERT and RIDDLE 1990).

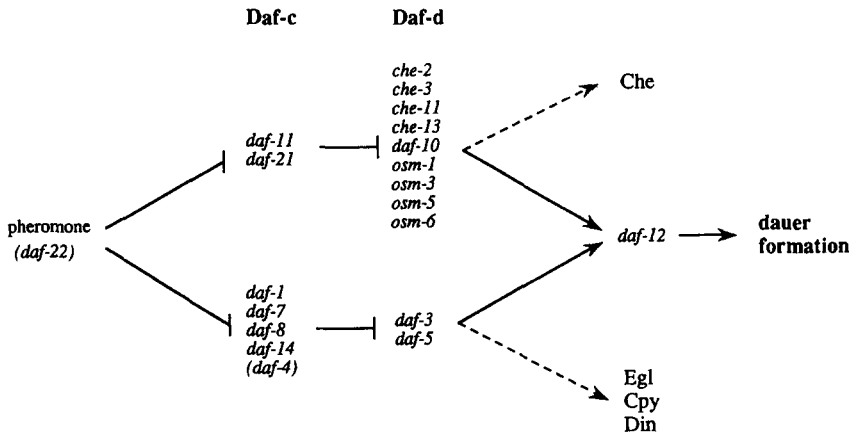


FIGURE 2.—Parallel model for sensory control of dauer formation. Arrows indicate positive regulatory interactions and lines ending with bars indicate negative interactions. *daf-4* is shown in parentheses to signify that it is part of the dauer pathway but does not cause the Egl, Cpy and Din phenotypes of *daf-1*, *daf-7*, *daf-8* and *daf-14*. The model does not pretend to be complete. For example, *daf-6* acts upstream of both branches of the pathway (see VOWELS and THOMAS 1992) and the cilium-structure genes may act upstream of the lower branch in addition to their role downstream of *daf-11* and *21* in the upper branch. We also emphasize that the depiction of these genetic interactions is strictly a formalism designed to summarize the genetic interaction data and does not imply direct molecular interactions of any kind.

The *daf-11* and *daf-21* genes might function in parallel with these genes in these same neurons, or they might function in distinct cells that act in concert with ADF and ASI to control dauer formation. It should be possible to distinguish these alternatives by further cell-killing experiments.

None of our data offer compelling proof of the existence of the hypothesized parallel pathways. It seems likely that conclusive evidence for or against such a model will be forthcoming only when more cellular and molecular detail about the relevant genes is available. The only currently viable alternative model is that shown in Figure 4 of VOWELS and THOMAS (1992), with the addition of the newly characterized gene *daf-21* at the same point in the pathway as *daf-11*. For the reasons discussed above, we think that the parallel model currently offers the best explanation of the available data.

***daf-12*, *daf-2* and *daf-19*:** Daf-d mutations in *daf-12* suppress the Daf-c phenotype of mutations in both group 1 and group 2 genes, and even of double mutants between the two groups. Assuming that *daf-12* functions at one point in the pathway, this result indicates that *daf-12* must act downstream of the point at which the parallel pathways converge (Figure 2). *daf-12* and the Daf-c gene *daf-2* have been shown to have lethal interactions, a result that is inconsistent with ordering these two genes in a simple linear pathway (VOWELS and THOMAS 1992) and that suggests the two genes are functionally closely related. *daf-2* mutations were not analyzed in this study, primarily because they are epistatic to both the cilium-structure genes and *daf-3* and *daf-5* (VOWELS and THOMAS 1992). Interestingly, *daf-2* is also unique in having both fully penetrant Daf-c alleles and incompletely penetrant alleles that are not pheromone responsive (GOLDEN and RIDDLE 1984b; RIDDLE 1988). These results together suggest that *daf-2* also acts downstream of the point at which the parallel pathways converge, although in unclear relation to *daf-12*. It is unclear how one additional Daf-c gene, *daf-19*, fits into the model in Figure 2. This gene is of some interest since the one mutation in *daf-19*, *m86*, causes a cilium-structure defect but, in contrast to all other cilium-structure genes, confers a Daf-c phenotype (PERKINS *et al.* 1986). The epistatic relationships between *daf-19(m86)* and the Daf-d genes have not been reported.

**General implications of our findings:** Despite extensive analysis of gene interactions among dauer-formation genes, there previously existed remarkably little evidence that contradicted a simple linear pathway for the action of the genes considered in this work (VOWELS and THOMAS 1992). The reason for this is that any convergent branched pathway may appear linear by epistasis criteria unless genes of both the constitutive and defective type are found on *both* branches of the pathway. If genes of only one type are found on one branch of the pathway, epistatic interactions may never be able to distinguish whether these genes act upstream in a linear pathway or on a converging branch of a parallel pathway.

How might such parallelism be recognized in other cases of apparent simple linear pathways? A suggestive feature of many mutations we analyzed in this work is that they caused an incompletely penetrant Daf-c (*daf-1*, *daf-4*, *daf-7*, *daf-8*, *daf-11*, *daf-14* and *daf-21*) or Daf-d (*daf-3* and *daf-5*) phenotype. While incomplete penetrance of phenotype will not necessarily characterize such branched pathways (*e.g.*, if each branch is absolutely required for some process, or if the branches are fully redundant), it may be a common feature. Mutations in genes that control complex regulatory networks, such as those described here, often display incomplete penetrance. In some of these cases, analysis similar to that presented here has suggested that such genes act on parallel pathways. These processes are as diverse as cell engulfment during programmed cell death (ELLIS, JACOBSON, and HORVITZ 1991), response to a mating pheromone (ELION, BRILL

and FINK 1991), and control of the cell cycle by $G_1$ cyclins (*e.g.*, LEW, MARINI and REED 1992). In many other cases, the possibility that incompletely penetrant mutant defects reflect partial redundancy of parallel pathways has not been ruled out, for example for the many incompletely penetrant mutations affecting sex determination in *C. elegans*. We suggest that the systematic construction of double mutants between mutations with *like* phenotypes is highly desirable for pathways in which such incomplete penetrance is observed.

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