Copyright © 1992 by the Genetics Society of America

Genetic Analysis of Chemosensory Control of Dauer Formation in *Caenorhabditis elegans*

Jennifer J. Vowels and James H. Thomas

Department of Genetics, University of Washington, Seattle, Washington 98195

Manuscript received May 29, 1991
Accepted for publication September 27, 1991

ABSTRACT

Dauer larva formation in *Caenorhabditis elegans* is controlled by chemosensory cells that respond to environmental cues. Genetic interactions among mutations in 23 genes that affect dauer larva formation were investigated. Mutations in seven genes that cause constitutive dauer formation, and mutations in 16 genes that either block dauer formation or result in the formation of abnormal dauers, were analyzed. Double mutants between dauer-constitutive and dauer-defective mutations were constructed and characterized for their capacity to form dauer larvae. Many of the genes could be interpreted to lie in a simple linear epistasis pathway. Three genes, *daf-16*, *daf-18* and *daf-20*, may affect downstream steps in a branched part of the pathway. Three other genes, *daf-2*, *daf-3* and *daf-5*, displayed partial or complex epistasis interactions that were difficult to interpret as part of a simple linear pathway. Dauer-defective mutations in nine genes cause structurally defective chemosensory cilia, thereby blocking chemosensation. Mutations in all nine of these genes appear to fall at a single step in the epistasis pathway. Dauer-constitutive mutations in one gene, *daf-11*, were strongly suppressed for dauer formation by mutations in the nine cilium-structure genes. Mutations in the other six dauer-constitutive genes caused dauer formation despite the absence of functional chemosensory endings. These results suggest that *daf-11* is directly involved in chemosensory transduction essential for dauer formation, while the other Daf-c genes play roles downstream of the chemosensory step.

UNDER permissive environmental conditions, the postembryonic life cycle of *Caenorhabditis elegans* consists of four larval stages (L1 through L4) and the adult, each stage separated by a molt. Under certain less favorable environmental conditions, at the L2 molt the nematode can enter an alternate 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 life cycle at the 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 dauer pheromone is composed of a closely related family of very stable nonvolatile compounds that have chemical characteristics similar to hydroxylated fatty acids and bile acids (GOLDEN and RIDDLE 1984c). 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). However, high levels of pheromone can induce dauer formation even at low temperatures in abundant food (GOLDEN and RIDDLE 1984a). Conversely, in a *daf-22* mutant, which fails to synthesize dauer pheromone, high temperature and starvation fail to induce any dauer formation, but exogenous pheromone induces normal dauer formation (GOLDEN and RIDDLE 1985; our unpublished observations). These results suggest that the presence of dauer pheromone is a necessary condition for induction of dauer formation, and that high levels of pheromone are sufficient to override other environmental stimuli.

We are interested in identifying and characterizing genes involved in the chemosensory transduction that controls 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 these genes cause two types of abnormal development. Temperature-sensitive dauer-constitutive (Daf-c) mutations cause dauers to form at restrictive temperature even when environmental conditions are noninducing for dauer formation (no pheromone, abundant food). 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 (RIDDLE, SWANSON and ALBERT 1981). Presumably, the sequence of the genes

Downloaded from https://academic.oup.com/genetics/article/130/1/105/6007131 by guest on 23 April 2024

in this pathway corresponds to a sequence of physiological events involved in formation of a dauer larva. However, with two exceptions, the particular anatomical site of function of the genes in the dauer pathway is unknown. The two exceptions are *daf-6* and *daf-10*, which are required for production of normal chemosensory amphid and phasmid sensilla (ALBERT, BROWN and RIDDLE 1981; PERKINS *et al.* 1986). Although these two genes both function in chemosensation, they lie at very different positions in the published epistasis pathway (RIDDLE, SWANSON and ALBERT 1981). Thus it was unclear which of the many genes that affect dauer formation might be involved in chemosensory transduction, and which might act further downstream in the pathway.

Chemosensation in *C. elegans* is thought to be mediated by three main groups of chemosensory neurons, or sensilla, which are exposed to the external environment through pores in the cuticle. All of these chemosensory neurons have dendritic endings containing a nonmotile cilium that projects into a channel formed by two support cells, a socket cell and a sheath cell. The socket cell lines the pore in the cuticle, while the sheath cell surrounds the sensory endings just proximal to the socket, probably creating an environment essential for sensory cilium function (WARD *et al.* 1975; BARGMANN, THOMAS and HORVITZ 1990). The bilateral amphid sensilla in the head each contain an identical set of twelve sensory neurons (WARD *et al.* 1975; and WARE *et al.* 1975). The sensory endings of eight of these amphid neurons are exposed directly to the external environment through the amphidial pore (WARD *et al.* 1975). The inner labial (IL) sensilla in the head, consist of a pair of neurons (IL1 and IL2) in each of the six lips around the mouth. The sensory endings of the IL2, but not IL1, neurons are exposed directly to the environment through the IL pore (WARD *et al.* 1975). The bilateral phasmid sensilla in the tail each contain sensory endings from the neurons PHA and PHB, both of which are exposed directly to the environment (SULSTON, ALBERTSON and THOMSON 1980; WHITE *et al.* 1986).

Dauer-defective mutations in nine genes have been identified that cause structurally abnormal cilia in one or more of the three chemosensory sensilla (PERKINS *et al.* 1986; LEWIS and HODGKIN 1977; ALBERT, BROWN and RIDDLE 1981). For convenience, we will refer to these as the "cilium structure" mutants. Based on the results of electron-microscopic studies of both chemosensory and mechanosensory sensilla in the head, mutants for these nine genes have been divided into four phenotypic groups. These groups vary in the number of classes of affected sensory neurons, and in the exact morphology of the defective cilia (PERKINS *et al.*, 1986; ALBERT, BROWN and RIDDLE 1981). In mutants for all nine genes, both the amphid and the phasmid cilia are abnormal, although the exact structural defect has not been determined for the phasmid cilia (PERKINS *et al.* 1986). The least pleiotropic cilium-structure mutant, *osm-3*, has defects only in the amphid and phasmid cilia, and is dauer defective. In mutants for two other genes, *che-11* and *daf-10*, the only affected chemosensory endings are in the amphids and phasmids, but some mechanosensors are also affected. Dauer-defective mutations in another gene, *daf-6*, cause abnormal amphid and phasmid sensilla (ALBERT, BROWN and RIDDLE 1981; HERMAN 1984). In the second-larval stage, when dauer larva formation is controlled, the structure and arrangement of the amphid cilia is approximately normal in *daf-6* mutant animals, but the amphid pore is occluded by an abnormal amphid sheath cell (ALBERT, BROWN and RIDDLE 1981). These results suggest that the amphid and/or phasmid neurons are necessary for dauer pheromone sensation, and consequently dauer formation.

Here, we determine the position of the nine cilium-structure genes in the dauer formation epistasis pathway, with the goal of distinguishing genes likely to mediate chemosensory transduction from those that act downstream of this step. We also analyze in more detail the phenotypes of previously analyzed double mutants (RIDDLE, SWANSON and ALBERT 1981) and considerably refine our understanding of the epistatic relationships among the dauer-formation genes. We show that function of only one of the dauer-constitutive genes, *daf-11*, depends on normal chemosensory endings, making *daf-11* a good candidate for encoding a component of the chemosensory mechanism that controls dauer formation.

MATERIALS AND METHODS

General genetic methods: General methods of maintenance of *C. elegans* are as described by BRENNER (1974). The standard wild-type strain, N2, and most other nematode strains used in this work, were provided by the Caenorhabditis Genetics Center, which is funded by the NIH National Center for Research Resources (NCRR). Many of the dauer mutant strains were obtained from D. RIDDLE, University of Missouri. The alleles used were generally the canonical reference alleles, except where additional alleles were used to confirm results. This paper follows the standard *C. elegans* nomenclature (HORVITZ *et al.* 1979).

Marker mutations used:

LGI: dpy-5(e61), unc-13(e51), unc-29(e1072), unc-75(e950).

LGII: unc-52(e444), mnC1.

LGIII: unc-32(e189), sma-4(e729), dpy-1(e1), unc-36(e251).

LGIV: unc-33(e204), unc-22(e66), dpy-20(e1282), dpy-9(e12).

LGV: dpy-11(e224), unc-76(e911).

LGX: egl-17(e1313), egl-15(n484), dpy-6(e14), lon-2(e678), unc-3(e151), lin-15(n765).

Complementation testing and map data: During the course of this work we learned that *daf-16(m26)* and what was previously called *daf-17(m27)* fail to complement for the

Downloaded from https://academic.oup.com/genetics/article/130/1/105/6007131 by guest on 23 April 2024

phenotype of suppression of *daf-2* (S. GOTTLIEB and G. RUVKUN, personal communication). This result together with the similarity in phenotype caused by *m26* and *m27*, and the fact that the two mutations map to the same region of chromosome *I*, prompted us to test further whether the two mutations might be alleles of the same gene. Male stocks of JT5490 *m27; daf-2(e1370)* and JT5478 *daf-8(e1393) m27* were generated by heat shock and inbreeding. Males from each stock were mated, respectively, to JT5489 *m26; daf-2(e1370)* and JT5477 *daf-8(e1393) m26* for one day at 20° with large numbers of males and the parents were transferred to a fresh plate at 25° the next day, to ensure efficient mating. The *daf-2* cross was scored for the production of suppressed (non-dauer) male progeny and the *daf-8* cross was scored for the production of male partial dauers (the males must be cross progeny). Both crosses were efficient as judged by the production of males, and *m26* and *m27* failed to complement by both criteria. For the *daf-8* cross, the partial dauers were observed using Nomarski optics and their phenotype was approximately intermediate between the slightly different partial dauers produced by *m26* and *m27* (see Results). Control crosses using *daf-2* and *daf-8* males showed that both *m26* and *m27* are fully recessive for both the suppression and partial dauer phenotypes. We conclude that *m26* and *m27* are allelic and choose to reassign *m27* to the prior gene name *daf-16*. In the process of constructing the *daf-8* double mutants with *m26* and *m27* we also gathered more map data for these mutations. One Daf-c non Unc recombinant from heterozygotes of genotype *daf-8 unc-29/m26* segregated *m26*, and one Daf-c non Unc recombinant from *daf-8 unc-29/m27* segregated *m27*. These data suggest that both *m26* and *m27* map to the right of *daf-8*.

We gathered additional map data for other genes in this study. *daf-20* was mapped to the right end of *X* left of *unc-3*. Animals of genotype *daf-20/mnDf19* were constructed and their broods formed plentiful dauers when allowed to starve, indicating that *daf-20* is not deleted by *mnDf19*. Animals of genotype *daf-8; daf-20/unc-3 lin-15* were constructed in order to map *daf-20* as a suppressor of *daf-8*. The heterozygotes were picked to plates at 25° and observed to segregate about 1/4 non-dauer progeny. Fifteen non-dauer progeny were picked singly to plates at 25°. Progeny testing confirmed that *daf-20* was homozygous and one of the fifteen segregated about 1/4 Unc Lin progeny and one segregated 1/4 Lin progeny. These data indicate that *daf-20* is a few map units to the left of *unc-3*. *che-11* was mapped with respect to *daf-11* and *unc-76*: 5 of 61 Daf-c non Unc recombinants from *daf-11 unc-76/che-11* segregated *che-11* (scored as Osm defective), and 0 of 15 Unc non Daf-c recombinants segregated *che-11*. *osm-6* was also mapped with respect to *daf-11* and *unc-76*: 2 of 66 Daf-c non Unc recombinants from *daf-11 unc-76/osm-6* segregated *osm-6* (scored as Osm defective). These data map both *che-11* and *osm-6* close to the right of *daf-11*.

Construction of double mutants: The double mutants between Daf-c and Daf-d mutations were constructed in one of several ways. The first method was used for cases in which the Daf-d mutation was expected to suppress the Daf-c mutation (RIDDLE, SWANSON and ALBERT 1981). *daf-c*/+ males were mated to *daf-d* hermaphrodites and several F_1 hermaphrodite progeny were picked singly to plates at 25°. From plates that segregated some dauer-constitutive progeny, pre-dauer (L2d) or dauer progeny were picked to plates at 15°, allowed to mature, and then picked singly to plates at 25°. L2d animals are a distinct pre-dauer form of the L2 stage that were recognized on the basis of their dark appearance (GOLDEN and RIDDLE 1984a). About 3 days later the homozygosity of the Daf-c mutation was confirmed by observing the segregation of about 3/4 dauers. From such a plate several non-dauer (suppressed) L4 animals were picked to individual plates at 25°. From such suppressed animals that produced all or mostly non-dauer progeny the double mutant strain was established. In the rare cases in which the penetrance of suppression by the Daf-d mutation was less than about 2/3, the Daf-c mutation and the Daf-c suppressor mutations were tested for breeding true by observing the progeny types from 16 parents picked singly to plates at 25°. In all cases the approximate parental ratio of dauer larvae to non-dauer larvae was observed, indicating that the Daf-c mutation and the suppressor of Daf-c were both homozygous. In cases in which the Daf-d mutation mapped to the *X* chromosome the method of crossing was reversed: *daf-d/0* males were mated to *daf-c* hermaphrodites; all subsequent steps were the same.

In several cases, the suppression was not expected (RIDDLE, SWANSON and ALBERT 1981), or the assumption of suppression by the Daf-d mutation proved incorrect (no suppressed animals segregated). In these cases a marked balancer chromosome was used in *trans* to the Daf-d mutation. In each case the construction followed one of two similar protocols. If the Daf-d gene was *X*-linked, *daf-c*/+ males were mated to *daf-d* hermaphrodites and the progeny males were mated to the balancing marker. Cross progeny were picked to 25° and from those that segregated dauers, L2d and dauer progeny were picked to 15° and allowed to mature, and were then picked singly to plates at 15° or 20°. Whenever possible, one of these animals that failed to segregate the markers was kept as a presumed *daf-d* homozygote. If no such animal was found, the markers were eliminated in the next generation. If the Daf-d gene was not X-linked a similar method was used, but the crosses were initiated by mating *daf-c*/+ males to the marker mutant and heterozygous male progeny were mated to the *daf-d* mutant.The markers used were *egl-17 lon-2* (*daf-3*), *unc-52* (*daf-5*), *unc-3 lin-15* (*daf-6*), *dpy-6 egl-15* (*daf-12*), *dpy-5 unc-75* (*daf-16*), *dpy-9* (*daf-18*) and *lin-2 unc-3* (*daf-20*).

Another method was used for most of the *daf-6* constructions. *daf-6* mutants exhibit pleiotropic defects in chemosensation (PERKINS *et al.* 1986), including osmotic avoidance (Osm). None of the Daf-c mutants analyzed in this study display Osm defects. The Osm phenotype of *daf-6* mutants was used to follow the *daf-6* mutation in crosses. Specifically, *daf-c*/+ males were mated to *daf-6* mutant hermaphrodites. F_1 progeny of this cross were picked to 25° and, from one that segregated dauers, Osm animals were picked (CULOTTI and RUSSEL 1978) singly to fresh plates at 25°. From a plate that segregated about 1/4 dauer progeny (heterozygous for the Daf-c mutation), the homozygosity of the *daf-6* mutation was confirmed by progeny testing. From a *daf-6* homozygote several L2d progeny were picked to a plate at 15°, allowed to mature, and were then picked singly to plates at 15°. Alternatively, the Daf-c mutation was made homozygous first, by picking L2d and dauer progeny of the F_1, and Osm segregants were subsequently picked from their progeny.

Construction of double mutants with the chemosensory cilium mutations: These constructions were made in several ways. At first some double mutants were constructed using Daf-c mutations linked in *cis* to marker mutations. Subsequently, all constructions were designed to eliminate marker mutations in the double mutant, for fear that these might influence the phenotype of the double mutant. Many particular double-mutant combinations were made by both methods and the results were always the same, indicating that marker effects were not a common problem.

In the first method, *daf-c M*/+ + males (*M* = marker)

Downloaded from https://academic.oup.com/genetics/article/130/1/105/6007131 by guest on 23 April 2024

were mated to homozygous *daf-d* hermaphrodites. Hermaphrodite cross progeny were picked singly to 25°. From animals that segregated the Daf-c mutation, homozygous Daf-d F_2 progeny were selected either by their Osm phenotype or by the inability of their amphid sensory neurons to take up fluorescein isothiocyanate (FITC) (HEDGECOCK *et al.* 1985). These homozygous Daf-d mutants were picked individually to plates at 15° and F_3 progeny homozygous for the Daf-c linked marker were picked. These individuals were allowed to lay eggs overnight at 15° (to establish a stock), and were then transferred to 25° to confirm the homozygosity of the Daf-c mutation. In all cases except *daf-11*, the Daf-c mutation was epistatic to the cilium-structure mutant for dauer formation. The homozygosity of *daf-11* was confirmed by complementation testing (see next section).

Many of the marker-carrying double mutants were also constructed using unmarked Daf-c mutants. The initial double mutant constructs (those using a Daf-c mutation with a linked marker) had established that in each case, the Daf-c mutation was epistatic to the Daf-d mutation. This tentative knowledge of the epistatic relationships simplified the double mutant construction. *daf-c*/+ males were mated to homozygous *daf-d* hermaphrodites. Hermaphrodite progeny were picked singly to 25°. From those that segregated the Daf-c mutation, F_2 progeny homozygous for the Daf-d mutation were selected by their Osm or FITC-filling defect, and were picked individually to 25°. The homozygosity of the Daf-d mutation was confirmed by testing the brood by the same method. Among these, Daf-c heterozygotes were recognized by the segregation of dauers, but these dauers recover poorly at any temperature (ALBERT, BROWN and RIDDLE 1981; our unpublished observations). Therefore non-dauer F_3 animals, which are homozygous wild type or heterozygous for the Daf-c mutation, were singly picked and allowed to lay eggs at 15°. After 1–2 days, these F_3 individuals were transferred to 25° to identify the Daf-c heterozygotes by observing their progeny. Once heterozygotes were identified, twelve of their progeny (F_4) grown at 15° were singly picked to plates at 15° overnight (to establish a stock), and then transferred to 25° to identify the homozygous Daf-c strains. Finally, the presence of the cilium-structure mutation was again confirmed by failure of the amphid and phasmid sensory neurons to fill with FITC.

All *daf-11; daf-d* double mutant strains were initially constructed as described previously with a *daf-11*-linked marker mutation. These initial double mutants suggested that the cilium-structure Daf-d mutations were epistatic to *daf-11*, a result unique among the Daf-c genes. To rule out the possibility that the linked marker mutation was affecting the results, these strains were reconstructed without marker mutations, but provisionally assuming the above epistatic relationship. For these constructions, heterozygous *daf-11* males were mated to homozygous Daf-d hermaphrodites at 20°. Hermaphrodite progeny were picked singly to 25°. Heterozygotes for *daf-11* were recognized by the segregation of dauers. L2ds or dauers from these plates were picked to 15° to allow recovery. Two to 5 days later, the recovered L2ds or dauers were picked singly to plates at 25°. Nondauer F_3 progeny were selected as putative Daf-d homozygotes. To confirm the genotype, all strains were tested for the presence of the *daf-11* mutation by a complementation test, and for the presence of the Daf-d mutation by its osmotic avoidance defect or inability to take up FITC, as well as by the epistasis to *daf-11*.

A few double mutant strains were highly efficient at forming dauers even at 15°, making it impossible to establish a stock of a doubly homozygous mutant strain (see Table 4). In these cases, the Daf-c mutation was balanced in *trans* to a linked marker mutation. Strain construction was essentially as described above, except that *daf-c*/+ males were mated to homozygous *daf-d* hermaphrodites carrying a marker mutation on the same linkage group as the Daf-c mutation. Doubly heterozygous progeny (*daf-c/marker; daf-d*/+) were allowed to self fertilize at 25°, and were distinguished by the segregation of dauers. Homozygous Daf-d F_2 individuals were selected as previously described. Among the homozygous Daf-d individuals, heterozygotes for the Daf-c mutation were easily distinguished at 25° from homozygotes for either the Daf-c or linked marker mutations.

Linked *daf-c daf-d* double mutants were constructed by mating singly or doubly marked heterozygous *daf-c* males to homozygous *daf-d* hermaphrodites. Heterozygous hermaphrodites were allowed to self fertilize, and dauer progeny with recombination of outside markers were individually picked to 15°. Among recombinants, homozygous Daf-d mutants were selected as previously described. The presence of both Daf-c and Daf-d mutations were confirmed as described below.

Confirmation of double-mutant genotypes: If the presence of the Daf-c mutation was not clear from the phenotype of the double mutant, the putative double-mutant strain was tested for failure to complement the appropriate Daf-c mutation in a test cross. For all of the Daf-c genes, the canonical mutation was constructed as a double mutant with *him-5* or *him-8* for the production of Daf-c homozygous males. These males were mated to the *daf-c; daf-d* double mutant for 1 day at 25°, then the parents were moved to a fresh plate at 25°. The plates from the second day of mating were assessed for the fraction dauer formation. In all cases at least 75% cross progeny were demanded (estimated by the fraction of males produced or the production of nearly all dauer progeny; if the cross was less efficient it was repeated), and in nearly all cases the cross was over 90% efficient. In cases in which the Daf-d was autosomal, failure to complement was indicated by the production of nearly all dauers, and very few or no male non-dauers. In cases in which the Daf-d was *X*-linked, failure to complement was indicated by the segregation of approximately 50% dauers, with nearly all the non-dauers being male (they are hemizygous for the *X*-linked Daf-d mutation that suppresses the Daf-c). This strategy failed for *daf-14* and *daf-4* for an interesting reason: males in all the *daf-c; him* double mutants were much more prone to form dauers even at 15° than the hermaphrodites (the unlikely possibility that all the Daf-cs are instead suppressing the Him phenotype was ruled out by the observation of approximately normal numbers of triplo-X dumpy animals). In the cases of *daf-14* and *daf-4* this effect was the most extreme, and it was difficult to find any males to make the test crosses. Therefore, in these two cases the genotypes were confirmed using *daf-c*/+ males. *daf-7* males were also rare and some tests were conducted with heterozygous males.

Since the Daf-c mutation was shown, by the above means, to be present and homozygous in each double mutant, the presence of each Daf-d mutation was often demonstrated by its distinctive effect on the Daf-c. In the cases of *daf-3*, *daf-5* and *daf-12*, this consisted of acting as a strong suppressor of the dauer-constitutive phenotype. In the cases of *daf-16*, *daf-18* and *daf-20* this consisted of efficient production of only the partial dauers characteristic of these mutations, or suppression of the Daf-c phenotype (see Table 5). The *daf-6*, *daf-10*, *che-2*, *che-3*, *che-11*, *che-13*, *osm-1*, *osm-3*, *osm-5* and *osm-6* mutations cause their own phenotypes: failure to avoid high osmotic strength and failure to fill with FITC. In all double mutant constructions one or the other

Downloaded from https://academic.oup.com/genetics/article/130/1/105/6007131 by guest on 23 April 2024

of these phenotypes was used to confirm the presence of these Daf-d mutations.

In the few cases in which the presence of the Daf-d mutation was not directly apparent from the phenotype of the double mutant (*daf-3* and *daf-5* double mutants with *daf-2*), males heterozygous for a marker linked to the Daf-d gene were mated to the putative double mutant, and F_1 progeny of the genotype *daf-d/M; daf-c/+* were picked. A large number of F_2 progeny of this F_1 were picked singly to plates at 25° and those that did not carry the Daf-c mutation (segregated no dauers) and had lost the marker were assessed for forming dauers upon starvation. If all such animals were dauer defective the homozygous presence of the Daf-d mutation in the double mutant was considered confirmed.

Construction of triple mutants: A *daf-16(m27); daf-7(e1372); daf-12(m20)* triple mutant was constructed by the following method. Wild-type males were mated to *daf-7; daf-12* hermaphrodites and the male progeny (hemizygous for the X-linked *daf-12*) were mated to *daf-16(m27); daf-7* at 20°. Unmated Egl progeny (*daf-7* homozygotes were picked singly to plates at 15°. Their genotype must be *daf-16/+; daf-7; daf-12/+*. Random self progeny of these animals were picked to 25° and their progeny classes were carefully observed. The expected frequency (about 1 out of 6) of parental animals produced 3/4 partial dauer and 1/4 non-dauer progeny. These parents must be *daf-7; daf-16; daf-12/+*, based on the known phenotypes of the double mutant parents. The non-dauer progeny of this parent were picked and confirmed to produce all non-dauer progeny at 25°. A complementation test was used to confirm the presence of a homozygous *daf-7* mutation in the final strain (see above). A *daf-16(m26); daf-7(e1372); daf-12(m20)* triple mutant was constructed by the same method.

Phenotype counts: The formation of dauers or partial dauers was assessed under dauer-inducing and noninducing conditions. Noninducing conditions were uncrowded animals (no more than about 200 larvae) on well-seeded NG agar plates. The fraction of dauers or dauer-like animals for each strain was determined using partially synchronized populations in order to avoid miscounting transient dauers. Between 1 and 10 L4 to young adult hermaphrodites (depending on the expected brood size) grown at 15° or 20° were placed on a plate at 25°. No more than 24 hr after egg laying began at 25° the parents were removed. This degree of synchrony is sufficient to distinguish dauers, transient dauers, and animals that do not form dauers. 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 plastic sides of the dish for trapped dauers. For counts at 15° parents were allowed to lay eggs for up to 2 days before removal, producing the same degree of synchrony, since development is twice as slow as at 25°. The double mutants between *daf-4* and *daf-14* and some of the cilium-structure mutants were so highly dauer constitutive even at 15° that it proved impossible to establish a homozygous strain. These double mutants were constructed and maintained with the cilium-structure mutant homozygous and the dauer-constitutive mutation balanced in *trans* to a linked marker. A slight maternal rescue of the dauer-constitutive homozygotes from the heterozygous parents permitted us to perform counts on the progeny of *daf-c; daf-d* homozygotes for most of these strains. The heterozygous parents were grown at 15° and the occasional maternally rescued *daf-c* homozygous progeny were recognized on the basis of a second phenotype (for *daf-4* they are Sma and for *daf-14* they are Egl) or the presence of a *cis* linked marker. These putative homozygotes were picked singly to the appropriate test temperature and transferred daily (25°) or once each two days (15°) for partially synchronized counts of the whole brood. As expected, occasional putative Daf-c homozygotes picked were recombinants rather than true homozygotes, as evidenced by the segregation of the *trans* marker and few dauers, and these broods were eliminated from the counts. A few double mutants displayed no dauer escapers even from the heterozygous parent grown at 15°. In these cases counts were made by determining fractions of dauers segregating from a heterozygous parent (see Tables 2 and 4).

Formation of dauers under inducing conditions was determined less quantitatively. Each strain was grown to starvation on plates at 25°, and from 2 to 4 days after starvation, the plates were scanned for the approximate frequency of dauer formation. On these plates the animals are exposed to strong dauer inducing conditions: high levels of dauer pheromone (due to crowding), low levels of food, and high temperature. Due to variation in the efficiency of dauer formation by the wild type under these conditions, it was not possible to be very quantitative in this test. The variability in dauer formation on starved plates probably results from variation in the number of animals that chance to be at the competent stage for dauer formation (L2) when food is depleted. For some tests and strain constructions, animals were exposed to crudely purified dauer pheromone by the method of GOLDEN and RIDDLE (1984a), except that 50 μg/ml streptomycin was added to the plates rather than the bacterial slurry.

All but a few of the strains analyzed for dauer formation carried no marker mutations. The following double mutants carried *dpy-5(e61)*: *daf-8(e1393); che-11(e1810)*, *daf-8(e1393); osm-5(p813)* and *daf-8(e1393) che-13(e1805)*. The following double mutants carried *dpy-1(e1)*: *daf-7(e1372); che-13(e1805)*, *daf-7(e1372); che-11(e1810)*, *daf-7(e1372); daf-10(e1387)*, *daf-7(e1372); che-3(e1124)* and *daf-7(e1372); osm-1(p808)*. The following double mutants carried *unc-32(e189)*: *daf-2(e1370); osm-1(p808)*, *daf-7(e1372); che-11(e1810)*, *daf-4(e1364); osm-1(p808)*, *daf-7(e1372); che-3(e1124)*. The double mutant *che-3(e1124) daf-8(e1393)* carried *unc-29(e1072)*. The double mutant *daf-14(m77); osm-1(p808)* carried *unc-22(e66)*. In each case the parental marked strain was analyzed to confirm that the marker did not substantially interfere with the dauer phenotype.

Nomarski observations and photography: Observations using Nomarski optics were done as described by SULSTON and HORVITZ (1977). Nomarski photomicrographs were taken as described by STERNBERG and HORVITZ (1986).

RESULTS

Rationale for epistasis analysis: Dauer-defective (Daf-d) mutations in a group of nine genes cause known anatomical defects in the structure of chemosensory cilia (PERKINS *et al.* 1986), but only one of these genes, *daf-10*, had been placed in the dauer-formation pathway (RIDDLE, SWANSON and ALBERT 1981). We reasoned that positioning the remaining cilium-structure mutants in the dauer pathway by epistasis analysis might divide the pathway into those genes acting upstream or in the chemosensory endings, and those acting downstream of the chemosensory endings. In order to analyze the epistatic inter-

Downloaded from https://academic.oup.com/genetics/article/130/1/105/6007131 by guest on 23 April 2024

actions among these genes, we constructed double mutants between the Daf-c mutations and the cilium-structure Daf-d mutations (see MATERIALS AND METHODS). In addition, we constructed the double mutants between the previously ordered Daf-c and Daf-d mutations in order to make more detailed analyses of their phenotypes. Since the Daf-c and Daf-d mutations cause opposite phenotypes, their epistatic relationships are formally represented as a negative regulatory pathway (RIDDLE 1977; RIDDLE, SWANSON and ALBERT 1981). If the double mutant between a particular pair of Daf-c and Daf-d mutations displays a dauer-formation phenotype similar to that of one of the single mutants, we infer that the epistatic mutation affects the downstream gene in the dauer pathway. The phenotypes of the double mutants were analyzed in several ways. First, the percent dauer formation of the double mutant under non-dauer-inducing conditions was determined. This test was performed with synchronous populations of uncrowded animals in the presence of plentiful food at the restrictive temperature (25°) for the Daf-c mutations. Second, the ability to form dauers under strong dauer-inducing conditions was determined. This qualitative determination was made with crowded and starved populations at 25°. In addition, various other phenotypes were noted. In particular, the ability to form the predauer second-larval stage (L2d) was noted (GOLDEN and RIDDLE 1984a), and any secondary phenotypes caused by the mutations were observed (*e.g.*, many Daf-c mutants are egg-laying defective).

Cilium-structure mutants: Since there are Daf-d mutations in nine genes that affect cilium structure, and Daf-c mutations in seven genes, the number of double mutants that might be analyzed is daunting. However, the Daf-d cilium-structure mutants fall into only four phenotypic classes, based on the detailed ultrastructural defects displayed by their sensory endings (PERKINS *et al.* 1986). Therefore we undertook to construct the double mutants between a mutation in each Daf-c gene and a mutation in one member of each of the four cilium-structure mutant classes. Mutations in the largest class of genes, composed of *che-2*, *che-13*, *osm-1*, *osm-5* and *osm-6* cause the middle and distal segments of all cilia to be absent. Mutations in the second class of genes, composed of *che-11* and *daf-10*, cause cilia that are nearly normal in length but have irregular contours and abnormal electron-dense material in their centers. The other two classes consist of only one gene each. *che-3* mutations cause the distal segments of the cilia to be absent while the middle segments are reduced to bulb-shaped endings. *osm-3* mutations cause only the distal segments of the cilia to be absent, the middle segments and other features appear normal. Mutations in all of these genes cause defects not only in dauer formation, but also in chemotaxis toward soluble chemoattractants and avoidance of noxious chemical stimuli (PERKINS *et al.* 1986). Despite the varying severity of their cilium-structure defects, all of these mutants appear completely deficient for all of these chemosensory responses.

TABLE 1

Percent constitutive dauer formation at 25° of *daf-11* double mutants with cilium-structure mutations

	daf-11 allele		
Daf-d gene	*daf-11(m47)*	*daf-11(m84)*	*daf-11(m87)*
+	74 (1375)	99 (1771)	97 (1003)
osm-1(p808)	1.3 (314)	3 (525)	3 (176)
osm-3(p802)	0.2 (571)	47 (153)	37 (125)
osm-3(el806)	6 (710)	13 (349)	0 (158)
osm-3(mn391)	ND	4.5 (288)	ND
osm-5(p813)	0 (286)	0 (199)	ND
osm-6(p811)	21 (262)	0 (356)	ND
che-2(e1033)	0.3 (286)	0 (456)	ND
che-3(e1124)	0.6 (338)	0 (93)	ND
che-11(e1810)	0 (594)	0 (288)	ND
che-13(e1805)	0 (165)	0 (323)	ND
daf-10(e1387)	0 (241)	0.8 (265)	ND

In all tables the percentage for a particular count is followed by the number of animals counted in parentheses.

One member of each mutant class was chosen for this analysis: *osm-5(p813)*, *che-11(e1810)*, *che-3(e1124)* and *osm-3(p802)*. A second member of the largest class of cilium-structure genes, *osm-1(p808)*, was also analyzed. The method of construction of the double mutants is described in detail in Materials and Methods. In brief, in most cases we followed the independently scorable phenotypes of the Daf-d mutations (osmotic avoidance defective and FITC dye-filling defective) and the Daf-c mutations (dauer constitutive) in the constructions. In some double mutants the phenotype of the Daf-c mutation was masked by the presence of the Daf-d mutation and in these cases the presence of the Daf-c mutation was confirmed by complementation testing.

The resulting double mutants were tested for the Daf-c phenotype (dauer formation under non-inducing conditions) and for dauer formation when crowded and starved (inducing conditions), as described in Materials and Methods. The results for the Daf-c phenotype are shown in Tables 1 and 2. The results can be summarized quite simply. Mutations in all of the Daf-c genes, except *daf-11*, were fully epistatic to all of the cilium-structure Daf-d mutations tested. These double mutants all showed the same high levels of dauer constitutivity at 25° that was displayed by the Daf-c single mutant (Table 2). In addition to those shown in Table 2, double mutants between the Daf-c genes *daf-7* and *daf-8* and the other four cilium structure genes, *che-2*, *che-13*, *daf-10* and *osm-6* were constructed. These double mutants were

Downloaded from https://academic.oup.com/genetics/article/130/1/105/6007131 by guest on 23 April 2024

TABLE 2

Percent constitutive dauer formation at 25° of double mutants between other *daf-c* mutations and cilium-structure mutations

		Daf-d gene				
Daf-c gene	+	*che-3* (*e1124*)	*che-11* (*e1810*)	*osm-1* (*p808*)	*osm-3* (*p802*)	*osm-5* (*p813*)
daf-1(m40)	99 (312)	97 (283)	100 (949)	100 (173)	99 (140)	100 (175)
daf-2(e1370)	100 (146)	100 (96)[a]	100 (87)[a]	100 (62)[a]	100 (134)[a]	100 (77)[a]
daf-4(e1364)	100 (177)	100 (83)	100 (477)	100 (91)	100 (153)	100 (76)
daf-7(e1372)	100 (316)	96 (208)	95 (131)	99 (586)	100 (183)	100 (200)
daf-8(e1393)	97 (343)	100 (727)	95 (158)	99 (415)	95 (133)	97 (259)
daf-14(m77)	99 (409)	100 (285)	≥93 (101)[b]	100 (103)[b]	100 (251)	100 (171)

In addition to data shown: *daf-8(e1393); che-2(e1033)* was 100% Daf-c (N = 204), *daf-8(e1393); osm-6(p811)* was 100% Daf-c (N = 277), *daf-7(e1372); che-2(e1033)* was 100% Daf-c (N = 141), and *daf-7(e1372); osm-6(p811)* was 99.7% Daf-c (N = 334), all counts at 25°.

[a] These percentages are of animals that reached at least the dauer or L3 stage. In addition, from 50% to 75% of all animals arrested as eggs or L1 larvae. This arrest was not seen at 15°.

[b] Since the homozygous double mutant formed close to 100% dauers even at 15°, these counts are made on broods from heterozygotes for the Daf-c mutation. From *daf-14/+; osm-1* parents 114/381 progeny were dauers, which is 110% of the expected 1/4 dauers, presumably due to random fluctuation.

also fully dauer constitutive in phenotype (data not shown). As expected, all of these double mutants also formed plentiful dauers under dauer-inducing conditions. In contrast, mutations in all nine cilium-structure genes were completely or mostly epistatic to the Daf-c phenotype of the *daf-11* mutation initially tested (*daf-11(m47)*, Table 1). These results suggest that the cilium-structure genes all fall at a single step in the dauer pathway, upstream of *daf-1, daf-2, daf-4, daf-7, daf-8* and *daf-14*, but downstream of *daf-11*. Since a cilium-structure gene in each phenotypic class fell in the same spot in the pathway, we presume that the other cilium-structure genes would likewise fall at the same step (also see below).

When interpreting these epistasis results we were concerned that none of the mutants analyzed are known to be null mutations and that only a single allele of many of the genes had been analyzed. This concern seemed particularly severe in the case of the Daf-c mutations, which are all temperature sensitive, although the null phenotype of these genes might be temperature sensitive (GOLDEN and RIDDLE 1984b). This concern is allayed by several features of the results. First, all five cilium-structure mutations gave virtually identical results in all of these double mutants, suggesting that variability in strength of mutant phenotype is not contributing to the results. Second, the possibility that the downstream Daf-c mutations are not nulls seems unlikely to be problematic, since even the Ts mutation tested is fully epistatic to the cilium-structure mutations. Finally, the results were very clear, with no significant quantitative effects of upstream mutations on downstream mutations.

Our major concern in this analysis was the placement of *daf-11* upstream of the cilium-structure mutants. *m47*, the first allele of *daf-11* that we tested, was found subsequently to be phenotypically weaker than some other *daf-11* mutations (Table 1). Therefore we constructed double mutants between both *m47* and the stronger *daf-11* mutation *m84*, and mutations in all nine cilium-structure genes. We also tested a second strong *daf-11* allele, *m87*, with two cilium structure genes, and a second mutation in *osm-3* with each of the three *daf-11* mutations. The results are shown in Table 1. In every double mutant there is clear suppression of the Daf-c phenotype by the cilium-structure mutation, but it is not always complete. In particular, *osm-3(p802)* gave quite substantial numbers of dauers in double mutants with *daf-11(m84)* and *daf-11(m87)*, while *osm-3(e1806)* and *osm-3(mn391)* were much stronger in their suppression. The *osm-6* mutation tested was also incomplete in its suppression of *m47*. We have also constructed the double mutants between three other *daf-11* mutations (*m51, m124* and *m1294*) and *osm-1(p808)* and similarly find strong suppression of dauer constitutivity in each case (data not shown). Dauer formation in each of these double mutants was also qualitatively assessed under dauer-inducing conditions (crowded and starved). In each double mutant a low level of dauers were formed, indicating that none of the cilium-structure mutations are fully epistatic to *daf-11* mutations. In all cases the production of dauers under inducing conditions was reduced in the double mutants compared to the *daf-11* single mutants, consistent with the results for the Daf-c phenotype (Table 1). The interpretation of these results is considered more fully in discussion.

Double mutants with other dauer-defective mutations: We also constructed double mutant combinations of at least one mutation in each of the seven Daf-c genes (*daf-1, daf-2, daf-4, daf-7, daf-8, daf-11, daf-14*) and at least one mutation in each of the six Daf-d genes that have apparently normal amphid sensory endings (*daf-3, daf-5, daf-12, daf-16, daf-18* and *daf-*

Downloaded from https://academic.oup.com/genetics/article/130/1/105/6007131 by guest on 23 April 2024

TABLE 3

Percent constitutive dauer formation at 25° of other *daf-c*; *daf-d* double mutants

Daf-c gene	+	Daf-d gene				
		daf-3(e1376)	*daf-5(e1386)*	*daf-5(e1385)*	*daf-6(e1377)*	*daf-12(m20)*
daf-1(m40)	99 (312)	0 (323)	0 (310)	0 (419)	100 (156)	0 (238)
daf-1(e1287)	99 (167)	0 (425)	0 (339)	ND	ND	0 (116)
daf-2(e1370)	100 (306)	100 (85)	100 (184)	ND	100 (121)	0 (246)[a]
daf-2(e1286)[b]	100 (209)	ND	ND	100 (229)	ND	0 (87)[a]
daf-4(e1364)	100 (177)	0 (274)	4 (290)	0 (241)	100 (303)	0 (98)
daf-4(m72)	100 (203)	0 (295)	0 (191)	ND	ND	0 (152)
daf-7(e1372)	100 (316)	0 (322)	0 (315)	0 (308)	100 (388)	0 (218)
daf-7(m62)	100 (281)	0 (264)	0 (256)	ND	ND	0 (176)
daf-8(e1393)	97 (343)	0 (309)	31 (313)	0 (181)[c]	98 (171)	0 (161)
daf-11(m47)	74 (1375)	13 (293)	20 (173)	ND	47 (259)[d]	0 (307)
daf-11(m84)	99 (1771)	ND	ND	ND	96 (247)	ND
daf-11(m87)	97 (1003)	32 (281)	71 (281)	25 (336)	ND[e]	0 (181)
daf-14(m77)	99 (409)	1 (361)	2 (299)	0 (136)	97 (247)	0 (114)

[a] For *daf-2(e1370)*; *daf-12* at 25°, 175 animals arrested at about the L1 molt, the remaining 71 arrested at about the L2 molt but were not dauers. For *daf-2(e1286)*; *daf-12* at 23°, all 87 animals arrested at the L2 molt, and at 25° nearly all animals at about the L1 molt.

[b] *daf-2(e1286)* causes early larval lethality at 25°, so all counts of double mutants containing *e1286* were performed at 23°.

[c] An additional 38 animals arrested as eggs or early larvae (not counted for table).

[d] *daf-11(m47)* was also constructed with *daf-6(n1543)* and the double mutant was 94% Daf-c at 25° ($N = 139$).

[e] This double mutant was constructed but was too strongly constitutive even at 15° to be propagated.

20) and *daf-6*. One double mutant was omitted, that between *daf-1* and *daf-18*, because it proved highly problematic to construct due to *daf-1* maternal effect and tight genetic linkage. The structure of the sensory cilia of mutants for the first six Daf-d genes has not been directly determined by electron microscopy, but the amphid cells are known to function normally in FITC uptake (PERKINS *et al.* 1986) and in avoidance responses (our unpublished data). In *daf-6* mutants the amphid sensory cilia in the L2 are normal in morphology, but are displaced posteriorly and are blocked from exposure to the environment by abnormal amphid sheath cells (ALBERT, BROWN and RIDDLE 1981). The double mutants were constructed without marker mutations and genotypes were carefully confirmed after construction (see MATERIALS AND METHODS). Each double mutant was tested for dauer formation under inducing and noninducing conditions. In the course of these studies we realized that three of the Daf-d genes are distinct from the others in that they are not fully dauer defective, instead giving rise to abnormal dauers in response to dauer pathway activation. These genes, *daf-16*, *daf-18*, and *daf-20* are considered separately in the next section.

The formation of dauers under noninducing conditions by double mutants between all Daf-c genes and *daf-3*, *daf-5*, *daf-6* and *daf-12* are shown in Table 3. The formation of dauers under inducing conditions followed a similar pattern: all double mutants that were dauer constitutive formed dauers at high frequency, while those that showed no constitutivity formed no dauers, and those that gave an intermediate level of dauer constitutivity also formed some dauers. The results indicate that *daf-6* is upstream of all the Daf-c genes, since double mutants with *daf-6* were still fully Daf-c. *daf-12* was downstream of all the Daf-c genes except *daf-2* (see below), since none of the *daf-12* double mutants formed any dauers. *daf-3* and *daf-5* were likewise probably downstream of all the genes except *daf-2*, but their Daf-d blocks were less complete in some double mutants.

As mentioned earlier, we were particularly concerned in this analysis with the possibility that the mutations used might not be null, or that mutant interactions observed might be allele specific. To address this concern, we constructed many of the double mutants using a second allele of the various genes, as shown in Tables 2 and 3. Unfortunately, *daf-8* and *daf-14* each has only one known mutation, and second mutations in *daf-3* and *daf-12* were unavailable to us. Nevertheless, the results obtained are fairly compelling. We were able to confirm with second mutations the positions of *daf-1*, *daf-4*, *daf-7* and *daf-11* upstream of *daf-12*, *daf-3* and *daf-5*. The interactions of the second *daf-2* mutation with all the Daf-d genes were similar to the first *daf-2* mutation (Tables 3 and 5). The position of *daf-3* and *daf-5* as downstream of *daf-1*, *daf-7*, *daf-8* and *daf-14* is also implied by their interactions for the egg-laying defective phenotype of the Daf-c mutations (see below).

Phenotypes at permissive temperature: The percent constitutive dauer formation for many of the single and double mutant strains was also determined at 20° and 15°. For all other double mutants similar,

Downloaded from https://academic.oup.com/genetics/article/130/1/105/6007131 by guest on 23 April 2024

TABLE 4

Percent dauer formation at 15° of double mutants between Daf-c mutations and cilium-structure mutations

		Daf-d gene				
Daf-c gene	+	*che-3* (*e1124*)	*che-11* (*e1805*)	*osm-1* (*p808*)	*osm-3* (*p802*)	*osm-5* (*p813*)
daf-1(m40)	0.6 (179)	14 (337)	0.6 (327)	2 (134)	0 (354)	40 (164)
daf-2(e1370)	0 (123)	0 (587)	0 (378)	0 (151)	0 (287)	0 (288)
daf-4(e1364)	49 (175)	100 (18)	100 (477)	100 (221)	52 (140)	100 (191)
daf-7(e1372)	14 (140)	61 (117)	43 (280)	25 (217)	57 (555)	31 (306)
daf-8(e1393)	1.4 (138)	67 (651)	1 (183)	36 (308)	4 (333)	48 (324)
daf-11(m47)	10 (1165)	0 (277)	0 (433)	0.2 (614)	0 (416)	0 (346)
daf-14(m77)	53 (445)	42 (127)	≥92 (132)[a,b]	90 (134)[a]	85 (182)	74 (94)

[a] Since these double mutants formed nearly 100% dauers even at 15° these counts were made of progeny of heterozygotes for the Daf-c mutation.

[b] The parental heterozygote also produced 10 dead eggs which are probably the *daf-14*; *che-11* double mutant since neither single mutant makes dead eggs.

but less quantitative, observations were made. All of the Daf-c mutations studied here, except those in *daf-2*, display some dauer-constitutive phenotype even at 15°, our lowest permissive temperature (Table 4). *daf-4(e1364)*, *daf-7(e1372)*, *daf-11(m47)* and *daf-14(m77)* show this affect most clearly, while *daf-1(m40)* and *daf-8(e1393)* are only very weakly Daf-c at 15°. This result indicates that the dauer-constitutive genes, except possibly *daf-2*, are not fully functional even at the permissive temperature. This observation is consistent with previous reports that the temperature sensitivity of the dauer-constitutive mutants is not due to production of temperature-sensitive gene products; rather the process of dauer formation itself is temperature sensitive (GOLDEN and RIDDLE 1984b). The dauer constitutivity of the double mutants generally dropped at lower temperatures, as expected from the fact that the Daf-c mutations are temperature sensitive. In all cases, when a *daf-c; daf-d* double mutant was dauer defective at restrictive temperature it was similarly dauer defective at permissive temperature (data not shown). With some interesting exceptions (see below), when a *daf-c; daf-d* double mutant was dauer constitutive at restrictive temperature, it was much less so or not at all at permissive temperature (data not shown).

Strangely, a few of the *daf-c; daf-d* double mutants actually show more dauer formation at 15° than does the corresponding *daf-c* single mutant. All cases in which this was found are shown in Table 4. Certain *daf-1*, *daf-4*, *daf-7*, *daf-8*, and *daf-14* double mutants with cilium-structure mutations, showed this enhancement at 15°. The enhancement was not found for any *daf-2* double mutant. The enhancement effect was most consistently shown by *che-3* and *osm-5* mutations, but other cilium-structure mutations produced some enhancement in other particular double mutants. No obvious pattern was apparent that might explain the variations among the double mutants. We speculate that the enhancement of constitutivity might be due to aberrant food and/or temperature sensation by the cilium-structure mutants, since both temperature and the availability of food modulate the efficiency of dauer formation.

Mutants that form partial dauers: Dauers differ from the alternative L3 stage in a number of ways that are discernible using Nomarski optics or a dissecting microscope (CASSADA and RUSSELL 1975; and our observations). Dauers possess cuticular alae made by the hypodermis, which are absent in the L3. Dauers have a structurally remodeled pharynx that is slimmer than that of the L3 and does not pump. Dauers have large highly refractile bodies present in the hypodermal cells laterally (and also anterior to the nerve ring dorsally and ventrally), which are absent in the L3. Dauers have highly refractile intestinal cells that appear dark using a dissecting microscope, while the L3 intestine appears lighter. In most cases, the dauers formed by the double mutants in our studies appeared fully dauer like by all of these criteria. However, there were three exceptions. Dauers formed by double mutants carrying mutations in the Daf-d genes *daf-16*, *daf-18* or *daf-20* produced abnormal dauers. Under dauer-inducing conditions, mutants for these genes produce dauers with some tissues that appear fully dauer-like, while others are either wholly L3-like or intermediate in phenotype. We refer to these abnormal dauer larvae as partial dauers, since they display only some features of the dauer state. No strains carrying mutations in *daf-16*, *daf-18* or *daf-20* were ever observed to produce a normal dauer, instead they form either partial dauers or L3s of normal appearance.

The *daf-18* partial dauers form readily not only in double mutants with Daf-c mutations, but also in the *daf-18* single mutant in response to dauer pheromone (Table 5). A comparison of three anatomical features of wild-type dauers and L3s with those of *daf-18*

Downloaded from https://academic.oup.com/genetics/article/130/1/105/6007131 by guest on 23 April 2024

TABLE 5

Percent partial dauer formation at 25° in double mutants between Daf-c mutations and partial Daf-d mutations

Daf-c gene	Partial Daf-d gene				
	+[a]	*daf-16(m26)*	*daf-16(m27)*	*daf-18(e1375)*	*daf-20(m25)*
+	0	0	0	0	0
+, pheromone treated[b]	75 (155)	<1 (348)	28 (292)	46 (165)	<1 (403)
daf-1(m40)	99 (312)	98 (236)	100 (106)	ND[c]	27 (216)
daf-2(e1370)	100 (306)	41 (235)	0 (313)	100 (328)	100 (113)[d]
daf-2(e1286)[e]	100 (209)	0 (488)	0 (597)	57 (277)	ND
daf-4(e1364)	100 (177)	99 (235)	100 (243)	100 (112)	88 (147)
daf-7(e1372)	100 (316)	98 (110)	100 (145)	100 (163)	85 (303)
daf-8(e1393)	97 (343)	83 (175)	95 (198)	82 (241)	0 (211)
daf-11(m47)	74 (1375)	15 (229)	63 (268)	74 (200)	0 (203)
daf-14(m77)	99 (409)	79 (165)	97 (111)	97 (155)	0 (196)

[a] The dauer larvae formed by the wild type in response to pheromone, and by each of the Daf-c single mutants, were fully dauer-like. All others in the table were partial dauers.

[b] Larvae grown in the presence of 64 μl of crude dauer pheromone per plate at 25°, as described in Materials and Methods. The dauers formed by wild type were fully dauer-like. Although *daf-16(m26)* and *daf-20(m25)* mutants responded poorly to pheromone, occasional partial dauers could be found and appeared morphologically similar to those formed in Daf-c double mutants.

[c] These two mutations are closely linked, so the double mutant was problematic to construct (see MATERIALS AND METHODS).

[d] In addition to 113 partial dauers, there were 19 arrested L1s and 46 dead eggs.

[e] As described in Table 2, all counts of strains carrying *daf-2(e1286)* were performed at 23°.

partial dauers is found in Figures 1, 2 and 3. The distinctive cuticular alae and the lateral hypodermal bodies characteristic of wild-type dauers are formed by *daf-18* mutant dauers (Figures 1D and 2D). The pharynx remains completely unremodeled in *daf-18* dauers when compared to the slimmed wild-type dauer pharynx (Figure 3, A and D). In addition, the pharynx pumps often in *daf-18* dauers, in contrast to the wild-type dauer which lacks pumping. *daf-18* dauers appear of intermediate darkness, suggesting that the intestine is neither fully dauer nor fully L3 in type. The *daf-18* partial dauers formed by exposure to pheromone (Figures 1D, 2D and 3D) and at restrictive temperature in double mutants with *daf-4*, *daf-7* or *daf-14* (not shown) all appear similar.

The *daf-16(m26)* or *daf-16(m27)* partial dauers form with high efficiency only in double mutants with Daf-c mutations.The *daf-16(m26)* single mutant responds only weakly to pheromone, while the *daf-16(m27)* mutant responds somewhat more strongly (Table 5). In both mutants the partial dauers appear only at reduced levels on starved crowded plates. The anatomy of *daf-16(m26)* partial dauers is shown in Figures 1, 2 and 3 in comparison with normal dauers and L3s. The dauer cuticular alae are present and the lateral hypodermal bodies are present (Figures 1C and 2C). The *daf-16* alae are typically less refractile than normal dauer alae and do not always have a completely normal morphology. The pharynx appears morphologically closer to that of the L3 than the dauer larva and pumps often. The *daf-16* dauers are also incompletely darkened, suggesting that the intestine is of intermediate phenotype. *daf-16(m27)* dauers (not shown) are similar to *daf-16(m26)* dauers, except that the pharynx tends to be even closer to L3 in type, suggesting that the *m27* block in dauer pharynx formation may be more complete. The *daf-16* partial dauers formed by exposure to pheromone, or by growth at restrictive temperature in double mutants with *daf-4*, *daf-7*, or *daf-14* all appear similar (not shown).

daf-20(m25) partial dauers form efficiently only in some double mutants with dauer-constitutive mutations (Table 5). The *daf-20* single mutant forms only rare partial dauers when exposed to dauer pheromone (Table 5). Nevertheless, the *daf-20* partial dauers formed by exposure to pheromone, or by growth at restrictive temperature in double mutants with *daf-4* or *daf-7* all appear similar (not shown). In *daf-20* dauers, the dauer alae and lateral hypodermal bodies are present but the pharynx is incompletely remodeled (Figures 1E, 2E and 3E). The *daf-20* alae are typically less refractile than in normal dauers and are morphologically more variable in appearance. The pharynx pumps in *daf-20* dauers but less often than in *daf-16* and *daf-18* dauers.

As shown in Table 5, *daf-16*, *daf-18* and *daf-20* mutations, in addition to causing the formation of partial dauers, also reduce the frequency of dauer formation by some upstream dauer-constitutive mutations. Both *daf-16* alleles reduce dauer formation by *daf-2* and *daf-11*, while *daf-18(e1375)* reduces the frequency of dauers formed by *daf-2(e1286)* but not *daf-2(e1370)*. *daf-20(m25)* reduces the frequency of dauer formation by *daf-1* and completely prevents dauer formation by *daf-8*, *daf-11* and *daf-14*. Thus, mutations in each gene tend to block entry to the dauer state, as well as prevent the production of fully differ-

Downloaded from https://academic.oup.com/genetics/article/130/1/105/6007131 by guest on 23 April 2024

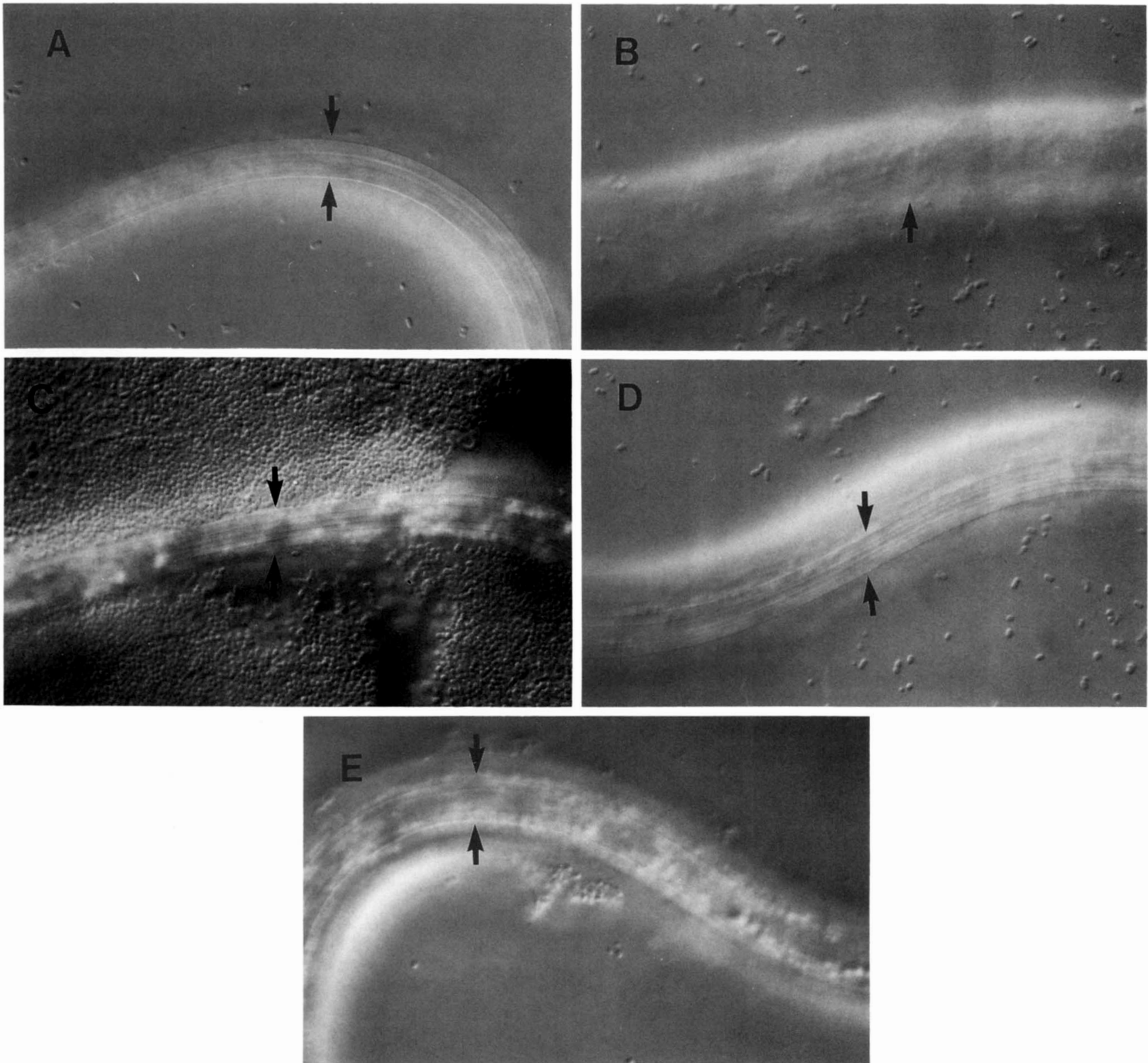

FIGURE 1.—Nomarski micrographs of the lateral cuticle in wild-type and mutant dauers and the wild-type L3. A, Wild-type dauer; B, wild-type L3; C, *daf-16(m26)* dauer; D, *daf-18(e1375)* dauer; E, *daf-20(m25)* dauer. The arrows indicate the longitudinal raised ridges which are the alae (or where they are absent in the L3). The alae in the Daf mutants tend to be somewhat indistinct, especially in *daf-20* mutants, but are clearly present on all animals. The dauers here and in Figures 2 and 3 were formed under the same conditions, by exposure to pheromone on limiting food at 25°. The wild-type L3 was from a well fed population in the presence of low levels of pheromone.

entiated dauers. We do not know whether these two phenotypes arise from the same underlying defect, but it seems plausible to suppose that some tissue that is important for entry to dauer formation fails to be transformed to the dauer type in these mutants.

Interactions with *daf-2*: Daf-c mutations in *daf-2* interact with some Daf-d mutations quite differently from any other Daf-c gene. As found previously (RIDDLE, SWANSON and ALBERT 1981), it is difficult to sensibly fit *daf-2* into a simple epistasis pathway. Nevertheless some useful conclusions may be drawn. First, *daf-2* clearly behaves as a relatively downstream gene, since *daf-2* mutations are fully epistatic not only to the cilium-structure mutations but also to *daf-3* and *daf-5* mutations (Tables 2 and 3). Second, its interaction with *daf-12* indicates that *daf-2* may have a function beyond its role in dauer formation.The double mutant between these two genes has a larval-arrest phenotype, which is characteristic of neither single mutant. Some animals arrest in the early L1 while others arrest at about the L2 molt. The detailed phenotype of the arrested animals was not characterized, but they show no sign of dauer character as determined by Nomarski microscopy. When two mu-

Downloaded from https://academic.oup.com/genetics/article/130/1/105/6007131 by guest on 23 April 2024

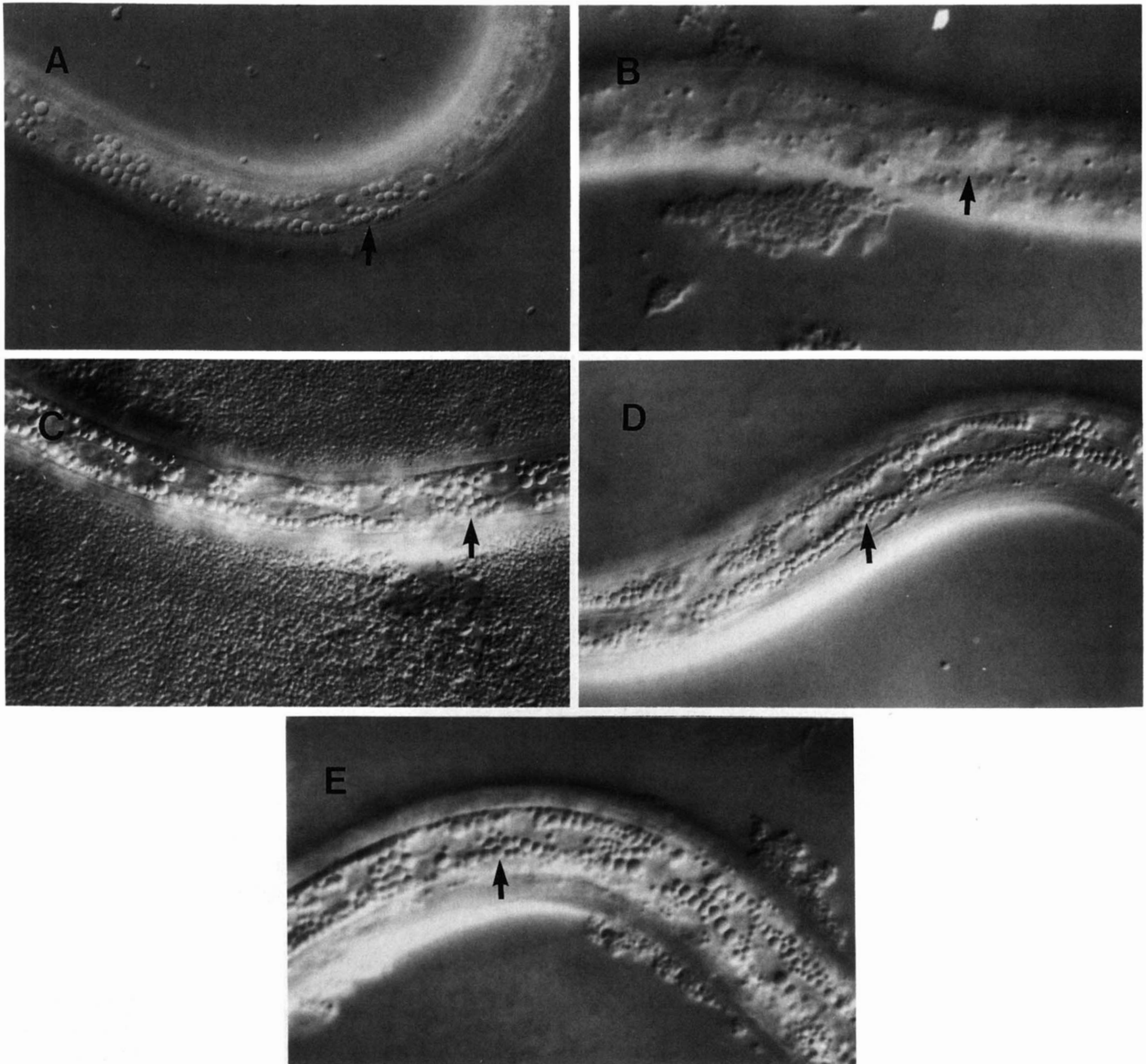

FIGURE 2.—Nomarski micrographs of the lateral hypodermal cells in wild-type and mutant dauers and the wild-type L3. A, Wild-type dauer; B, wild-type L3; C, *daf-16(m26)* dauer; D, *daf-18(e1375)* dauer; E, *daf-20(m25)* dauer. The arrows indicate the larger, more prevalent, refractile bodies that are present in the hypodermal cells of dauers. In the L3 hypodermis there is a much lower density of smaller, less distinct, bodies. The arrow for the L3 indicates the region in which the dauer hypodermal bodies would be found.

Downloaded from https://academic.oup.com/genetics/article/130/1/105/6007131 by guest on 23 April 2024

tations together result in the production of a novel phenotype, this indicates that the genes function, at least in part, in independent pathways (HEREFORD and HARTWELL 1974). Since *daf-12* appears by all criteria to fit simply into the dauer-formation pathway, this result suggests that *daf-2* performs some other function (alternatively *daf-12* might play a role outside of its function in dauer formation). One speculative possibility is that *daf-2* is required for developmental progression to the L3 larva, while *daf-12* is required for progression to the dauer larva. The *daf-2* phenotype of dauer constitutivity might arise as a default state due to failure in L3 progression.

A similar novel phenotype was noticed in double mutants between *daf-2* and each of the cilium-structure genes, *osm-5*, *che-11*, *che-3*, *osm-3* and *osm-1*. In each double mutant a substantial fraction of the animals arrested as early L1s at 25° but not at 15°. The counts in Table 2 were performed on that portion of these double mutants that escaped this L1 arrest (these all went on to form dauers). Since this arrest phenotype is not seen in any of the single mutants, it must arise from an interaction between these genes. The interaction is not allele or gene specific, since mutations in all five cilium-structure genes tested show a similar effect. This result again suggests that *daf-2* is

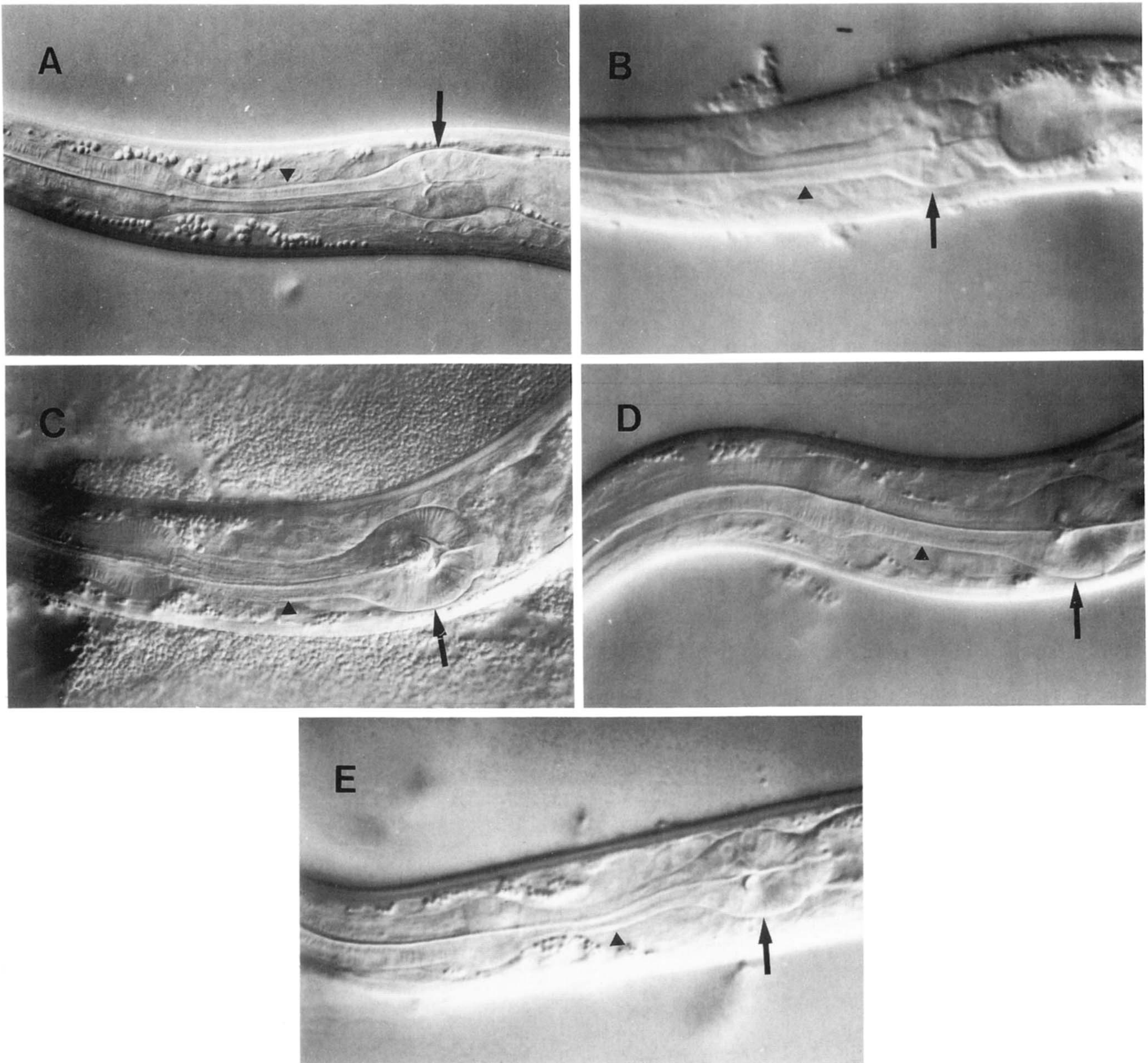

FIGURE 3.—Nomarski micrographs of the pharynx in wild-type and mutant dauers and the wild-type L3. A, wild-type dauer; B, wild-type L3; C, *daf-16(m26)* dauer; D, *daf-18(e1375)* dauer; E, *daf-20(m25)* dauer. The arrows indicate the posterior bulb of the pharynx and the arrowheads indicate the isthmus of the pharynx, both of which are slimmer in the normal dauer larva than in the L3. This slimming of the pharynx is somewhat obscured by the fact that the dauer larva is much thinner than the corresponding L3, making the pharynx occupy a larger relative width of the animal. The *daf-16* and *daf-18* pharynges are very similar to the wild-type L3nd the *daf-20* pharynx appears to be intermediate in shape. In addition to pharyngeal morphology, the normal dauer pharynx never pumps, while the *daf-16*, *daf-18* and to a lesser extent, the *daf-20* pharynxes pump.

required for some process other than dauer formation.

The interactions of *daf-2* and the partial dauer genes *daf-16*, *daf-18* and *daf-20* are difficult to interpret as well. Dauer formation by *daf-2* under noninducing conditions is partially or completely suppressed by *daf-16* mutations depending on the allelic combinations used (Table 5). In contrast, both *daf-2* mutations tested form a high percentage of partial dauers in double mutants with *daf-18* and *daf-20* (Table 5). The placement of *daf-2* in the epistasis pathway is considered further in discussion.

Other observations: Mutants for the Daf-c genes *daf-1*, *daf-4*, *daf-7*, *daf-8* and *daf-14* are all egg-laying defective in addition to being temperature-sensitive dauer constitutive (TRENT, TSUNG and HORVITZ 1983). In every case except *daf-8* the egg-laying defect is not temperature-sensitive, suggesting that the Daf-c mutations are not actually temperature sensitive, but instead reveal a temperature-sensitive process

Downloaded from https://academic.oup.com/genetics/article/130/1/105/6007131 by guest on 23 April 2024

(GOLDEN and RIDDLE 1984b). *e1393*, the single known mutation in *daf-8*, may cause a *bona fide* temperature-sensitive gene product, since both its egg-laying and dauer-constitutive phenotypes are temperature sensitive (our data, not shown). We find that mutations in *daf-3* and *daf-5*, but no other Daf-d genes, fully suppress the egg-laying defects of all of these Daf-c genes except *daf-4*. Suppression of the egg-laying defect of *daf-7* and *daf-14* by a *daf-3* mutation has been previously noted (TRENT, TSUNG and HORVITZ 1983). These results suggest that the egg-laying defects caused by *daf-1*, *daf-7*, *daf-8* and *daf-14* are functionally closely related and that each gene interacts similarly with *daf-3* and *daf-5* in both the dauer-formation and egg-laying systems. It also provides further evidence that *daf-3* and *daf-5* act downstream of these Daf-c genes. The fact that *daf-3* and *daf-5* do not suppress the egg-laying defect of *daf-4* may not be significant for the dauer-formation pathway, since *daf-4* mutations confer a different class of egg-laying defect from the other Daf-c mutations (TRENT, TSUNG and HORVITZ 1983). *daf-4* mutants are small in addition to being egg-laying defective, presumably because of severe nutritional deficiency due to its intestinal endocytosis defect (L. JACOBSON, personal communication). The small phenotype is not affected by any of the Daf-d mutations.

daf-12 mutants are unique among the Daf-d mutants analyzed in that, with the appropriate dauer-forming stimulus, they form what appear to be normal second larval stage predauer larvae (L2d) but fail to proceed to the dauer larva. All the other fully Daf-d mutants fail to form both the L2d and the dauer larva. *daf-12* mutants form the L2d in response both to pheromone treatment and in double mutants with all upstream dauer constitutives, suggesting that *daf-12* is required for progression from the L2d to the dauer stage, but not entry to the L2d stage.

DISCUSSION

Genes required for structurally normal chemosensory cilia occupy an upstream position in the dauer pathway: Dauer formation is induced by high levels of dauer pheromone, and is modulated by temperature and the abundance of food. The dauer-inducing stimulus has been presumed to act on specific chemosensory cells, since mutants that affect the ciliated sensory endings of these cells are dauer-formation defective (ALBERT, BROWN and RIDDLE 1981; PERKINS *et al.* 1986). If these cilium-structure mutations prevent dauer formation by blocking chemosensation of the inducing stimulus, they should occupy a relatively upstream position in the epistasis pathway. Our observations satisfy this prediction. Dauer-constitutive mutations in each Daf-c gene except *daf-11* are fully epistatic to defects in the sensory cilia. We conclude that the activation of dauer formation by these Daf-c mutations is independent of chemosensory stimuli. We have also confirmed the pathway position of the dauer-defective mutation in *daf-22* (GOLDEN and RIDDLE 1985) as being upstream of all dauer constitutives (data not shown). The *daf-22* mutant fails to synthesize dauer pheromone but responds normally to exogenous pheromone (GOLDEN and RIDDLE 1985). Thus the general features of our pathway are plausible: synthesis of pheromone is the first known required step in dauer formation while chemosensation is the next step. Downstream steps in the pathway presumably act to respond to and convey information from the sensory transduction process.

daf-11 probably acts in the amphid sensory endings: *daf-11* occupies a unique position in the epistasis pathway among identified Daf-c genes. Recessive defects in *daf-11* cause constitutive dauer formation and the expression of that phenotype is dependent on the cilium-structure genes. We depict *daf-11* as upstream of the cilium-structure genes in Figure 4 to signify this suppression of *daf-11*. However, the suppression in some double mutants is incomplete (Table 1), which we have indicated with a dotted line from *daf-11* bypassing the cilium-structure genes. An alternative depiction would show *daf-11* and the cilium-structure genes acting in parallel, reflecting a coexpression of mutant phenotypes in the double mutants. All the *daf-11* mutations are fully recessive, suggesting that they cause loss of gene function, and yet they result in constitutive activation of dauer formation. These considerations suggest that the *daf-11* gene normally acts negatively on another gene whose function promotes dauer formation. Since the *daf-11* mutant phenotype is suppressed by the cilium-structure mutations, the dauer-promoting function of the gene that *daf-11* negatively regulates must depend on those cilia. A simple explanation of these results is that the products of both *daf-11* and the gene it negatively regulates function in the sensory endings themselves, presumably as part of the chemosensory transduction mechanism that controls dauer formation. An alternative, but less plausible, explanation is that both genes function downstream of the sensory endings, but that their function is dependent on the structural integrity of the sensory endings even in the absence of environmental stimuli.

In light of this model for *daf-11* function, the interactions with *daf-6* are interesting. *daf-6* is unique among the genes studied in that it affects the amphid structure, but not by directly altering sensory cilium structure. Instead, in *daf-6* mutant animals, the amphid pore fails to form properly, leaving the amphid cilia unexposed to the external environment (ALBERT, BROWN and RIDDLE 1981). Genetic mosaic analysis

Downloaded from https://academic.oup.com/genetics/article/130/1/105/6007131 by guest on 23 April 2024

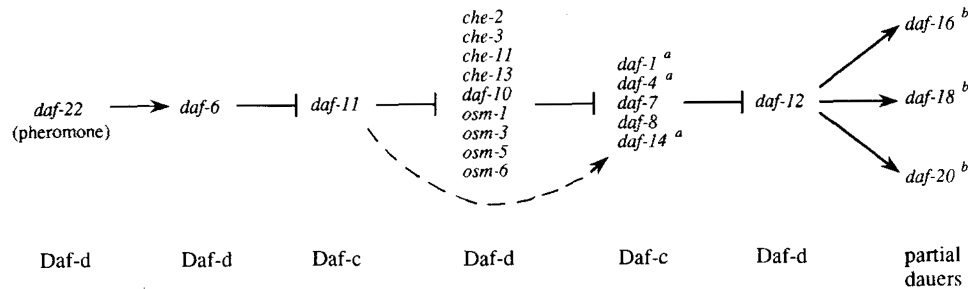

FIGURE 4.—A model for the function of dauer-formation genes. Lines terminating in a vertical bar indicate negative regulatory interactions, while those terminating in arrows indicate a positive regulatory interaction. The dashed line from *daf-11* to the *daf-1* cluster of genes indicates that the epistasis of some of the cilium-structure genes to *daf-11* was not quite complete. *daf-2*, *daf-3* and *daf-5* are not indicated on this pathway because of uncertainty about their proper position (see text). *daf-3* and *daf-5* probably lie at the *daf-12* step. [a]*daf-1*, *daf-4* and *daf-14* have been ordered with respect to *che-3*, *che-11*, *osm-1*, *osm-3* and *osm-5*, but not with respect to the other four cilium structure genes (see text). [b]*daf-16*, *daf-18* and *daf-20* are placed on branches downstream of *daf-12*, not on the basis of epistasis results, but rather as the simplest explanation of their tissue specificity (see text). The fact that the three genes are drawn on separate branches is not intended to signify independent action; the relationships among these branches was not investigated.

(HERMAN, 1984) and electron microscopic studies (ALBERT, BROWN and RIDDLE 1981) indicate that the *daf-6* amphid sheath cell degenerates and that this defect is probably responsible for its chemosensory mutant defects. We presume that the amphid sheath defect of *daf-6* causes the defect in dauer pheromone responsiveness. If the *daf-6* phenotype were this simple, then one might predict that it would lie most upstream in the dauer pathway, and indeed it appears to. All Daf-c mutations are fully epistatic to *daf-6* except possibly *daf-11(m47)*. *daf-6(e1377)* may weakly suppress the dauer constitutivity of *daf-11(m47)*, but a second allele of *daf-6* showed no suppression (Table 3). Suppression of stronger *daf-11* mutations by *daf-6* is insignificant (Table 3), leading us to tentatively conclude that *daf-6* is upstream of *daf-11*.

Differences from previous reports: The epistasis pathway (Figure 4) inferred from these results is substantially different from that previously determined (Figure 4 in RIDDLE, SWANSON and ALBERT 1981). The differences between our results cannot be explained on the basis of different dauer-formation mutations used, since we analyzed exactly the same mutations used in the previous study. The major differences between our pathways result from the repositioning of the dauer-defective gene *daf-6* and the partial-dauer genes *daf-16*, *daf-18* and *daf-20* (and *daf-17(m27)*, now known to be allelic to *daf-16*). Mutations in all of these genes were previously classified as dauer defective, and were found to divide the dauer-constitutive genes *daf-1*, *daf-4*, *daf-7*, *daf-8* and *daf-14* into several distinct negative regulatory steps (RIDDLE, SWANSON and ALBERT 1981). We find no compelling evidence that these Daf-d genes meaningfully distinguish among these dauer-constitutive genes. Thus, all five of these Daf-c genes lie at a single place in the dauer pathway, and the mutants that previously were interpreted as distinguishing them lie either upstream (*daf-6*) or downstream (*daf-16*, *daf-18* and *daf-20*).

Several considerations lead us to believe that our analysis more closely approximates the truth. The previously reported epistasis results were inferred by analysis of segregation of progeny classes from heterozygous parents. Each Ts Daf-c mutation was linked to one or more recessive marker mutations, and crossed to a Daf-d mutant. Double heterozygotes of genotype *daf-c marker*/+ +; *daf-d*/+ were identified at permissive temperature and fifteen marked progeny of this parent were picked to restrictive temperature. These marked (presumptive *daf-c* homozygote) animals were observed for the segregation among their progeny of suppressed (non-dauer) animals. If non-dauer progeny were observed in many of the broods, the Daf-d mutation was inferred to be epistatic to the Daf-c mutation. If no non-dauer forming progeny were observed in any of the broods, the Daf-c mutation was inferred to be epistatic. In a few cases, when the Daf-c and Daf-d mutations were genetically linked, the double mutants were constructed by a directed recombination method. This method for epistasis testing has some difficulties of interpretation, a few of which we mention here. First, the marker mutations used might influence the outcome of the test. Second, lethal interactions between the Daf-d and Daf-c mutations or maternal rescue of the Daf-d mutation can easily confound the results. Third, the animals scored for dauer formation were not synchronized in growth, making it difficult to distinguish between failure to form dauers and premature recovery from a transient dauer state. Finally, it is difficult or impossible to engage in a detailed observation of the double mutant phenotype or confirmation of the double mutant genotype since the double mutant is never actually isolated and grown as a homozygote for both mutations.

Of the difficulties listed above, the last three prob-

Downloaded from https://academic.oup.com/genetics/article/130/1/105/6007131 by guest on 23 April 2024

ably contribute to the differences between our findings. The difficulty of detailed phenotype determination is likely to explain our different assignment of *daf-16*, *daf-18* and *daf-20*. Mutations in each of these genes, although previously characterized as dauer defective, actually result under dauer-inducing conditions in the production of animals with some clear dauer-like characters, but lacking others. Since these partial dauers are formed in response to all of the upstream dauer constitutives (there are a few exceptions for *daf-20*, see below for discussion), we infer that they act late in the dauer-forming pathway. The difficulty, when analyzing asynchronous populations of animals, in distinguishing between a dauer entry defect and the formation of transient dauers is likely to explain several other points of difference. Many of the double mutants between *daf-1*, *daf-4*, *daf-7*, *daf-8*, *daf-11* and *daf-14* and the partial-dauer genes *daf-16*, *daf-18* and *daf-20* form partial dauers with high efficiency (Table 5) but a substantial fraction of these dauers quickly exit the dauer state (often within 24 hr) and grow to adulthood, even when kept at 25°. In the previous study this result probably would have been scored as suppression, since some animals in the asynchronous brood would recover from the partial dauer state and appear to have escaped dauer formation. Since our epistasis pathway reflects the genetic interactions leading to dauer formation, rather than recovery from the dauer state, the formation of transient dauers should be interpreted as dauer constitutivity. We have not undertaken systematic quantitative study of the phenomenon of premature recovery, but we have found that it is restricted to the partial-dauer double mutants and is variable depending both on the Daf-c and the partial-dauer genes in the particular double mutant. This variability may explain why the *daf-16*, *daf-18* and *daf-20* genes appeared to "suppress" some dauer-constitutive mutations and not others (RIDDLE, SWANSON and ALBERT 1981). The fact that some of these dauers recover from the dauer state suggests that the pathway for dauer entry is not identical to that for dauer exit. Finally, we find that one double mutant *daf-2; daf-12* is neither dauer forming nor L3 forming at restrictive temperature for *daf-2*, instead the animals arrest at the first or second larval stage. This fact explains why *daf-12* did not appear as a suppressor of *daf-2* (RIDDLE, SWANSON and ALBERT 1981).

A few of our conflicts cannot be explained by these differences in our studies. *daf-11* was previously determined to be epistatic to *daf-10* (RIDDLE, SWANSON and ALBERT 1981) whereas we find the opposite result. Our ordering of these two genes has been independently confirmed (D. RIDDLE, personal communication). *daf-6* was previously reported to be downstream of *daf-7*, *daf-8* and *daf-14* (RIDDLE, SWANSON and ALBERT, 1981). We find mutations in all three of these Daf-c genes to be fully epistatic to *daf-6*.We are confident of the genotype of our double mutants since we can independently test the presence of *daf-6* by its osmotic-avoidance and FITC-filling defects. Because the double mutants in the previous study were never actually constructed and retained as a strain, the genotypes and phenotypes can not be rechecked for errors. We also note that the suppression of *daf-8* and *daf-14* by *daf-6* was reported to be quite weak (RIDDLE, SWANSON and ALBERT 1981). In addition to these difficulties, the previous study used only single mutations of each gene. In our study, whenever possible, second alleles of genes were used to confirm functional order (Tables 1, 3 and 5). However only one mutation exists for some of the genes, and for other genes second alleles were not available to us for this study. Until at least second mutations in all genes can be tested, and preferably known null mutations, the pathway shown in Figure 4 must be considered tentative.

The downstream genes: *daf-16* and *daf-18* are inferred to be downstream steps in the pathway for two reasons. First, mutations in each gene block the formation of only some dauer tissues. Assuming the mutations in these genes are phenotypically strong or null, these genes are presumably required for the differentiation of the affected tissues rather than for the induction of dauer formation *per se*. Second, mutations in each gene show their characteristic block of particular dauer tissues in double mutants with all the Daf-c genes. We confirmed the placement of *daf-16* as downstream of *daf-12* by building the triple mutant *daf-16; daf-12; daf-7* with each allele of *daf-16* (the dauer-constitutive mutation in *daf-7* was included to conveniently activate the dauer pathway). Both triple mutants fail to form partial dauers (data not shown), indicating that *daf-12* blocks the induction of partial dauer formation in these mutants.We interpret these genes as components of the tissue specific differentiation events that occur in response to activation of the dauer pathway. *daf-20* probably also belongs in this class of genes, but its placement is less certain. *daf-20* causes the production of partial dauers, like *daf-16* and *daf-18*, but it also prevents formation of dauers in double mutants with *daf-8*, *daf-11* and *daf-14*. Since *daf-20* mutants form partial dauers, suggesting a downstream step in the pathway, and no other data support the interpretation that *daf-8* and *daf-14* lie upstream of *daf-1*, *daf-4* and *daf-7*, we have refrained from interpreting this result.

We place the partial-dauer forming genes *daf-16* and *daf-18* in the pathway in Figure 4 on the basis of their partial blocks of the pathway. The simplest explanation of this phenotype is that the genes function downstream in a branched part of the pathway. The

Downloaded from https://academic.oup.com/genetics/article/130/1/105/6007131 by guest on 23 April 2024

placement of each gene on a separate downstream branch is not intended to have functional significance, since we have not investigated how the branches controlled by each gene are related to each other. We also note that the phenotype of most of the *daf-16* and *daf-18* (as well as *daf-20*) double mutants with Daf-c mutations (Table 5) is partial-dauer formation under noninducing conditions, a phenotype distinct from that of either single mutant. This result can be interpreted in various ways, including independent functions (HEREFORD and HARTWELL 1974), a branched pathway, or more complex relations. Yet another alternative explanation for *daf-16*, *daf-18* and *daf-20* is that the mutations analyzed are not null, and that the tissues affected are simply those most sensitive to partial loss of gene function. Clearly these genes require further analysis in order to adequately test their role in the model presented in Figure 4.

Mutations in two genes not analyzed in this study, *daf-9* and *daf-15*, cause the constitutive production of dauer-like larvae (ALBERT and RIDDLE 1988). Electron microscopy indicates that arrested *daf-9* larvae have a dauer-like cuticle and hypodermis, but pharyngeal pumping continues sporadically (ALBERT and RIDDLE 1988). These animals may be similar to those produced by pheromone treatment of *daf-16* and *daf-18* mutants, but direct comparison is difficult because different criteria for tissue type were applied in the two studies. Arrested *daf-15* larvae have non-dauer cuticle and hypodermis (ALBERT and RIDDLE 1988) and thus are clearly phenotypically distinct from mutants analyzed in this study.

A model for the genetic and cellular pathway for dauer formation: The pathway reported here is considerably simpler and to us seems more biologically sensible than that reported previously. The number of negative regulatory steps has been drastically reduced, because many genes are now seen to lie at single steps in a relatively short pathway. Our formal genetic pathway also fits well with the findings in a study of the sensory cells that control dauer formation (BARGMANN and HORVITZ 1991). This study found that killing three amphid sensory neurons, ADF, ASI and ASG, causes a dauer-constitutive phenotype. This laser-induced dauer-constitutive phenotype is independent of dauer pheromone, since the same treatment causes dauer formation in the pheromoneless *daf-22* mutant (BARGMANN and HORVITZ 1991).The authors suggest that ADF, ASI and ASG act to repress dauer formation in the absence of dauer-inducing conditions, and that dauer-inducing conditions derepress dauer formation by acting through these three neurons. This model provides a partial cellular basis for our genetic pathway findings. We propose that the nine genes required for normal cilium structure prevent dauer-inducing conditions from derepressing dauer formation, presumably because the sensory endings fail to function properly. This model is supported by the fact that when ADF, ASI and ASG are killed in cilium structure-defective mutants, dauers are formed under noninducing conditions (BARGMANN and HORVITZ 1991; our unpublished data). We propose that the *daf-11(+)* gene product acts in the sensory endings to block dauer formation, and that dauer-inducing conditions act on the sensory cells by inhibiting the function of *daf-11*.

The phenotype caused by killing ADF, ASI and ASG has been tested in various dauer-defective mutants, in essence providing a partial epistasis analysis for the laser-induced Daf-c phenotype (BARGMANN and HORVITZ 1991). This analysis in general fits well with our results. With one exception, the laser kill fits into the genetic pathway at the same position as the Daf-c genes *daf-1*, *daf-4*, *daf-7*, *daf-8* and *daf-14* (BARGMANN and HORVITZ 1991). This coincidence is intriguing, since the repression of dauer formation by ADF, ASI and ASG presumably must act through a set of gene products. These five Daf-c genes are good candidates to encode functions required for the repression of dauer formation by ADF, ASI and ASG, either presynaptically or postsynaptically. The one possible exception to the good fit between our findings and those of BARGMANN and HORVITZ (1991), is *daf-6*, which we find to be upstream in the genetic pathway, but which showed inexplicable variability in response to ADF, ASI and ASG kills. Further experiments are required to determine the true position of *daf-6* with respect to the cell kills.

In addition to the genes tentatively ordered in a pathway in Figure 4, some reasonable, but less conclusive, inferences can be made about other genes in the dauer pathway. *daf-3* and *daf-5* probably lie at a similar position in the pathway as *daf-12*, but two considerations make their placement somewhat uncertain. First, all three mutations available to us, two in *daf-5* and one in *daf-3*, block dauer formation incompletely. Both in response to strong dauer-inducing conditions (starvation on a crowded plate at 25°), and in double mutants with certain Daf-c mutations, they produce a small percentage of dauers (Table 3). In particular, the double mutants with two different *daf-11* mutations and with *daf-8* produce an anomalously high percentage of dauers, despite other evidence that *daf-11* is the most upstream Daf-c gene. The *daf-12* mutation analyzed, in contrast, is completely penetrant in its block of dauer formation as a single mutant and in all double mutants. The second way *daf-3* and *daf-5* differ from *daf-12* concerns the formation of the second-larval predauer stage, L2d (GOLDEN and RIDDLE 1984a). *daf-12* mutants in response to dauer pheromone and *daf-12* double mutants with all upstream Daf-c mutations efficiently produce what appear to be

Downloaded from https://academic.oup.com/genetics/article/130/1/105/6007131 by guest on 23 April 2024

normal L2d animals, but these L2ds fail to produce dauers at the L2 molt. Presumably *daf-12* is required for the progression from L2d to dauer, but not from the L1 to L2d. In contrast, *daf-3* and *daf-5* block L2d formation in addition to dauer formation. Since dauer formation in the wild type is dependent on passage through the L2d stage (GOLDEN and RIDDLE 1984a), the defect in dauer formation in *daf-3* and *daf-5* mutants may be in L2d formation. It would therefore be potentially confusing to show these genes acting at the same step in the pathway as *daf-12*.

Interestingly, mutations in both *daf-3* and *daf-5* suppress the egg-laying defect of *daf-1*, *daf-7*, *daf-8* and *daf-14*. The *daf-12* mutation shows no sign of suppressing the egg-laying defect of any of these mutants, despite its stronger dauer-formation block. This result, together with the dauer epistasis results, suggests that *daf-3* and *daf-5* are probably best interpreted as affecting the same formal step as *daf-12*, but with some clear difference between their roles. One model would be that *daf-3* and *daf-5* together function in the L1 to L2d progression in a manner similar to *daf-12* for the L2d to dauer progression. The suppression of the egg-laying defect of *daf-1*, *daf-7*, *daf-8* and *daf-14* mutations by *daf-3* and *daf-5* mutations suggests that all of these genes play a second role in sensory regulation of egg laying, and that they function in this second role in the same relative order as for dauer formation.

As found previously (RIDDLE, SWANSON and ALBERT 1981), *daf-2* is difficult to interpret as a component of the simple linear part of the dauer pathway. For example, some combinations of *daf-2* mutations with *daf-16* and *daf-18* mutations fail to form even partial dauers, suggesting that *daf-2* might affect only a downstream branch of the pathway that is controlled by *daf-16* and *daf-18*. However, *daf-2* single mutant dauers are fully dauer-like for all features we can score. *daf-2* also shows a strong lethal interaction with the *daf-12* mutation, and a weaker lethal interaction with the cilium-structure mutations, interactions unique among the Daf-c genes. Finally *daf-2* mutations are fully epistatic to *daf-3* and *daf-5* mutations, but not to the *daf-12* mutation, which is unique among dauer constitutives. This result might suggest that *daf-2* splits the *daf-3/5* step from *daf-12*, with *daf-3* and *daf-5* upstream, except that the *daf-2* suppression by *daf-16* and *daf-18* then becomes inexplicable. In short, it is currently not possible to fit *daf-2* into the simple linear pathway shown in Figure 4. Its function clearly enters fairly far downstream in the dauer pathway, but the precise role that *daf-2* plays may be different or more complex than for the other genes.

We thank SHOSHANNA GOTTLIEB and GARY RUVKUN for communicating unpublished data suggesting that *m26* and *m27* are allelic.We thank CORI BARGMANN, LEON AVERY, LEE HARTWELL, BARBARA WAKIMOTO and BONNIE BREWER for helpful discussions of the dauer pathway, and LEON AVERY and CHRISTINE STEWART for critical reading of the manuscript. We thank DON RIDDLE and PATRICE ALBERT for providing many of the mutations used in this study. This work was supported by the Searle Scholars Program/The Chicago Community Trust and by U.S. Public Health Service research grant GM39868.

LITERATURE CITED

ALBERT, P. S., AND D. L. RIDDLE, 1988 Mutants of *Caenorhabditis elegans* that form dauer-like larvae. Dev. Biol. **126:** 270–293.

ALBERT, P. S., S. J. BROWN and D. L. RIDDLE, 1981 Sensory control of dauer larva formation in *Caenorhabditis elegans*. J. Comp. Neurol. **198:** 435–451.

BARGMANN, C., and H. R. HORVITZ, 1991 Control of larval development by chemosensory neurons in *Caenorhabditis elegans*. Science **251:** 1243–1246.

BARGMANN, C., J. H. THOMAS and H. R. HORVITZ, 1990 Chemosensory cell function in the behavior and development of *Caenorhabditis elegans*. Cold Spring Harbor Symp. **55:** 529–538.

BRENNER, S., 1974 The genetics of *Caenorhabditis elegans*. Genetics **77:** 71–94.

CASSADA, R., and R. L. RUSSELL, 1975 The dauerlarva, a post-embryonic developmental variant of the nematode *Caenorhabditis elegans*. Dev. Biol. **46:** 326–342.

CULOTTI, J. G., and R. L. RUSSELL, 1978 Osmotic avoidance defective mutants of the nematode *Caenorhabditis elegans*. Genetics **90:** 243–256.

GOLDEN, J., and D. L. RIDDLE, 1982 A pheromone influences larval development in the nematode *Caenorhabditis elegans*. Science **218:** 578–580.

GOLDEN, J., and D. L. RIDDLE, 1984a The *Caenorhabditis elegans* dauer larva: Developmental effects of pheromone, food and temperature. Dev. Biol. **102:** 368–378.

GOLDEN, J., and D. L. RIDDLE, 1984b A pheromone-induced developmental switch in *Caenorhabditis elegans*: Temperature-sensitive mutants reveal a wild-type temperature-dependent process. Proc. Nat. Acad. Sci. **81:** 819–823.

GOLDEN, J., and D. L. RIDDLE, 1984c A *Caenorhabditis elegans* dauer-inducing pheromone and an antagonistic component of the food supply. J. Chem. Ecol. **10:** 1265–1280.

GOLDEN, J., and D. L. RIDDLE, 1985 A gene affecting production of the *Caenorhabditis elegans* dauer-inducing pheromone. Mol. Gen. Genet. **198:** 534–536.

HEDGECOCK, E. M., J. G. CULOTTI, J. N. THOMSON and L. A. PERKINS, 1985 Axonal guidance mutants of *Caenorhabditis elegans* identified by filling sensory neurons with fluorescein dyes. Dev. Biol. **111:** 158–170.

HEREFORD, L. M., and L. H. HARTWELL, 1974 Sequential gene function in the initiation of *Saccharomyces cerevisiae* DNA synthesis. J. Mol. Biol. **84:** 445–461.

HERMAN, R. K., 1984 Analysis of genetic mosaics of the nematode *Caenorhabditis elegans*. Genetics **108:** 165–180.

HORVITZ, H. R., S. BRENNER, J. HODGKIN and R. K. HERMAN, 1979 A uniform genetic nomenclature for the nematode *Caenorhabditis elegans*. Mol. Gen. Genet. **175:** 129–133.

LEWIS, J. A., and J. A. HODGKIN, 1977 Specific neuroanatomical changes in chemosensory mutants of the nematode *Caenorhabditis elegans*. J. Comp. Neurol. **172:** 489–510.

PERKINS, L. A., E. M. HEDGECOCK, J. N. THOMSON and J. G. CULOTTI, 1986 Mutant sensory cilia in the nematode *Caenorhabditis elegans*. Dev. Biol. **117:** 456–487.

Downloaded from https://academic.oup.com/genetics/article/130/1/105/6007131 by guest on 23 April 2024

RIDDLE, D. L., 1977 A genetic pathway for dauer larva formation in *Caenorhabditis elegans*. Stadler Genet. Symp. **9:** 101–120.

RIDDLE, D. L., M. M. SWANSON and P. S. ALBERT, 1981 Interacting genes in nematode dauer larva formation. Nature **290:** 268–271.

STERNBERG, P., and H. R. HORVITZ, 1986 Pattern formation during vulval development in *Caenorhabditis elegans*. Cell **44:** 761–772.

SULSTON, J. E., and H. R. HORVITZ, 1977 Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans*. Dev. Biol. **56:** 110–156.

SULSTON, J. E., D. G. ALBERTSON and J. N. THOMSON, 1980 The *Caenorhabditis elegans* male: postembryonic development of nongonadal structures. Dev. Biol. **78:** 542–576.

TRENT, C., N. TSUNG and H. R. HORVITZ, 1983 Egg-laying defective mutants of the nematode *Caenorhabditis elegans*. Genetics **104:** 619–647.

WARD, S., N. Thomson, J. G. White and S. BRENNER, 1975 Electron microscopical reconstruction of the anterior sensory anatomy of the nematode *Caenorhabditis elegans*. J. Comp, Neurol. **160:** 313–338.

WARE, R. W., D. CLARK, K. CROSSLAND and R. L. RUSSELL, 1975 The nerve ring of the nematode *Caenorhabditis elegans*: sensory input and motor output. J. Comp. Neurol. **162:** 71–110.

WHITE, J. G., E. SOUTHGATE, J. N. THOMSON and S. BRENNER, 1986 The structure of the nervous system of the nematode *Caenorhabditis elegans*. Phil. Trans. R. Soc. Lond. **314:** 1–340.

Communicating editor: R. K. HERMAN

Downloaded from https://academic.oup.com/genetics/article/130/1/105/6007131 by guest on 23 April 2024