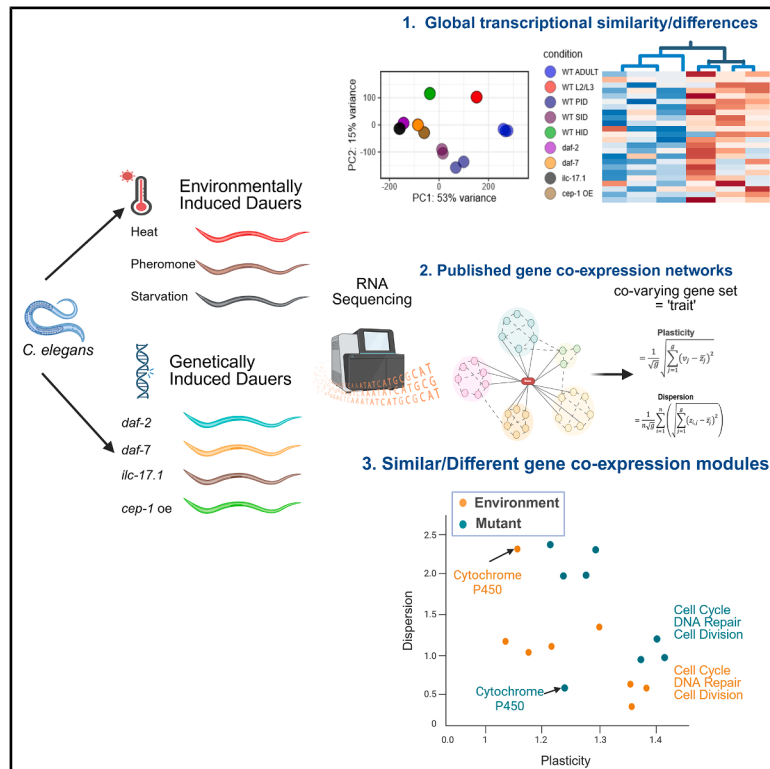


Pathway-specific canalization and plasticity of gene expression during *C. elegans* dauer development

Graphical abstract



Authors

Johnny Cruz Corchado,
Kavinila Selvarasu, Veena Prahlad

Correspondence

johnny.cruzcorchado@roswellpark.org
(J.C.C.),
veena.prahlad@roswellpark.org (V.P.)

In brief

Developmental biology; Genetics;
Molecular biology; Omics

Highlights

- Analysis of >100 co-varying gene sets (traits) in seven different *C. elegans* dauers
- Transcriptomes and traits differ depending on the dauer-inducing stimulus
- Traits were less conserved among environmentally induced wild-type dauers
- Cell cycle and DNA repair gene expression conserved; cytochrome P450 most variable



Article

Pathway-specific canalization and plasticity of gene expression during *C. elegans* dauer development

Johnny Cruz Corchado,^{1,2,*} Kavinila Selvarasu,¹ and Veena Prahlad^{1,*}¹Department of Cell Stress Biology, Roswell Park - Comprehensive Cancer Center, Elm and Carlton Streets, CGP-BLSC L3-307, Buffalo, NY 14263, USA²Lead contact*Correspondence: johnny.cruzcorchado@roswellpark.org (J.C.C.), veena.prahlad@roswellpark.org (V.P.)<https://doi.org/10.1016/j.isci.2026.115058>

SUMMARY

How robustness and plasticity—mechanisms that restrain or promote variation—interact to generate faithful developmental outcomes remains unclear. *Caenorhabditis elegans* development, where different environmental and genetic stimuli each can induce larvae to switch from continuous growth to a seemingly identical dauer (dormancy) state, provides a tractable model to explore this question. To identify dauer features that differ or are invariant, we compared gene expression of seven dauers generated by different dauer-inducing conditions, globally and within >100 sets of curated covarying genes or “modules” enriched for functional features such as “protein expression,” “mitochondria,” “germline,” etc., through gene co-expression network analyses. We found that most modules varied between dauers. Yet, a subset of modules associated with dormancy—DNA repair, cell cycle, and cell division—were invariant. We propose that the robust expression of genes that regulate a few core traits governing dormancy accommodates variation in others, supporting dauers’ adaptability and resilience.

INTRODUCTION

Robustness (canalization) and phenotypic plasticity are pervasive features of developmental programs. Canalization—the remarkable tendency for a specific genotype to generate an invariant phenotype, even when environmental conditions or genetic backgrounds vary—preserves developmental integrity.^{1–5} Phenotypic plasticity—the process by which a single genotype can yield multiple phenotypes—allows organisms to adapt their physiology, metabolism, and morphology to a changing and unpredictable environment.^{6,7} How plasticity mechanisms that promote flexible responses to stimuli and canalization mechanisms that limit variability interact to shape phenotypic outcomes remains poorly understood.^{2,3,7–11} Indeed, depending on the timing, regulatory nodes, and transcriptome dynamics, canalization mechanisms can buffer variations to limit plastic responses to stimuli,^{4,7,10–17} or act cooperatively with mechanisms that induce phenotypic plasticity to generate and stabilize the discrete phenotypic outcomes that arise in response to a continuously varying stimulus.^{6,11,18–23}

Here, we utilized the dauer stage in *Caenorhabditis elegans*, an alternative to the larval stage 3 (L3), as an excellent system to explore how canalization and plasticity mechanisms shape the gene expression landscape to produce the discrete and stable dauer outcome. The dauer arrest is an example of extreme phenotypic plasticity.^{24–33} The decision to enter dauer state, instead of developing continuously into the adult stage, is trig-

gered during larval stage 1 (L1) by several independent, unfavorable environmental conditions such as starvation, crowding, or extreme temperatures. Seminal studies have shown that the decision to enter dauer state is triggered by the loss of growth-promoting signals required to license continued development.^{24–33} Thus, when food is abundant, insulins act through the sole insulin-like receptor DAF-2 in the insulin signaling pathway (ILS) to antagonize the Forkhead transcription factor DAF-16/FOXO and permit continuous growth; food scarcity inhibits DAF-2 signaling, activates DAF-16, and triggers dauer entry.^{24,26,32,34} Likewise, DAF-7 antagonizes the DAF-3/SMAD-DAF-5/Ski transcription factor complex by acting through TGF- β receptors, DAF-1/DAF-4, to permit continuous growth under favorable conditions. Inhibition of DAF-7 signaling, which occurs, for example, under high population densities, activates DAF-3/SMAD-DAF-5/Ski and leads to dauer stage arrest.^{24,26,30,32,35} In addition, the recently discovered cytokine interleukin IL-17 ortholog, ILC-17.1, is another growth-promoting signal, whose loss triggers dauer arrest by activating the *C. elegans* p53 ortholog p53/CEP-1 but also requires DAF-16/FOXO, DAF-3/SMAD-DAF-5/Ski transcription factors and can be mimicked by overexpressing p53/CEP-1 (p53/CEP-1 OE).³⁶

The dauer signaling pathways act as redundant switches to initiate dauer (for example, the ILS/DAF-2 and TGF- β /DAF-7 pathways are genetically independent). Yet, all pathways funnel through a common node—the steroid hormone signaling pathway—where the nuclear hormone receptor DAF-12, which



responds to the steroid hormone ligand DAF-9 produced by cytochrome P450 (CYP450), under favorable environments, promotes continuous growth, and when absent, triggers dormancy.^{24,32,37–39} As a result, dauer larvae induced by different environmental means or genetic mutations share similar physiological, metabolic, behavioral, and growth-arresting mechanisms that postpone growth under unfavorable conditions. However, several recent studies have shown that dauers induced by different stimuli also vary in many respects.^{40–44} For instance, the stereotypic hitchhiking behavior differs across different dauer types,⁴² there is natural variation in nictation behavior,^{43,45} and like other developmental processes, dauer development is also variable.⁴⁴ Furthermore, while the dauer stage itself has been defined based on the acquisition of specific characteristics like detergent tolerance, cessation of pharyngeal pumping, development of specialized cuticular structures called alae, and unique behaviors such as nictation, the dauers continue to respond to stimuli and alter gene expression and physiology, even when dormant.^{46,47} In addition, although the dauer stage has retained its critical role in the life history across nematode species, it has evolved important differences.^{31,48,49} For instance, the mechanisms of activation or inhibition of the *Pristionchus pacificus* steroid hormone pathway appear to differ,⁵⁰ copy number variation and gene duplications in the orphan nuclear hormone receptors suggest that DAF-12 function may be fast-evolving,⁵¹ and the role of the TGF- β pathway in dauer formation is not conserved.^{49,52} These similarities and differences between dauers, both within *C. elegans* and across related species, raise open questions^{33,53,54} as to which (if any) traits are conserved in all dauers, whether dauer phenotypes or traits vary depending on the dauer-inducing mechanism, and how the signaling pathways that promote dauer formation and generate stereotypic dauer characteristics, also, in some cases, yield incomplete dauers that vary in their expression of specific dauer features.^{32,37,55–58} In addition, because dauer-inducing stimuli and pathways have pleiotropic effects on aspects of organismal physiology unrelated to dauer entry, it is unclear whether the gene expression changes induced by these pathways en route to dauer arrest are retained during the dauer stage, or whether dauer arrest resets these gene expression differences to a more “similar,” conserved, or vestigial state.

Here, we aimed to address these key questions. First, we asked how the route into dauer influences dauer gene expression. To answer this, we sequenced mRNA from seven different dauers induced either by environmental (temperature, starvation, and pheromone) or genetic (ILS, TGF- β , cytokine/ILC-17.1, and CEP-1/p53) stimuli and compared their gene expression patterns. Next, we asked which biological or functional features of dauers were more or less variable. For this, we leveraged published data from gene co-expression analyses (e.g., gene expression “mountains” identified by Kim et al. (2001)⁵⁹, and “metabolic pathways” from iCEL1314 compiled by Nanda et al. (2023),⁶⁰ and identified over 100 gene sets or modules,^{59–61} each containing genes that were spatially and temporally co-regulated and enriched for pathways such as “protein expression,” “mitochondria,” “germline,” “fatty acid degradation,” etc., and examined which modules were most conserved and

which were most variable across the different dauer types (e.g., environmental or genetic). To compare modules across dauer types, we introduced two metrics: dispersion index, which is a measure of the similarity (canalization) of a module between sets of dauers, and plasticity index, which captures gene expression changes that occurred in that module during dauer entry (see [results](#) and [STAR Methods](#)). Finally, to examine how the mechanisms of robustness and plasticity interacted to shape gene expression during dauer development, we evaluated whether gene co-expression modules that underwent the most or least plastic gene expression changes during dauer transition were more or less variable (dispersed) among dauers.

Our studies show that the dauers generated by different mechanisms differ in their genome-wide gene expression patterns and in the expression of genes within the various modules. Nevertheless, a small subset of modules that control cell cycle progression (DNA repair, cell cycle, and cell division) were conserved across all dauers. We also found that the constraints over the expression of genes within the different modules appeared to differ based on the dauer-inducing stimulus. In dauers triggered to arrest through genetic means, modules that were the most plastic (i.e., had changed the most during dauer entry) were also the most conserved. In contrast, among the environmentally induced wild-type dauers, canalization and plasticity of modules were either uncoupled or inversely correlated, and gene expression was generally less canalized and less plastic than in the genetically induced dauers. This suggests that dauer entry through genetic triggers may minimize gene expression variability, whereas environmental triggers may result in larger variability and a more graded change in gene expression related to the different dauer features (albeit there are caveats; see [results](#)). Intriguingly, key modules involving genes integral to the dauer pathway—CYP450 family of genes, DAF-12, DAF-16, and “insulins”—were among the most variable modules in environmentally induced wild-type dauers, whereas they were more conserved in genetically induced dauers. Together, these findings led us to propose that the transcriptomic architecture of dauer larvae, in which gene expressions within a limited set of core cellular modules central for dormancy progression are held invariant and sufficient to support the dauer phenotype, might allow dauers to accommodate and retain larger variability in gene expression caused by different routes into dauer, which enable their remarkable diversity and adaptability.

RESULTS

Dauer larvae generated through different triggers

C. elegans larvae were induced to arrest as dauers through different means and harvested for RNA sequencing ([Figure 1A](#)). We used seven different dauer triggers: three environmental stimuli (starvation, pheromone, and high temperatures),²⁹ and four genetic stimuli (loss of function mutations in *daf-2* (*e1370*) *III* [ILS pathway], *daf-7* (*e1372*) *III* [TGF- β pathway], *ilc-17.1* (*syb5296*) *X* [cytokine IL-17 pathway], and overexpression of *cep-1* [*cep-1 OE*]).^{26,29,30,36} Starvation-induced dauers (SIDs) were generated by allowing wild-type animals to exhaust their bacterial food at 20°C for over 12 days. Pheromone-induced dauers (PIDs) were generated by allowing defined numbers of

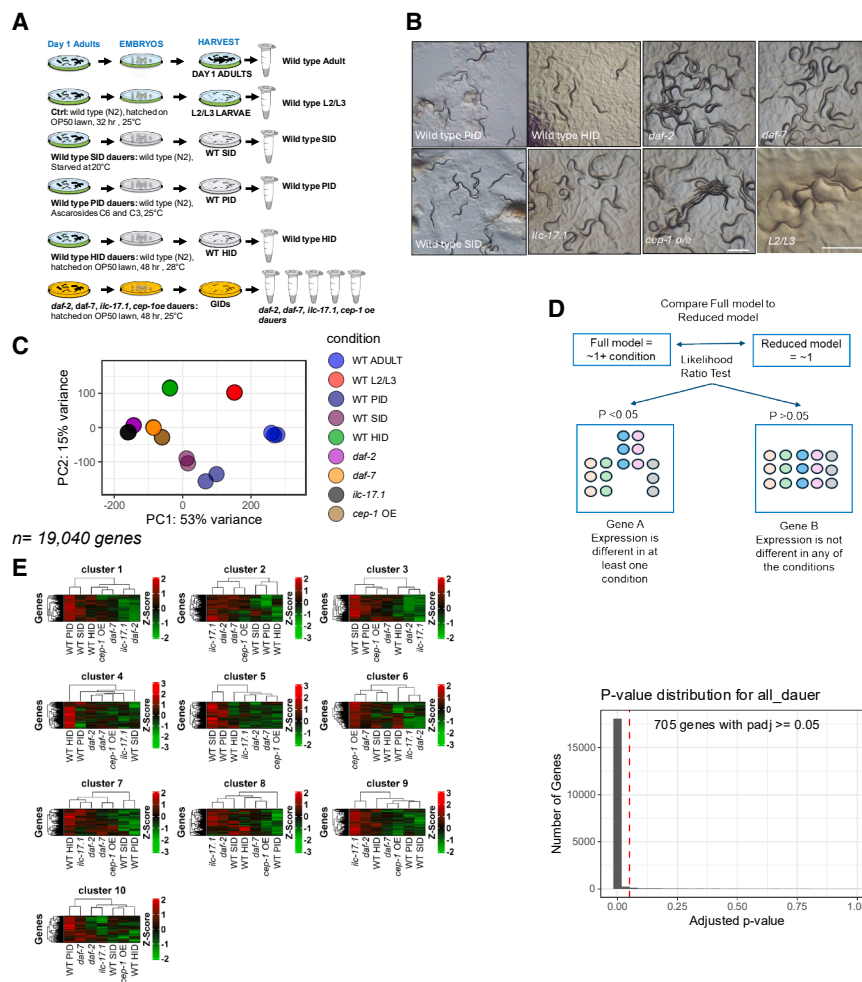


Figure 1. Dauers induced through different genetic or environmental means are transcriptionally distinct
(A) Schematic of the experimental design: Embryos from day-one adults (WT-adults) grown at 20°C were bleached-synchronized and induced to arrest as dauers or harvested at L2/early L3 stage, or as WT-adults for RNA-sequencing. Dauers: (1) High temperature-induced dauers (HIDs): wild-type (N2) HIDs or wild-type HIDs generated by growth at 27°C for 48 h. (2) Pheromone-induced dauers (PIDs): wild-type (N2) PIDs or wild-type PIDs, generated by growth at 25°C in the presence of pheromones for 10–12 days. (3) Starvation-induced dauers (SIDs): wild type (N2) SIDs or wild-type SIDs, generated by growth at 20°C until food exhaustion for 12 days. (4) *daf-2* (*e1370*) III. (5) *daf-7*(*e1372*) III. (6) *ilc-17.1* (*syb5296*) X dauers. (7) *cep-1* overexpressing (OE) dauers, generated by growth at 25°C for 48 h, post-hatching. Wild-type (N2) L2/L3 larvae that were grown for 32 h post-hatching at 25°C, and WT-adults were grown for 48–52 h post-hatching. Samples were collected as described in the text. (B) Micrographs of dauer larvae and WT (N2) L2/L3. Scale bars, 1 mm. (C) Principal component analysis (PCA; VST-scaled data) of the RNA-sequencing data from three repeats of WT-adults, WT L2/L3, WT HID, *daf-2* (*e1370*) III, *daf-7*(*e1372*) III, *ilc-17.1* (*syb5296*) X, and *cep-1* (*cep-1* OE) and two replicates of WT PID and WT SID. Many of the replicates occupy overlapping positions and are not visible in this PCA plot. Genes included in the PCA: $n = 19,040$. (D) Top: Schematic of likelihood ratio test (LRT). Gene expression in each of the seven dauers was subjected to an LRT test under the null hypothesis that dauer gene expression was similar. Bottom: Histogram with adjusted p value (Holm) results of LRT.

wild-type embryos to hatch and develop in the presence of ascarosides (a mixture of ascarosides C6 and C3) for 10–12 days.⁵⁸ Heat-induced dauers (HIDs) were generated by allowing wild-type embryos to hatch and develop for 48 h at 27°C. Genetically induced dauers, *daf-2* (*e1370*) III, *daf-7* (*e1372*) III, *ilc-17.1* (*syb5296*) X, and *cep-1* OE backgrounds, were generated by allowing embryos to develop at 25°C for 48 h following hatching. Dauer arrest in all strains was confirmed phenotypically and through resistance to 1% SDS.²⁶ We chose to harvest dauers at the earliest time when >99% of the genetically induced population and approximately 75% of the environmentally induced population had entered the dauer stage. The larvae that escaped dauer arrest in all populations were either visible as larval stage 4 (L4) and manually removed prior to harvesting for mRNA extraction, or, as in the case of SIDs and PIDs, separated by fractionation following detergent solubilization (1% SDS) of non-dauers. Wild-type larvae grown at 25°C for 30–32 h to larval stage 2/very early larval stage 3 (L2/L3) were used for a comparison, considering that they represented a

developmentally equivalent stage to dauers. Wild-type day-one animals were harvested as the endpoint of wild-type development.

Gene expression differs between dauers

A sample distance matrix produced by comparing expression levels across all genes between the different dauers, the continuously growing wild-type L2/L3 (WT L2/L3), and adult samples demonstrated excellent agreement among biological replicates (Figure S1A). Dauers induced by these different methods showed strong phenotypic similarities and differed from L2/L3 (Figure 1B), as could be expected.^{26,27,30} A pairwise Pearson's distance matrix (1-Pearson's correlation coefficient, PCC; Figure S1B) and Spearman's correlation tests (Figure S1C) indicated that all the samples shared a strong linear and monotonic relationship ($\rho = 0.80$ to >0.9). Perhaps, because of the clonal nature of *C. elegans*, determinate development of this species, and tendency of many genes to not be regulated dramatically at the expression level, this was also true for WT L2/L3 and adults

(Figure S1C). Nevertheless, despite these strong co-linear expression patterns,⁶² dauers generated through the different genetic and environmental triggers were transcriptionally different and differed from WT L2/L3 and day-one adults (WT-adults). This was evident in a principal component analysis (PCA) using all genes (19,040 genes filtered to exclude low expression of <10 counts; see STAR Methods; Figure 1C). The different dauer larvae occupied distinct regions in transcriptional space and separated from each other, with WT-L2/L3 and WT-adults segregating along the first principal component (PC1), which captured most (53%) of the variance. Dauers separated among themselves along the second principal component (PC2), which captured 15% of the variance between the samples (Figure 1C). Notably, the wild-type dauers were more dispersed from one another, with the PIDs and SIDs exhibiting more similarity and marked differences from the HIDs. The genetically induced dauers were closer in gene expression space and separated into two groups: *daf-2* and *ilc-17.1*, and *daf-7* and *cep-1* OE. A PCA of only the dauers also confirmed these relationships (Figure S1D). A likelihood ratio test (LRT) for hypothesis testing further indicated that the expression of the majority of genes was dissimilar among the dauers: the null hypothesis, namely that all dauers were similar ($p_{adj} < 0.05$) in gene expression patterns, was rejected for most genes and found to be valid only for 705 of the 19,040 genes (Figure 1D and Table S1).

To identify shared pathways between dauers, we conducted unsupervised hierarchical clustering of gene expression (rlog) in all dauers (Figures 1E and S2A; Table S2) and also separately in environmentally induced, wild-type dauers (SIDs, PIDs, and HIDs), and genetic dauers (*daf-7*, *daf-2*, *ilc-17.1*, and *cep-1* OE) (Figures S2B–S2E; Tables S3 and S4). This analysis, when conducted for all dauers, further supported the existence of different dauer types but did not provide a clear understanding of pathway similarities or differences. Genes expressed in all dauer types separated optimally into ten clusters (Figure S2A), and different dauers shared expression patterns within different, overlapping clusters enriched for different gene ontology (GO) pathways (Figure S3 and Table S5). Surprisingly, we did not observe clustering of *daf-2* with SIDs or *daf-7* with PIDs, as we had expected, given the roles of ILS in food sensing and TGF- β in the pheromone response during dauer induction. Likewise, the *cep-1* OE dauers and *ilc-17.1* dauers did not cluster together, which is also surprising because *cep-1* functions in an epistatic relationship to *ilc-17.1*³⁶ (Figure 1E and Table S5).

Hierarchical clustering of wild-type (Figures S2B and S2D) or genetically induced dauers (Figures S2C and S2E) separately was more informative. The best-studied wild-type dauers, PIDs, and SIDs shared similar expression of genes in clusters 4, 5, 6, and 10, differing from HIDs, and these genes were enriched in GO pathways related to stress responses, larval development, post-embryonic development, nervous system development, ion channel activity, ATP-dependent enzymes, ATP-hydrolysis, etc. (Figures S2D and S4; Table S5). The HIDs and SIDs were similar for clusters 1, 3, and 9, dissimilar from PIDs, and were mainly enriched for genes regulating protein biogenesis, unfolded protein response, and protein phosphatases (translation, peptide biosynthesis process, mRNA metabolic process, IRE1-mediated unfolded protein response, ribo-

some, eukaryotic translation initiation factor 3 complex, phosphatase activity, metalloproteinase activity, etc.; Figures S2D and S4; Table S5). Genes with similar expression patterns in HIDs and PIDs—clusters 2, 7, and 8—were enriched for sensory perception, nervous system processes, ion channel activity, cilium assembly and organization, as well as translation (Figures S2D and S4; Table S5). Importantly, none of the three wild-type dauers clustered more frequently together. Genetically induced dauers clustered into two distinct groups: *daf-2* separated more often with *ilc-17.1*, and *cep-1* OE with *daf-7* dauers (Figure S2E and Table S5). The two groups also showed clear differences in the expression patterns of genes enriched for developmental processes altered during dauer stage, such as “regulation of multicellular organismal development,” “animal organ morphogenesis,” “mitotic cell cycle process,” and stress responses (“response to endoplasmic reticulum stress,” “regulation of cellular response to stress,” etc.; clusters 2 and 7; Figures S2E and S5; Table S5). Cluster 1, which was clearly distinct in *ilc-17.1* dauers compared to the other three dauers, was predominantly enriched for genes involved in the innate immune response, consistent with the role of ILC-17.1 as a conserved cytokine (e.g., “innate immune response,” “immune response,” “immune system process,” “defense response to other organism,” “response to biotic stimulus,” “response to external biotic stimulus,” “response to other organism,” “defense response,” etc.; Figures S2E and S5; Table S5). Surprisingly, cluster 3 genes, which differed in *cep-1* OE dauers, were related to nervous system function (“nervous system process,” “sensory perception of chemical stimulus,” “sensory perception,” “detection of chemical stimulus involved in sensory perception,” “detection of chemical stimulus,” “neuropeptide signaling pathway,” “G protein-coupled receptor activity,” “olfactory receptor activity,” etc.). Cluster 9 genes (“rRNA processing,” “rRNA metabolic process,” “ribosome biogenesis,” and “ncRNA processing”) also distinctly expressed in *cep-1* OE dauers, consistent with the known roles of p53 (CEP-1) in nucleolar function and rRNA biogenesis.⁶³ Cluster 6 gene expression was different in *daf-2* and enriched for chromosomal and nucleotide metabolism (“meiotic cell cycle,” “chromosome localization,” “meiotic cell cycle process,” “mitotic metaphase plate congression,” etc.) and several processes related to rRNA and ribosome biogenesis (“nucleolus,” “preribosome,” “90S preribosome,” “ribosome biogenesis,” “rRNA processing,” etc.). Gene expression in cluster 4, distinct in *daf-7* from the other dauers, was enriched for aspects of phosphorylation and reproductive processes (“cell cycle process,” “multicellular organismal reproductive process,” “gamete generation,” “nuclear division,” “eggshell formation,” etc.; Figures S2E and S5).

Together, these data showed that different dauer triggers led to dauers with distinct transcriptomes, with the transcriptomic differences aligning, in part, with what is already known about the different pathways and dauer types. These differences could arise because each trigger activated a different hypothetical “core set of genes” essential for dauer stage entry, and/or because they affected additional genes not directly related to dauer, and/or because dauer gene expression is so labile that even unavoidable differences in harvesting methods, post-dauer arrest, caused large changes in the transcriptome, even while

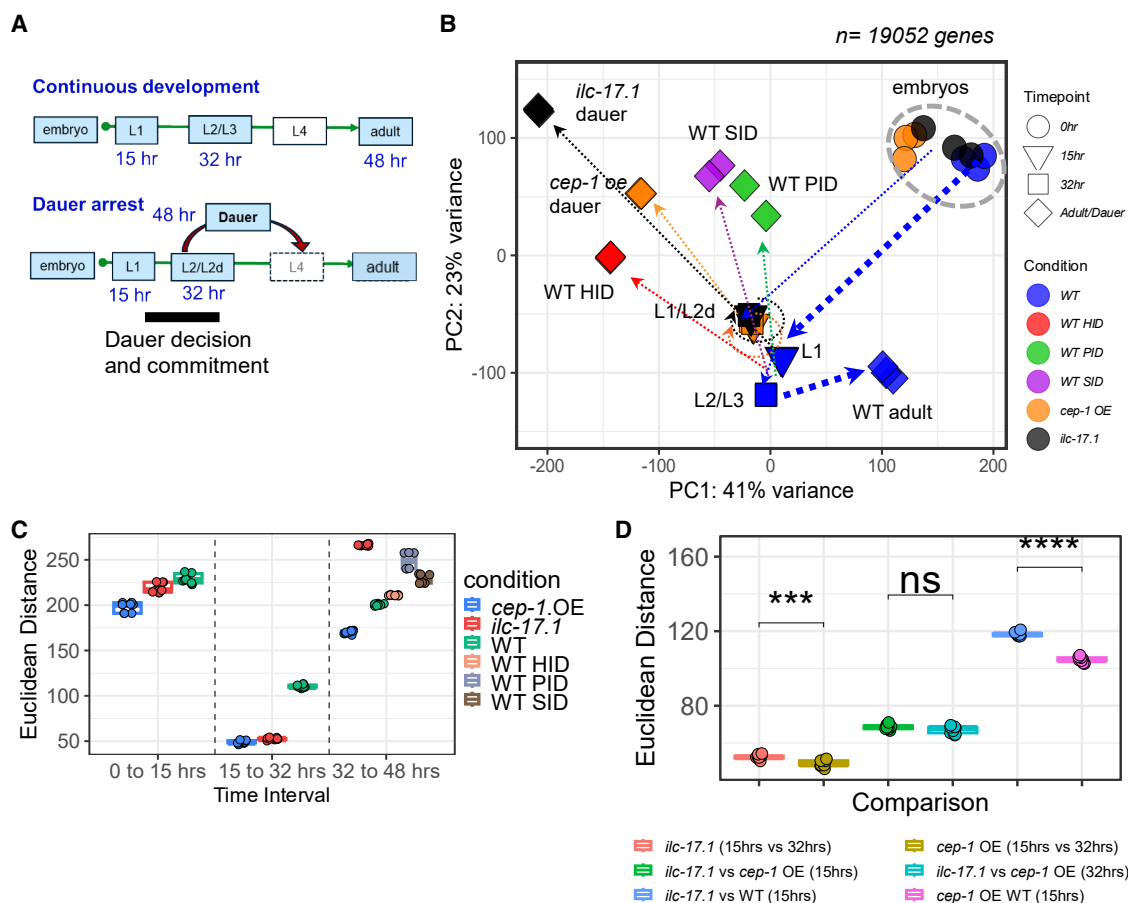


Figure 2. Transcriptomic differences between dauers emerged during their transition from L1/L2d to the dauer state

(A) Schematic of *C. elegans* development. Top: continuous growth (wild type). Bottom: dauer arrest. Developmental timepoints at which the time-series RNA-seq samples were collected are highlighted in blue. Estimated dauer decision and commitment point is shown with black bar.

(B) Principal component analysis (PCA; VST-scaled data; $n = 19,052$ genes) and trajectory of the developmental time series RNA-sequencing data. Colors: strains. Symbols: time point. Arrows show the developmental trajectory. Note: L1 and L2/L2d stages of *ilc-17.1* and *cep-1* OE occupy overlapping positions in the PCA.

(C) Euclidean distance between samples during different development time points (intervals of time). Boxplots show pairwise distance between replicates of each sample in each comparison. Significance: t test: 15–32 h vs. 32–48 h (p value = 1.19×10^{-28}).

(D) Euclidean distance between *ilc-17.1* and *cep-1* OE larvae between L1 (15 h) and L2d (32 h). Boxplots show pairwise distance between biological replicates in each sample (replicates per sample = 3, comparisons, $n = 9$). Significance: t test; **** p value < 0.0001; *** p value < 0.001; ** p value < 0.01, * p value < 0.05.

the larvae remained as dauers. Nevertheless, these studies suggested that dauer entry did not reset global gene expression to a common shared expression pattern, or if all dauers did share a common set of “dauer signature genes,” they could not be identified using this clustering analysis. Instead, different pairs of dauers appeared to share similar expression patterns in different subsets of genes.

Gene expression differences between the dauers arise during their formation

Previous work has shown that the decision to enter dauer stage is made during L1 (~14–15 h post-embryogenesis, at 25°C) but is reversible; commitment to dauer occurs at ~32–40 h at 25°C if growth challenges persist.^{24,30,32,56,64,65} L1 larvae then alter metabolism and growth to enter L2d, instead of L2 (L2d is a specialized larval stage 2 or L2, committed to dauer;

Figure 2A). Therefore, to understand when dauers acquire their gene expression differences, we compared the transcriptomes of L1, L2/L2d, and dauer larvae from wild-type, *ilc-17.1*, and *cep-1* OE strains. If dauer-associated gene expression changes develop gradually over the decision and commitment phases, we would expect L1, L2/L2d, and dauer samples to be positioned roughly equidistant from each other in PCA space. Conversely, major gene expression differences arising primarily during dauer entry from the L2/L2d stage would be reflected by a greater separation between L1/L2/L2d larvae and dauers in the PCA. We chose to evaluate *ilc-17.1* and *cep-1* OE strains for this study, in part, because they were the most different of the genetically induced dauers (Figure S1C), making them ideally suited to determine when gene expression differences arose.

A PCA of the gene expression of (1) embryos, (2) 15-h (L1) larvae of all strains near the L1 time point when the reversible

dauer decision can be made, (3) 32-h (L2) wild-type larvae fated to develop into adults, (4) 32-h (L2/L2d) *ilc-17.1* and *cep-1 OE* larvae fated to arrest as dauers, and (5) dauers (wild type, PIDs, HIDs, SIDs, *ilc-17.1*, and *cep-1 OE*), showed that, in all strains, the L1, L2, and L2d larvae were more similar in gene expression space than they were to the corresponding dauers (Figure 2B). In fact, among wild-type larvae, even though they were undergoing continuous development, L1 and L2 stages were more similar than the wild-type HIDs and wild-type SIDs or PIDs. Likewise, for the *ilc-17.1* and *cep-1 OE* larvae that proceeded to arrest as dauers, gene expression in the L1 and L2d stages was barely resolvable in the PCA, whereas the dauer stages were distinctly separated (Figure 2B). These relationships were also apparent upon computing the transcriptome-wide Euclidean distance between the different stages. Within each strain, gene expression differences between the L1 and L2/L2d were markedly smaller than those between L2/L2d and dauers or adults (Figures 2C and 2D), suggesting that the main gene expression patterns of dauers developed after the L2/L2d stage. This suggests that the differences in “final” gene expression profiles of the dauer types were likely acquired during their transition into the dauer state from L2d and that during the L1-to-L2/L2d transition, even in larvae not committed to dauer, gene expression underwent relatively few changes.

Plasticity in the dauer transcriptome

In all dauers, entry into the dauer stage involved an almost complete alteration of the transcriptome: compared with the continuously growing L2/L3, between 79.6% and 87.7% of the 19,040 genes showed differential expression (PIDs [$n = 15,162$], SIDs [$n = 15,647$], HIDs [$n = 15,789$], *daf-2* [$n = 16,700$], *daf-7* [$n = 16,070$], *ilc-17.1* [$n = 16,273$], and *cep-1 OE* [$n = 15,979$]; $p_{adj} < 0.05$; mean-difference [MA] plots; Figure S6; Tables S6 and S7). Nevertheless, consistent with the results of the LRT (Figure 1D), only 50% of these differentially expressed genes (DEGs) were shared by all dauers ($n = 9,332$; Figure 3A; Tables S6 and S7). Despite this, GO enrichment analysis showed that all dauers exhibited downregulated processes required for dormancy, such as DNA metabolism (DNA repair, DNA replication, chromatin organization, etc.), cell cycle (mitosis, mitotic cell cycle, and meiosis), protein synthesis (ribosome biogenesis, regulation of translation, tRNA processing, etc.), developmental progression (oogenesis, spermatogenesis, embryonic morphogenesis, nematode larval development, etc.), and transcription (mRNA processing, mRNA splicing, rRNA processing, mRNA transport, etc.), albeit to different extents (for GO of DEGs in each dauer, see Figure 3B; for GOs of shared DEGs, see Figure S7 and Table S8). These processes are known to be arrested reversibly upon dauer entry.^{24,26,27,65} Upregulated DEGs were enriched for neuropeptide signaling and several pathways involved in neuronal and sensory functions, also consistent with previous reports regarding the dauers' high responsiveness to their environments^{41,66–69} (Figure 3C and Table S8). A more detailed examination of genes within each pathway revealed that in many cases, the DEGs in the different dauer types were distinct but perhaps functionally equivalent, being enriched in similar biological, cellular, or molecular pathways. For instance, while upregulated genes across all dauer types were enriched for components of the neuro-

peptide signaling pathway, even across the three WT dauer types (HIDs, PIDs, and SIDs), many of the specific neuropeptide-encoding genes differed (Figure S8 and Table S10). Likewise, some of the specific genes altered in the downregulated GO categories, like “regulation of translation,” differed between the dauers (Figure S8B and Table S10). A subset of DEGs were unique to each of the dauers, with HIDs and SIDs showing the largest numbers of DEGs, and *daf-7* and *daf-2* the smallest (Figure 3A). These genes were enriched for very few annotated GO pathways, particularly for HIDs, *ilc-17.1*, and *cep-1 OE* (Figure 3D and Table S9). Interestingly, dauers differed in the GO category related to cuticle components (Figure 3B), which is responsible for increased stress resistance and mechanosensation.^{32,70,71}

These studies indicated that plasticity (gene expression changes) among dauers also differed, but dauers shared approximately half of their DEGs and showed similar enrichment for a large number of pathways related to the anabolic and catabolic changes that accompany dauer entry. Even among the shared pathways, dauers differed in the specific genes that contributed to the enriched pathways, and in each dauer, a unique set of genes were differentially expressed.

Using gene co-expression networks to infer similarities and differences between the different dauer larvae

The goals of this study were to understand how the route into dauer influenced dauer traits and identify which traits (if any) were invariant and conserved among all dauers. Therefore, to infer biological features (or traits) of dauers from their gene expression patterns,^{72–74} we utilized published *C. elegans* gene co-expression networks, i.e., networks of genes that were spatially or temporally co-regulated (modules) and enriched for specific organismal, cellular, or molecular pathways (Table S11). Two main gene co-expression networks have been described in *C. elegans*: (1) Gene expression “mountains”: A set of 34 co-expression modules described by Kim et al. (2001),⁵⁹ derived from the analysis of 5,361 *C. elegans* genes across numerous experiments involving wild-type and mutant animals at various developmental stages. The modules in this network are called “mountains,” and represent functionally or spatially related gene groups, enriched in pathways such as “protein expression,” “histone,” “mitochondria,” “germline,” “G-protein receptors,” “heat shock,” and others. (2) Metabolic pathways from iCEL1314: A set of 85 modules identified through co-expression analyses and curated in the iCEL1314 metabolic network model by Nanda et al. (2023),⁶⁰ which comprise 1,799 metabolism-related genes. In addition to these, we curated the following modules from the published literature: (3) Dauer-inducing pathways: Key genes from the primary dauer-signaling pathways, namely the ILS pathway (insulins and DAF-16 targets), the TGF- β pathway, and the DAF-12/steroid hormone signaling pathway^{26,28,35,75–77}; (4) Growth arrest pathways: Genes involved in executing the dauer-specific developmental arrest. These included genes that function together to regulate the cell cycle (e.g., cyclins, anaphase-promoting complex, cyclin-dependent kinases, and inhibitors) and energy metabolism (e.g., glycolysis and the electron transport chain).^{78–83} Omitting co-expression network modules containing fewer than 8 genes yielded a list of 106 “modules,” each encoded by 8–1,416 genes.

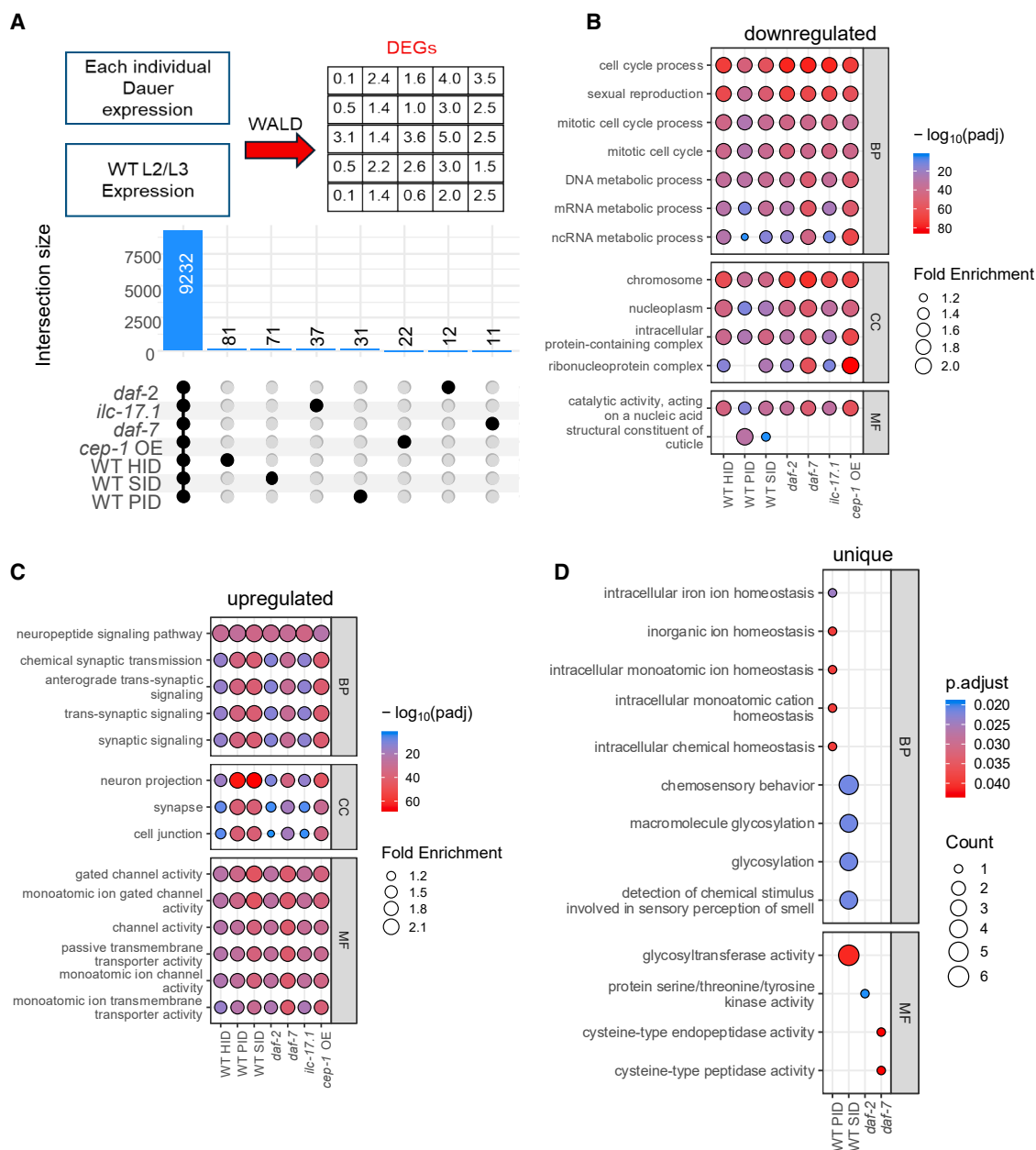


Figure 3. Differentially expressed genes in dauers formed by different triggers

(A) Top: Schematic of Wald test used to estimate differential expression between dauers and WT L2/L3. Bottom: UpSet plot showing the shared and unique differentially expressed genes (DEGs; dauers compared to WT L2/L3 larvae; $p_{adj} < 0.05$) in WT HID, WT PID, WT SID, *daf-2*, *daf-7*, *ilc-17.1*, and *cep-1* OE dauers. Number of genes in each comparison is shown. Matrix: dauer or combination of dauers is compared.

(B) Dot plot showing comparison of enrichment between downregulated DEGs in WT HID, WT PID, WT SID, *daf-2*, *daf-7*, *ilc-17.1*, and *cep-1* OE dauers. Three types of ontologies are shown. Top: biological process (BP); middle: cellular component (CC); bottom: molecular function (MF). y axis: names of GO categories. x axis: dauers. Color bar: $-\log_{10}(\text{adjusted } p \text{ values})$ (Benjamini-Hochberg corrected, $p < 0.05$). Circle size: Fold enrichment (gene ratio/background ratio). Only some GO categories per ontology are shown in the graph due to space constraints. See Table S9 for complete GO categories.

(C) Dot plot showing comparison of enrichment between upregulated DEGs in WT HID, WT PID, WT SID, *daf-2*, *daf-7*, *ilc-17.1*, and *cep-1* OE dauers, as in (B). (D) Dot plot showing comparison of enrichment between unique DEGs for each dauer. Three types of ontologies are shown. Top: biological process (BP); middle: cellular component (CC); bottom: molecular function (MF). y axis shows the names of GO categories, and x axis represents the types of dauers. Color bar, adjusted p values (Benjamini-Hochberg corrected, $p < 0.05$); circle size, number of genes.

See Table S8 for complete GO categories.

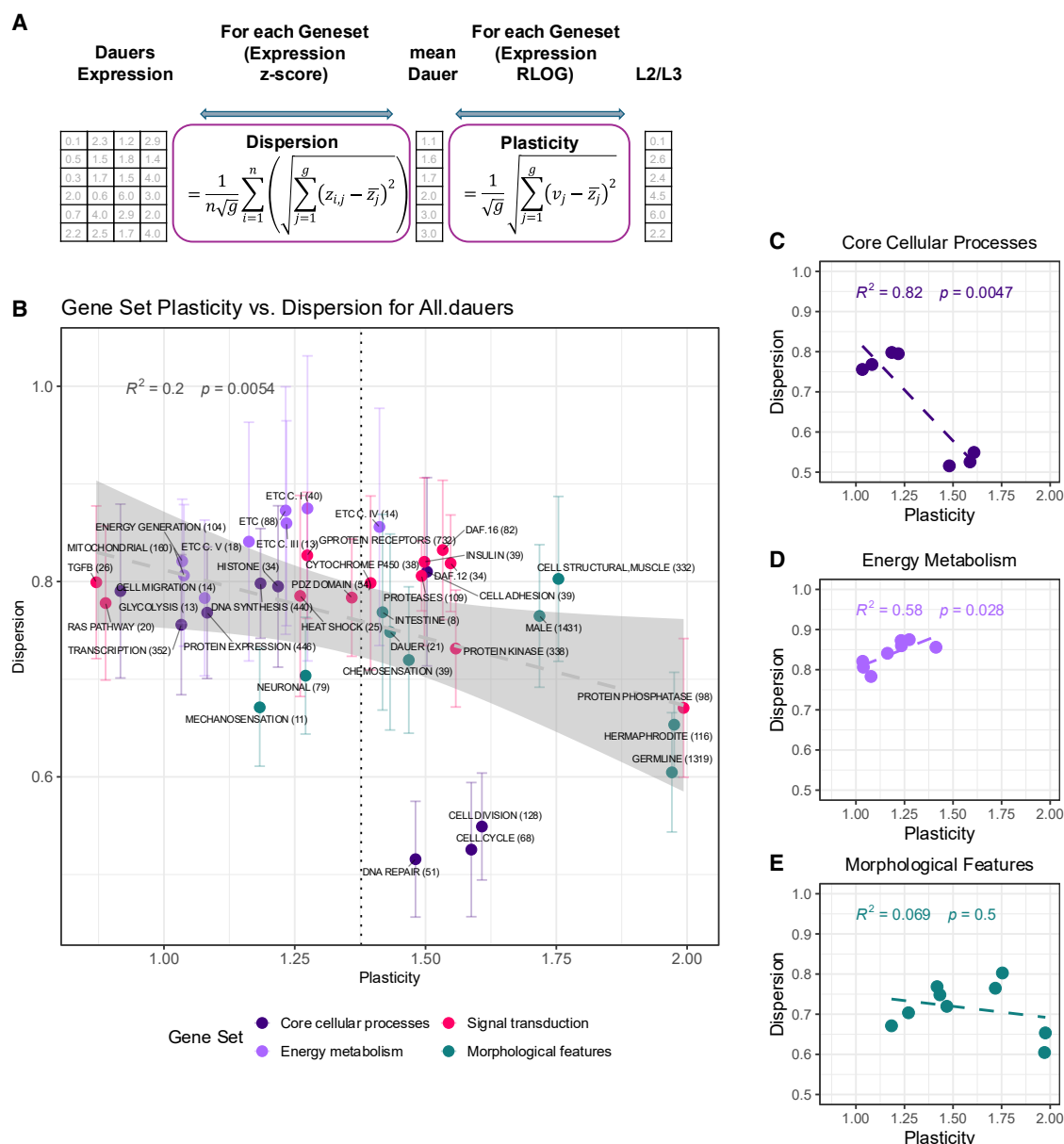


Figure 4. The relationship between plasticity and canalization within gene co-expression networks in all dauers

(A) Schematic: Covarying gene sets were defined as “modules.” Dispersion index and plasticity index for each of the “modules” were computed. Equations used are shown. Scaled gene expression used as an input was either Z-scored expression values (dispersion index) or rlog values (plasticity index).

(B) Scatterplot showing dispersion vs. plasticity of all modules for all dauers. Each data point represents a gene set or “module”; data are represented as the mean $\pm$ standard deviation of dispersion.

(C–E) Scatterplots showing dispersion vs. plasticity of subgroups for (C) core cellular processes, (D) energy metabolism, and (E) morphological features. R^2 values for subgroups are shown in the indicated color. Color represents the grouping of modules based on related biological processes. Numbers in brackets indicate the number of genes that contribute to each module. Confidence interval (99%) of linear regression of all the pathways is shown in gray. Vertical dotted line indicates the median plasticity index value. R^2 values for all the modules are shown.

Next, to compare these modules across different dauer types, we computed two metrics: plasticity index and dispersion index. To calculate the plasticity index, a measure of gene expression changes within a module, we first computed a mean expression value for each gene (from individual rlog values) within a “module,” across dauer types, calculated its Euclidean distance

from the WT L2/L3, integrated (square root of the sum of distances) these distance values across all genes within the module, and normalized the result by the number of genes within that module (Plasticity = $\frac{1}{\sqrt{g}} \sqrt{\sum_{j=1}^g (v_j - \bar{z}_j)^2}$; Figure 4A; Table S12). Dispersion index, a measure of how dauers differed in their expression of a given module, was computed by first

calculating a mean value for each gene within a module (meanDauer) and deriving the Euclidean distance for the expression of that gene between each dauer and the “meanDauer.” This was done for all genes within a module, integrated as described for the plasticity index, to yield one value/dauer/module. These values were averaged between the dauers and normalized by the number of genes within the network (Figure 4A; Dispersion = $\frac{1}{n\sqrt{g}} \sum_{i=1}^n \left(\sqrt{\sum_{j=1}^g (z_{ij} - \bar{z}_j)^2} \right)$; Table S12). To minimize the influence exerted by the magnitude of gene expression on dispersion calculations, for the dispersion index, we converted the expression values of each gene across the dauers into Z scores prior to calculating the Euclidean distances, allowing Euclidean distance to be interpreted as a measure of similarity (i.e., because Z score normalized, squared Euclidean Distance is equal to Pearson’s correlation coefficient).

The dispersion index allowed us to infer, with a given level of confidence, the degree to which a particular module varied between dauer types. The plasticity index measured how much that particular module changed during dauer entry relative to continuously developing conspecifics (L2/L3). A higher plasticity index value indicated that gene expression within a particular module was more different from that in L2/L3, and thus, more likely to reflect dauer-specific expression; modules that had relatively low plasticity indices were considered more similar to L2/L3, and their expression was deemed more typical of the developmental stage. To account for the fact that gene expression in all modules is unlikely to be equally flexible or plastic, we chose the median plasticity index as an approximate threshold to evaluate the plasticity index values. Modules, and by inference the associated GO pathways, with low dispersion (less variable between dauers) and high plasticity (dauer specific) indices were considered the most conserved (canalized) among a set of dauers. Conversely, modules and their associated pathways with high dispersion and high plasticity indices were considered to be the more variable features of dauers.

The modules were plotted on scatterplots, with the plasticity index as the x coordinate, and dispersion index as the y coordinate; all modules in all dauers were plotted, with environmentally induced dauers and genetically induced dauers plotted separately (Figures 4B–4E and 5A–5D; see also Table S12). Modules outside the confidence interval (CI) generated for the dispersion indices were considered significantly different from the general gene expression trend. Modules to the right of the median plasticity index were considered specific to dauers. Besides identifying modules that were the most conserved or varying (and more and less dauer-specific), these scatterplots also provided insights into the relationship between the plasticity and dispersion of all modules within dauers induced by different types of triggers.

Identifying the overall distribution of gene co-expression modules based on dauer-inducing stimuli

When plotted for all seven dauer types, the dispersion of the 106 modules and their plasticity were negatively correlated ($R^2 = 0.2$, $p = 0.0054$) (Figure 4B). This implied that modules where gene expression had changed the most during the transition into dauer (i.e., more plastic and “dauer specific”) were also the least

variable among dauers. However, separating the dauers by their trigger—environmental or genetic—revealed that this negative correlation was driven by the genetically induced dauers ($R^2 = 0.21$, $p = 0.004$; Figure 5B) and was not evident among the environmentally induced wild-type dauers ($R^2 = 0.0043$, $p = 0.69$; Figure 5A). Thus, for the dauers induced through genetic means, large gene expression changes driven by any of the genetic dauer-inducing pathways (i.e., loss of *daf-2*, *daf-7*, *ilc-17.1*, or *cep-1* overexpression), resulted in a very similar outcome. This is consistent with our understanding that these dauer-inducing pathways, although redundant, converge on the common steroid hormone pathway to induce dauer arrest. Surprisingly, this was not the rule among environmentally induced wild-type dauers, which are also expected to activate the same genetic signal transduction pathways (i.e., ILS, TGF- β , etc.) during dauer entry and converge on the DAF-9/DAF-12 steroid hormone pathway. Instead, for the environmentally induced dauers, canalization and plasticity mechanisms appeared largely uncoupled ($R^2 = 0.0043$, $p = 0.69$; Figure 5A). Moreover, among these environmentally induced dauers, gene expression within modules directly related to dauer signaling pathways themselves—DAF-12, DAF-16, “dauer,” “insulin,” and “cytochrome P450”—was more variable. In addition, dauer-specific modules like “germline” and “protein phosphatase,” which had large, similar plasticity indices as genetically induced dauers, and thus were equally “dauer specific,” were also more variable.

The difference between genetically and environmentally induced dauers was more apparent among modules that regulate metabolism (Figure S9). These metabolic modules showed a higher overall variability across all dauers and remained negatively correlated with plasticity among genetically induced dauers ($R^2 = 0.12$, $p = 0.0046$; Figure S9B) but positively correlated among wild-type dauers ($R^2 = 0.12$, $p = 0.0031$; Figure S9A). Thus, for environmentally induced dauers, the more the “dauer-like” was a metabolic module, the higher was the variability. For instance, mitochondrial and peroxisomal fatty acid degradation, which modulate the duration of the dauer diapause,^{84–86} and ascaroside biosynthesis, which controls almost all features of dauer biology,^{87–89} were more dispersed among wild-type dauers but more conserved across genetically induced dauers. Some pathways like ubiquinone biosynthesis and vacuolar ATPase showed the opposite trend^{90,91} (Figures S9A and S9B; see also Table S12), but these were more L2/L3-like and accounted for a minority of the modules.

Together, these analyses show that the dauer trigger influences how consistently a dauer phenotypic trait is expressed, at least as inferred from gene co-expression networks. Any one of the genetic triggers for dauers (*daf-2*, *daf-7*, *ilc-17.1*, or overexpression of *cep-1*) resulted in a similar gene expression pattern within all gene co-expression modules—metabolic and other—based on the extent to which that module differed from L2/L3: the larger its gene expression change, the more the module was conserved. Among the wild-type dauers, the environmental triggers for dauers resulted in either greater variability in gene expression, and for metabolic modules, the greater the change in gene expression from L2/L3 (i.e., the more dauer specific it seemed), the more it varied between the dauers. Arguably, this greater variability could be due to additional (non-dauer)

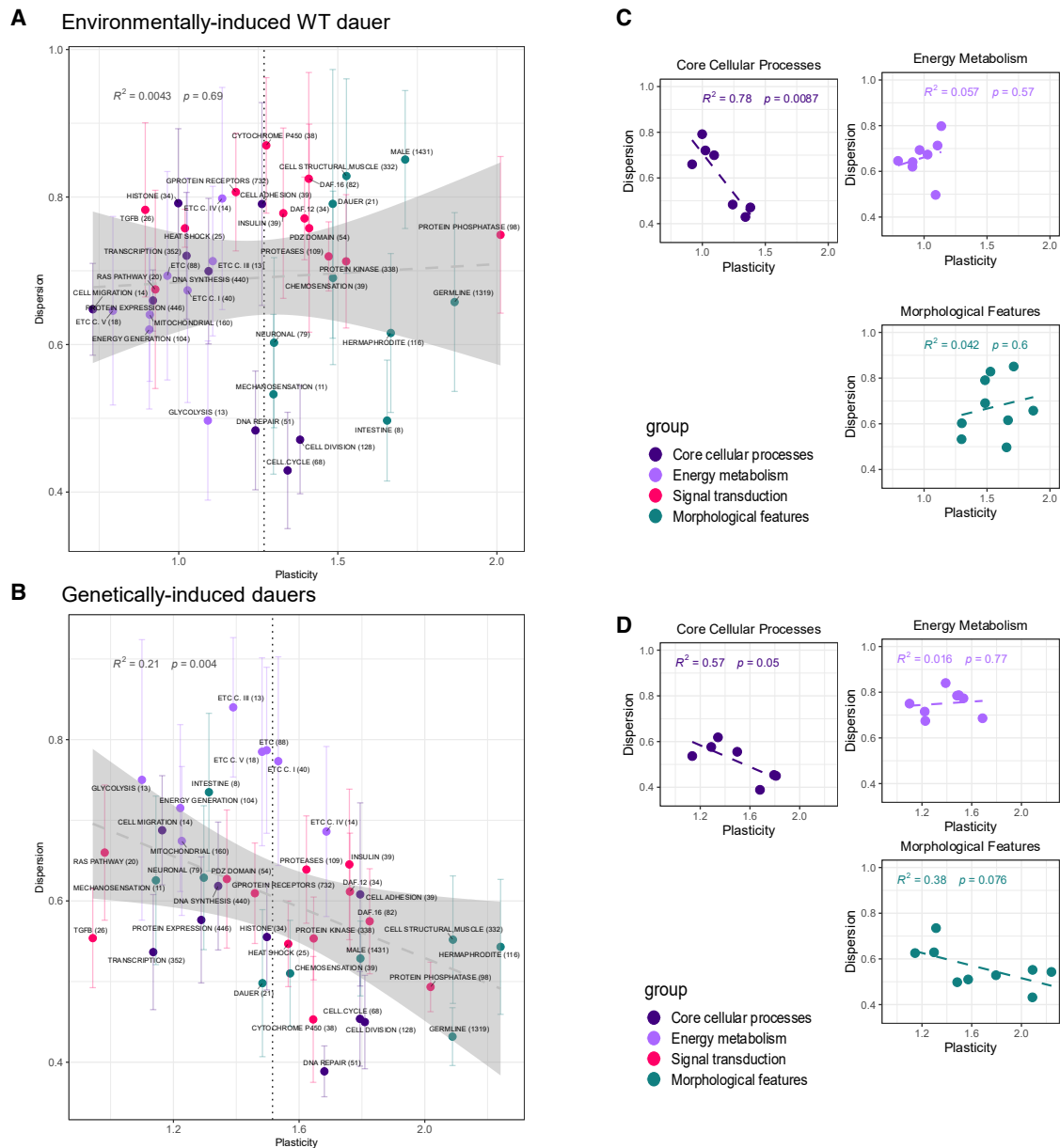


Figure 5. The relationship between plasticity and canalization within modules in environmentally induced wild-type dauers and genetically induced dauers

(A) Scatterplot showing dispersion vs. plasticity (environmentally induced wild-type dauers) of all modules. Each point represents a module; data are represented as the mean $\pm$ standard deviation of dispersion. Color represents the grouping of pathways into subgroups. Confidence interval (99%) of linear regression of all the traits. Vertical dotted line indicates the median plasticity index value. R^2 values for all modules are shown.

(B) Similar scatterplot, for the same modules as in (A), but for genetically induced dauers.

(C) Scatterplots showing dispersion vs. plasticity (environmentally induced wild-type dauers) of subgroups of modules: (1) core cellular processes, (2) energy metabolism, and (3) morphological features. R^2 values for subgroup of modules are shown.

(D) Similar scatterplots, for the same modules as in (C), but for genetically induced dauers.

effects of environmental exposure on gene expression within a module. However, if so, it is unclear why a similar variability was not evident among genetically induced dauers, where mutations in dauer-related pathways also influence organismal functions beyond dauer. Thus, while the behavior of the genetically induced dauers is consistent with all dauer pathways funneling

through one common mechanism, we hypothesize that gene expression changes of the environmentally induced dauers, considered together with the variability in key dauer-inducing modules themselves (e.g., DAF-16, insulins, etc.), are suggestive of simultaneous influence of several signal transduction pathways that could generate the observed variability.

Conserved and variable dauer modules

To identify modules and associated biological pathways that were conserved, or varied the most, across the dauer types, we identified modules that fell outside the 99% CI of the regression line and had plasticity indices higher than (or equal to) the median value. Among these, we considered the modules with low dispersion index values as the most conserved among dauers and those with high dispersion index values as the most varying (Figures 4 and 5). When compared across all dauers or between only wild-type or genetically induced dauers, modules related to core cellular processes that regulate cell cycle progression—DNA repair, cell cycle, and cell division—were found to be the most conserved. Thus, these processes, essential for dormancy, appeared to be conserved irrespective of the dauer-inducing mechanism. Gene expression contributing to “mechanosensation,” was also conserved across all dauers, but this appeared to be a consequence of the developmental stage of all dauers and L2/L3, and not necessarily because gene expression related to mechanosensation was conserved during dauer arrest. The “glycolysis” module was also conserved among dauers, but, again, appeared to be expressed similar to L2/L3 larvae, and was, therefore, not considered a dauer-specific module. However, when evaluated only among the wild-type dauers, “mechanosensation” and “intestine,” were found to be conserved and displayed relatively high plasticity values, suggesting that here, gene expression in these modules was dauer specific. Among genetically induced dauers, besides “DNA repair,” “cell cycle,” and “cell division,” the only other dauer-specific module that was conserved was “cytochrome P450,” which, intriguingly, was among the most variable of the dauer-specific modules among the environmentally induced dauers.

We noticed that modules could be readily grouped based on their roles in related biological processes (Figures 4C–4E and Table S12), such as (1) core cellular processes (DNA repair, DNA synthesis, histone, transcription, protein expression, cell division, and cell cycle), (2) mitochondrial processes (ATP generation and energy metabolism—“energy generation,” “mitochondrial,” “glycolysis,” “ETC complex I,” “ETC complex III,” “ETC complex IV,” “ETC complex V,” and “ETC”), and (3) organ- and tissue-level structure/function (“mechanosensation,” “neuronal,” “intestine,” “chemosensation,” “dauer,” “germline,” “cell structural, muscle,” “male,” and “hermaphrodite”). The distribution of these modules showed differences between wild-type and genetically induced dauers but also revealed similarities among them (Figures 5C and 5D). Notably, as described above, among all dauer types, independent of the dauer trigger, modules that were the most plastic within the subgroup “core cellular processes” were the most canalized and conserved (Figures 4C, 5C, and 5D). Modules related to mitochondrial processes or morphological features were not significantly correlated in either environmentally induced wild-type dauers or genetically induced dauers, although the genetically induced dauer subgroup continued to show a modest (but not significant; $p = 0.076$) negative correlation between plasticity and dispersion (Figures 5C and 5D). Because these modules were somewhat subjectively grouped based on their readily apparent roles in related biological processes, such relationships could be extended in future studies to examine other modules whose re-

lationships are not yet immediately evident. The differences between the genetically and environmentally induced dauers were also apparent when both groups were plotted on a common scatterplot. Here, except for the modules related to morphological traits, gene expression in the wild-type dauers showed a lower plasticity (i.e., higher similarity to L2/L3) and greater dispersion than those in the genetically induced dauers (Figure 6).

Together, these analyses identified conserved and varying modules enriched for specific biological pathways in the different dauer types. Among these, only the modules governing dormancy appeared to be invariant; the remaining modules showed dauer trigger-dependent differences. In addition, gene expression related to “cytochrome P450” showed marked differences between genetically and environmentally induced dauers. Given the central role played by *daf-9*, one of the CP450s,^{38,39} in the biosynthesis of the steroid hormone ligand, a tantalizing possibility is that wild-type dauers might utilize alternative, environment- or trait-specific CP450s, besides *daf-9*, for entry in dauer phase. Moreover, lower plasticity in several of the modules in environmentally induced dauers suggests that the features encoded by these modules may undergo a more graded change in these dauers during the transition to dauer phase.

DISCUSSION

Given the deep knowledge regarding the epistasis relationships between the dauer-inducing pathways, where the major upstream signaling pathways—ILS and TGF- β , and even the less well-studied ILC-17.1 and CEP-1/p53 pathways—funnel through a common node (the steroid hormone receptor, DAF-12), we naively expected all the genetically induced dauers to be highly canalized, and, if this translated into gene expression, to possess very similar gene expression profiles. Moreover, since the same genetic pathways also underlie dauer formation in wild-type animals, we expected the similarities to extend to wild-type environmentally induced dauers. In sum, we expected all dauers to possess similar gene expression patterns, even with the variability introduced by unavoidable differences in the harvesting methods (since we expected this stochastic variability to fall out of our analyses). This was not the case: an LRT test showed that the expression of all but 705 of the 19,040 genes differed across dauers, and, instead, there appeared to be as many transcriptomes as there were dauers in our study. Nevertheless, some of the gene expression patterns could be explained by known epistasis relationships. For instance, gene expression in the *daf-2*, *daf-7*, *ilc-17.1*, and *cep-1* OE dauers was more similar, and these dauers clustered together in a PCA, even though wild-type environmentally induced dauers were more distant in the gene expression space. The *ilc-17.1* and *daf-2* dauers, both triggered by pathways that respond to nutrient (glucose)³⁶ availability, were also more similar to one another. Most telling, perhaps, our analyses of dispersion between covarying gene sets or modules, which we used as a working definition of dauer traits or features, showed that a large magnitude of gene expression change induced by any of the genetic pathways—loss of *daf-2*, *daf-7*, *ilc-17.1*, or *cep-1* OE—resulted in a broadly similar expression of that module in all

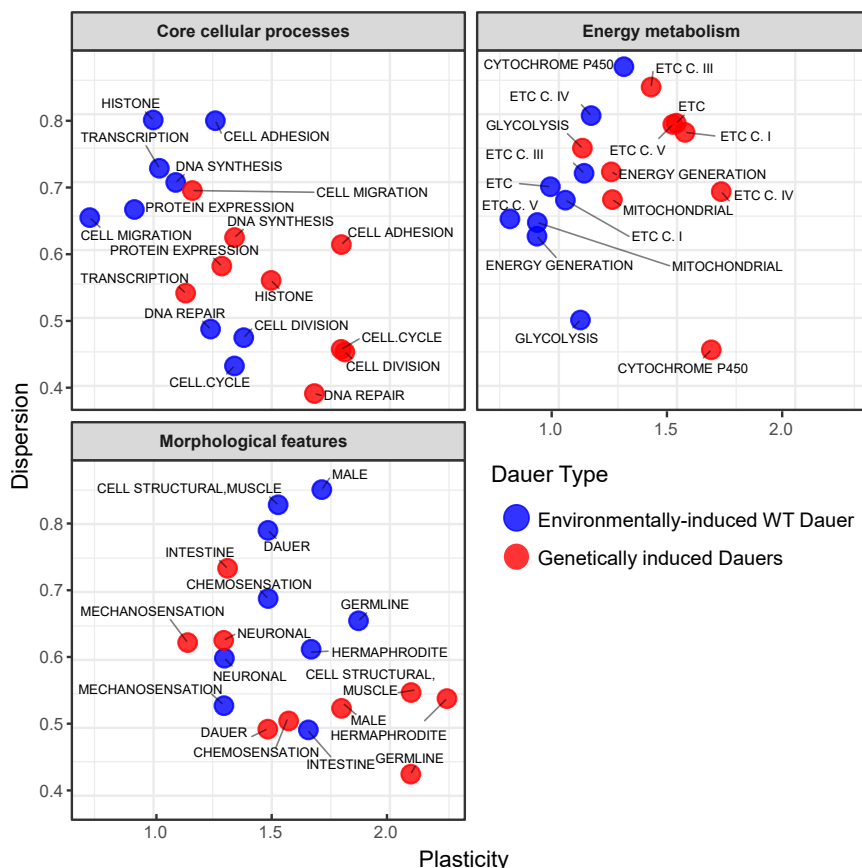


Figure 6. Direct comparison of dispersion vs. plasticity of modules in environmentally induced wild-type dauers and genetically induced dauers

Scatterplot showing dispersion vs. plasticity in both wild-type and genetically induced dauers. The three subgroups of modules, core cellular process, energy metabolism, and morphological features, are shown. Each point represents a module. Color represents the dauer type: red, genetically induced dauer; blue, wild-type dauers.

others were not obvious. Thus, together, these observations highlight the challenges in inferring gene expression similarities and differences from genetic epistasis studies.

Our analysis of covarying gene sets, or modules, was more revealing. These analyses also showed that the path into dauer influences the variability of gene expression related to most biological traits. Nevertheless, gene co-expression modules governing core cellular functions, key to dormancy mechanisms in all animals—DNA repair, cell cycle, and cell division—were highly canalized regardless of the dauer-inducing signal. In addition, almost all modules displayed lesser plasticity in environmentally induced dauers than in the genetically

induced dauers, as could be expected, with these pathways converging on a common node.

Other observations did not conform to expectations from epistasis studies. For one, the genetically induced dauers varied among themselves and further separated into two groups: *daf-2* and *ilc-17.1*, and *cep-1* OE and *daf-7*. This particular grouping was also surprising because published studies have suggested that *ilc-17.1* and *cep-1* OE should be more similar, given that both pathways triggered dauer entry by acting upstream of both DAF-16/FOXO and DAF-3/SMAD-DAF-5/Ski, and *ilc-17.1* was epistatic to *cep-1*, whereas *daf-2* was not.³⁶ Also inconsistent with epistasis studies, gene expressions in *ilc-17.1* and *cep-1* OE were the least similar among the genetically induced dauers. Despite this, at the L1/L2d stage of both these dauers (though we hypothesize that this may extend to all dauers), gene expression converged and became remarkably similar, before diverging again to generate the final distinct dauer transcriptomes. This disconnect in the timing of developmental canalization and the development of the dauer phenotype might explain some of the discrepancies between genetic epistasis relationships and the final transcriptomic outcomes that we observed. Hierarchical clustering and PCA also revealed several distinct and shared characteristics of the dauers, suggesting that an individual dauer trigger shaped the final gene expression outcome. In these analyses too, some specific gene expression patterns could be predicted by the dauer-inducing stimulus, but

induced dauers, suggesting that the dauer state among wild-type larvae may be a more graded process, with dauers undergoing varying magnitudes of gene expression changes compared with L2/L3 larvae. Environmental changes—temperature, pheromone, and starvation—induced greater gene expression variability than genetic manipulations among dauers. While some of this variability could undoubtedly arise from the pleiotropic effects of the environment on dauer-related and dauer-unrelated gene expression, our analyses suggest that this alone may not fully explain the different degrees of gene expression variability among dauers. Indeed, among environmentally induced wild-type dauers, there appeared to be greater variability among several of the dauer-inducing signaling pathways themselves. Most notable was the difference in canalization of the CP450 family, of which *daf-9*, a CP450 of the CYP2 class, that produces steroid hormone for DAF-12 steroid hormone receptor⁹²; this family was highly dispersed among wild-type dauers but canalized among genetically induced dauers. This led us to hypothesize that perhaps among the wild-type dauers, different members of the CP450 family may modulate dauer development, as well as contribute to dauer traits.⁵¹ This lack of conservation in CP450 gene expression among the wild-type environmentally induced dauers is reminiscent of the fast-evolving nature of “dauerless,” the orphan receptor that contributes to natural variation in *Pristionchus*.⁵¹ In further support, in *C. elegans*, another CP450 family member, *dach-1*, regulates

neurotransmission during *daf-2* dauer entry,⁹³ suggesting that the ligands generated by these powerful enzymes could lead to the graded variation in dauer traits, and ultimately, the different types of dauers.

During normal development, gene expression is thought to follow a trajectory that gradually converges on the final differentiated expression state.^{1,2,5,9,11} Developmental plasticity enables organisms to persist in and exploit changing environments. However, whether developmental or phenotypic plasticity potentiates or limits evolutionary change remains unresolved.^{7,94–96} Previous studies have described different canalization mechanisms that ensure developmental integrity.^{3,7,11,14,15} Some mechanisms, such as the actions of the molecular chaperone Hsp90, microRNAs, or DNA methylation, buffer stochastic variation in molecular processes like protein folding or transcription, dampening the variance associated with these molecular processes but likely inhibiting plasticity if and when active during the same developmental window. Other mechanisms, such as the activation of “developmental switch genes” to implement a developmentally plastic response to a stimulus, could allow canalization and plasticity to co-exist and reinforce each other to generate discrete traits.^{7,11} Entry into dauer phase involves such a binary switch triggered by the upstream signal transduction pathways. Our data suggest that because canalization and plasticity mechanisms appear to function during different developmental windows—canalization during the decision and commitment phase, and plasticity during L2d-to-dauer formation—dauer transcriptomes might differ. Yet, this still leaves open the question how the L2d-to-dauer transition results in transcriptome-wide changes.

The ability to remain dormant under environmental stress and postpone development and reproduction until conditions become favorable confers significant fitness advantages for an individual and is likely to contribute to strong positive selection. Thus, if indeed very few gene co-expression networks regulating core cellular traits are sufficient to support the dauer phenotype, this pattern of simultaneous conservation of core dormancy traits while harboring differences in other traits, particularly in key mediators of the dauer program, could facilitate fixation of the dauer signature traits by natural selection while also promoting co-selection and stabilization of the associated pathways.

Limitations of the study

(1) The choice of the “age” at which to harvest dauers was somewhat arbitrary. Both the genetically and environmentally induced dauers were harvested soon after the majority of larvae entered dauer state (approximately >99% for the former, and >75% for the latter). Yet, if gene expression within dauers can change while they persist in the dauer state, as has been shown in some cases, the transcriptomic analyses here are but one snapshot of the differences between dauers. (2) The choice of gene co-expression network modules to infer phenotypic features, or traits, is incomplete, and given the difficulties inherent in mapping genotype to phenotype, may be misleading; arguably, other ways of defining traits to complement these studies would strengthen the analyses and may yield additional insights. (3) The grouping of dauers into environmentally induced wild-type dauers and genetic dauers was not optimal and did not allow

us to distinguish whether the differences arose from the response to the environment or because of the wild-type genome. More experiments and data from mutant dauers subjected to stressful environments could unravel this more precisely. (4) We could have conceived other groupings of dauers, such as dauers induced by food limitations (*SID*, *daf-2*, and *ilc-17.1*) and only mutants (*daf-2*, *daf-7*, and *ilc-17.1*), which might have yielded additional insights into how the route into dauer influences dauer phenotypes. (5) Finally, unavoidable technical differences in the way the environmental and genetic dauers were produced (liquid versus plate; with liquid culture being stressful), could contribute to some of the observed variability in gene expression. (6) The results are applicable to hermaphrodite populations given the small numbers of males that spontaneously arise, and the influence of sex on the results of the study was not examined.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Johnny Cruz Corchado (johnny-cruzcorchado@roswellpark.org).

Materials availability

This study did not generate new, unique reagents.

Data and code availability

- Data: The mutant and HID dauers' expression data generated in this study have been deposited in NCBI's Gene Expression Omnibus (GEO) under accession number GEO: GSE274872. The pheromone and starvation dauer datasets generated for this study have been deposited in the GEO under accession number GEO: GSE318477. The WT L2/L3 data were previously published and are available in GEO under accession numbers GEO: GSE218596 and GSE229132.
- Code: Plasticity and dispersion function on R is available in Figshare at <https://doi.org/10.6084/m9.figshare.29421131.v2>.
- Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request.

ACKNOWLEDGMENTS

We thank Bin He (University of Iowa) for extremely valuable discussions, particularly on RNA-seq data transformation for PCA and differential gene expression (DGE) analysis, devising average PCC metrics among datasets, and helping us understand how to navigate complex datasets. We thank the V.P. laboratory and Dr. Gidalevitz (Drexel University) for their comments. We thank Abhishiktha Godthi for RNA extraction. We thank the reviewers for their helpful comments, which significantly strengthened the manuscript. Nematode strains were provided by the Caenorhabditis Genetics Center (CGC) (funded by the NIH Infrastructure Programs P40 OD010440). This work was supported by R01 MH126282 (V.P.) and the National Cancer Institute (NCI) grant P30CA016056, involving the use of Roswell Park Comprehensive Cancer Center's Pathology Network, Genomic, and Biomedical Research Informatics Shared Resources.

AUTHOR CONTRIBUTIONS

All authors designed the study and performed experiments. J.C.C. and V.P. designed the analyses; J.C.C. conducted data analysis; and J.C.C., K.S., and V.P. drafted the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the authors used ChatGPT in order to check for grammar and sentence construction. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS
 - *C. elegans* strains
- METHOD DETAILS
 - Synchronization and staging of *C. elegans*
 - Obtaining embryos, larvae and dauers
 - Day-one adult *C. elegans*:
 - RNA extraction, library preparation, and sequencing
 - Pre-processing and alignment of RNA-seq
 - Data normalization and analysis
 - Principal Component Analysis (PCA)
 - Differential Expression
 - Gene Ontology
 - Likelihood Ratio Test (LRT)
 - Hierarchical clustering
 - Distance metrics and correlation analysis
 - Analysis of canalization and plasticity
 - Data visualization
- QUANTIFICATION AND STATISTICAL ANALYSIS
 - General statistical analysis

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2026.115058>.

Received: June 27, 2025

Revised: November 5, 2025

Accepted: February 12, 2026

Published: February 17, 2026

REFERENCES

1. Waddington, C.H. (1959). Canalization of development and genetic assimilation of acquired characters. *Nature* 183, 1654–1655. <https://doi.org/10.1038/1831654a0>.
2. Hallgrímsson, B., Green, R.M., Katz, D.C., Fish, J.L., Bernier, F.P., Roseman, C.C., Young, N.M., Cheverud, J.M., and Marcucio, R.S. (2019). The developmental-genetics of canalization. *Semin. Cell Dev. Biol.* 88, 67–79. <https://doi.org/10.1016/j.semcdb.2018.05.019>.
3. Kerszberg, M. (2004). Noise, delays, robustness, canalization and all that. *Curr. Opin. Genet. Dev.* 14, 440–445. <https://doi.org/10.1016/j.gde.2004.06.001>.
4. Wagner, G.P., Booth, G., and Bagheri-Chaichian, H. (1997). A Population Genetic Theory of Canalization. *Evolution* 51, 329–347. <https://doi.org/10.1111/j.1558-5646.1997.tb02420.x>.
5. Qiu, B., Dai, X., Li, P., Larsen, R.S., Li, R., Price, A.L., Ding, G., Texada, M.J., Zhang, X., Zuo, D., et al. (2022). Canalized gene expression during development mediates caste differentiation in ants. *Nat. Ecol. Evol.* 6, 1753–1765. <https://doi.org/10.1038/s41559-022-01884-y>.
6. Levy, S.F., and Siegal, M.L. (2012). The robustness continuum. *Adv. Exp. Med. Biol.* 751, 431–452. https://doi.org/10.1007/978-1-4614-3567-9_20.
7. Sommer, R.J. (2020). Phenotypic Plasticity: From Theory and Genetics to Current and Future Challenges. *Genetics* 215, 1–13. <https://doi.org/10.1534/genetics.120.303163>.
8. Abley, K., Locke, J.C.W., and Leyser, H.M.O. (2016). Developmental mechanisms underlying variable, invariant and plastic phenotypes. *Ann. Bot.* 117, 733–748. <https://doi.org/10.1093/aob/mcw016>.
9. Wong, M., and Gilmour, D. (2020). Getting back on track: exploiting canalization to uncover the mechanisms of developmental robustness. *Curr. Opin. Genet. Dev.* 63, 53–60. <https://doi.org/10.1016/j.gde.2020.04.001>.
10. Sieriebriennikov, B., Markov, G.V., Witte, H., and Sommer, R.J. (2017). The Role of DAF-21/Hsp90 in Mouth-Form Plasticity in *Pristionchus pacificus*. *Mol. Biol. Evol.* 34, 1644–1653. <https://doi.org/10.1093/molbev/msx106>.
11. Takahashi, K.H. (2019). Multiple modes of canalization: Links between genetic, environmental canalizations and developmental stability, and their trait-specificity. *Semin. Cell Dev. Biol.* 88, 14–20. <https://doi.org/10.1016/j.semcdb.2018.05.018>.
12. Moris, N., Pina, C., and Arias, A.M. (2016). Transition states and cell fate decisions in epigenetic landscapes. *Nat. Rev. Genet.* 17, 693–703. <https://doi.org/10.1038/nrg.2016.98>.
13. Jordan, D.J., and Miska, E.A. (2023). Canalisation and plasticity on the developmental manifold of *Caenorhabditis elegans*. *Mol. Syst. Biol.* 19, e11835. <https://doi.org/10.15252/msb.202311835>.
14. Kaneko, K. (2009). Relationship among phenotypic plasticity, phenotypic fluctuations, robustness, and evolvability; Waddington's legacy revisited under the spirit of Einstein. *J. Bio. Sci.* 34, 529–542. <https://doi.org/10.1007/s12038-009-0072-9>.
15. Zabinsky, R.A., Mason, G.A., Queitsch, C., and Jarosz, D.F. (2019). It's not magic - Hsp90 and its effects on genetic and epigenetic variation. *Semin. Cell Dev. Biol.* 88, 21–35. <https://doi.org/10.1016/j.semcdb.2018.05.015>.
16. Elgart, M., Snir, O., and Soen, Y. (2015). Stress-mediated tuning of developmental robustness and plasticity in flies. *Biochim. Acta* 1849, 462–466. <https://doi.org/10.1016/j.bbagr.2014.08.004>.
17. Wojciechowski, M., Lowe, R., Maleszka, J., Conn, D., Maleszka, R., and Hurd, P.J. (2018). Phenotypically distinct female castes in honey bees are defined by alternative chromatin states during larval development. *Genome Res.* 28, 1532–1542. <https://doi.org/10.1101/gr.236497.118>.
18. Casasa, S., Katsougia, E., and Ragsdale, E.J. (2023). A Mediator subunit imparts robustness to a polyphenism decision. *Proc. Natl. Acad. Sci. USA* 120, e2308816120. <https://doi.org/10.1073/pnas.2308816120>.
19. Sieriebriennikov, B., and Sommer, R.J. (2018). Developmental Plasticity and Robustness of a Nematode Mouth-Form Polyphenism. *Front. Genet.* 9, 382. <https://doi.org/10.3389/fgene.2018.00382>.
20. Wang, S., and Callaway, R.M. (2024). Associations Between Developmental Stability, Canalization, and Phenotypic Plasticity in Response to Heterogeneous Experience. *Ecol. Evol.* 14, e70436. <https://doi.org/10.1002/ece3.70436>.
21. Wang, S., and Zhou, D.W. (2021). Morphological canalization, integration, and plasticity in response to population density in *Abutilon theophrasti*: Influences of soil conditions and growth stages. *Ecol. Evol.* 11, 11945–11959. <https://doi.org/10.1002/ece3.7960>.
22. Willmore, K.E., Klingenberg, C.P., and Hallgrímsson, B. (2005). The relationship between fluctuating asymmetry and environmental variance in rhesus macaque skulls. *Evolution* 59, 898–909.
23. Siegal, M.L., and Leu, J.Y. (2014). On the Nature and Evolutionary Impact of Phenotypic Robustness Mechanisms. *Annu. Rev. Ecol. Evol. Syst.* 45, 496–517. <https://doi.org/10.1146/annurev-ecolsys-120213-091705>.

24. Fielenbach, N., and Antebi, A. (2008). *C. elegans* dauer formation and the molecular basis of plasticity. *Genes Dev.* 22, 2149–2165. <https://doi.org/10.1101/gad.1701508>.
25. Baugh, L.R., and Hu, P.J. (2020). Starvation Responses Throughout the *Caenorhabditis elegans* Life Cycle. *Genetics* 216, 837–878. <https://doi.org/10.1534/genetics.120.303565>.
26. Hu, P.J. (2007). Dauer (WormBook), pp. 1–19. <https://doi.org/10.1895/wormbook.1.144.1>.
27. Karp, X. (2018). Working with dauer larvae. *WormBook 2018*. WormBook 2018, 1–19. <https://doi.org/10.1895/wormbook.1.180.1>.
28. Kiontke, K., and Sudhaus, W. (2006). Ecology of *Caenorhabditis* Species (WormBook), pp. 1–14. <https://doi.org/10.1895/wormbook.1.37.1>.
29. Ailion, M., and Thomas, J.H. (2000). Dauer formation induced by high temperatures in *Caenorhabditis elegans*. *Genetics* 156, 1047–1067. <https://doi.org/10.1093/genetics/156.3.1047>.
30. Riddle, D.L., and Albert, P.S. (1997). In *Genetic and Environmental Regulation of Dauer Larva Development*, C. elegans II, D.L. Riddle, T. Blumenthal, B.J. Meyer, and J.R. Priess, eds. (Cold Spring Harbor Laboratory Press).
31. Vlaar, L.E., Bertran, A., Rahimi, M., Dong, L., Kammenga, J.E., Helder, J., Goverse, A., and Bouwmeester, H.J. (2021). On the role of dauer in the adaptation of nematodes to a parasitic lifestyle. *Parasit. Vectors* 14, 554. <https://doi.org/10.1186/s13071-021-04953-6>.
32. Androwski, R.J., Flatt, K.M., and Schroeder, N.E. (2017). Phenotypic plasticity and remodeling in the stress-induced *Caenorhabditis elegans* dauer. *Wiley Interdiscip. Rev. Dev. Biol.* 6. <https://doi.org/10.1002/wdev.278>.
33. Diaz, S.A., and Viney, M. (2015). The evolution of plasticity of dauer larva developmental arrest in the nematode *Caenorhabditis elegans*. *Ecol. Evol.* 5, 1343–1353. <https://doi.org/10.1002/ece3.1436>.
34. Wolkow, C.A., Muñoz, M.J., Riddle, D.L., and Ruvkun, G. (2002). Insulin receptor substrate and p55 orthologous adaptor proteins function in the *Caenorhabditis elegans* daf-2/insulin-like signaling pathway. *J. Biol. Chem.* 277, 49591–49597. <https://doi.org/10.1074/jbc.M207866200>.
35. Gunther, C.V., Georgi, L.L., and Riddle, D.L. (2000). A *Caenorhabditis elegans* type I TGF beta receptor can function in the absence of type II kinase to promote larval development. *Development* 127, 3337–3347. <https://doi.org/10.1242/dev.127.15.3337>.
36. Godthi, A., Min, S., Das, S., Cruz-Corchado, J., Deonaraine, A., Misel-Wuchter, K., Issuree, P.D., and Prahlad, V. (2024). Neuronal IL-17 controls *Caenorhabditis elegans* developmental diapause through CEP-1/p53. *Proc. Natl. Acad. Sci. USA* 121, e2315248121. <https://doi.org/10.1073/pnas.2315248121>.
37. Lee, S.S., and Schroeder, F.C. (2012). Steroids as central regulators of organismal development and lifespan. *PLoS Biol.* 10, e1001307. <https://doi.org/10.1371/journal.pbio.1001307>.
38. Gerisch, B., Weitzel, C., Kober-Eisermann, C., Rottiers, V., and Antebi, A. (2001). A hormonal signaling pathway influencing *C. elegans* metabolism, reproductive development, and life span. *Dev. Cell* 1, 841–851. [https://doi.org/10.1016/s1534-5807\(01\)00085-5](https://doi.org/10.1016/s1534-5807(01)00085-5).
39. Jia, K., Albert, P.S., and Riddle, D.L. (2002). DAF-9, a cytochrome P450 regulating *C. elegans* larval development and adult longevity. *Development* 129, 221–231. <https://doi.org/10.1242/dev.129.1.221>.
40. Narbonne, P., and Roy, R. (2009). *Caenorhabditis elegans* dauers need LKB1/AMPK to ration lipid reserves and ensure long-term survival. *Nature* 457, 210–214. <https://doi.org/10.1038/nature07536>.
41. Lee, J.S., Shih, P.Y., Schaedel, O.N., Quintero-Cadena, P., Rogers, A.K., and Sternberg, P.W. (2017). FMRamide-like peptides expand the behavioral repertoire of a densely connected nervous system. *Proc. Natl. Acad. Sci. USA* 114, E10726–E10735. <https://doi.org/10.1073/pnas.1710374114>.
42. Lee, D., Lee, H., Kim, N., Lim, D.S., and Lee, J. (2017). Regulation of a hitchhiking behavior by neuronal insulin and TGF-beta signaling in the nematode *Caenorhabditis elegans*. *Biochem. Biophys. Res. Commun.* 484, 323–330. <https://doi.org/10.1016/j.bbrc.2017.01.113>.
43. Lee, D., Yang, H., Kim, J., Brady, S., Zdravljec, S., Zamanian, M., Kim, H., Paik, Y.K., Kruglyak, L., Andersen, E.C., and Lee, J. (2017). The genetic basis of natural variation in a phoretic behavior. *Nat. Commun.* 8, 273. <https://doi.org/10.1038/s41467-017-00386-x>.
44. Yim, H., Choe, D.T., Bae, J.A., Choi, M.K., Kang, H.M., Nguyen, K.C.Q., Ahn, S., Bahn, S.K., Yang, H., Hall, D.H., et al. (2024). Comparative connectomics of dauer reveals developmental plasticity. *Nat. Commun.* 15, 1546. <https://doi.org/10.1038/s41467-024-45943-3>.
45. Le, T.A., Temmerman, L., and Roy, C. (2025). Nictation behavior in nematodes. *BMC Biol.* 23, 356. <https://doi.org/10.1186/s12915-025-02443-0>.
46. Erkut, C., Vasilj, A., Boland, S., Habermann, B., Shevchenko, A., and Kurzchalia, T.V. (2013). Molecular strategies of the *Caenorhabditis elegans* dauer larva to survive extreme desiccation. *PLoS One* 8, e82473. <https://doi.org/10.1371/journal.pone.0082473>.
47. Erkut, C., Penkov, S., Fahmy, K., and Kurzchalia, T.V. (2012). How worms survive desiccation: Trehalose pro water. *Worm* 1, 61–65. <https://doi.org/10.4161/worm.19040>.
48. Crook, M. (2014). The dauer hypothesis and the evolution of parasitism: 20 years on and still going strong. *Int. J. Parasitol.* 44, 1–8. <https://doi.org/10.1016/j.ijpara.2013.08.004>.
49. Crook, M., Thompson, F.J., Grant, W.N., and Viney, M.E. (2005). daf-7 and the development of *Strongyloides ratti* and *Parastrongyloides trichosuri*. *Mol. Biochem. Parasitol.* 139, 213–223. <https://doi.org/10.1016/j.molbiopara.2004.11.010>.
50. Carstensen, H.R., and Hong, R.L. (2025). Dafadine Does Not Promote Dauer Development in *Pristionchus pacificus*. *MicroPubl. Biol.* 2025. <https://doi.org/10.17912/micropub.biology.001470>.
51. Mayer, M.G., Rödelberger, C., Witte, H., Riebesell, M., and Sommer, R.J. (2015). The Orphan Gene dauerless Regulates Dauer Development and Intraspecific Competition in Nematodes by Copy Number Variation. *PLoS Genet.* 11, e1005146. <https://doi.org/10.1371/journal.pgen.1005146>.
52. Lo, W.S., Roca, M., Dardiry, M., Mackie, M., Eberhardt, G., Witte, H., Hong, R., Sommer, R.J., and Lightfoot, J.W. (2022). Evolution and Diversity of TGF-beta Pathways are Linked with Novel Developmental and Behavioral Traits. *Mol. Biol. Evol.* 39, msac252. <https://doi.org/10.1093/molbev/msac252>.
53. Viney, M., and Diaz, A. (2012). Phenotypic plasticity in nematodes: Evolutionary and ecological significance. *Worm* 1, 98–106. <https://doi.org/10.4161/worm.21086>.
54. Viney, M., and Morris, R. (2022). Approaches to studying the developmental switch of *Strongyloides* - Moving beyond the dauer hypothesis. *Mol. Biochem. Parasitol.* 249, 111477. <https://doi.org/10.1016/j.molbiopara.2022.111477>.
55. Albert, P.S., and Riddle, D.L. (1988). Mutants of *Caenorhabditis elegans* that form dauer-like larvae. *Dev. Biol.* 126, 270–293. [https://doi.org/10.1016/0012-1606\(88\)90138-8](https://doi.org/10.1016/0012-1606(88)90138-8).
56. Zhang, M.G., and Sternberg, P.W. (2022). Both entry to and exit from diapause arrest in *Caenorhabditis elegans* are regulated by a steroid hormone pathway. *Development* 149, dev200173. <https://doi.org/10.1242/dev.200173>.
57. Antebi, A. (2015). Nuclear Receptor Signal Transduction in *C. elegans* (WormBook), pp. 1–49. <https://doi.org/10.1895/wormbook.1.64.2>.
58. Dogra, D., Kulalert, W., Schroeder, F.C., and Kim, D.H. (2022). Neuronal KGB-1 JNK MAPK signaling regulates the dauer developmental decision in response to environmental stress in *Caenorhabditis elegans*. *Genetics* 220, iyab186. <https://doi.org/10.1093/genetics/iyab186>.
59. Kim, S.K., Lund, J., Kiraly, M., Duke, K., Jiang, M., Stuart, J.M., Eizinger, A., Wylie, B.N., and Davidson, G.S. (2001). A gene expression map for *Caenorhabditis elegans*. *Science* 293, 2087–2092. <https://doi.org/10.1126/science.1061603>.

60. Nanda, S., Jacques, M.A., Wang, W., Myers, C.L., Yilmaz, L.S., and Walhout, A.J. (2023). Systems-level transcriptional regulation of *Caenorhabditis elegans* metabolism. *Mol. Syst. Biol.* 19, e11443. <https://doi.org/10.15252/msb.202211443>.
61. Langfelder, P., and Horvath, S. (2008). WGCNA: an R package for weighted correlation network analysis. *BMC Bioinf.* 9, 559. <https://doi.org/10.1186/1471-2105-9-559>.
62. Pereira, V., Waxman, D., and Eyre-Walker, A. (2009). A problem with the correlation coefficient as a measure of gene expression divergence. *Genetics* 183, 1597–1600. <https://doi.org/10.1534/genetics.109.110247>.
63. Calo, E., Gu, B., Bowen, M.E., Aryan, F., Zalc, A., Liang, J., Flynn, R.A., Swigut, T., Chang, H.Y., Attardi, L.D., and Wysocka, J. (2018). Tissue-selective effects of nucleolar stress and rDNA damage in developmental disorders. *Nature* 554, 112–117. <https://doi.org/10.1038/nature25449>.
64. Cohen, S.M., Sun, J.J., Schroeder, F.C., and Sternberg, P.W. (2021). Transcriptional Response to a Dauer-Inducing Ascaroside Cocktail in Late L1 in *C. elegans*. *MicroPubl. Biol.* 2021. <https://doi.org/10.17912/micropub.biology.000397>.
65. Golden, J.W., and Riddle, D.L. (1984). The *Caenorhabditis elegans* dauer larva: developmental effects of pheromone, food, and temperature. *Dev. Biol.* 102, 368–378. [https://doi.org/10.1016/0012-1606\(84\)90201-x](https://doi.org/10.1016/0012-1606(84)90201-x).
66. Banerjee, N., Rojas Palato, E.J., Shih, P.Y., Sternberg, P.W., and Hallem, E.A. (2024). Distinct neurogenetic mechanisms establish the same chemosensory valence state at different life stages in *Caenorhabditis elegans*. *G3 (Bethesda)* 14. <https://doi.org/10.1093/g3journal/jkac271>.
67. Zhang, M.G., Seyedolmohadesin, M., Hawk, S.M., Tauffenberger, A., Park, H., Finnen, N., Schroeder, F.C., Venkatachalam, V., and Sternberg, P.W. (2024). Sensory integration of food and population density during the diapause exit decision involves insulin-like signaling in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* 121, e2405391121. <https://doi.org/10.1073/pnas.2405391121>.
68. Cockx, B., Van Bael, S., Boelen, R., Vandeweyer, E., Yang, H., Le, T.A., Dalzell, J.J., Beets, I., Ludwig, C., Lee, J., and Temmerman, L. (2023). Mass Spectrometry-Driven Discovery of Neuropeptides Mediating Nicotinic Behavior of Nematodes. *Mol. Cell. Proteomics* 22, 100479. <https://doi.org/10.1016/j.mcpro.2022.100479>.
69. Yang, H., Lee, B.Y., Yim, H., and Lee, J. (2020). Neurogenetics of nictation, a dispersal strategy in nematodes. *J. Neurogenet.* 34, 510–517. <https://doi.org/10.1080/01677063.2020.1788552>.
70. Wang, Z., Garcia, F., Ehlers, R.U., and Molina, C. (2024). Dauer juvenile recovery transcriptome of two contrasting EMS mutants of the entomopathogenic nematode *Heterorhabditis bacteriophora*. *World J. Microbiol. Biotechnol.* 40, 128. <https://doi.org/10.1007/s11274-024-03902-6>.
71. Ewald, C.Y., Landis, J.N., Porter Abate, J., Murphy, C.T., and Blackwell, T.K. (2015). Dauer-independent insulin/IGF-1-signalling implicates collagen remodelling in longevity. *Nature* 519, 97–101. <https://doi.org/10.1038/nature14021>.
72. Boyle, E.A., Li, Y.L., and Pritchard, J.K. (2017). An Expanded View of Complex Traits: From Polygenic to Omnigenic. *Cell* 169, 1177–1186. <https://doi.org/10.1016/j.cell.2017.05.038>.
73. Vidal, M., Cusick, M.E., and Barabási, A.L. (2011). Interactome networks and human disease. *Cell* 144, 986–998. <https://doi.org/10.1016/j.cell.2011.02.016>.
74. Radulescu, E., Chen, Q., Pergola, G., Di Carlo, P., Han, S., Shin, J.H., Hyde, T.M., Kleinman, J.E., and Weinberger, D.R. (2023). Investigating trait variability of gene co-expression network architecture in brain by controlling for genomic risk of schizophrenia. *PLoS Genet.* 19, e1010989. <https://doi.org/10.1371/journal.pgen.1010989>.
75. Inoue, T., and Thomas, J.H. (2000). Targets of TGF- β signaling in *Caenorhabditis elegans* dauer formation. *Dev. Biol.* 217, 192–204. <https://doi.org/10.1006/dbio.1999.9545>.
76. Li, H.Y., Lin, X.W., Geng, S.L., and Xu, W.H. (2018). TGF- β and BMP signals regulate insect diapause through Smad1-POU-TFAM pathway. *Biochim. Biophys. Acta Mol. Cell Res.* 1865, 1239–1249. <https://doi.org/10.1016/j.bbamcr.2018.06.002>.
77. Patterson, G.I., Kowek, A., Wong, A., Liu, Y., and Ruvkun, G. (1997). The DAF-3 Smad protein antagonizes TGF- β -related receptor signaling in the *Caenorhabditis elegans* dauer pathway. *Genes Dev.* 11, 2679–2690. <https://doi.org/10.1101/gad.11.20.2679>.
78. Gartner, A., and Engebrecht, J. (2022). DNA repair, recombination, and damage signaling. *Genetics* 220, iyab178. <https://doi.org/10.1093/genetics/iyab178>.
79. Kipreos, E.T., and van den Heuvel, S. (2019). Developmental Control of the Cell Cycle: Insights from *Caenorhabditis elegans*. *Genetics* 211, 797–829. <https://doi.org/10.1534/genetics.118.301643>.
80. O'Neil, N., and Rose, A. (2006). DNA Repair (WormBook), pp. 1–12. <https://doi.org/10.1895/wormbook.1.54.1>.
81. Pintard, L., and Bowerman, B. (2019). Mitotic Cell Division in *Caenorhabditis elegans*. *Genetics* 211, 35–73. <https://doi.org/10.1534/genetics.118.301367>.
82. van den Heuvel, S. (2005). Cell-cycle Regulation (WormBook), pp. 1–16. <https://doi.org/10.1895/wormbook.1.28.1>.
83. van der Bliek, A.M., Sedensky, M.M., and Morgan, P.G. (2017). Cell Biology of the Mitochondrion. *Genetics* 207, 843–871. <https://doi.org/10.1534/genetics.117.300262>.
84. Zhou, Y., Wang, Y., Zhang, X., Bhar, S., Jones Lipinski, R.A., Han, J., Feng, L., and Butcher, R.A. (2018). Biosynthetic tailoring of existing ascaroside pheromones alters their biological function in *C. elegans*. *eLife* 7, e33286. <https://doi.org/10.7554/eLife.33286>.
85. Park, S., and Paik, Y.K. (2017). Genetic deficiency in neuronal peroxisomal fatty acid β -oxidation causes the interruption of dauer development in *Caenorhabditis elegans*. *Sci. Rep.* 7, 9358. <https://doi.org/10.1038/s41598-017-10020-x>.
86. Zhang, X., Feng, L., Chinta, S., Singh, P., Wang, Y., Nunnery, J.K., and Butcher, R.A. (2015). Acyl-CoA oxidase complexes control the chemical message produced by *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* 112, 3955–3960. <https://doi.org/10.1073/pnas.1423951112>.
87. Ludwig, A.H., and Schroeder, F.C. (2013). Ascaroside signaling in *C. elegans*. *WormBook*, 1–22. <https://doi.org/10.1895/wormbook.1.155.1>.
88. Edison, A.S. (2009). *Caenorhabditis elegans* pheromones regulate multiple complex behaviors. *Curr. Opin. Neurobiol.* 19, 378–388. <https://doi.org/10.1016/j.conb.2009.07.007>.
89. Lee, D., Fox, B.W., Palomino, D.F., Panda, O., Tenjo, F.J., Koury, E.J., Evans, K.S., Stevens, L., Rodrigues, P.R., Kolodziej, A.R., et al. (2023). Natural genetic variation in the pheromone production of *C. elegans*. *Proc. Natl. Acad. Sci. USA* 120, e2221150120. <https://doi.org/10.1073/pnas.2221150120>.
90. Jonassen, T., Larsen, P.L., and Clarke, C.F. (2001). A dietary source of coenzyme Q is essential for growth of long-lived *Caenorhabditis elegans* clk-1 mutants. *Proc. Natl. Acad. Sci. USA* 98, 421–426. <https://doi.org/10.1073/pnas.98.2.421>.
91. Li, T.Y., Gao, A.W., Li, X., Li, H., Liu, Y.J., Lalou, A., Neelagandan, N., Naef, F., Schoonjans, K., and Auwerx, J. (2023). V-ATPase/TORC1-mediated ATFS-1 translation directs mitochondrial UPR activation in *C. elegans*. *J. Cell Biol.* 222, e202205045. <https://doi.org/10.1083/jcb.202205045>.
92. Rottiers, V., Motola, D.L., Gerisch, B., Cummins, C.L., Nishiwaki, K., Mangelsdorf, D.J., and Antebi, A. (2006). Hormonal control of *C. elegans* dauer formation and life span by a Rieske-like oxygenase. *Dev. Cell* 10, 473–482. <https://doi.org/10.1016/j.devcel.2006.02.008>.
93. Son, S., Choi, M.K., Lim, D.S., Shim, J., and Lee, J. (2022). A genetic screen for aldicarb resistance of *Caenorhabditis elegans* dauer larvae uncovers 2 alleles of dach-1, a cytochrome P450 gene. *G3 (Bethesda)* 12. <https://doi.org/10.1093/g3journal/jkac266>.
94. Levis, N.A., and Pfennig, D.W. (2019). Phenotypic plasticity, canalization, and the origins of novelty: Evidence and mechanisms from amphibians.

- Semin. Cell Dev. Biol. 88, 80–90. <https://doi.org/10.1016/j.semcdb.2018.01.012>.
95. Oostra, V., Saastamoinen, M., Zwaan, B.J., and Wheat, C.W. (2018). Strong phenotypic plasticity limits potential for evolutionary responses to climate change. *Nat. Commun.* 9, 1005. <https://doi.org/10.1038/s41467-018-03384-9>.
96. West-Eberhard, M.J. (2005). Developmental plasticity and the origin of species differences. *Proc. Natl. Acad. Sci. USA* 102, 6543–6549. <https://doi.org/10.1073/pnas.0501844102>.
97. Ewels, P.A., Peltzer, A., Fillinger, S., Patel, H., Alneberg, J., Wilm, A., Garcia, M.U., Di Tommaso, P., and Nahnsen, S. (2020). The nf-core framework for community-curated bioinformatics pipelines. *Nat. Biotechnol.* 38, 276–278. <https://doi.org/10.1038/s41587-020-0439-x>.
98. Di Tommaso, P., Chatzou, M., Floden, E.W., Barja, P.P., Palumbo, E., and Notredame, C. (2017). Nextflow enables reproducible computational workflows. *Nat. Biotechnol.* 35, 316–319. <https://doi.org/10.1038/nbt.3820>.
99. Andrews, S. (2010). FastQC: A Quality Control Tool for High Throughput Sequence Data (Babraham Institute: Babraham Bioinformatics). <http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>.
100. Krueger, F. (2010). TrimGalore. GitHub repository. <https://github.com/FelixKrueger/TrimGalore>.
101. Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., and Gingeras, T.R. (2013). STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 29, 15–21. <https://doi.org/10.1093/bioinformatics/bts635>.
102. Love, M.I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 15, 550. <https://doi.org/10.1186/s13059-014-0550-8>.
103. R Core Team (2023). R: A language and environment for statistical computing.
104. Wu, T., Hu, E., Xu, S., Chen, M., Guo, P., Dai, Z., Feng, T., Zhou, L., Tang, W., Zhan, L., et al. (2021). clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. *Innovation* 2, 100141. <https://doi.org/10.1016/j.xinn.2021.100141>.
105. Gu, Z. (2022). Complex heatmap visualization. *iMeta* 1, e43. <https://doi.org/10.1002/imt2.43>.
106. Kolde, R. (2019). pheatmap: Pretty heatmaps.
107. Blighe, K., Rana, S., and Lewis, M. (2024). EnhancedVolcano: publication-ready volcano plots with enhanced colouring and labeling.
108. Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis.
109. Crowley, B.S. (2024). Ggally: Extension to 'ggplot2'.
110. Neal, S.J., Kim, K., and Sengupta, P. (2013). Quantitative assessment of pheromone-induced Dauer formation in *Caenorhabditis elegans*. *Methods Mol. Biol.* 1068, 273–283. https://doi.org/10.1007/978-1-62703-619-1_20.
111. Yates, A.D., Allen, J., Amode, R.M., Azov, A.G., Barba, M., Becerra, A., Bhai, J., Campbell, L.I., Carbajo Martinez, M., Chakiachvili, M., et al. (2022). Ensembl Genomes 2022: an expanding genome resource for non-vertebrates. *Nucleic Acids Res.* 50, D996–D1003. <https://doi.org/10.1093/nar/gkab1007>.
112. Garcia-Alcalde, F., Okonechnikov, K., Carbonell, J., Cruz, L.M., Gotz, S., Tarazona, S., Dopazo, J., Meyer, T.F., and Conesa, A. (2012). Qualimap: evaluating next-generation sequencing alignment data. *Bioinformatics* 28, 2678–2679. <https://doi.org/10.1093/bioinformatics/bts503>.
113. Wang, L., Wang, S., and Li, W. (2012). RSeQC: quality control of RNA-seq experiments. *Bioinformatics* 28, 2184–2185. <https://doi.org/10.1093/bioinformatics/bts356>.
114. Benjamini, Y., and Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *J. Roy. Stat. Soc. B* 57, 289–300. <https://doi.org/10.1111/j.2517-6161.1995.tb02031.x>.
115. Yu, G., Wang, L.G., Han, Y., and He, Q.Y. (2012). clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS* 16, 284–287. <https://doi.org/10.1089/omi.2011.0118>.
116. Carlson, M. (2023). [org.Ce.eg.db: Genome wide annotation for Worm](https://www.ebi.ac.uk/Ensembl/GenomeWideAnnotation).
117. Tepper, R.G., Ashraf, J., Kaletsky, R., Kleemann, G., Murphy, C.T., and Bussemaker, H.J. (2013). PQM-1 complements DAF-16 as a key transcriptional regulator of DAF-2-mediated development and longevity. *Cell* 154, 676–690. <https://doi.org/10.1016/j.cell.2013.07.006>.
118. Shostak, Y., Van Gilst, M.R., Antebi, A., and Yamamoto, K.R. (2004). Identification of *C. elegans* DAF-12-binding sites, response elements, and target genes. *Genes Dev.* 18, 2529–2544. <https://doi.org/10.1101/gad.1218504>.
119. Oh, S.W., Mukhopadhyay, A., Dixit, B.L., Raha, T., Green, M.R., and Tissenbaum, H.A. (2006). Identification of direct DAF-16 targets controlling longevity, metabolism and diapause by chromatin immunoprecipitation. *Nat. Genet.* 38, 251–257. <https://doi.org/10.1038/ng1723>.
120. Gumienny, T.L., and Savage-Dunn, C. (2013). TGF-beta signaling in *C. elegans*. *WormBook*, 1–34. <https://doi.org/10.1895/wormbook.1.22.2>.
121. Ashrafi, K. (2007). Obesity and the regulation of fat metabolism. *WormBook*, 1–20. <https://doi.org/10.1895/wormbook.1.130.1>.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
<i>Escherichia coli</i> OP50	<i>Caenorhabditis elegans</i> Genetics Center	WB Cat#WBStrain00041969
Chemicals, peptides, and recombinant proteins		
Ascr#2	MedChemExpress	CAS:946524-24-9
Ascr#3	MedChemExpress	CAS: 946524-26-1
Ascr#5	TargetMol	N/A
Nematode Growth Media (NGM)	In-house preparation	N/A
M9 buffer	In-house preparation	N/A
Critical commercial assays		
Direct-zol RNA Miniprep	Zymo Research	RRID:SCR_021935 Cat# R2050
Illumina TruSeq Stranded mRNA kit	Illumina	N/A
Deposited data		
Dauer Raw data	This Paper	NCBI GEO: GSE274872 and GSE318477
WT L2/L3 Raw and analyzed data	Godthi et al. ³⁶	NCBI GEO: GSE218596 and GSE229132
Experimental models: Organisms/strains		
<i>C. elegans</i> N2 (Wild-type, Bristol)	<i>Caenorhabditis</i> Genetics Center (CGC)	N2
<i>C. elegans</i> CB1370, daf-2(e1370) III	<i>Caenorhabditis</i> Genetics Center (CGC)	CB1370
<i>C. elegans</i> VEP032, ilc-17.1(syb5296) X	Prahlad Lab / SunyBiotech	VEP032
<i>C. elegans</i> VEP036, unc-119(ed4); gtIs1[CEP-1::GFP]	Prahlad Lab	VEP036
<i>C. elegans</i> CB1372, daf-7(e1372) III	<i>Caenorhabditis</i> Genetics Center (CGC)	CB1372
Software and algorithms		
nf-core/rnaseq v3.12.0	Ewels et al. ⁹⁷	doi: https://doi.org/10.5281/zenodo.1400710
Nextflow	Di Tomasso et al. ⁹⁸	RRID:SCR_018365; https://www.nextflow.io/ ;
Fastqc v0.11.9	Babraham Institute ⁹⁹	https://www.bioinformatics.babraham.ac.uk/projects/fastqc/
Trimgalore v0.67	Babraham Institute ¹⁰⁰	https://github.com/FelixKrueger/TrimGalore
STAR v.2.7.9a	Dobin et al. ¹⁰¹	https://github.com/alexdobin/STAR
Salmon	Combine-lab	https://github.com/COMBINE-lab/salmon
Qualimap v2.2.2	-	http://qualimap.conesalab.org/
RseQC v3.0.1	-	https://rseqc.sourceforge.net/
DESeq2 v1.42.1	Love et al. ¹⁰²	DOI: https://doi.org/10.18129/B9.bioc.DESeq2
tximport	Love et al. ¹⁰²	DOI: https://doi.org/10.18129/B9.bioc.tximport
R	The R Foundation ¹⁰³	N/A
clusterProfiler v4.0	Wu et al. ¹⁰⁴	DOI: https://doi.org/10.18129/B9.bioc.clusterProfiler
ComplexHeatmap v2.1.8.0	Gu, Zhang ¹⁰⁵	DOI: https://doi.org/10.18129/B9.bioc.ComplexHeatmap
Pheatmap v1.0.12	Kolde, Raivo ¹⁰⁶	https://github.com/raivokolde/pheatmapcom
EnhancedVolcano v1.13.2	Blinghe et al. ¹⁰⁷	DOI: https://doi.org/10.18129/B9.bioc.EnhancedVolcano
ggplot2 v3.5.1	Wickman et al. ¹⁰⁸	https://ggplot2.tidyverse.org
GGally	Barret et al. ¹⁰⁹	https://ggobi.github.io/ggally/
Plasticity and Dispersion Function on R	This paper.	https://doi.org/10.6084/m9.figshare.29421131.v2

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

C. elegans strains

C. elegans strains used in this study are listed in the [key resources table](#). Strains were obtained from the Caenorhabditis Genetics Center (CGC, Twin Cities, MN), generated in-house, or by Suny Biotech (Suzhou, Jiangsu, China). All strains were grown and maintained at 20°C unless otherwise mentioned. Animals were grown in 20°C incubators (humidity controlled) on 60mm nematode growth media (NGM) plates by passaging 8-15 L4s (depending on the strain) onto a fresh plate. Extra care was taken to ensure equal worm densities across all strains. Animals were fed *Escherichia coli* OP50 obtained from Caenorhabditis Genetics Center (CGC) that were seeded ($OD_{600}=1.5$, strictly maintained in all experiments) onto culture plates 2 days before use. The NGM plate thickness was controlled by pouring 8.9ml of autoclaved liquid NGM per 60mm plate. Laboratory temperature was maintained at 20°C and monitored throughout. For all experiments, age-matched day-one hermaphrodites, or larvae timed to reach specific developmental stages as mentioned in the text or figure legend, were used.

METHOD DETAILS

Synchronization and staging of *C. elegans*

Populations of 250-300 gravid adults were generated by passaging L4s on NGM plates. These plates were used for obtaining synchronized embryos by bleach-induced solubilization of the adults to then obtain larvae for harvesting mRNA. Specifically, animals were washed off the plates with 1X PBS and pelleted by centrifuging at 2665Xg for 30s. The PBS was removed carefully, and worms were gently vortexed in the presence of bleaching solution [250μl 1N NaOH, 200μl standard (regular) bleach and 550μl sterile water] until all the worm bodies had dissolved (approximately 5-6 minutes), and only eggs were viable. The eggs were pelleted by centrifugation (2665Xg for 45s), bleaching solution was carefully removed and then embryos were washed with sterile water 4-5 times and counted under the microscope. The desired number of embryos were seeded on fresh OP50 plates and allowed to grow at 25°C or 27°C for specific time periods depending on the experimental need. If >5% of eggs remained unhatched, these plates were discarded.

Obtaining embryos, larvae and dauers

Mixed stage embryos

Embryos obtained through bleach synchronization were pelleted by centrifugation (2665Xg for 45s), bleaching solution was carefully removed, embryos were washed with sterile water 4-5 times and pipetted onto unseeded NGM plates for 30 minutes at 25°C. This latter step enabled the complete removal of any traces of bleach that may have remained. Embryos were washed off the NGM plates and processed for RNA extraction.

15 hrs L1 larvae/ 32 hrs L2/L3 larvae

Day-one adult worms grown at 20°C (I-36LLVL Incubator) were bleach-hatched and ~3200 eggs/genotype (~800 eggs/plate and 4 plates/genotype) were seeded on fresh OP50 plates and allowed to grow for either 15 hours (L1 stage) or 32 hours (L2/L3 or L2d stage) at 25°C in Echotherm incubator IN30-2. Worms were washed with sterile water, and total RNA was extracted.

Day-one adult *C. elegans*:

~3200 embryos (~800 eggs/plate and 4 plates) of wild type day-one adults obtained through bleach synchronization were grown at 20°C (I-36LLVL Incubator) on fresh OP50 plates and allowed to grow to day-one adults in Echotherm incubator IN30-2. Worms were washed with sterile water, and total RNA was extracted from biological triplicates using the Direct-zol RNA Miniprep (catalog no. R2050, Zymo Research).

Genetically-induced dauers

Day-one adult worms of the different genetic backgrounds that lead to constitutive dauer formation were grown at 20°C (I-36LLVL Incubator), bleached to obtain ~3200 eggs/genotype (~800 eggs/plate and 4 plates/genotype), allowed to hatch on fresh OP50 plates and grow for 48 hours at 25°C Echotherm incubator IN30-2. The population developed into dauers (>99.5% dauers). The population was harvested at 48hrs after seeding the embryos onto plates. Any non-dauer worms were picked off the plate before collection. Worms were washed with sterile water, and total RNA was extracted from biological triplicates using the Trizol extraction method.

Heat induced dauer larvae (HID)

Heat-induced dauers (Wild-type HID) were obtained from N2 (wildtype control) eggs incubated for 48 hours at 27°C in New Brunswick Galaxy 170S Incubator. These plates consisted of >96% dauers and all non-dauer worms were picked off to avoid variable staged worms in the RNA prep. Worms were washed with sterile water, and total RNA was extracted.

Pheromone induced dauer larvae (PID)

We followed the protocol described by Neal et al.¹¹⁰ Briefly, ~1000 embryos were bleach hatched from Gravid Adults and grown in six-well plates with a mixture 3ml of M9 buffer, 10μl of OP50 bacteria, and 100μl of 20μM ascarosides (#2, #3, #5) mixture at 25°C for 10 days.¹¹⁰ 1% SDS was used to select dauers; the dauers were washed and processed for RNA extraction.

Starvation induced dauer larvae (SID)

Two day-1 adults that were moved onto seeded OP50 plated, and the population was allowed to exhaust food over 10-12 days. Plates were washed off with sterile water, 1% SDS was used to select dauers; the dauers were washed and processed for RNA extraction.

RNA extraction, library preparation, and sequencing

Total RNA was extracted from biological triplicates (duplicates in the case of Pheromone and Starvation dauers) using either the Direct-zol RNA Miniprep kit (Zymo Research, R2050) following the manufacturer's protocol, including an on-column DNase I digestion step, or a standard Trizol extraction method for dauer larvae. mRNA libraries were prepared using the Illumina TruSeq Stranded mRNA kit. All samples were sequenced on a single lane of an Illumina NovaSeq 6000, generating 2x100bp paired-end reads.

Pre-processing and alignment of RNA-seq

- RNA-seq analysis (dauers): RNA seq data was analyzed and processed using nf-core/rnaseq (v3.12.0),⁹⁷ and the pipeline was executed with Nextflow (v22.10.6).⁹⁸ In short, the quality of the sequences was assessed with Fastqc (v.0.11.9),⁹⁹ and Trimalore (v.0.67)¹⁰⁰ was used to filter low quality sequence reads and remove adapters. The processed reads were aligned to *C. elegans* genome (WBCel235 Ensembl release 111),¹¹¹ using STAR (v.2.7.9a)¹⁰¹ with default settings. Alignments were quantified with Salmon using the WBCel235 annotation. Quality control of the alignment was performed with Qualimap (v.2.2.2)¹¹² and RseqQC (v.3.0.1).¹¹³
- RNA-seq analysis (embryos, larvae and adults): RNA seq was processed using nf-core/rnaseq, v3.12.0 with parameters previously described.^{36,97}
- RNA-seq analysis (combining dauer and larvae): For the analysis that required data from combining all developmental stages, i.e. the dauers, embryos, larvae, and adults, the counts matrix of each study was concatenated and merged, then the data was normalized with DESeq2 (v.1.42.1).¹⁰²

Data normalization and analysis

Transcript-level estimates were summarized to gene-level counts with tximport. Genes with low counts ($n < 10$ in two or more replicates per sample) were filtered, leaving 19040 genes for downstream analysis, unless otherwise indicated. For the Time Series analysis this number of genes was calculated again leaving 19052 genes. Regularized log transformation (rlog)¹⁰² and variance stabilization transformation (VST)¹⁰² were used to normalize and compare gene-level expression. For each sample, rlog expression of its replicates ($n=3$ or $n=2$) was averaged to generate the rlog mean-expression. Z-score of the rlog mean-expression was calculated by scaling and centering the data, row-wise (scaling the expression values of genes).

Principal Component Analysis (PCA)

PCA was performed on VST-transformed gene counts using the prcomp function in R to assess variance between conditions. Combining the different samples resulted in a slight variation in the number of genes used for each PCA.

- Figure 1C: Genes filtered for low counts (19040) were used to compare dauers, WT L2/L3 larvae, and WT adults.
- Figure S1D (Dauers Only): The same genes used above were used but the VST matrix was filtered to include only data from the seven dauer strains.
- Figure 2B (Time Series): Genes filtered for low counts (19052) genes were used to compare WT (including induced Dauers), *ilc-17.1*, and *cep-1* OE at 0, 15, 32, and 48 hours.

Differential Expression

Differential expression between dauers and L2/L3 larvae was computed with DESeq2²⁸ by using the WALD test.¹⁰² Changes in expression were presented as log₂ fold-change, and genes with an adjusted p-value of < 0.05 (after correction with Benjamini & Hochberg),¹¹⁴ were considered significant.

Gene Ontology

Gene Ontology (GO) enrichment analysis was performed using Over Representation Analysis (ORA) with the clusterProfiler¹¹⁵ R package using annotations from the package org.Ce.eg.db.¹¹⁶ GO terms with an adjusted p-value < 0.05 (Benjamini & Hochberg)¹¹⁴ were considered significant. Adjusted p-values were converted to $-\log_{10}$ (adjusted p-value) for plotting, and the Fold-Enrichment was calculated as the ratio of the frequency of input genes annotated in a GO term to the frequency of all genes annotated to that term.¹⁰⁴ In Figures 3B and 3C, for the comparison between Dauers and WT L2/L3, the significantly upregulated and downregulated genes were used as input for the enrichment test.

Likelihood Ratio Test (LRT)

To determine whether gene expression was similar between dauers, we performed a Likelihood Ratio test,¹⁰² using the DESeq2 package. Specifically, for the gene expression in the seven dauers, the model in DESeq2, $expression \sim 1 + condition$ was compared

to the reduced model $expression = \sim 1$, representing the null model where the expression is constant across all the samples. Genes with an adjusted p-value < 0.05 (Benjamini & Hochberg)¹¹⁴ were considered to have differences in expression in at least one of the seven dauers. Genes with p-value > 0.05 , were considered not to be significantly different between the dauers.

Hierarchical clustering

Hierarchical clustering (Euclidean distance, complete linkage) was performed on Z-scores of rlog mean-expression. The optimal number of clusters was determined by using the elbow method.

Distance metrics and correlation analysis

Distance

In all distance metrics a lower value indicates proximity between the two samples. Unless otherwise indicated the rlog mean-expression was used as gene expression input. The sample-sample pairwise distances were calculated by determining (i) the Euclidean distance and (ii) the 1-Pearson Correlation.

Correlation analysis

Spearman's correlation and Pearson correlations were calculated on VST normalized data using the R packages stats and Ggally.^{103,109} Correlations were considered significant if the p-value < 0.05 .

Analysis of canalization and plasticity

Selection of gene Co-expression networks and pathways (traits)

A subset of pathways (106) curated from the literature were selected for the Distance analysis. This included: (i) Co-expression clusters (Kim- Mounts, 34 pathways from 5,361 *C. elegans* genes)⁵⁹; (ii) Metabolic Pathways (85 pathways, comprising 1,799 metabolism-related genes)⁶⁰; (iii) Dauer Inducing Pathways (Insulin, daf-12, daf-16, TGF β)^{26,27,117–120} and (iv) growth arrest pathways (Energy metabolism, cell cycle).^{82,121} Pathways with less than 8 genes were excluded from analysis. For visualization and linear regression analysis, the pathways were grouped together into four-super sets "Core cellular processes", "Energy_metabolism_mito", "Morphological_features", "Signal_transduction", and "Metabolic_features".

Calculating a plasticity index

To calculate the level of Plasticity, for each geneset (pathway or co-expression network, which we defined as a 'trait'), the rlog mean-expression was used. The Plasticity Index was meant as a way to compute one value per trait for a group of dauers that would reflect the gene expression change for genes within that trait, when compared to continuously growing L2/L3. Briefly, the Plasticity Index, was computed, by calculating a mean expression value (meanDauer) for each gene within a gene co-expression network, or 'trait', across all dauers, or the two groups of dauers (environmentally-induced, and genetically-induced). The Euclidean distance, between this meanDauer value for each gene and the wild type L2/L3 expression value was computed. These distances were integrated (by using the square root of the sum of distances) across all genes that contributed to a trait. Finally, the index was normalized by dividing the result by square root of the number of genes within that trait

The general formula is shown below

$$Plasticity = \frac{1}{\sqrt{g}} dist(\mathbf{V}_{L2/L3}, \overline{\mathbf{Z}}_{dauer}) \quad (\text{Equation 1})$$

- (i) g are the number of genes in the geneset analyzed.
- (ii) $\mathbf{V}_{L2/L3}$ is the expression vector for the genes in the WT L2/L3 sample (rlog values)
- (iii) $\overline{\mathbf{Z}}_{dauer}$ is the mean expression vector (centroid) of all dauer samples (rlog values), referred to in the text as the meanDauer.
- (iv) $dist$ represents the calculation of Euclidean distance between the two vectors and its integrations, which is shown in more detail in the next formula

$$Plasticity = \frac{1}{\sqrt{g}} \sqrt{\sum_{j=1}^g (v_j - \bar{z}_j)^2} \quad (\text{Equation 2})$$

- (i) g are the number of genes in the geneset analyzed.
- (ii) v_j is the expression value of the j -th gene in the $\mathbf{V}_{L2/L3}$ vector.
- (iii) $\bar{z}_j$ represents the mean rlog of the j -th gene across meanDauer.

Calculating a dispersion index

To calculate the level of dispersion between the dauers, for each geneset (pathway or co-expression network), the z-score expression was used as input. The Dispersion Index was computed by first calculating a mean value for each gene within a trait (meanDauer)

and then deriving the Euclidean distance for the expression of that gene between each dauer and the 'meanDauer'. This procedure was done for all genes within a trait, these distances were integrated (by using the square root of the sum of distances) to obtain one value per dauer/trait. The values were averaged by the number of dauers, obtaining one value for a group of dauers for a given trait. Finally, this value was normalized by dividing the result by square root of the number of genes within the network to obtain a Dispersion Index value across a given set of dauers for each of the traits.

The general formula is shown below

$$Dispersion = \frac{1}{n\sqrt{g}} \sum_{i=1}^n dist(\mathbf{Z}_i, \overline{\mathbf{Z}_{dauer}}) \quad (\text{Equation 3})$$

- i. n is the number of dauers samples in the group (For example 7 dauers for all.dauers group)
- ii. g are the number of genes in the geneset analyzed.
- iii. $\overline{\mathbf{Z}_{dauer}}$ is the mean vector (centroid) of all Z-scored dauer samples, referred on the text as the meanDauer.
- iv. $\mathbf{Z}_i$ is the Z-scored expression vector for the i -th individual dauer sample.
- v. $dist$ represents the calculation of Euclidean distance between the two vectors and its integrations, which is shown in more detail in the next formula

$$Dispersion = \frac{1}{n\sqrt{g}} \sum_{i=1}^n \left(\sqrt{\sum_{j=1}^g (z_{ij} - \bar{z}_j)^2} \right) \quad (\text{Equation 4})$$

- i. $\bar{z}_j$ represents the mean Z-score of the j -th gene across meanDauer.
- ii. z_{ij} represent the Z-score of the j -th gene in the i -th dauer sample
- iii. The left term $\frac{1}{n\sqrt{g}} \sum_{i=1}^n$ represents the operation of averaging the results of the by the number of dauers and then normalizing by the number of genes

Data visualization

Heatmaps were generated using the R packages ComplexHeatmap (v.2.1.8.0) and pheatmap (v.1.0.12).^{105,106} Volcano Plots were created with Enhanced Volcano (v.1.13.2).¹⁰⁷ Additional plots were generated using ggplot2 (v.3.5.1).¹⁰⁸

QUANTIFICATION AND STATISTICAL ANALYSIS

General statistical analysis

Student's t-test was used for comparing Euclidean distance distributions and the difference between the means was considered significant if the p -value < 0.05 ; in the figures the stars represent: **** $p < 0.0001$; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$. Linear regression was performed using the function `lm` in R,¹⁰³ with a confidence interval level of 99% and a significance threshold of $p < 0.05$. All the statistical analyses were conducted in R.¹⁰³ Additional statistical details of experiments can be found in the methods and figure legends.