**Figure 1. A)** Experimental design for our RNA-seq studies. *C. elegans* hermaphrodites were raised until they grew to pre-reproductive adulthood (50hrs) or reproductive adulthood (58hrs) in the presence or absence of ascr#10. **B)** Venn diagram of differentially expressed genes in response to ascr#10 exposure at 50hrs and 58hrs. A total of 12,223 genes were reliably detected across all experiments. **C)** For genes DE at both times in response to ascr#10 exposure, the great majority of genes are positively correlated. A small (and we argue, insignificant) population was anti-correlated. **D)** We detected significant topological correlations among genes, potentially indicative of transcription-factor driven enhancer activity. Additionally, genes in chromosome IV were over-represented (p < 10-2) among the commonly differentially expressed genes, whereas genes in chromosome X were depleted (p < 10-7). Shaded region represents the correlations observed in 95% of 10,000 randomized shuffled datasets.

**Figure 2. A)** Volcano plot of enriched tissues at 58hrs. Briefly, we asked whether genes expressed in specific tissues tended to change in one direction more often than expected by random chance. Statistical significance was measured using a binomial test; magnitude of enrichment was measured using regulatory enrichment, defined as the difference between the observed and expected fraction of differentially upregulated genes, normalized to the fraction of upregulated genes. Tissues that have a net excess (>0) regulatory enrichment have genes that tend to be upregulated more than expected by chance. At 58hrs, the only downregulated tissue was *sperm*. **B)** Q-values for all the enriched tissues at 58hrs, and their corresponding values at 50hrs. There is broad concordance in the significance and direction of the values, with a notable improvement in the germ line signals. **C)** log2(Fold Change) values for specific groups of genes (vitellogenin, major sperm protein and ribosomal protein subunit genes), showing these groups behave as functional units, frequently changing in the same direction. The small increase in ribosomal protein subunits could reflect increased protein genesis, whereas the depletion in major sperm protein mRNA reflects feminization of the germline. **D)** Visualization of the positive and negative ascr#10 signature using the single-cell RNA-seq dataset from Cao *et al.* Signatures are defined as genes that were commonly DE in both conditions and in the same direction and contain 100-200 genes. The positive signature shows enrichment in the female germ line, some enrichment in somatic reproductive tissue and in the pharynx. The negative signature shows enrichment in sperm, hypodermis and intestine. A random signature is shown for comparison.

**Figure 3.** WILL WRITE.

**Figure 4. A)** PQM-1-bound gene enrichment in up- or downregulated genes at 50hrs and 58hrs. PQM-1-bound genes were enriched only in genes upregulated in response to ascr#10 at both timepoints. **B)** A subset of PQM-1-bound genes that are upregulated at 50hrs in response to ascr#10 are downregulated in a *pqm-1* mutant exposed to ascr#10 at 50hrs, showing that a subset of PQM-1-bound genes alter their responses to ascr#10 when *pqm-1* is lost. Thus, *pqm-1* quantitatively modulates and refines the ascr#10 response. **C)** In the absence of *pqm-1*, the germ line is still feminized in response to ascr#10, but the other tissue signals are scrambled, suggesting that *pqm-1* coordinates and refines the tissue-specific responses to ascr#10. **D)** Vitellogenin genes are down-regulated at 50hrs in a *pqm-1* mutant, in contrast to wild type animals at 50hrs where these genes are upregulated. Major sperm protein genes are downregulated at 50hrs in *pqm-1* mutants (in wild type animals, they are slightly down-regulated overall). Whereas wild type animals have increased levels of ribosomal protein subunit mRNAs, in a *pqm-1* these genes do not change on average. **E)** Loss of *pqm-1* ablates certain ascr#10 mediated phenotypes. Germline progenitor cells do not proliferate in response to ascr#10 in the absence of *pqm-1.* Similarly, *pqm-1* mutants show no effect in their pharyngeal pumping rates. *pqm-1* mutants do not move more in response to ascr#10.

**A male pheromone reprograms an organism’s life trajectory**

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**Introduction**

*Pheromones and their known roles in the animal kingdom*

*ascr#10 is a male signal that alters reproductive strategies in* C. elegans

*Signal transduction likely involves multiple tissues?*

**Results**

*The response to ascr#10 is temporally and sequentially correlated in* C. elegans *hermaphrodites*

Previously (Aprison et al, 2022), we sequenced wild-type *C. elegans* N2 animals that had reached pre-reproductive adulthood (at approximately 50 hrs post-L1 starvation) or post-reproductive adulthood, as assessed by the first egg-laying (at 58 hrs post-L1 starvation) that had been exposed to nothing or to a physiologically relevant concentration of ascr#10 (Fig 1A). Previously, we used *DESeq2* to perform a differential expression analysis that identified genes that change expression in response to ascr#10 exposure at either timepoint (Fig 1B). The magnitude of differential expression was limited in both cases, with the great majority of genes changing less than 2-fold. There were more (3,628) differentially expressed genes (DEGs) at 58hrs than at 50hrs (1,663), which could suggest that ascr#10 response strengthens with time. However, we also had greater statistical power to detect gene expression changes at 58hrs because the 58hr experiment was performed twice, since the first experiment recovered fewer reads than desired. Of the 1,663 DEGs at 50hrs, 741 DEGs were shared with the 58hr condition—we refer to this set of genes as ascr#10-responsive genes (DO WE NEED A P-VALUE? I prefer not to have it). Given the high false negative rate associated with RNA-seq (XXX), this overlap suggests excellent agreement in the transcriptomic response at both timepoints. Additionally, the 741 DEGs are largely positively correlated with similar magnitudes (Fig 1C).

Pre-reproductive adults exposed to ascr#10 do not show responses along several measured phenotypes, in contrast to post-reproductive adults at 58hrs, which do show responses (); however, these results demonstrate that the ascr#10 receptors and signaling pathways are present and active at 50hrs. Another possibility is that the ascr#10 response induces distinct responses at 50hrs and at 58hrs, but the significant overlap between DEGs suggests that two different responses, if different, largely involve the same genes. Taken together, these results suggest that the ascr#10 is refined and augmented through time to mediate its physiological effects.

To better understand how ascr#10 effects organismal-level responses through molecular pathways, we explored the spatial distribution and correlation among ascr#10 responsive genes. Ascr#10-responsive genes were enriched in chromosome IV and depleted in chromosome X (PVALS HERE). We found significant spatial structure among the ascr#10 response, particularly along chromosomes I, IV and V (Fig 1D). We interpret this spatial structure as evidence of enhancer activity, and by extension, evidence of at least one transcription factor modulating the ascr#10 response.

*Exposure to ascr#10 feminizes the* C. elegans *germline*

We dissected the effects that ascr#10 has on the various *C. elegans* tissues using the gene expression patterns provided by WormBase (CITATION). Briefly, for each tissue, we tested whether differentially expressed genes associated with this tissue tended to be upregulated (downregulated) more often than expected by random chance. We termed the extent of excess upregulation (downregulation) “regulatory enrichment”. Regulatory enrichment is defined as the difference between the observed and expected fraction of upregulated genes, normalized to the expected fraction of upregulated genes. This analysis revealed that, at 50 and 58hrs, the germ line was greatly upregulated, whereas sperm were the only cells with regulatory enrichment far below that expected by chance (Fig 2A-B).

We explored a small set of gene programs to further understand ascr#10 effects. All vitellogenin genes increased expression at 50hrs, but not at 58hrs. Major sperm protein genes did not change expression on average at 50hrs, but they were almost entirely downregulated at 58hrs. ascr#10 also caused a small but consistent increase in the expression of all ribosomal protein subunits, large and small, suggesting that translational activity is increased in animals exposed to ascr#10 (Fig 2C) (INCIDENTALLY, THIS IS PROBABLY WHY THERE’S AN ENORMOUS CHAPERONE RESPONSE DEPLOYED IN ASCR#10 EXPOSED ANIMALS).

A potential drawback of our tissue analyses so far is that they rely on manually

annotated, though expertly curated, gene expression patterns derived largely from GFP promoter constructs. To validate our results, we reanalyzed the single-cell dataset provided by (Cao, Murray). We derived positive and negative ascr#10 signatures of ascr#10 responsive genes by identifying genes that are significantly differentially expressed at 50hrs and 58hrs with a q-value <0.01, and which are sign concordant across both time points. For comparison, we also generated a random signature. Using these signatures, we computed an enrichment score per cell for each signature. The positive signature was enriched in the germ line and the somatic reproductive tissues, whereas the negative signature showed the greatest score in sperm cells (Fig 2D). Taken together, these results demonstrate that ascr#10 feminizes *C. elegans* tissues.

*Hermaphrodites exposed to ascr#10 suffer increased rates of pharyngeal hypertrophy*

Our tissue enrichment analyses also identified the pharynx as another tissue that showed regulatory enrichment. The pharynx was also highlighted in the single-cell data using the positive ascr#10 signature. **Previous work has shown that hermaphrodites exposed to ascr#10 suffer from a shortened lifespan; separately, pharyngeal hypertrophy has been identified as a cause of death in *C. elegans* animals (CITATION). The upregulation of pharyngeal-associated genetic programs in early adulthood made us consider whether ascr#10 causes increased pharyngeal use leading to hypertrophy, potentially explaining the life-shortening effects of ascr#10 exposure**. To test this hypothesis, we raised animals in the presence or absence of ascr#10. At 7 days post-hatch (ILYA CHECK), we measured the size of the posterior pharyngeal bulb and observed that the size of this bulb was increased in animals exposed to ascr#10 (XX in control animals; YY in animals exposed to ascr#10; p-value < 5 \* 10-6, non-parametric bootstrap). We also measured an increased pumping rate in animals exposed to ascr#10 relative to control animals (XX in control animals; YY in animals exposed to ascr#10; p-value = 0.012, non-parametric bootstrap). A hallmark of pharyngeal hypertrophy is increased bacterial colonization in the intestine (DID THE ORIGINAL PAPER SHOW INCREASED COLONIZATION IN THE PHARYNX?). We confirmed that the increased posterior bulb area and the elevated pharyngeal pumping rate in animals exposed to ascr#10 was pathological by measuring live bacterial accumulation in the intestine at 7 days post-hatching (p-value = 0.011, non-parametric bootstrap). Therefore, we conclude that exposure to ascr#10 increases the pharyngeal pumping rate in *C. elegans* hermaphrodites, leading to accelerated pharyngeal deterioration and hypertrophy. The effects of ascr#10 on the pharynx are detectable through RNA-seq as early as 50 hours post-L1 starvation, highlighting the power of transcriptomes as physiologically relevant phenotypes.

pqm-1 *is a major regulator of the ascr#10* *response*

The strong spatial correlation among the ascr#10 response (Fig 1D) strongly suggested the activity of at least one major transcription factor. One of us (IR) recognized that some of the differentially expressed genes have been previously associated with PQM-1 binding sites and control (). We therefore tested our ascr#10 responses for enrichment of PQM-1 bound genes using a previously published dataset (). We found a strong enrichment of PQM-1 bound genes in the upregulated transcriptomic responses at both timepoints (Fig 4A), suggesting that *pqm-1* may be an important regulator of the ascr#10 response. *pqm-1* expression levels are slightly up-regulated at 50 hrs (log2(Fold Change) = 0.017, q-value=0.042).

We tested the hypothesis that *pqm-1* is required for appropriate response to ascr#10 by exposing animals that are null mutants for *pqm*-1 (allele: XXX, strain: ) to ascr#10 at 50hrs. *pqm-1* mutants were ascr#10-responsive: we identified 8,835 differentially expressed genes in response to ascr#10 exposure at 50 hrs. We focused on the positive ascr#10-responsive genes at 50 hrs. Of these genes, approximately half maintained a response like the N2 response at 50 hrs, displaying a very slight upregulation. The remaining genes changed direction—instead of becoming up-regulated like the N2 response, they were strongly downregulated (Fig 4A). *pqm-1* transcripts themselves are downregulated by 70% (q-value < 10^-9), which could indicate that *pqm-1* activity promotes its own expression, though we cannot rule out that the decreased expression is the result of nonsense-mediated decay. Non-PQM-1-bound, ascr#10-responsive genes correlated well between *pqm-1* mutants and N2 (Supp Info). We interpret this as evidence that there is at least one other major transcription factor controlling ascr#10 response in addition to *pqm-1*. Therefore, *pqm-1* activity promotes the expression of a set of genes in response to ascr#10 exposure, and it inhibits responsiveness of ~6,000 genes at 50 hrs. We conclude that *pqm-1* is a transcriptional modulator of the ascr#10 response.

To explore the importance of *pqm-1* for appropriate ascr#10 activity, we asked whether the regulatory enrichment profile of *pqm*-*1* mutants exposed to ascr#10 was like that of N2 animals. Using the entire set of differentially expressed genes in *pqm-1* mutants, we observed that *pqm-1* animals still mount a feminization response in the germline and sperm cells (Fig 4C); however, regulatory enrichment of all other tissues is scrambled below significance. Similarly, the vitellogenin program is not activated at 50 hours in *pqm-1* mutants exposed to ascr#10, major sperm protein gene expression is downregulated in a phenotype that resembles a heterochronic phenotype (major sperm protein genes are very weakly downregulated at 50 hrs in N2 animals exposed to ascr#10, but are downregulated at 58 hrs), and the ribosomal subunits genes do not show any expression change on average (Fig 4D). Therefore, loss of *pqm-1* scrambles the appropriate ascr#10 response of *C. elegans* hermaphrodite’s somatic tissues at the level of the transcriptome.

Although the transcriptome may show significant scrambling, there is not widespread acceptance that such changes necessarily result in physiological consequences (transcriptomes are not phenotypes). To establish the degree to which loss of *pqm-1* results in the loss of cellular and behavioral responses to ascr#10, we measured the ability of *pqm-1* mutants to respond to ascr#10 by measuring the number of germline proliferating cells (GPCs) per gonad arm; the number of pumps per minute; and, area explored per (UNIT TIME???). In all cases, loss of *pqm-1* abrogated the response to ascr#10 observed in N2 animals, establishing its necessity for ascr#10-mediated phenotypes.

**Discussion**

*A model for ascr#10 signal transduction in* C. elegans

Our work supports a model whereby ascr#10 exposure triggers a transcriptional response even in early pre-reproductive adulthood. This transcriptional response is mediated by an unknown receptor, probably in a sensory neuron. SOMETHING HERE TO LINK TO 5HT WORK. The receptor triggers a signal cascade culminating in translocation of PQM-1 to the nucleus. This translocation happens within intestinal cells (Supp Figure), and potentially in other tissues with *pqm-1* expression. PQM-1 signaling acts, cell autonomously or non-autonomously, to promote or repress expression of a set of ~6,000 genes. This signaling almost certainly happens concurrently as another, currently unknown, factor or factors act to repress the effects of *pqm-1*. A potential candidate that might also be involved in signaling is *daf-16*, given its previously known and well-characterized relationship to *pqm-1*.

Regardless of their specific molecular identity, a group of factors modulate a transcriptomic response to a male pheromone that is very likely trivial when considered on an individual gene basis because they either constitute minute changes in organism-wide expression or moderate changes in small numbers of cells. However, these quantitative changes occur across entire gene programs leading to a robust organismal response that is permissive to a set of macroscopic responses upon a licensing event. The transcriptomic reprogramming leads to feminization of the hermaphroditic germline, likely upregulates proteogenesis (indicated by the increased mRNA levels of all ribosomal protein subunits and ER chaperones), and increases pharyngeal usage. Given the role of *pqm-1* in this response, it is likely that a heat shock response is also deployed in hermaphrodites (all the markers reported by Von Ootje *et al*, …, are altered in our ascr#10 conditions), though whether the role of this response is to fold new proteins or to protect against a predicted stress is unclear.

*Pheromones as signaling molecules that modulate organismal states*

Pheromone signaling is an important aspect of animal biology, typically associated with acute changes in mating behavior. Here, we show that pheromone signaling at the appropriate moment in an organism’s life can have profound consequences reaching far beyond mating behavior by fundamentally reprogramming the germline to enhance oocyte quality; shutting down hermaphroditic sperm; changing eating and moving behaviors of the organism leading to an accelerated death; and causing a general upregulation of their immune system. Pheromone signaling in physiologically relevant scenarios likely occurs at sub-nanomolar concentrations (as performed in this study), and sensing of the pheromone is likely to occur through a limited number of GPCRs (likely a single receptor) in specialized sensory cells. Despite the limited number of signaling molecules, and the limited number of recipient cells, it appears that pheromone exposure can nonetheless permanently alter the life trajectory of an organism in much the same way as sperm-depletion(), exposure to seminal fluids() or food starvation().

**Methods:**

We used data from (Aprison *et al*, 2022) to carry out all analyses. Briefly, in (Aprison *et al*, 2022), animals were synchronized and exposed to place or ascr#10 when they became pre-reproductive adults at 50hrs post-L1 starvation, and immediately after they became reproductive adults following the first egg-lay at 58hrs. Reads were aligned to the *C. elegans* N2 genome using the RNA-seq Nextflow pipeline version XXXXXX (all software cited here), and differential expression analyses were performed using DESeq2 version XXXXX (citation here). We used the same pipelines and methods to analyze the data we collected for *pqm-1(ALLELE HERE)* mutants at 50hrs post-L1 starvation. *pqm-1* mutant data was deposited into GEO [accession code here].

We restricted our analyses only to those genes that were identified across all three conditions. Consequently, the number of differentially expressed genes in our analyses is smaller than previously reported, but the fold-changes and q-values are identical with (Aprison *et al*, 2022).

All the code required to completely reproduce our analyses is available in a GitHub repository [LINK HERE]. Statistical functions and large pieces of code are written as python v.3.7(citation) scripts that can be loaded locally; the analyses are carried out in annotated Jupyter notebooks (Jupyter citation) which constitute our supplementary information (S1, S2, S3, S4, S5, S6).

We computed hypergeometric p-values to test for up- or downregulated genes for enrichment across chromosomes. We used a non-parametric bootstrap test to test whether intergenic distances across chromosomes were shorter than expected by chance. Briefly, for each chromosome, we generated a null distribution of intergenic distances by re-sampling the set of measured genes using the number of genes commonly differentially expressed across 50 hrs and 58 hrs 10,000 times. We calculated the median intergenic distance across this set of genes per chromosome. Then, we computed the fraction of median distances that were less than or equal to the observed distance to obtain a p-value. To search for enhancer signatures, we performed a running window correlation across ordered, differentially expressed genes at 50 hrs or 58 hrs. We identified significant peaks by performing a non-parametric bootstrap with 1,000 random draws. For each chromosome, we shuffled the positions assigned to each gene and re-computed the running window correlations. We used the 95% percentile as a cut-off to visually identify clusters of genes more correlated than expected through chance. We used a hypergeometric test to search for enrichment of PQM-1-bound genes at 50 hrs and 58 hrs in up- and downregulated subsets.

We tested tissues for directional enrichment using a binomial test. We downloaded the complete tissue expression annotations from WormBase (citation). We restricted the set of tissues to those tissues that had at least 5 annotated genes. We did not use genes if they had promiscuous tissue expression–we dropped any genes that were annotated in 30 or more terms. If a term had annotated genes that were all considered promiscuous, that term was also dropped. We tested terms for regulatory enrichment by using a binomial test, where the proportion of genes expected to increase their expression was given by the net fraction of genes that were upregulated in each condition, followed by a Benjamini-Hochberg correction. Exclusively for visualization purposes, we removed redundant tissue terms by identifying terms that were more than 75% similar by Jaccard similarity and removing the term with fewer annotated gene expression levels.

We re-analyzed the single-cell *C. elegans* atlas as from Cao *et al* and as provided by (cite murray etc.. here) using scanpy (citation). For our analyses, we removed all cells annotated as ‘Unknown’, ‘Unannotated’ or ‘Muscle\_mesoderm’. We removed ‘Muscle\_mesoderm’ cells because they clustered very different from the remaining cell types and distorted the UMAP diagrams; however, removing this tissue does not alter our conclusions. We filtered cells that showed expression of less than 150 genes, and we removed any genes expressed in less than 50 cells. We also removed cells that had fewer than 250 total counts, mitochondrial content <1% or >10%, riboprotein content <1% or >20%, and NADH enzyme content <.3% or >3%. If a cell was annotated as a neuron, we removed cells that had < 800 total counts, because we observed that neurons had much greater read content than the other cell types. Given the low read content, we normalized counts in cells to counts per thousand, as suggested in (Nimweigen et al).

To generate UMAPs, we subsampled the dataset to equally represent all tissues with 2000 cells (or less if fewer cells were annotated in the dataset). This allows the UMAP to show better separation among tissues. We reduced the dimensionality of the dataset using PCA and keeping the first 15 principal components. Next, we computed the 100 nearest neighbors per cell, and computed the UMAP using scanpy’s UMAP function, initializing the UMAP with pre-computed PAGA positions (Wolf et al). Next, we computed signature enrichment scores. We identified the genes that were differentially expressed at 50 hrs and 58 hrs, that changed in the same direction and that increased (decreased) expression in response to ascr#10 to create a positive (negative) ascr#10 signature. We also generated a random signature with 159 randomly selected genes for comparison. We calculated an enrichment score using scanpy’s `score\_genes` function.

For area measurements, we measured the posterior pharyngeal bulb of (INSERT AGE) nematodes under a DIC microscope. We measured pumps per minute … . We measured bacterial abundance by measuring the fluorescence … (ILYA should write).

Significance for all assays was assessed by a non-parametric bootstrap testing equality of means with 1 million draws.