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C. elegans males optimize mate-preference decisions via sex-specific responses to multimodal sensory cues

Highlights

- *C. elegans* males can detect a potential mate's sex, stage, health, and mating history
- Diverse chemical and physical signals enable stepwise evaluation of potential mates
- Males prefer virgin, well-fed mates, likely by using honest indicators of fitness
- Genetic masculinization of hermaphrodite neurons elicits male-like mate preference

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In brief

The selection of optimal mates is central to animal fitness and evolution. Here, Luo et al. find that diverse sensory signals inform *C. elegans* males about the sex, stage, feeding status, and mating history of potential mates. Sex-specific detection and integration of these signals enables a stepwise program that subserves male mate preference.

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C. elegans males optimize mate-preference decisions via sex-specific responses to multimodal sensory cues

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SUMMARY

For sexually reproducing animals, selecting optimal mates is important for maximizing reproductive fitness. In the nematode *C. elegans*, populations reproduce largely by hermaphrodite self-fertilization, but the cross-fertilization of hermaphrodites by males also occurs. Males' ability to recognize hermaphrodites involves several sensory cues, but an integrated view of the ways males use these cues in their native context to assess characteristics of potential mates has been elusive. Here, we examine the mate-preference behavior of *C. elegans* males evoked by natively produced cues. We find that males use a combination of volatile sex pheromones (VSPs), ascaroside sex pheromones, surface-associated cues, and other signals to assess multiple features of potential mates. Specific aspects of mate preference are communicated by distinct signals: developmental stage and sex are signaled by ascaroside pheromones and surface cues, whereas the presence of a self-sperm-depleted hermaphrodite is likely signaled by VSPs. Furthermore, males prefer to interact with virgin over mated, and well-fed over food-deprived, hermaphrodites; these preferences are likely adaptive and are also mediated by ascarosides and other cues. Sex-typical mate-preference behavior depends on the sexual state of the nervous system, such that pan-neuronal genetic masculinization in hermaphrodites generates male-typical social behavior. We also identify an unexpected role for the sex-shared ASH sensory neurons in male attraction to ascaroside sex pheromones. Our findings lead to an integrated view in which the distinct physical properties of various mate-preference cues guide a flexible, stepwise behavioral program by which males assess multiple features of potential mates to optimize mate preference.

INTRODUCTION

Behaviors that influence mate selection have central roles in sexual selection and reproductive isolation.[1] While much is known about the mate-preference behaviors of diverse species,[2] their neurobiological underpinnings are poorly understood,[3] and the degree to which they are adaptive is under debate.[2,4] Innate mate-preference behaviors provide an opportunity to understand how genetic programs shape the function of neural circuits; they are also an entry point for understanding how and why biological sex influences the structure and function of the nervous system.

Though studies of mate choice traditionally focus on evaluation of courtship displays and other features of males by female choosers, recent work has shown that both sexes can make mate-choice decisions.[2] Here, we study mate preference in the nematode *C. elegans*, a model system in which little is known about this process. Several unusual features of this species likely influence mate preference.[5,6] There are no true females in *C. elegans*; rather, XX individuals are hermaphrodites that produce a limited pool of self-sperm (~300),[7] allowing them to reproduce by self-fertilization. *C. elegans* hermaphrodites cannot mate with each other, but they can be cross-fertilized by X0 males. Young adult hermaphrodites typically resist mating attempts,[8,9] presumably a consequence of the genetic cost of, and damage incurred by, mating.[10–14] Thus, for healthy young hermaphrodites, self-fertilization is likely the optimal reproductive strategy.

C. elegans males face different pressures.[5] Males are rare in self-fertilizing populations[15]; those that do arise can likely choose from many possible mates. While the cost of sex to males is poorly understood,[5,16] this suggests that mate choice could be important for male reproductive success. Consistent with this, males can detect features of hermaphrodites related to their quality as potential mates: the fertility status of hermaphrodites affects the type and strength of chemical cues they produce,[17–19] and males can detect viral infection in a potential mate.[20]

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As in other species,[21] mate choice in *C. elegans* likely involves the detection and integration of multiple stimuli. Accordingly, previous studies have identified multiple hermaphrodite-derived secreted and surface-associated cues that attract or retain males.[18,22–25] The best characterized of these are the ascarosides,[26–30] some of which, including ascr#3 and ascr#8, are produced in markedly higher amounts by hermaphrodites.[31,32] These compounds strongly retain males[24,33] and, depending on context, can repel hermaphrodites.[24,34–36] As ascarosides are thought to have low volatility[24,37] and limited diffusion,[38] they likely retain males in the vicinity of hermaphrodites. A distinct class of pheromones, the volatile sex pheromones (VSPs), potently attracts males at a distance.[18,25] These compounds, whose molecular structure is unknown, are typically produced by hermaphrodites only after their self-sperm are depleted,[17,18,25] when they become functionally female and might benefit from attracting a mate. Sex pheromones produced by younger hermaphrodites under conditions of stress[39] might also be related to this family.

Peri-mating signals[2] also likely influence *C. elegans* mate choice. Once a male has located a potential mate, additional sensory cues determine whether it will initiate and complete the copulatory behavior program. Contact-dependent signals, likely sensed by sex-specific sensilla in the male tail, are particularly important for this.[40–45] These cues, likely both chemical and mechanical, influence initiation of the first step of male mating behavior, contact response, and persistence in the second step, scanning.[40,46] If males do not receive favorable signals during this phase, they cease scanning and resume searching for a potential mate.

Rosenthal[2] defines mate choice as "any aspect of an animal's phenotype that leads to its being more likely to engage in sexual activity with certain individuals than with others." Mate preference, the relative ranking of prospective mates,[47,48] is an important component of this.[2] For *C. elegans* males, mate preference can be gauged by the degree of interaction with a potential mate, which captures both pre-mating (pheromone-guided navigation) and peri-mating (remaining close to or in contact with a mate) behaviors. As both of these are heavily dependent on the presentation of multiple cues in their native context,[2,21] measuring responses to conditioned media and synthetic pheromones do not provide a robust view of mate-choice behavior.

Here, we use a simple, choice-based social interaction assay to study the features of potential mates that males can assess, the signals that carry information about these features, and the mechanisms by which males detect and integrate these signals. We approach these issues in a native context by disrupting the production and/or detection of multiple types of cues, allowing us to assess the contributions of individual signals under a variety of conditions. Together, our results illuminate a flexible, stepwise behavioral program by which males evaluate multiple aspects of potential mates and optimize their mate-preference decisions.

RESULTS

Adult *C. elegans* males robustly distinguish the sex and stage of potential mates

To assess male mate preference, we used a quadrant-format assay in which ten sexually naive male "searchers" were allowed to interact with two distinct classes of conspecific "targets" located in adjacent quadrants (Figure 1A). To prevent targets from migrating during the assay, they carried mutations in the muscle myosin *unc-54*.[49] After placing searchers on the plate, we scored their locations at 30, 60, and 90 min to calculate a mate preference index (MPI) (Figure 1A). Unless noted otherwise, this approach does not mask time-dependent trends (STAR Methods). By focusing on the tendency to approach and stay with potential mates, this assay measures mate preference without considering progeny production, which is subject to the separate and potentially confounding influences of copulation efficiency and hermaphrodite fertility. Moreover, this assay allows us to assess decisions based on target sex or developmental stage.

To validate the mate-preference assay (MPA), we first asked whether it could capture males' ability to identify the sex of potential mates. When male searchers chose between adult male and hermaphrodite targets, they exhibited a strong preference for hermaphrodites, with roughly 75% of searchers localizing to the hermaphrodite-containing quadrants (mean MPI = 0.49 ± 0.23; Figure 1B). We also carried out assays in which we let male and hermaphrodite targets condition the agar for 2.5 h; we then removed target animals before placing searchers on the plate. Consistent with previous results,[22] males still preferentially localized to the hermaphrodite-conditioned quadrants (Figure S1A). However, the mean preference index (0.20 ± 0.27) was markedly lower than that seen under standard assay conditions, indicating that intact animals provide stronger mate-preference cues.

We next asked whether this assay could detect another feature of mate-preference behavior, males' ability to distinguish between larval and adult targets. Indeed, males showed a robust preference for adult over L4-stage larval hermaphrodites (Figure 1C). The developmental stage of targets also influences males' ability to determine sex: males had a clear preference for hermaphrodites when presented with L4 targets, but not with L3s (Figure 1D). Since larvae cannot mate, this shows that the ability to copulate does not drive males' preference for interacting with hermaphrodites. Further, the production of at least some mate-preference cues used by males is developmentally regulated.

Finally, we asked whether behavior in this assay differed by sex, another typical feature of mate-preference behavior. Consistent with this, we found that the preference for interacting with hermaphrodites was exhibited only by males (Figure 1E). In contrast, adult hermaphrodites showed no apparent preference for interaction with males over hermaphrodites (Figure 1E), consistent with the idea that these animals prefer to self-fertilize rather than mate with males.[8,9] While the MPA does not assess searchers' granular navigational decisions, these results taken together indicate that it provides a reliable and rapid measure of males' relative preference for interacting with different classes of potential mates.

Ascaroside pheromones contribute to mate preference

To identify signals mediating mate preference, we first considered the ascaroside pheromones. Several ascarosides, particularly ascr#3 and ascr#8, are produced primarily by hermaphrodites and, as synthetic compounds, potently attract or retain

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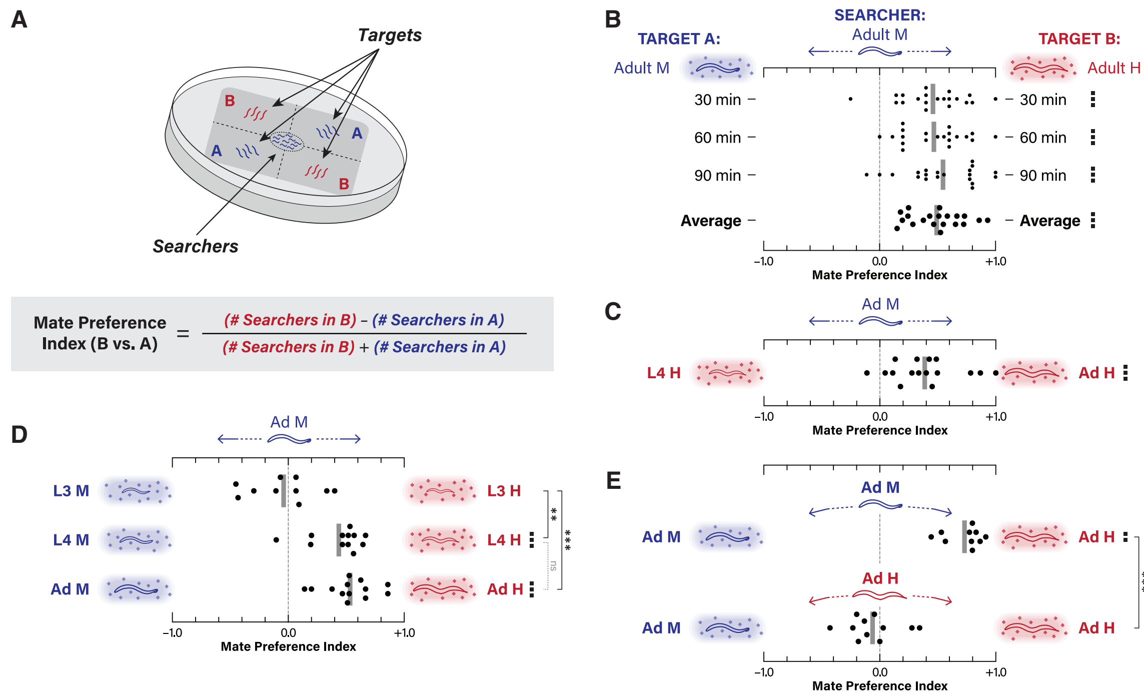

Figure 1. *C. elegans* males can determine the sex and developmental stage of potential mates

(A) The mate-preference assay (MPA). Immobilized targets of two classes are placed in two opposing quadrants; after 30 min, searchers are placed in the center. Their positions over the following 90 min are used to calculate a mate preference index (MPI) (STAR Methods).

(B) MPA with control adult male searchers paired with adult male and adult hermaphrodite targets. MPI values at individual time points, as well as their average, are shown.

(C) MPA with male searchers paired with L4 and adult hermaphrodite targets.

(D) MPA with male searchers paired with male and hermaphrodite targets at L3, L4, and adult stages.

(E) MPA with control adult male and hermaphrodite searchers paired with adult male and hermaphrodite targets.

In all figures, schematic diagrams depict the cues potentially produced by targets: VSPs are indicated by gradient shading, ascarosides by small hexagons, and surface cues by the outline of the body. Red or "H" indicates hermaphrodite and blue or "M" indicates male. Each data point represents an individual MPI derived from a single assay with ~10 searchers; the vertical gray bar indicates the mean MPI. For each condition, a Wilcoxon signed-rank test was used to ask whether the MPI differed from zero (i.e., whether males had a significant preference for either target class). Results are shown with black squares at the right of each row: ■p ≤ 0.05; ■■p ≤ 0.005; ■■■p ≤ 0.001. To compare MPI values between assays, we used Mann-Whitney tests or Kruskal-Wallis tests with Dunn's correction. Asterisks indicate the associated p values: *p ≤ 0.05; **p ≤ 0.005; ***p ≤ 0.001. Comparisons that did not reveal statistical significance are shown with gray dotted lines and are labeled "ns."

See also Figure S1.

males.[24,31,32] However, the contribution of endogenously produced ascarosides to male mate-preference decisions has not been carefully examined. To address this, we used targets carrying mutations in *daf-22*, an enzyme necessary for the synthesis of short-chain ascarosides, including all known ascaroside sex pheromones (Figure 2A).[50–52] Interestingly, males readily recognized the sex of *daf-22* adults, strongly preferring to interact with *daf-22* hermaphrodites over *daf-22* males (Figure 2B). Thus, ascaroside pheromones are not essential for the sex discrimination aspect of mate preference. However, males did not distinguish the sex of L4-stage *daf-22* animals (Figure 2B). We infer that ascaroside sex pheromones are produced by both L4 and adult hermaphrodites, but that additional, *daf-22*-independent signals are also produced by adults.

While ascarosides are dispensable for adult sex discrimination, males preferred to interact with control over *daf-22* adult hermaphrodites (Figure 2C). Consistent with previous results,[33] this indicates that ascarosides contribute to mate choice. This also allowed us to ask how behavior in the MPA is related to the rate of fertilization. Using searchers with fluorescently marked sperm,[53] we scored the fraction of control and *daf-22* targets that contained male sperm at 30, 60, and 90 min. At all three time points tested, mean frequencies of mated hermaphrodites were higher for control than *daf-22* targets, though this difference was not statistically significant at 90 min (Figure 2D). The reduced effect size at 90 min likely arises because this assay does not distinguish single matings from the multiple matings that will occur more frequently with control targets than *daf-22* targets as the assay progresses (STAR Methods). However, we cannot rule out the possibility that males become less selective in their mating behavior during the experiment or that the link between social interaction and mating frequency changes in some other way with time. Regardless, these results indicate that behavior in the MPA is related to mate choice and mating frequency.

Ascarosides, glycosides of the sugar ascarylose, have extremely low volatility.[24,37] To confirm this, we asked whether males could distinguish between control and *daf-22* targets

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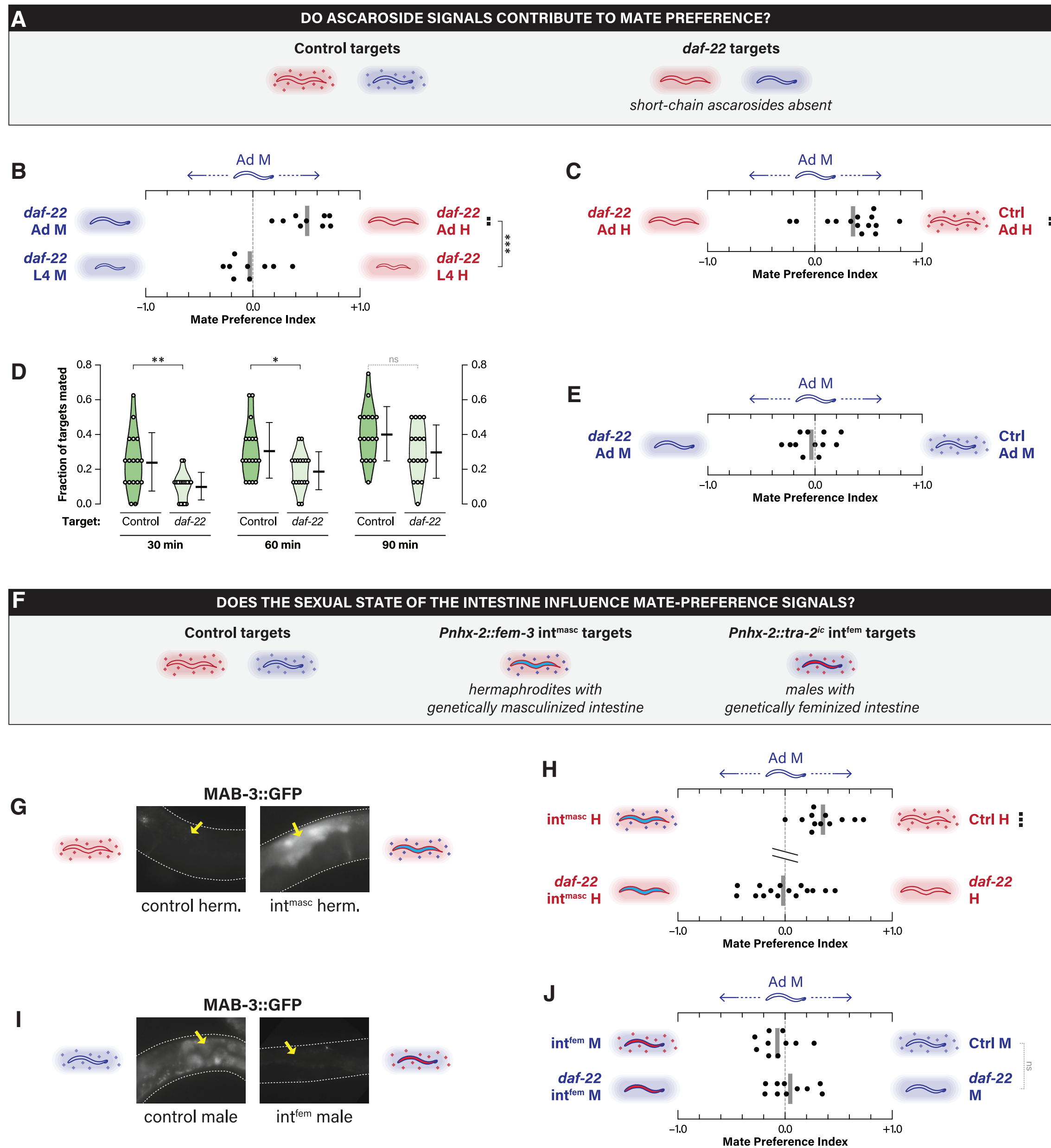

Figure 2. Ascaroside pheromones have central roles in sex and stage discrimination

(A and F) Schematic depiction of targets.

(B, C, E, H, and J) MPAs with control male searchers paired with targets of the indicated sexes, stages, and genotypes.

(D) Fraction of targets mated assessed using searchers with mNeonGreen-labeled sperm. Data points indicate individual assays; mean and standard deviation are also shown.

(G and I) Epifluorescence images of *MAB-3::GFP* in animals of the indicated sex and genotype. Dashed white lines outline the body; yellow arrows indicate the intestine.

The break in the vertical axis in (H) is used here and in subsequent figures to separate experiments that were not carried out in parallel and therefore cannot be directly compared.

See also Figure S2.

placed on inverted agar plugs suspended ∼3 mm above the assay plate's surface. Males showed no tendency to localize underneath either group of targets (Figure S2A), consistent with the idea that ascarosides are not volatile. Thus, ascaroside sex pheromones probably do not attract males from a distance but rather retain them in the vicinity of hermaphrodites.

We also tested male preferences for control vs. *daf-22* male targets. Males showed no apparent preference (Figure 2E), indicating that male-enriched ascarosides such as

ascr#10[31] do not detectably repel males from each other in this setting.

The sex specificity of ascaroside production depends on the sexual state of the intestine

Ascarosides are produced in the intestine, where differential expression of peroxisomal enzymes is thought to cause sexual dimorphism in ascaroside production.[31,32,54–57] To ask whether the sexual state of the intestine influences the attractiveness of a potential mate, we used "int^{masc}" hermaphrodites, in which the sexual state of the intestine is masculinized through expression of the male sexual regulator *fem-3* (Figure 2F).[32,58] Int^{masc} hermaphrodites expressed the male-specific intestinal marker *MAB-3::GFP*,[59] validating this manipulation (Figure 2G). Recent metabolomics studies have shown that these animals produce male-typical ascaroside profiles.[32] Accordingly, we found that males robustly preferred to interact with control over int^{masc} hermaphrodite targets (Figure 2H). This preference was completely dependent on *daf-22* (Figure 2H). As some neurons also express *daf-22*,[60] we tested neuro^{masc} targets (hermaphrodites carrying a pan-neuronal masculinization transgene[61]). This manipulation had no apparent effect (Figure S2B). Thus, in hermaphrodites, the sexual state of the intestine is necessary to produce functional, sex-typical patterns of ascarosides.

We also asked whether genetic feminization of the intestine would cause males to produce hermaphrodite-like pheromones. To create int^{fem} males, we expressed an activated form of the hermaphrodite sexual regulator *tra-2*[62] in the intestine. *MAB-3::GFP* was repressed in int^{fem} males (Figure 2I), but searchers showed no preference for these over control males (Figure 2J). Feminization of the intestine may be insufficient to trigger production of hermaphrodite-typical pheromone profiles; alternatively, the effects of any hermaphrodite pheromones produced by int^{fem} males might be dominated by unknown, non-ascaroside aversive cues produced by males.

The ability to copulate does not drive mate-preference decisions

We next considered roles for non-ascaroside cues in mate preference. Because a role for these is most apparent in adult targets (Figure 2B), we asked whether the hermaphrodite vulva, an adult-specific structure, contributes to mate preference (Figure 3A). However, males showed no detectable preference for control over vulvaless *lin-39*[63] adult hermaphrodites (Figure 3B). Therefore, neither the vulva itself, nor the ability to copulate, is a principal determinant of mate-preference behavior. However, when the influence of ascaroside pheromones was removed, males displayed a weak preference for control over vulvaless hermaphrodites (Figure 3C). This could be a by-product of the increased time males spend in copulation with control hermaphrodites. The vulva might also have a role in generating a signal that attracts or retains males independently of copulation; however, such a signal would likely have a minor role in mate-preference decisions.

The hermaphrodite cuticle provides mate-preference cues

Contact-associated cues, both chemical and mechanical, likely play important roles in male recognition of hermaphrodites.[40–44,64] Recent work has demonstrated a key role for body stiffness, a physical cue detected when males scan the hermaphrodite cuticle, in this process.[45] Surface glycoproteins are also likely to be involved, as males spend less time in contact with hermaphrodites in which cuticle protein glycosylation is disrupted.[44]

To ask whether surface glycoproteins have a role in mate preference, we used animals carrying mutations in *bus-2*, a putative glycosyltransferase implicated in the O-linked glycosylation of surface proteins and the production of cues used by males to identify hermaphrodites (Figure 3D).[44,65] Accordingly,[44] we found that males had a slight preference for interaction with wild-type over *bus-2* hermaphrodites (Figure 3E). Importantly, *bus-2* loss alters but does not abolish surface protein glycosylation,[65] so it is unlikely that these animals entirely lack such cues. *bus-2* activity in targets had no apparent role in male-male interactions (Figure 3E). *bus-2* likely functions in the hypodermis to promote hermaphrodite attraction, since hypodermal-specific expression rescued the *bus-2* attractiveness phenotype (Figure S3A), consistent with the expression of *bus-2* in hypodermal seam cells.[65]

Interestingly, sex discrimination ability was significantly reduced in a *bus-2* mutant background (Figure 3F). To probe the relative contributions of surface signals and ascaroside pheromones, we assayed mate preference with *daf-22; bus-2* targets of both sexes. Males' preference for hermaphrodites was not further reduced compared with either single mutant (Figure 3F), demonstrating that yet other cues are sufficient for sex discrimination in this context. We also compared mate preference with *daf-22* vs. *daf-22; bus-2* hermaphrodites and *bus-2* vs. *daf-22; bus-2* hermaphrodites. The double mutants were significantly less attractive than either single mutant (Figure 3G), indicating that each gene promotes attractiveness independently of the other, consistent with their distinct biochemical functions and sites of action. (For the *bus-2* vs. *daf-22; bus-2* experiment, we detected a significant effect of time [$p = 0.039$] across the three time points measured. However, the pattern of variation does not suggest a biologically meaningful change in behavior over time [Figure S3B].) Thus, ascaroside and *bus-2*-dependent surface cues converge to optimize male mate preference.

Peri-mating signals such as contact response, the first step of male mating behavior,[40] seemed especially vulnerable to changes in surface cues. Indeed, we found that males exhibited significantly less contact-response behavior with *bus-2* compared with control hermaphrodites (Figure 3H). In contrast, loss of *daf-22* in target hermaphrodites did not significantly reduce contact-response behavior, either in control or *bus-2* backgrounds (Figure 3H), consistent with the idea that ascaroside pheromones influence mate-preference behavior by influencing earlier steps of mate preference.

The sexual state of the hypodermis controls the production of sex-specific surface cues

The sex specificity of surface cues might be instructed by the sexual state of the hypodermis, which synthesizes the worm's cuticle. Therefore, we asked whether feminization of the male hypodermis might make these animals more attractive to males. We examined a property particularly sensitive to contact signals, the ability of hermaphrodites to retain males on a small food

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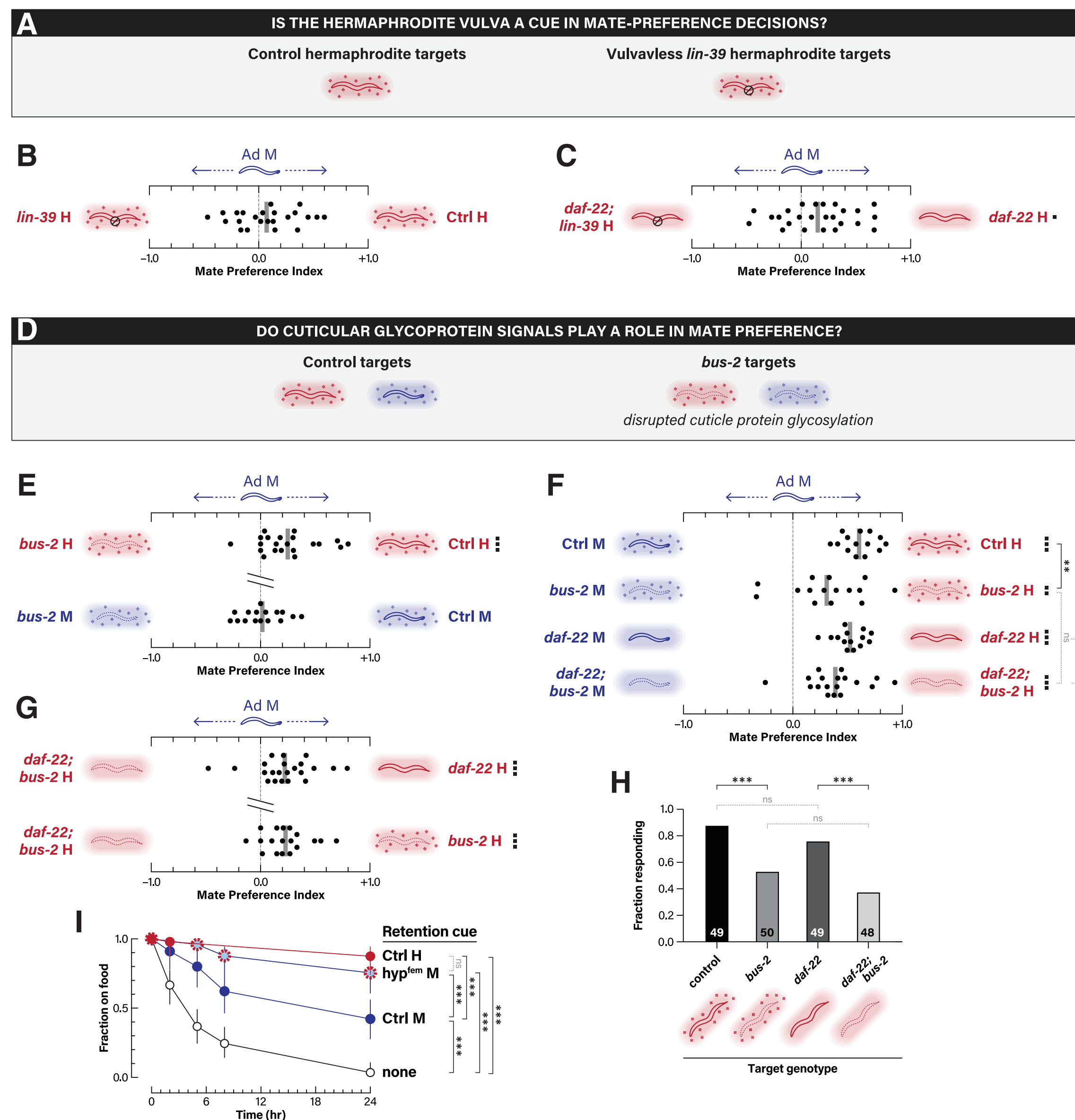

Figure 3. Morphological and surface cues also contribute to sex discrimination

(A and D) Schematic depiction of targets.

(B, C, E, F, and G) MPAs with control male searchers paired with targets of the indicated sexes and genotypes.

(H) Contact-response frequency of control males paired with hermaphrodites of the indicated genotypes.

(I) Male food-leaving assay measuring retention by different classes of targets (none, control males, control hermaphrodites, and hyp^{fem} males). Data points show the fraction of males remaining on a food spot harboring the indicated retention cue over 24 h. Error bars indicate standard deviation.

See also Figure S3.

patch.[41,42] As expected,[42] we found that control males retained males to some degree; however, hyp^{fem} males retained other males nearly as effectively as wild-type hermaphrodites (Figure 3I). Thus, the genetic sex of the hypodermis is an important determinant of signals that guide mate preference.

Male mate-preference decisions can integrate volatile sex pheromones

Additional potential mate-preference signals for *C. elegans* males are the VSPs, which are produced by hermaphrodites once their supply of self-sperm is depleted.[17,18,25] While the molecular nature of VSPs is unknown, their production is independent of *daf-22*, and they are unlikely to be chemically related to ascarosides.[17,25] Males detect at least some VSPs using the chemoreceptor *srd-1*, which is expressed male-specifically in the sex-shared AWA olfactory neurons.[25]

To explore a role for VSPs in mate preference (Figure 4A), we used *daf-22*; *bus-2* targets to minimize cue redundancy. *srd-1* mutant males readily distinguished the sex of these adults (Figure S4A), confirming that young adult hermaphrodites produce

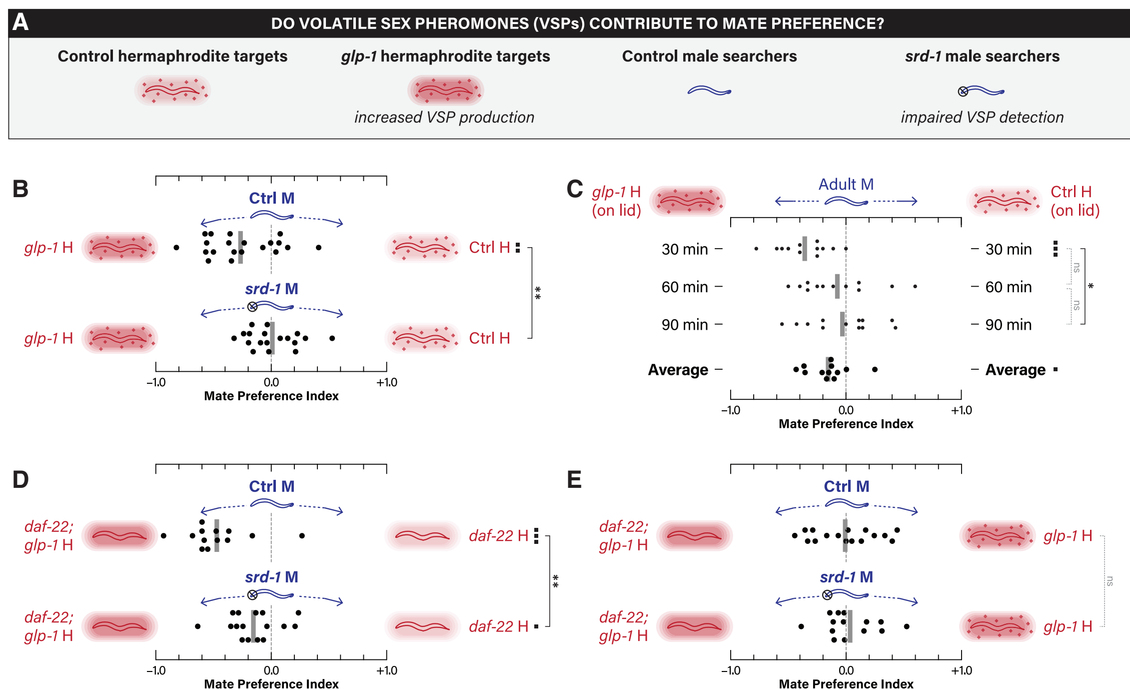

Figure 4. Volatile sex pheromones (VSPs) that act through *srd-1* are powerful mate-preference signals
(A) Schematic depiction of targets.
(B–E) MPAs with control or *srd-1* mutant male searchers paired with targets of the indicated sexes and genotypes. In (C), targets were placed on small agar plugs attached to the lid of the assay plate. The lid was then inverted to cover the assay plate, such that targets were suspended ∼3 mm above the surface on which searchers navigated.
See also Figure S4.

negligible amounts of VSP.[18,25,66] VSPs are expected to be produced by older hermaphrodites, but because older *unc-54* hermaphrodites retain eggs and become unhealthy, they are unsuitable for use in the MPA. To circumvent this, we used germline-less *glp-1* mutants, which produce VSPs as young adults.[18,25] Males showed a significant preference for interacting with *glp-1* over control hermaphrodites (Figure 4B), suggesting that VSPs can have a strong influence on mate preference. This effect was absent at 15°C, the permissive temperature for *glp-1*[67] (Figure S4B). The increased attractiveness of *glp-1* hermaphrodites was completely dependent on male *srd-1* function (Figure 4B), demonstrating that this attraction is mediated solely by *srd-1*-detected VSPs. To confirm that these cues were volatile, we placed targets on agar plugs suspended ∼3 mm above the plate and found that males clearly accumulated underneath the *glp-1* testers (Figure 4C). Interestingly, this effect was limited to the 30-min time point, suggesting that males might rapidly adapt to VSPs and, without other cues to retain them, migrate away from the *glp-1* quadrants. Such adaptation is a well-known feature of *C. elegans* olfaction.[68] Together with previous findings, this result indicates that VSPs attract animals from a distance, while more highly localized cues (i.e., ascarosides and/or surface cues) are likely important for close-range retention.

When we removed the contribution of ascaroside pheromones, VSPs appeared to play an even larger role and, unexpectedly, their effect was only partially dependent on *srd-1* (Figure 4D). This suggests that *glp-1* hermaphrodites produce additional cues, perhaps including VSPs whose detection is *srd-1*-independent, and that the activity of these is apparent only when the influence of ascaroside pheromones is removed. Next, we allowed males to choose between *daf-22; glp-1* and *glp-1* hermaphrodite targets. Surprisingly, males had no apparent preference (Figure 4E), indicating that the long-range potency of VSPs overwhelms the short-range effects of ascaroside pheromones. This result held even when males lacked *srd-1* (Figure 4E), again suggesting that *srd-1* detects only a subset of VSPs. An alternative possibility, that sterile *glp-1* hermaphrodites produce lower amounts of attractive ascarosides or higher amounts repellent ones, is less likely, as the germline does not have a strong influence on ascaroside biosynthesis.[32] Together, our results indicate that mate-preference decisions involving wild-type young adult hermaphrodites do not depend on VSPs but instead that these molecules are powerful cues that promote mating with sperm-depleted hermaphrodites. Further, our results confirm a key role for *srd-1* in detecting VSPs but also implicate additional chemoreceptor(s) in detecting these molecules.

Ascaroside and other signals allow males to assess the nutritional status of potential mates

Beyond sex and developmental stage, the extent to which *C. elegans* males can assess other characteristics of potential mates is poorly understood. We first asked whether males can detect a hermaphrodite's nutritional status (Figure 5A), as this can strongly influence oocyte quality and number.[69] When

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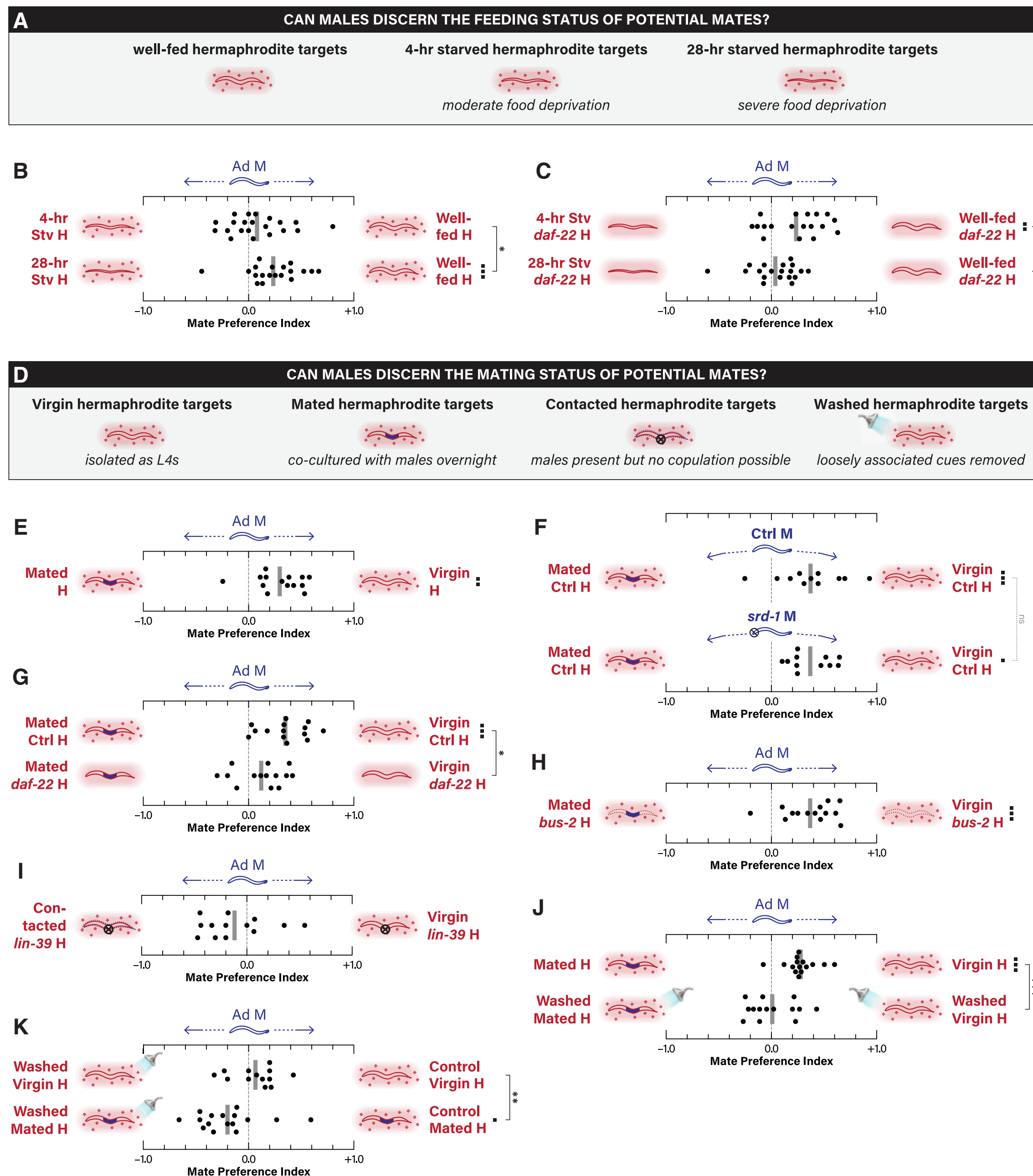

Figure 5. *C. elegans* males assess target nutritional state and reproductive history in mate-preference decisions

(A and D) Schematic depiction of targets.

(B, C, and E–K) MPAs with control or *srd-1* mutant male searchers paired with targets of the specified sexes, genotypes, and conditions. In (B) and (C), some targets were deprived of food for 4 or 28 h prior to the beginning of the assay. In (E)–(K), targets were either virgin (cultured overnight with sterile males) or mated (cultured overnight with wild-type males). In (J) and (K), some targets were briefly washed with sterile water before placing them on the assay plate.

choosing between well-fed adult hermaphrodites and those starved for 4 h, males showed no discernable preference (Figure 5B). However, males clearly preferred well-fed to 28-h starved targets (Figure 5B), demonstrating that they can detect severe starvation. To ask whether this preference stems from differences in ascaroside biosynthesis, we used *daf-22* targets. Unexpectedly, males exhibited a robust preference for well-fed over 4-h starved *daf-22* hermaphrodites (Figure 5C); further, this effect was absent at 28 h (Figure 5C). We infer that the aversive effects of long-term fasting could be mediated by ascaroside pheromones. Consistent with this, starvation of young L1 larvae increases production of the aversive ascaroside osas#9 and decreases levels of ascr#3.[52,70] Further, the rapid production of aversive cues by 4-h starved *daf-22* hermaphrodites might reflect a sensitization to nutrient deprivation arising from their altered fatty acid metabolism,[56,71] potentially reducing the

production of unknown attractive, non-short-chain ascaroside pheromones, or increasing the production of unknown aversive cues.

Males deposit an aversive cue that decreases hermaphrodite attractiveness

Another feature of hermaphrodite physiology that impacts reproduction is mating history. Both virgin and mated young adult hermaphrodites are fertile; however, males might prefer virgins so that their sperm are not competing with that of other males, particularly since male sperm outcompete hermaphrodite self-sperm.[72] Consistent with this, males are slightly more attracted to conditioned media produced by virgin adult hermaphrodites compared with mated ones[19] and mating decreases the potency of VSPs produced by sperm-less *fog-2* hermaphrodites.[18]

To probe the role of mating status (Figure 5D), we let males choose between young adult hermaphrodites that had previously been co-cultured with young adult *him-5* males for 16 h or instead with sterile *unc-54; him-5* males, controlling for exposure to male pheromones, which can alter hermaphrodite physiology.[73,74] Males displayed a clear preference for virgin over mated hermaphrodites (Figure 5E); further, this ability did not require the VSP chemoreceptor *srd-1* (Figure 5F). We also asked whether males could distinguish mating status in targets lacking *daf-22* or *bus-2*. The virgin-vs.-mated interaction preference was significantly lower with *daf-22* than with control targets (Figure 5G), suggesting that ascaroside biosynthesis or release changes in mated hermaphrodites. In contrast, males detected the fertilization status of *bus-2* hermaphrodites comparably to controls (Figure 5H).

Because males had a marginally significant ($p = 0.079$) ability to detect mating status in the absence of ascaroside pheromones (Figure 5G), we asked whether they might detect cue(s) deposited by a previous mate on the hermaphrodite body. In many nematodes, including some natural *C. elegans* isolates, males deposit a mating plug, potentially decreasing their mate's attractiveness.[75] Although laboratory strain N2 has lost this ability,[75] males could still leave evidence of their encounter with a hermaphrodite while their tail is scanning the hermaphrodite's body. To test this, we used vulvaless hermaphrodite targets that had been co-cultured with males. However, we found no evidence that males avoid these previously contacted, but not mated, hermaphrodites (Figure 5I).

Males could also deposit cues on the hermaphrodite body during copulation itself.[76,77] Further, copulation damages the hermaphrodite cuticle, which could cause release of a signal.[13] Therefore, we washed virgin or previously mated hermaphrodites before placing them on the assay plate. Under these conditions, preference toward virgin hermaphrodites was abolished (Figure 5J), strongly suggesting a role for a surface cue. To explore this further, we directly compared washed vs. control animals. Washing virgin hermaphrodites had no effect on their attractiveness to males, but washing previously mated hermaphrodites eliminated males' ability to discern mating status (Figure 5K). We infer that an aversive surface-associated cue decreases the attractiveness of mated hermaphrodites. Because any effects of altered ascaroside production should still apply here, the decreased attractiveness of mated hermaphrodites might depend on an interaction between hermaphrodite ascarosides and post-mating aversive cues.

The male state of sex-shared neurons is essential for responses to multiple classes of sex discrimination cues

We next investigated mechanisms that could underlie the sex differences in mate-preference behavior. First, we focused on overt sexual dimorphisms in *C. elegans* neuroanatomy. Previous work has shown that the CEM neurons, the only male-specific sensory neurons in the head,[78] can detect ascarosides.[24,79] *ceh-30* mutant males, which lack CEM neurons,[80,81] could still efficiently distinguish target sex (Figure S5A). Further, *ceh-30* males behaved comparably to control males when choosing between wild-type and *daf-22* hermaphrodites (Figure S5B). However, there was a marked increase in their behavioral variability ($p = 0.0016$, F test). Thus, ascaroside detection by CEMs likely contributes to the robustness of mate-preference decisions, but consistent with earlier studies,[33] CEM function is not essential for some behavioral responses to ascaroside pheromones. *ceh-30* mutants also performed comparably to controls in distinguishing target sex in the absence of ascarosides (Figure S5C). We also tested *pkd-2* mutant males, in which the functions of CEMs and the male-specific RnB and HOB tail sensory neurons are disrupted.[82] These animals were fully able to distinguish target sex in the absence of ascaroside pheromones (Figure S5D).

Sex differences in *C. elegans* behavior can also emerge from sex-specific properties of neurons and circuits shared by both sexes.[83,84] Thus, we asked whether male-specific features of shared neurons were important for mate-preference behavior (Figure 6A). Indeed, males carrying a pan-neural feminization transgene ("neuro^{fem}" males)[62] completely lost their preference for hermaphrodites (Figure 6B). Moreover, masculinizing the hermaphrodite nervous system was sufficient to masculinize behavior: neuro^{masc} hermaphrodites[23,85] showed a clear preference for interacting with hermaphrodites over males (Figure 6B). Because precursor lineages are not altered in these animals, neuro^{masc} hermaphrodites do not typically possess male-specific neurons; instead, sex-shared neurons adopt male-like properties.[23,85] Thus, male-specific properties of shared circuits are sufficient to establish a preference for interacting with hermaphrodites.

We next asked how the sexual state of the nervous system influences responses to different classes of mate-preference cues. Neuro^{fem} males lost their preference for control over *daf-22* hermaphrodites (Figure S6A), confirming a requirement for the male state of shared circuits in ascaroside detection.[33] Neuro^{masc} hermaphrodites behaved comparably to controls but did display a slight preference for interacting with control over *daf-22* hermaphrodites (Figure S6A). Previous work has shown that neuro^{masc} hermaphrodites are robustly attracted to synthetic ascarosides[33]; the weaker effects seen here suggest that additional male-specific features may be important for robust responses to natively produced ascarosides.

We also explored male responses to surface cues, which are thought to be detected by male-specific sensory structures.[40] Surprisingly, the ability to distinguish the sex of *daf-22* targets was abolished in neuro^{fem} males (Figure S6B), even though these animals retain male-specific sensory neurons. The

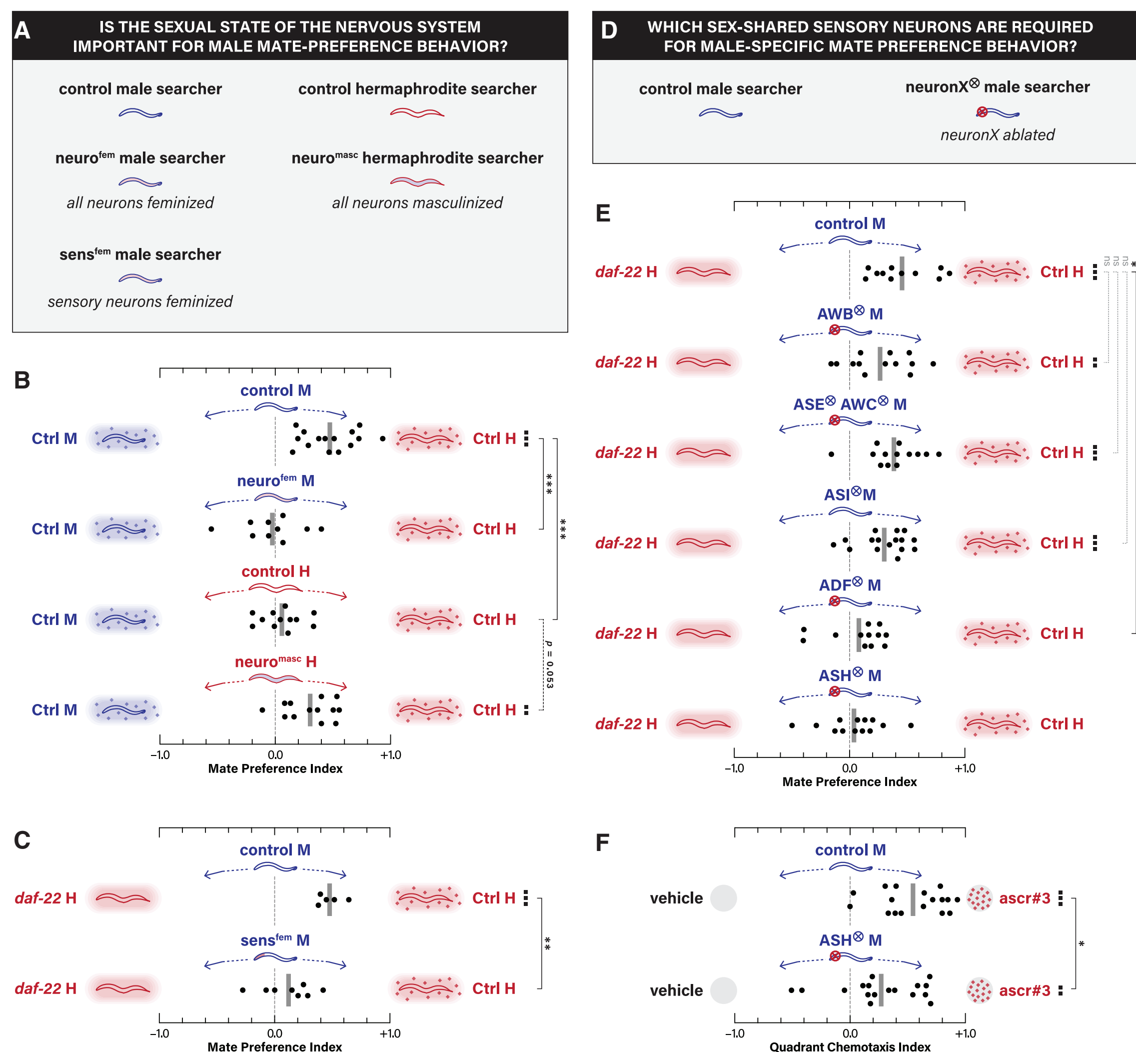

Figure 6. Sex-specific features of shared neurons and circuits guide sex-typical mate-choice decisions

(A and D) Schematic depiction of targets.

(B, C, and E) MPAs with control males, males bearing neuronal sex-reversal transgenes, or neuronal ablation transgenes, paired with targets of the indicated sex and genotype.

(F) Quadrant chemotaxis assay measuring ascr#3 attraction/retention using control or ASH-ablated males.

See also Figures S5–S7.

behavior of neuro^{masc} hermaphrodites was statistically indistinguishable from that of control hermaphrodites (Figure S6B). Interestingly, however, these animals displayed a slight preference for interacting with hermaphrodite targets (Figure S6B). Thus, the male state of the nervous system is likely necessary for the detection and/or interpretation of non-ascaroside cues and, in hermaphrodites, is sufficient to elicit a measurable response to these.

We also tested males' ability to distinguish between *daf-22; bus-2* and *daf-22* targets. Unlike control searchers, neuro^{fem} males had no preference (Figure S6C). Thus, male-specific features of shared neurons—either shared sensory neurons or interneurons receiving input from male-specific sensory neurons[86]—are likely important for detecting or interpreting information on the surface of potential mates.

The sex-shared ADF and ASH neurons have roles in male responses to natively produced pheromones

Sex-specific sensory tuning is a prominent feature of the *C. elegans* nervous system,[23,25,33,34,61,85,87] prompting us to explore roles for sensory neurons in detecting mate-preference cues. Feminization of all ciliated sensory neurons (sens^{fem}) significantly disrupted males' ability to distinguish *daf-22* from control targets (Figure 6C). We next explored roles for individual sensory neurons using genetic ablation (Figure 6D). Ablation of the sensory neurons ASK or ADL (Figures S7A and S7B), or AWB, ASE/AWC, or ASI (Figure 6E) did not significantly compromise male behavior. However, consistent with the male-specific ability of the shared ADF sensory neuron to detect hermaphrodite ascaroside pheromones,[33] ADF ablation severely impaired the preference for control over *daf-22* hermaphrodites (Figure 6E). ADF was not essential

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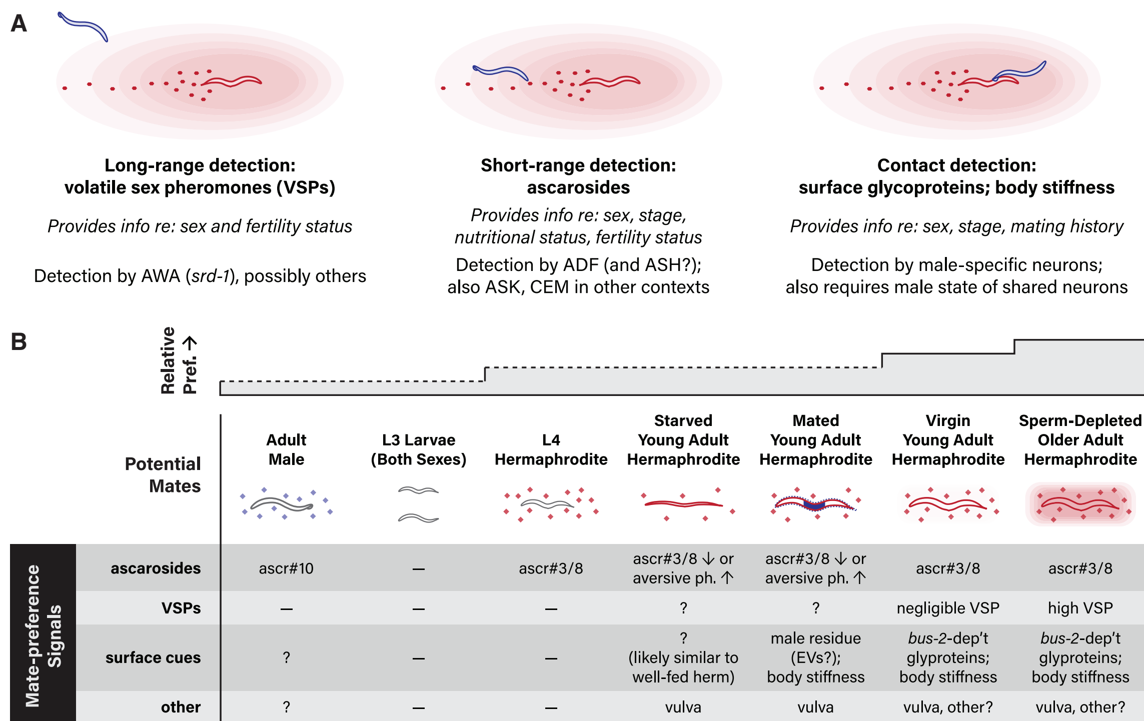

Figure 7. *C. elegans* males assess multiple features of potential mates using a stepwise behavioral strategy that incorporates multimodal sensory cues

(A) The stepwise nature of mate-preference behavior is determined by the physical properties of diverse mate-preference signals and the behaviors they elicit. In the scenario shown, a hermaphrodite has been migrating to the right and has recently transitioned from exploratory to dwelling behavior. A trail of ascaroside-containing deposits is indicated. As volatile cues,[18,25] VSPs likely attract males from a distance. Less diffusible ascaroside cues are thought to be deposited as excrement and are likely non-uniform in distribution.[38] This may allow males to identify regions in which hermaphrodites have recently been present and could trigger a transition out of an exploratory state. Detection and evaluation of peri-mating surface cues likely happens during the initial phase of mating behavior itself, as male-specific neurons in the male tail contact the mate's body during scanning behavior.[40] Each phase of mate-preference behavior allows males to assess multiple features of potential mates.

(B) A table summarizing various categories of potential mates, ordered according to male preference. Because we did not explicitly compare all classes of potential mates to each other, cases where the relative preference order is unknown are indicated with a dashed line. For each category, the role of different classes of mate-preference signals is indicated. "—" indicates that a signal is absent or does not play a detectable role. "?" indicates that the status of a signal is unknown.

for sex discrimination (Figures S7C and S7D), consistent with the redundancy of signals used in this behavior.

Unexpectedly, ablation of the polymodal nociceptive neuron ASH[88,89] also eliminated males' preference for control over *daf-22* targets (Figures 6E and S7E). Because ASH has not been implicated in ascaroside-evoked behavior, we tested the response of ASH-ablated males to synthetic ascr#3. These animals exhibited a marked decrease in attraction (Figure 6F), demonstrating that ASH is important, though not essential, for ascr#3 response. Similar to ADF, ASH was not required for males to distinguish target sex in control or *daf-22* animals (Figures S7F and S7G). Recent work has shown that sex differences in synaptic connectivity reduce ASH's contribution to avoidance behavior and permit robust mating in males.[90] Our results raise the possibility that reconfiguration of ASH physiology and/or connectivity might allow it to mediate an appetitive response in males.

DISCUSSION

While the copulatory behavior of *C. elegans* males has received significant attention,[40,91] less is known about the steps leading to copulation. Even the simplest aspects of mate choice—assessing the sex and stage of potential mates—have not been systematically examined in this species in the context of natively presented cues. Here, by studying behavior elicited by endogenous cues, we identify a sex-specific behavioral program in which males use volatile, soluble, and contact-dependent signals to locate and assess multiple features of potential mates (Figures 7A and 7B). When integrated with previous findings, our results provide a multistep framework for *C. elegans* male mate preference that incorporates diverse stimuli, the information they provide, and neuronal mechanisms that underlie their detection.

The distinct chemical and physical characteristics of mate-preference signals suggest a behavioral sequence guiding mate-preference behavior (Figures 7A and 7B). In many settings, the first salient mate-preference cue detected by males might be long-range olfactory signals from VSPs. Our results and others[18,66] indicate that young adult hermaphrodites produce negligible amounts of these cues. However, VSPs are essential mediators of the male preference for *glp-1* hermaphrodites, which mimic the increased attractiveness of

older sperm-depleted hermaphrodites.[17,18,66] VSPs are detected at least in part by the sex-shared AWA olfactory neurons via the receptor *srd-1*[25]; our studies indicate that additional VSPs whose detection is *srd-1*-independent might also exist. In addition to signaling sperm depletion, VSPs might also be used by younger hermaphrodites in contexts such as stress or pathogen exposure[39,92] that increase their motivation for outcrossing. Identifying the chemical structure of VSPs and the mechanisms that regulate their production will be important goals for the field.

At closer range, ascaroside pheromones, particularly ascr#3 and ascr#8, also inform mate-preference decisions (Figures 7A and 7B). These cues are not volatile[24,37] but instead are likely deposited as excrement and are only weakly diffusible.[38] As such, they could signal the presence of a nearby hermaphrodite and suppress exploration. Ascaroside biosynthesis is modulated not only by sex but also by developmental stage and feeding status[26–30]; accordingly, our data indicate that ascarosides contribute to males' ability to discern these features. Sex specificity in ascaroside production is influenced by the sexual state of the intestine, indicating that the master sexual regulator *tra-1*, possibly through its target *mab-3*,[59] modulates metabolic pathways in this tissue.[31,32] How other dimensions of internal state might influence ascaroside biosynthesis is less well understood.

Multiple sensory neurons have been implicated in ascaroside detection. In the assay used here, as in quadrant assays using synthetic pheromones,[33] the male-specific CEMs and the sex-shared ASKs make little apparent contribution to behavior. This might result from the relatively long time scale (90 min) of our assays, as opposed to other contexts (seconds to minutes) in which the CEM and ASK neurons predominate.[24] Differences in conditions like the amount of food present or the behavioral state of animals being tested might also contribute to these discrepancies. Instead, we find that the sex-shared ADF and ASH neurons are important for responses to natively presented ascarosides. A role for ADF in ascaroside detection is known[33]; however, to our knowledge, ASH has not been previously implicated. Interestingly, recent work has shown that the nociceptive function of ASH is blunted in males due in part to sex differences in synaptic connectivity.[90] Our results suggest that ASH might be repurposed in males, allowing it to contribute to appetitive responses.

The final phase of male mate-preference behavior depends on contact-dependent cues (Figures 7A and 7B). While the nature of these cues and the mechanisms by which they are detected is incompletely understood, body stiffness[45] and surface protein glycosylation[44] are important determinants of male recognition of hermaphrodites. We find that the surface protein glycosylation also plays an important role in males' ability to discriminate the sex of potential mates. The sex specificity of surface cues is influenced by the genetic sex of the hypodermis itself, but the nature of these dimorphisms and their control by the sex-determination pathway remain unclear. Surface cues are detected by male-specific tail sensory neurons[40,93]; interestingly, our results suggest that the male state of shared circuitry might be important for behavioral responses to these signals. Consistent with this, sex-shared neurons in the motor system are important mediators of male contact-response behavior.[94]

Other kinds of surface-associated cues are also important in male mate preference (Figure 7B). The hermaphrodite vulva might have a secondary role in promoting sustained interaction, providing a means to distinguish not only sex but also adults from larvae. Interestingly, surface-associated cues, either deposited by males or produced by hermaphrodites, also appear to contribute to the decreased attractiveness of mated hermaphrodites. Extracellular vesicles (EVs) are intriguing candidates for mediating this effect, as males shed neuronally derived EVs in the vicinity of the vulva during mating behavior.[95] However, male-derived EVs seem to enhance, rather than suppress, male mating behavior.[76] Further work will be necessary to understand how target mating status influences male mate preference.

Our findings in *C. elegans* have parallels in other systems. For example, in *Drosophila*, both sexes use partially redundant, multimodal cues to assess multiple features of potential mates.[96–98] *Drosophila* males prefer to mate with healthy over parasitized females[99]; insulin signaling influences the sexual attractiveness of *Drosophila* females, potentially indicating their physiological status[100]; and pheromones deposited by males on females deter subsequent mating by other males.[101,102] The use of multimodal cues in mate-choice decisions is also widespread in vertebrates, where there is evidence from several systems for hierarchical use of these cues.[21] While we do not suggest that there is an obligate hierarchy in the use of mate-preference cues by *C. elegans* males, the use of multiple signals in mate-choice decisions is a pervasive theme in animal behavior.

The reproductive behavior of the *C. elegans* male, particularly in its native habitat, remains enigmatic. *C. elegans* reproduces primarily by self-fertilization, and the species shows some hallmarks of "selfing syndrome," a degradation of traits that promote sexual reproduction.[5,103] However, population genetic studies reveal evidence of sporadic outcrossing in wild populations.[5] Thus, mate-preference behavior could be an important determinant of the contribution of cross-fertilization to fitness, particularly under times of stress.[20,92] Our finding that males accurately integrate hermaphrodite feeding and mating status into mate-choice decisions suggests that at least some of the cues used by males are honest indicators of fitness. How males integrate these indicators into their behavior, and how a male's own physiological status and reproductive experience influences mate choice, are among many worthwhile topics these studies raise for future research.

STAR★METHODS

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

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- METHOD DETAILS
  - Behavioral Assays
  - Construction of Transgenic Strains
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- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2024.02.036.

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AUTHOR CONTRIBUTIONS

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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REFERENCES

1. Darwin, C. (1888). The Descent of Man, and Selection in Relation to Sex (John Murray).
2. Rosenthal, G.G. (2017). Mate Choice: The Evolution of Sexual Decision Making from Microbes to Humans (Princeton University Press).
3. DeAngelis, R.S., and Hofmann, H.A. (2020). Neural and molecular mechanisms underlying female mate choice decisions in vertebrates. J. Exp. Biol. *223*, jeb207324.
4. Prum, R.O. (2010). The Lande-Kirkpatrick mechanism is the null model of evolution by intersexual selection: implications for meaning, honesty, and design in intersexual signals. Evolution *64*, 3085–3100.
5. Cutter, A.D., Morran, L.T., and Phillips, P.C. (2019). Males, outcrossing, and sexual selection in Caenorhabditis nematodes. Genetics *213*, 27–57.
6. Frézal, L., and Félix, M.A. (2015). C. elegans outside the Petri dish. eLife *4*, e05849.
7. Wood, W.B. (1988). The Nematode *Caenorhabditis elegans* (Cold Spring Harbor Laboratory Press).
8. Kleemann, G.A., and Basolo, A.L. (2007). Facultative decrease in mating resistance in hermaphroditic Caenorhabditis elegans with self-sperm depletion. Anim. Behav. *74*, 1339–1347.
9. Garcia, L.R., LeBoeuf, B., and Koo, P. (2007). Diversity in mating behavior of hermaphroditic and male-female Caenorhabditis nematodes. Genetics *175*, 1761–1771.
10. Gems, D., and Riddle, D.L. (1996). Longevity in Caenorhabditis elegans reduced by mating but not gamete production. Nature *379*, 723–725.
11. Maures, T.J., Booth, L.N., Benayoun, B.A., Izrayelit, Y., Schroeder, F.C., and Brunet, A. (2014). Males shorten the life span of *C. elegans* hermaphrodites via secreted compounds. Science *343*, 541–544.
12. Shi, C., and Murphy, C.T. (2014). Mating induces shrinking and death in *Caenorhabditis* mothers. Science *343*, 536–540.
13. Woodruff, G.C., Knauss, C.M., Maugel, T.K., and Haag, E.S. (2014). Mating damages the cuticle of *C. elegans* hermaphrodites. PloS One *9*, e104456.
14. Booth, L.N., Shi, C., Tantilert, C., Yeo, R.W., Miklas, J.W., Hebestreit, K., Hollenhorst, C.N., Maures, T.J., Buckley, M.T., Murphy, C.T., and Brunet, A. (2022). Males induce premature demise of the opposite sex by multifaceted strategies. Nat Aging *2*, 809–823.
15. Barrière, A., and Félix, M.-A. (2005). High local genetic diversity and low outcrossing rate in Caenorhabditis elegans natural populations. Curr. Biol. *15*, 1176–1184.
16. LeBoeuf, B., Correa, P., Jee, C., and García, L.R. (2014). Caenorhabditis elegans male sensory-motor neurons and dopaminergic support cells couple ejaculation and post-ejaculatory behaviors. eLife *3*, e02938.
17. Morsci, N.S., Haas, L.A., and Barr, M.M. (2011). Sperm status regulates sexual attraction in Caenorhabditis elegans. Genetics *189*, 1341–1346.
18. Leighton, D.H., Choe, A., Wu, S.Y., and Sternberg, P.W. (2014). Communication between oocytes and somatic cells regulates volatile pheromone production in Caenorhabditis elegans. Proc. Natl. Acad. Sci. USA *111*, 17905–17910.
19. Borne, F., Kasimatis, K.R., and Phillips, P.C. (2017). Quantifying male and female pheromone-based mate choice in Caenorhabditis nematodes using a novel microfluidic technique. PLoS One *12*, e0189679.
20. van Sluijs, L., Liu, J., Schrama, M., van Hamond, S., Vromans, S.P.J.M., Scholten, M.H., Žibrat, N., Riksen, J.A.G., Pijlman, G.P., Sterken, M.G., and Kammenga, J.E. (2021). Virus infection modulates male sexual behaviour in Caenorhabditis elegans. Mol. Ecol. *30*, 6776–6790.
21. Candolin, U. (2003). The use of multiple cues in mate choice. Biol. Rev. Camb. Philos. Soc. *78*, 575–595.
22. Simon, J.M., and Sternberg, P.W. (2002). Evidence of a mate-finding cue in the hermaphrodite nematode Caenorhabditis elegans. Proc. Natl. Acad. Sci. USA *99*, 1598–1603.
23. White, J.Q., Nicholas, T.J., Gritton, J., Truong, L., Davidson, E.R., and Jorgensen, E.M. (2007). The sensory circuitry for sexual attraction in C. elegans males. Curr. Biol. *17*, 1847–1857.
24. Srinivasan, J., Kaplan, F., Ajredini, R., Zachariah, C., Alborn, H.T., Teal, P.E., Malik, R.U., Edison, A.S., Sternberg, P.W., and Schroeder, F.C. (2008). A blend of small molecules regulates both mating and development in Caenorhabditis elegans. Nature *454*, 1115–1118.
25. Wan, X., Zhou, Y., Chan, C.M., Yang, H., Yeung, C., and Chow, K.L. (2019). SRD-1 in AWA neurons is the receptor for female volatile sex pheromones in C. elegans males. EMBO Rep. *20*, e46288.
26. Muirhead, C.S., and Srinivasan, J. (2020). Small molecule signals mediate social behaviors in C. elegans. J. Neurogenet. *34*, 395–403.
27. Butcher, R.A. (2017). Decoding chemical communication in nematodes. Nat. Prod. Rep. *34*, 472–477.
28. Park, J.Y., Joo, H.J., Park, S., and Paik, Y.K. (2019). Ascaroside pheromones: chemical biology and pleiotropic neuronal functions. Int. J. Mol. Sci. *20*, 3898.
29. McGrath, P.T., and Ruvinsky, I. (2019). A primer on pheromone signaling in Caenorhabditis elegans for systems biologists. Curr. Opin. Syst. Biol. *13*, 23–30.
30. Ludewig, A.H., and Schroeder, F.C. (2013). Ascaroside signaling in C. elegans. WormBook *18*, 1–22.
31. Izrayelit, Y., Srinivasan, J., Campbell, S.L., Jo, Y., von Reuss, S.H., Genoff, M.C., Sternberg, P.W., and Schroeder, F.C. (2012). Targeted

metabolomics reveals a male pheromone and sex-specific ascaroside biosynthesis in Caenorhabditis elegans. ACS Chem. Biol. *7*, 1321–1325.

32. Burkhardt, R.N., Artyukhin, A.B., Aprison, E.Z., Curtis, B.J., Fox, B.W., Ludewig, A.H., Palomino, D.F., Luo, J., Chaturbedi, A., Panda, O., et al. (2023). Sex-specificity of the C. elegans metabolome. Nat. Commun. *14*, 320.
33. Fagan, K.A., Luo, J., Lagoy, R.C., Schroeder, F.C., Albrecht, D.R., and Portman, D.S. (2018). A single-neuron chemosensory switch determines the valence of a sexually dimorphic sensory behavior. Curr. Biol. *28*, 902–914.e5.
34. Jang, H., Kim, K., Neal, S.J., Macosko, E., Kim, D., Butcher, R.A., Zeiger, D.M., Bargmann, C.I., and SenGupta, P. (2012). Neuromodulatory state and sex specify alternative behaviors through antagonistic synaptic pathways in C. elegans. Neuron *75*, 585–592.
35. Luo, J., and Portman, D.S. (2021). Sex-specific, pdfr-1-dependent modulation of pheromone avoidance by food abundance enables flexibility in C. elegans foraging behavior. Curr. Biol. *31*, 4449–4461.e4.
36. Ryu, L., Cheon, Y., Huh, Y.H., Pyo, S., Chinta, S., Choi, H., Butcher, R.A., and Kim, K. (2018). Feeding state regulates pheromone-mediated avoidance behavior via the insulin signaling pathway in Caenorhabditis elegans. EMBO J. *37*, e98402.
37. von Reuss, S.H. (2018). Exploring modular glycolipids involved in nematode chemical communication. Chimia (Aarau) *72*, 297–303.
38. Aprison, E.Z., and Ruvinsky, I. (2017). Counteracting ascarosides act through distinct neurons to determine the sexual identity of C. elegans pheromones. Curr. Biol. *27*, 2589–2599.e3.
39. Toker, I.A., Lev, I., Mor, Y., Gurevich, Y., Fisher, D., i-Zeevi, L., Antonova, O., Doron, H., Anava, S., Gingold, H., et al. (2022). Transgenerational inheritance of sexual attractiveness via small RNAs enhances evolvability in C. elegans. Dev. Cell *57*, 298–309.e9.
40. Liu, K.S., and Sternberg, P.W. (1995). Sensory regulation of male mating behavior in Caenorhabditis elegans. Neuron *14*, 79–89.
41. Barrios, A., Nurrish, S., and Emmons, S.W. (2008). Sensory regulation of C. elegans male mate-searching behavior. Curr. Biol. *18*, 1865–1871.
42. Lipton, J., Kleemann, G., Ghosh, R., Lints, R., and Emmons, S.W. (2004). Mate searching in Caenorhabditis elegans: a genetic model for sex drive in a simple invertebrate. J. Neurosci. *24*, 7427–7434.
43. Sakai, N., Iwata, R., Yokoi, S., Butcher, R.A., Clardy, J., Tomioka, M., and Iino, Y. (2013). A sexually conditioned switch of chemosensory behavior in C. elegans. PLoS One *8*, e68676.
44. Gravato-Nobre, M.J., Stroud, D., O'Rourke, D., Darby, C., and Hodgkin, J. (2011). Glycosylation genes expressed in seam cells determine complex surface properties and bacterial adhesion to the cuticle of Caenorhabditis elegans. Genetics *187*, 141–155.
45. Weng, J.W., Park, H., Valotteau, C., Chen, R.T., Essmann, C.L., Pujol, N., Sternberg, P.W., and Chen, C.H. (2023). Body stiffness is a mechanical property that facilitates contact-mediated mate recognition in Caenorhabditis elegans. Curr. Biol. *33*, 3585–3596.e5.
46. Jee, C., Goncalves, J.F., LeBoeuf, B., and Garcia, L.R. (2016). CRF-like receptor SEB-3 in sex-common interneurons potentiates stress handling and reproductive drive in C. elegans. Nat. Commun. *7*, 11957.
47. Jennions, M.D., and Petrie, M. (1997). Variation in mate choice and mating preferences: a review of causes and consequences. Biol. Rev. Camb. Philos. Soc. *72*, 283–327.
48. Neelon, D.P., Rodríguez, R.L., and Höbel, G. (2019). On the architecture of mate choice decisions: preference functions and choosiness are distinct traits. Proc. Biol. Sci. *286*, 20182830.
49. MacLeod, A.R., Waterston, R.H., and Brenner, S. (1977). An internal deletion mutant of a myosin heavy chain in Caenorhabditis elegans. Proc. Natl. Acad. Sci. USA *74*, 5336–5340.
50. Pungaliya, C., Srinivasan, J., Fox, B.W., Malik, R.U., Ludewig, A.H., Sternberg, P.W., and Schroeder, F.C. (2009). A shortcut to identifying small molecule signals that regulate behavior and development in Caenorhabditis elegans. Proc. Natl. Acad. Sci. USA *106*, 7708–7713.
51. Golden, J.W., and Riddle, D.L. (1985). A gene affecting production of the Caenorhabditis elegans dauer-inducing pheromone. Mol. Gen. Genet. *198*, 534–536.
52. von Reuss, S.H., Bose, N., Srinivasan, J., Yim, J.J., Judkins, J.C., Sternberg, P.W., and Schroeder, F.C. (2012). Comparative metabolomics reveals biogenesis of ascarosides, a modular library of small-molecule signals in C. elegans. J. Am. Chem. Soc. *134*, 1817–1824.
53. Krauchunas, A.R., Werner, M., Britt, N., Chen, D.S., Maddox, A.S., and Singson, A. (2020). C. elegans CYLC-2 localizes to sperm. MicroPubl. Biol. *2020*, https://doi.org/10.17912/micropub.biology.000314.
54. Le, H.H., Wrobel, C.J., Cohen, S.M., Yu, J., Park, H., Helf, M.J., Curtis, B.J., Kruempel, J.C., Rodrigues, P.R., Hu, P.J., et al. (2020). Modular metabolite assembly in Caenorhabditis elegans depends on carboxylesterases and formation of lysosome-related organelles. eLife *9*, e61886.
55. Faghih, N., Bhar, S., Zhou, Y., Dar, A.R., Mai, K., Bailey, L.S., Basso, K.B., and Butcher, R.A. (2020). A large family of enzymes responsible for the modular architecture of nematode pheromones. J. Am. Chem. Soc. *142*, 13645–13650.
56. Joo, H.J., Yim, Y.H., Jeong, P.Y., Jin, Y.X., Lee, J.E., Kim, H., Jeong, S.K., Chitwood, D.J., and Paik, Y.K. (2009). Caenorhabditis elegans utilizes dauer pheromone biosynthesis to dispose of toxic peroxisomal fatty acids for cellular homoeostasis. Biochem. J. *422*, 61–71.
57. Panda, O., Akagi, A.E., Artyukhin, A.B., Judkins, J.C., Le, H.H., Mahanti, P., Cohen, S.M., Sternberg, P.W., and Schroeder, F.C. (2017). Biosynthesis of modular ascarosides in C. elegans. Angew. Chem. Int. Ed. Engl. *56*, 4729–4733.
58. Mehra, A., Gaudet, J., Heck, L., Kuwabara, P.E., and Spence, A.M. (1999). Negative regulation of male development in Caenorhabditis elegans by a protein-protein interaction between TRA-2A and FEM-3. Genes Dev. *13*, 1453–1463.
59. Yi, W., Ross, J.M., and Zarkower, D. (2000). *mab-3* is a direct *tra-1* target gene regulating diverse aspects of *C. elegans* male sexual development and behavior. Development *127*, 4469–4480.
60. Taylor, S.R., Santpere, G., Weinreb, A., Barrett, A., Reilly, M.B., Xu, C., Varol, E., Oikonomou, P., Glenwinkel, L., McWhirter, R., et al. (2021). Molecular topography of an entire nervous system. Cell *184*, 4329–4347.e23.
61. Ryan, D.A., Miller, R.M., Lee, K., Neal, S.J., Fagan, K.A., SenGupta, P., and Portman, D.S. (2014). Sex, age, and hunger regulate behavioral prioritization through dynamic modulation of chemoreceptor expression. Curr. Biol. *24*, 2509–2517.
62. Mowrey, W.R., Bennett, J.R., and Portman, D.S. (2014). Distributed effects of biological sex define sex-typical motor behavior in Caenorhabditis elegans. J. Neurosci. *34*, 1579–1591.
63. Clark, S.G., Chisholm, A.D., and Horvitz, H.R. (1993). Control of cell fates in the central body region of C. elegans by the homeobox gene lin-39. Cell *74*, 43–55.
64. Baird, S.E., Sutherlin, M.E., and Emmons, S.W. (1992). Reproductive isolation in rhabditidae (Nematoda: Secernentea); mechanisms that isolate six species of three genera. Evolution *46*, 585–594.
65. Palaima, E., Leymarie, N., Stroud, D., Mizanur, R.M., Hodgkin, J., Gravato-Nobre, M.J., Costello, C.E., and Cipollo, J.F. (2010). The Caenorhabditis elegans bus-2 mutant reveals a new class of O-glycans affecting bacterial resistance. J. Biol. Chem. *285*, 17662–17672.
66. Chasnov, J.R., So, W.K., Chan, C.M., and Chow, K.L. (2007). The species, sex, and stage specificity of a Caenorhabditis sex pheromone. Proc. Natl. Acad. Sci. USA *104*, 6730–6735.
67. Austin, J., and Kimble, J. (1987). glp-1 is required in the germ line for regulation of the decision between mitosis and meiosis in C. elegans. Cell *51*, 589–599.
68. Colbert, H.A., and Bargmann, C.I. (1995). Odorant-specific adaptation pathways generate olfactory plasticity in C. elegans. Neuron *14*, 803–812.

69. Jordan, J.M., Hibshman, J.D., Webster, A.K., Kaplan, R.E.W., Leinroth, A., Guzman, R., Maxwell, C.S., Chitrakar, R., Bowman, E.A., Fry, A.L., et al. (2019). Insulin/IGF signaling and vitellogenin provisioning mediate intergenerational adaptation to nutrient stress. Curr. Biol. *29*, 2380–2388.e5.
70. Artyukhin, A.B., Yim, J.J., Srinivasan, J., Izrayelit, Y., Bose, N., von Reuss, S.H., Jo, Y., Jordan, J.M., Baugh, L.R., Cheong, M., et al. (2013). Succinylated octopamine ascarosides and a new pathway of biogenic amine metabolism in Caenorhabditis elegans. J. Biol. Chem. *288*, 18778–18783.
71. Zhang, S.O., Box, A.C., Xu, N., Le Men, J., Yu, J., Guo, F., Trimble, R., and Mak, H.Y. (2010). Genetic and dietary regulation of lipid droplet expansion in Caenorhabditis elegans. Proc. Natl. Acad. Sci. USA *107*, 4640–4645.
72. Ward, S., and Carrel, J.S. (1979). Fertilization and sperm competition in the nematode Caenorhabditis elegans. Dev. Biol. *73*, 304–321.
73. Aprison, E.Z., and Ruvinsky, I. (2016). Sexually antagonistic male signals manipulate germline and soma of C. elegans hermaphrodites. Curr. Biol. *26*, 2827–2833.
74. Angeles-Albores, D., Aprison, E.Z., Dzitoyeva, S., and Ruvinsky, I. (2023). A C. elegans male pheromone feminizes germline gene expression in hermaphrodites and imposes life-history costs. Preprint at bioRxiv. https://doi.org/10.1101/2023.02.17.528976.
75. Hodgkin, J., and Doniach, T. (1997). Natural variation and copulatory plug formation in Caenorhabditis elegans. Genetics *146*, 149–164.
76. Wang, J., Silva, M., Haas, L.A., Morsci, N.S., Nguyen, K.C., Hall, D.H., and Barr, M.M. (2014). C. elegans ciliated sensory neurons release extracellular vesicles that function in animal communication. Curr. Biol. *24*, 519–525.
77. Nikonorova, I.A., Wang, J., Cope, A.L., Tilton, P.E., Power, K.M., Walsh, J.D., Akella, J.S., Krauchunas, A.R., Shah, P., and Barr, M.M. (2022). Isolation, profiling, and tracking of extracellular vesicle cargo in Caenorhabditis elegans. Curr. Biol. *32*, 1924–1936.e6.
78. Barr, M.M., García, L.R., and Portman, D.S. (2018). Sexual dimorphism and sex differences in Caenorhabditis elegans neuronal development and behavior. Genetics *208*, 909–935.
79. Narayan, A., Venkatachalam, V., Durak, O., Reilly, D.K., Bose, N., Schroeder, F.C., Samuel, A.D., Srinivasan, J., and Sternberg, P.W. (2016). Contrasting responses within a single neuron class enable sex-specific attraction in Caenorhabditis elegans. Proc. Natl. Acad. Sci. USA *113*, E1392–E1401.
80. Peden, E., Kimberly, E., Gengyo-Ando, K., Mitani, S., and Xue, D. (2007). Control of sex-specific apoptosis in C. elegans by the BarH homeodomain protein CEH-30 and the transcriptional repressor UNC-37/Groucho. Genes Dev. *21*, 3195–3207.
81. Schwartz, H.T., and Horvitz, H.R. (2007). The C. elegans protein CEH-30 protects male-specific neurons from apoptosis independently of the Bcl-2 homolog CED-9. Genes Dev. *21*, 3181–3194.
82. Barr, M.M., DeModena, J., Braun, D., Nguyen, C.Q., Hall, D.H., and Sternberg, P.W. (2001). The *Caenorhabditis elegans* autosomal dominant polycystic kidney disease gene homologs *lov-1* and *pkd-2* act in the same pathway. Curr. Biol. *11*, 1341–1346.
83. Portman, D.S. (2017). Sexual modulation of sex-shared neurons and circuits in Caenorhabditis elegans. J. Neurosci. Res. *95*, 527–538.
84. Salzberg, Y., Gat, A., and Oren-Suissa, M. (2021). One template, two outcomes: how does the sex-shared nervous system generate sex-specific behaviors? Curr. Top. Dev. Biol. *144*, 245–268.
85. Lee, K., and Portman, D.S. (2007). Neural sex modifies the function of a C. elegans sensory circuit. Curr. Biol. *17*, 1858–1863.
86. Cook, S.J., Jarrell, T.A., Brittin, C.A., Wang, Y., Bloniarz, A.E., Yakovlev, M.A., Nguyen, K.C.Q., Tang, L.T., Bayer, E.A., Duerr, J.S., et al. (2019). Whole-animal connectomes of both Caenorhabditis elegans sexes. Nature *571*, 63–71.
87. Hilbert, Z.A., and Kim, D.H. (2017). Sexually dimorphic control of gene expression in sensory neurons regulates decision-making behavior in C. elegans. eLife *6*, e21166.
88. Yoshida, K., Hirotsu, T., Tagawa, T., Oda, S., Wakabayashi, T., Iino, Y., and Ishihara, T. (2012). Odour concentration-dependent olfactory preference change in C. elegans. Nat. Commun. *3*, 739.
89. Krzyzanowski, M.C., Woldemariam, S., Wood, J.F., Chaubey, A.H., Brueggemann, C., Bowitch, A., Bethke, M., L'Etoile, N.D., and Ferkey, D.M. (2016). Aversive behavior in the nematode C. elegans is modulated by cGMP and a neuronal gap junction network. PLoS Genet. *12*, e1006153.
90. Pechuk, V., Goldman, G., Salzberg, Y., Chaubey, A.H., Bola, R.A., Hoffman, J.R., Endreson, M.L., Miller, R.M., Reger, N.J., Portman, D.S., et al. (2022). Reprogramming the topology of the nociceptive circuit in C. elegans reshapes sexual behavior. Curr. Biol. *32*, 4372–4385.e7.
91. Garcia, L.R., Mehta, P., and Sternberg, P.W. (2001). Regulation of distinct muscle behaviors controls the C. elegans male's copulatory spicules during mating. Cell *107*, 777–788.
92. Wu, T., Ge, M., Wu, M., Duan, F., Liang, J., Chen, M., Gracida, X., Liu, H., Yang, W., Dar, A.R., et al. (2023). Pathogenic bacteria modulate pheromone response to promote mating. Nature *613*, 324–331.
93. Barr, M.M., García, L.R., and Portman, D.S. (2018). Sexual dimorphism and sex differences in *Caenorhabditis elegans* neuronal development and behavior. Genetics *208*, 909–935.
94. Sherlekar, A.L., Janssen, A., Siehr, M.S., Koo, P.K., Caflisch, L., Boggess, M., and Lints, R. (2013). The C. elegans male exercises directional control during mating through cholinergic regulation of sex-shared command interneurons. PLoS One *8*, e60597.
95. Wang, J., Nikonorova, I.A., Gu, A., Sternberg, P.W., and Barr, M.M. (2020). Release and targeting of polycystin-2-carrying ciliary extracellular vesicles. Curr. Biol. *30*, R755–R756.
96. Arbuthnott, D., Fedina, T.Y., Pletcher, S.D., and Promislow, D.E. (2017). Mate choice in fruit flies is rational and adaptive. Nat. Commun. *8*, 13953.
97. Vijendravarma, R.K., Narasimha, S., Steinfath, E., Clemens, J., and Leopold, P. (2022). Drosophila females have an acoustic preference for symmetric males. Proc. Natl. Acad. Sci. USA *119*, e2116136119.
98. Katayama, N., Abbott, J.K., Kjærandsen, J., Takahashi, Y., and Svensson, E.I. (2014). Sexual selection on wing interference patterns in Drosophila melanogaster. Proc. Natl. Acad. Sci. USA *111*, 15144–15148.
99. Wittman, T., and Fedorka, K.M. (2015). Male mate choice for unparasitized females in Drosophila melanogaster. J. Insect Behav. *28*, 37–43.
100. Kuo, T.H., Fedina, T.Y., Hansen, I., Dreisewerd, K., Dierick, H.A., Yew, J.Y., and Pletcher, S.D. (2012). Insulin signaling mediates sexual attractiveness in Drosophila. PLoS Genet. *8*, e1002684.
101. Lacaille, F., Hiroi, M., Twele, R., Inoshita, T., Umemoto, D., Manière, G., Marion-Poll, F., Ozaki, M., Francke, W., Cobb, M., et al. (2007). An inhibitory sex pheromone tastes bitter for Drosophila males. PLoS One *2*, e661.
102. Miyamoto, T., and Amrein, H. (2008). Suppression of male courtship by a Drosophila pheromone receptor. Nat. Neurosci. *11*, 874–876.
103. Cutter, A.D. (2008). Reproductive evolution: symptom of a selfing syndrome. Curr. Biol. *18*, R1056–R1058.
104. Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. Genetics *77*, 71–94.
105. Stiernagle, T. (2006). Maintenance of C. elegans. WormBook *11*, 1–11.
106. Barr, M.M., and Sternberg, P.W. (1999). A polycystic kidney-disease gene homologue required for male mating behaviour in *C. elegans*. Nature *401*, 386–389.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
E. coli OP50	*Caenorhabditis* Genetics Center (CGC)	OP50
Chemicals, peptides, and recombinant proteins		
Synthetic ascaroside#3 (ascr#3)	Synthesized and generously provided by F. Schroeder laboratory, Boyce Thompson Institute, Ithaca, NY	N/A
Experimental models: Organisms/strains		
him-5(e1490) V	CGC	DR466
unc-54(e190) I; him-5(e1490) V	This work and CGC (from DR466 and CB190)	UR1386
unc-54(e190) I; daf-22(m130) II; him-5(e1490) V	This work and CGC (from UR1386 and DR476)	UR1387
unc-54(e190) I; him-5(e1490) V; fsEx445[Pnhx-2::FEM-3(+)::SL2::mCherry + Psulp-3::GFP]	This work and CGC (from CB190 and UR936)	UR1382
him-5(e1490) V; fsEx445[Pnhx-2::FEM-3(+)::SL2::mCherry + Psulp-3::GFP]; fsEx526[MAB-3::GFP pDZ162 + Punc-122::GFP]	This work (From UR1130 and UR936)	UR1432
unc-54(e190) I; daf-22(m130) II; him-5(e1490) V; fsEx445[Pnhx-2::FEM-3(+)::SL2::mCherry + Psulp-3::GFP]	This work (from UR1382 and UR1387)	UR1383
unc-54(e190) I; him-5(e1490) V; fsEx443[Pnhx-2::TRA-2(ic)::SL2::mCherry + Psulp-3::GFP]	This work (from UR1386 and UR934)	UR1398
unc-54(e190) I; daf-22(m130) II; him-5(e1490) V; fsEx443[Pnhx-2::TRA-2(ic)::SL2::mCherry + Psulp-3::GFP]	This work (from UR1387 and UR934)	UR1399
him-5(e1490) V; fsEx443[Pnhx-2::TRA-2(ic)::SL2::mCherry + Psulp-3::GFP]; fsEx526[MAB-3::GFP pDZ162 + Punc-122::GFP]	This work (from UR1130 and UR934)	UR1433
unc-54(e190) I; lin-39(n1760) III; him-5(e1490) V	This work and CGC (from DR466, UR1386 and MT4009)	UR1420
unc-54(e190) I; daf-22(m130) II; lin-39(n1760) III; him-5(e1490) V	This work and CGC (from DR466, UR1387 and MT4009)	UR1426
unc-54(e190) I; bus-2(e2687) IV; him-5(e1490) V	This work and CGC (from DR466, UR1386 and CB5610)	UR1421
unc-54(e190) I; daf-22(m130) II; bus-2(e2687) IV; him-5(e1490) V	This work and CGC (from DR466, UR1387 and CB5610)	UR1395
unc-51(e369) V	CGC	CB369
unc-51(e369) him-5 (e1490) V; bxEx166[Pdpy-7::fem-3]	This work	EM1108
srd-1(eh1) II; him-5(e1490) V	This work and CGC (from DR466 and CB5414)	UR1404
unc-54(e190) I; glp-1(e2141ts) III; him-5(e1490) V	This work and CGC (from UR1386 and CB4037)	UR1402
unc-54(e190) I; daf-22(m130) II; glp-1(e2141ts) III; him-5(e1490) V	This work and CGC (from UR1387 and CB4037)	UR1403
nIs133[Ppkd-2::gfp] I; him-5(e1490) V	This laboratory, from MT13570	UR991
nIs133[Ppkd-2::gfp] I; him-5(e1467) V; ceh-30(n4289) X	Horvitz laboratory	MT13570
him-5(e1490) V; fsIs15[Prab-3p::FEM-3(+)::SL2::mCherry + Punc-122::GFP]	This laboratory	UR236
him-5(e1490) V; fsIs19[Prab-3::TRA-2(ic)::SL2::mCherry + Pelt-2::GFP]	This laboratory	UR1126
him-5; fsEx357[Posm-5::TRA-2(ic)::SL2::mCherry]	This laboratory	UR754
him-8(e1489) IV	CGC	CB1489
him-8(e1489) IV; qrIs2[sra-9::mCasp1] V	This work and CGC (from CB1489 and PS6025)	UR1216

(Continued on next page)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
him-5(e1490) V; Ex[srh-281p::mCasp1 + myo-3p::GFP]	This work and Dr. Hirotsu	UR1106
him-5(e1490) V; udEx428[elt-2p::GFP + srh-142p::GFP +srh-142p::CED-3(p15) + srh-142p::CED-3(p17)]	This laboratory	UR987
him-5(e1490) V; peIs1715[str-1p::mCasp1 + unc-122p::mCherry]	This work and Dr. Ishihara (from DR466 and JN1715)	UR1107
him-5(e1490) V; peIs1713[sra-6p::mCasp1 + unc-122p::mCherry]	This work and Dr. Ishihara (from DR466 and JN1713)	UR1108
him-5(e1490) V; udEx211[osm-10p::ced-3(p15), srb-6p::ced-3(p17), osm-10p::mCherry, elt-2p::gfp]	This work and Dr. Ferkey (from DR466 and FG521)	UR1396
him-5(e1490) V; oyIs85[Pceh-36::TU#813 + Pceh-36::TU#814 + Psrtx-1::GFP + Punc-122::dsRed]	This work and Dr. Sengupta (from DR466 and PY7502)	UR1109
him-5(e1490)V; oyIs84[gpa-4p::TU#813 + gcy-27p::TU#814 + gcy-27p::GFP + unc-122p::dsRed]	This work and Dr. Sengupta (from DR466 and PY7505)	UR1110
unc-54(e190) I; him-5(e1490) V; fsIs15[Prab-3p::FEM-3(+)::SL2::mCherry + Punc-122::GFP]	This work (from UR236 and UR1386)	UR1384
unc-54(e190) I; daf-22(m130) II; him-5(e1490) V; fsIs15[Prab-3p::FEM-3(+)::SL2::mCherry + Punc-122::GFP]	This work (from UR236 and UR1387)	UR1385
unc-54(e190) I; bus-2(e2687) IV; him-5(e1490) V; fsEx617[Pdpy-7::BUS-2(cDNA)::SL2::mCherry::unc-54 3'UTR, elt-2p::GFP]	This work	UR1423
pkd-2(sy606) IV; him-5(e1490) V	CGC	PT8
cylc-2(mon2[cylc-2::mNG::3xFLAG]) I; him-5(e1490) V	This work (from MDX44 and DR466)	UR1434
Oligonucleotides		
See Table S1	N/A	N/A

RESOURCE AVAILABLITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Douglas Portman (douglas.portman@rochester.edu).

Materials availability

Plasmids and nematode strains generated in the course of this work are freely available to interested academic researchers through the lead contact.

Data and code availability

- Source data obtained in the current study have not been deposited in a public repository but are available from the lead contact on request.
- This study did not generate code.
- Any additional information required to reanalyze the data reported in this work paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

All *C. elegans* strains were cultured using *E. coli* OP50 and NGM agar as described.[104,105] All strains were grown and assayed at 20°C, except for those containing *glp-1* mutations; for these, *glp-1* parents were cultured at 15°C from egg to young adult stage to permit germline development and then transferred to 25°C to generate germline-less *glp-1* targets, which were assayed at 20°C. All relevant control strains experienced identical temperature shifts. All target strains contained the mutation *unc-54(e190)* to prevent animals from migrating on the assay plate. All strains were derived from N2 and, unless otherwise noted, contained *him-5(e1490)* to increase the frequency of spontaneous males in self-fertilizing populations.

METHOD DETAILS

Behavioral Assays

Mate Preference Assay

One day prior to the assay, L4 animals (both searchers and targets) were picked to sex-segregated plates. To prepare assay plates, quadrant boundaries were marked on the bottom of unseeded 6-cm NGM plates with a custom-made stamp. The quadrant area was a square (3 cm on each edge) divided into four zones with a 1-cm diameter circle in the center. *E. coli* OP50 fresh culture (50 μL) was spread within the 3 cm square and plates were incubated at 20°C overnight. The next day, four target animals, now day 1 adults, were placed in the center of each quadrant (Figure 1A) at *t* = -30 mins. All targets carried *unc-54(e190)* to limit their mobility. While *unc-54* animals move poorly, they have wild-type morphology as larvae and young adults, and the chemical and physical cues they produce are likely unaffected by this mutation. At time *t* = 0, ten searcher animals were placed in the center circle; their positions were then recorded at 30 min intervals (t = 30 min, 60 min, and 90 min). Plates on which target animals moved outside their quadrant boundaries (an uncommon occurrence) were censored. We calculated Mate Preference Index values for each time point [Mate Preference Index = (# in "B" quadrants – # in "A" quadrants)/(# in "B" quadrants + # in "A" quadrants)] and averaged these to obtain a final Mate Preference Index. Thus, in the graphs shown, each data point represents the behavior of a group of ~10 searchers over 90 minutes. Occasionally, searchers remained at the origin spot or wandered outside the four-quadrant zone, in which case they were not counted. In most assays, this was the case for no more than two of the ten searchers. If there were more than 5 such animals in an assay (a rare occurrence), that trial was discarded. For all data obtained in this way, we confirmed that time made no detectable contribution to variation in the data (see quantification and statistical analysis section below), unless noted otherwise.

For experiments in which plates were pre-conditioned with targets, targets were placed on the assay plate for 2.5 hr and then removed before testers were placed on the assay plate. For experiments in which targets were suspended on the lid, one small chunk of food-containing NGM agar (~0.5 cm^2 area, ~0.4 cm height) was placed at the center of each quadrant on the inside surface of the lid of the assay plate, with the food surface pointing outward. Four targets were placed onto the chunk's food surface and the lid was placed over the assay plate. Using this method, targets and testers were separated by an air gap of ~3 mm.

Preparation of starved targets

To prepare starved targets, *unc-54* or *unc-54; daf-22* hermaphrodites were fed until the young adult stage and then hand-picked into a drop of sterile water freshly loaded on the surface of bacteria-free NGM agar. After soaking briefly (2-3 min), animals were hand-picked into bacteria-free NGM agar plate to initiate the fasting process (*t* = 0). At *t* = 4 hr, half of these animals were moved into assay plates to be used as 4hr-starved targets. The remainder continued to fast on the bacteria-free agar until *t* = 28 hr, when they were moved into assay plates to be used as 28h-starved targets. No bacterial growth was observed on the fasting plates at this time. To prepare well-fed controls, similar steps were performed at the same time points, except that animals were placed on OP50-seeded plates instead of bacteria-free ones after rinsing.

Preparation of mated and virgin targets

To prepare mated targets, 50 young adult *him-5* males and 50 L4 *unc-54* or *unc-54; daf-22* hermaphrodites were hand-picked to an NGM agar plate containing a small (~1 cm diameter) OP50 bacteria lawn. These plates were incubated for 24 hr at 20°C, after which time males will have mated with most hermaphrodites. To prepare control virgin targets, we incubated L4 hermaphrodites with *unc-54; him-5* males, which cannot mate[7] but still provide a source of male pheromones. For washed targets, targets were picked into a large drop of sterile water (~20 μL) loaded on the surface of bacteria-free NGM agar before they were placed into the assay plates.

Quadrant Assay with synthetic ascr#3

This assay was performed as described previously,[33] using 1 μM purified ascr#3, a gift of F. Schroeder (Boyce Thompson Institute and Cornell University).

Mate-retention using the food-leaving assay

The ability of a potential mate to suppress food-leaving behavior was assayed as previously described.[41,42]

Male contact-response assay

Response behavior was assayed as previously described,[106] except that hermaphrodites were *unc-54*.

Mating frequency assay

To measure mating frequency, we used a standard mate-preference assay, except that searcher males contained a marker (*cylc-2(mon2[cylc-2::mNG::3xFLAG])*[53]) that fluorescently labels their sperm. At the 30, 60, and 90 min time points, target hermaphrodites were scored for internal mNG fluorescence using a Leica MZFLIII microscope, allowing us to calculate the fraction of mated hermaphrodites. Because we did not quantify the mNG signal, this fraction does not reflect the number of times a hermaphrodite may have mated, but rather whether successful mating has ever taken place. At early time points, relatively few matings will have occurred, such that the frequency of mNG fluorescence in hermaphrodites will be proportional to the rate of mating. With time, however, increasing numbers of hermaphrodites will have mated multiple times. As this happens, the frequency of fluorescence in the population becomes an underestimate of the total number of mating events that have occurred. This effect occurs more rapidly for preferred than non-preferred mates, causing a reduction in the apparent effect size with time.

Construction of Transgenic Strains

Sex-reversal transgenes were generated using the Multisite Gateway Cloning System (Invitrogen). The intestine-specific *Pnhx-2* promoter was used to drive expression of *fem-3(+)* or *tra-2(ic)* to masculinize or feminize this tissue, respectively. *Pdpy-7* was used to

feminize the hypodermis. Transgenic animals were generated using injection mixes containing 50-80 ng/μL of the co-injection marker *Psulp-3::gfp* and 20-50 ng/μL of the *fem-3(+)* or *tra-2(ic)* expression construct.

Worm total genomic DNA was purified from mixed-stage DR466 with a commercial kit (Qiagen catalog number 51304) as the template of *dpy-7* promoter amplification. Worm total RNA was purified from mixed-stage DR466 with a commercial kit (Qiagen catalog number 74134), followed by *in vitro* cDNA synthesis (Invitrogen catalog number 18080-051), as the template of *bus-2* (isoform a) cDNA amplification. Other reagents used for the construction of expression plasmids include the MultiSite Gateway Three-fragment Vector Construction Kit (Invitrogen Catalog number 12537-023) and DH5α competent cells (ThermoScientific catalog number 18265017). Plasmid sequences were confirmed by Sanger sequencing (Genewiz). Standard microinjection procedures were used to generate transgenic animals with the desired plasmid and a fluorescent co-injection marker at a total DNA concentration of 100 ng/μL.

Microscopy

Animals were prepared for epifluorescence microscopy using standard procedures. DIC and fluorescence images were acquired using a Zeiss Axioplan II. Images were obtained using consistent exposure times and were not subjected to brightness or contrast manipulations.

QUANTIFICATION AND STATISTICAL ANALYSIS

In the mate-preference assay, for each tester-target condition, we carried out a Kruskal-Wallis test across the 30, 60, and 90 min time points to ask whether there was significant temporal variation in the data. Unless otherwise noted, no such variation was detected. We carried out a Wilcoxon signed-rank test for each condition to ask whether the Mate Preference Index differed from the null hypothesis that males had no preference for one class of targets over the other (*e.g.,* Mate Preference Index = 0). The associated *p* values are indicated with black squares to the right of each next to each row: ■, $p \leq 0.05$; ■■, $p \leq 0.005$; ■■■, $p \leq 0.001$. To compare MPI values between different classes of searchers, we used a Mann-Whitney test (to compare two genotypes) or a Kruskal-Wallis test with Dunn's correction (to compare more than two genotypes). Asterisks indicate *p* values associated with these tests: *$p \leq 0.05$; **$p \leq 0.005$; ***$p \leq 0.001$. We only carried out tests necessary to evaluate specific, pre-established hypotheses. For clarity, the brackets in each graph indicate all comparisons made, including those whose outcomes were not statistically significant ("n.s."). In cases where the variance in individual Mate Preference Index values appeared to be exceptionally high, we carried out F-tests to determine whether variance differed significantly from paired controls. All tests on data from the mate-preference assay were carried out using Prism 10.1 (GraphPad Software, LLC).

To analyze the fraction of targets mated, we used a Kruskal-Wallis test with Dunn's correction (Prism 10.1). To analyze the contact-response assays, we used Chi-square tests (Prism 10.1). For the food-leaving assay, P_L (probability of leaving per worm per hr) values for each genotype were calculated using R (www.rproject.org) to fit the censored data with an exponenCal parametric survival model, using maximum likelihood. The hazard values obtained were reported as the P_L values. To estimate P_L values, s.e.m., and the 95% confidence intervals, worms from each experimental treatment were pooled across replicas and contrasted against controls using maximum likelihood.