



**HAL**  
open science

# Body stiffness is a mechanical property that facilitates contact-mediated mate recognition in *Caenorhabditis elegans*

Jen-Wei Weng, Heenam Park, Claire Valotteau, Rui-Tsung Chen, Clara Essmann, Nathalie Pujol, Paul Sternberg, Chun-Hao Chen

## ► To cite this version:

Jen-Wei Weng, Heenam Park, Claire Valotteau, Rui-Tsung Chen, Clara Essmann, et al.. Body stiffness is a mechanical property that facilitates contact-mediated mate recognition in *Caenorhabditis elegans*. *Current Biology - CB*, 2023, 33 (17), pp.3585-3596.e5. 10.1016/j.cub.2023.07.020 . hal-04204502

**HAL Id: hal-04204502**

**<https://hal.science/hal-04204502>**

Submitted on 12 Sep 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

**Body Stiffness Is A Mechanical Property that Facilitates Contact-mediated Mate Recognition in *Caenorhabditis elegans***

Jen-Wei Weng<sup>1</sup>, Heenam Park<sup>2</sup>, Claire Valotteau<sup>3</sup>, Rui-Tsung Chen<sup>1</sup>, Clara L. Essmann<sup>4</sup>, Nathalie Pujol<sup>5</sup>, Paul W. Sternberg<sup>2\*</sup>, Chun-Hao Chen<sup>1,2,6\*</sup>

<sup>1</sup> Institute of Molecular and Cellular Biology, College of Life Science, National Taiwan University. No. 1, Sec. 4, Roosevelt Rd., Taipei 10617, Taiwan.

<sup>2</sup> Division of Biology and Biological Engineering, California Institute of Technology. 1200 E California Blvd, Pasadena, CA 91125, USA

<sup>3</sup> Aix-Marseille Univ, INSERM, CNRS, LAI, Turing Centre for Living Systems, 163 avenue de Luminy, 13009, Marseille, France

<sup>4</sup> Bio3/Bioinformatics and Molecular Genetics, Albert-Ludwigs-University, Schaezlestr. 1, 79104, Freiburg, Germany

<sup>5</sup> Aix Marseille Univ, INSERM, CNRS, CIML, Turing Centre for Living Systems, 163 avenue de Luminy, case 906, 13009, Marseille, France

<sup>6</sup> Lead contact

\* Correspondence: [pws@caltech.edu](mailto:pws@caltech.edu)

[chunhaochen@ntu.edu.tw](mailto:chunhaochen@ntu.edu.tw)

**Keywords:** Mate recognition, Mate searching, *C. elegans*, Collagen, Body stiffness

## Summary

1 Physical contact is prevalent in the animal kingdom to recognize suitable mates by  
2 decoding information about sex, species, and maturity. Although chemical cues for mate  
3 recognition have been extensively studied, the role of mechanical cues remains elusive.  
4 Here we show that *C. elegans* males recognize conspecific and reproductive mates  
5 through short-range cues, and the attractiveness of potential mates depends on the sex and  
6 developmental stages of the hypodermis. We find that a particular group of cuticular  
7 collagens is required for mate attractiveness. These collagens maintain body stiffness to  
8 sustain mate attractiveness but do not affect the surface properties that evokes the initial  
9 step of mate recognition, suggesting that males utilize multiple sensory mechanisms to  
10 recognize suitable mates. Manipulations of body stiffness via physical interventions,  
11 chemical treatments, and 3D-printed bionic worms indicate that body stiffness is a  
12 mechanical property for mate recognition and increases mating efficiency. Our study thus  
13 extends the repertoire of sensory cues of mate recognition in *C. elegans* and provides a  
14 paradigm to study the important role of mechanosensory cues in social behaviors.

15

16

17

18

19

## 20 **Introduction**

21 Mating is a social behavior conserved among metazoans that transfers genetic variation  
22 for phenotypic diversity within conspecific individuals. To maximize reproductive  
23 success and reduce the cost of energy for inappropriate mating, animals evolve different  
24 strategies to recognize conspecific and reproductive mates. Recognition of suitable mates  
25 requires accessing information from individuals through a broad array of sensory  
26 modalities <sup>1-4</sup>. Animals then integrate sensory information to decode the chemical and  
27 physical properties of mates through complex neural networks and respond appropriately  
28 according to their location, species, sex, maturity, and even physiological conditions <sup>3,5-7</sup>.  
29 Thus, identifying a complete set of sensory cues used in mate recognition is crucial to  
30 understanding the neural computation underlying social behaviors.

31 Studies of sensory cues for mate recognition mostly centered on chemical-based  
32 communication <sup>1,7,8</sup>, in which animals leave chemical marks in the environment or on  
33 their bodies that instruct suitable mates. However, in the wild, the mixture of chemical  
34 cues from different species or sex may disturb the transmission and interpretation of the  
35 chemical signal, which likely leads to lower efficiency. Furthermore, chemical cues can  
36 be mimicked by predators, as with carnivorous fungi that secrete pheromone-like  
37 chemicals to attract their *C. elegans* prey <sup>9</sup>. As a result, physical traits, such as colors,  
38 sounds, and body size, are used to enhance the specificity of mate recognition in many  
39 species <sup>10-12</sup>. For instance, pigmentation of eyes and body are visual cues for mate  
40 selections and courtship behaviors in *Drosophila* <sup>13,14</sup>. Males also use their forelegs to  
41 physically contact females to access cuticular pheromones for species and sex recognition  
42 <sup>15-17</sup>. After contacting females, males display “love songs” by extending and vibrating a

43 wing and finish copulation upon reception by females. Interestingly, cuticular extracts are  
44 insufficient to evoke mate recognition, suggesting the involvement of unidentified  
45 sensory cues to initiate courtship behaviors <sup>17</sup>. These findings highlight that integrating  
46 multiple sensory cues is pivotal for the precision of mate recognition.

47 *C. elegans* is hermaphroditic nematode with two sexes, males and self-reproductive  
48 hermaphrodites. Hermaphrodites secrete pheromones as long-range cues in the  
49 environment to attract males remotely <sup>2,6,18</sup>, and males move toward the source of  
50 pheromones sensed by olfactory and pheromone-sensing neurons <sup>19</sup>. In addition to  
51 pheromonal cues, physical contact provides short-range signals for mate recognition in *C.*  
52 *elegans* <sup>20,21</sup>. After contacting hermaphrodites, males restrict the locomotion area  
53 independent of pheromones, presumably increasing the chance of mating <sup>20</sup>. Moreover,  
54 physical contact with hermaphrodites is essential for the sexual conditioning of males that  
55 reverses the learned aversive behaviors associated with starvation <sup>20-22</sup>. Although physical  
56 contact is an essential step for contact-mediated mate recognition, Barrios et al. have  
57 shown that male-specific EF interneurons are dispensable for initial contact response to  
58 mates but are required for enduring changes of locomotion <sup>21</sup>. Further studies speculated  
59 that persistent scanning of suitable mates provides additional information to discriminate  
60 mates <sup>20,21</sup>. These findings highlight a complex neural basis with multiple steps in  
61 decoding mate information. However, the nature of contact-mediated sensory cue(s) for  
62 each step on hermaphrodites is unknown. Because physical contact is mainly controlled  
63 by male-specific ray sensory neurons, which are sensitive to mechanical stimulation <sup>23</sup>, it  
64 raises the possibility that mechanical cues transmit the short-range signal for mate  
65 recognition. Indeed, mechanosensory proteins have been implicated in mating but what

66 they sense is unknown <sup>24</sup>.

67 In nematodes, a collagen-rich apical extracellular matrix, namely cuticle, maintains  
68 body shape and serves as a physical barrier to prevent pathogenic infection <sup>25-27</sup>. The  
69 composition of collagens is crucial to determine surface structure <sup>28,29</sup>, and the physical  
70 constraints of cuticles maintain body stiffness supported by internal organs and muscle  
71 tones <sup>30-32</sup>. A recent study has shown that specific collagens in the cuticle are involved in  
72 maintaining body mechanical properties <sup>32</sup>, but their role in mate recognition has not been  
73 explored.

74 Here we showed that the body stiffness of mates is a mechanical property that  
75 facilitates mate recognition. We demonstrated that *C. elegans* males physically contacted  
76 potential mates to access the information of species, sex, and reproductive stages.  
77 Through a candidate genetic screen, we discovered that the furrow collagens, including  
78 *dpy-2*, *dpy-7*, and *dpy-10*, were generally required for mates to attract males independent  
79 of their known roles in controlling body size and surface texture. Interestingly, these  
80 collagens were dispensable for the first step in mate recognition by evoking contact  
81 responses in males. Instead, furrow collagens maintain body stiffness as recently reported  
82 <sup>32</sup> in the second step. We found that cuticular cues, which evoke contact response, and a  
83 specific range of body stiffness were inseparable sensory cues, suggesting a two-step  
84 sensory mechanism for contact-mediated mate recognition. Lastly, we showed that mate  
85 attractiveness was associated with mating efficiency, indicating the biological  
86 significance of contact-mediated mate recognition in social interactions. We therefore  
87 provide evidence that the mechanical property conferred by furrow collagens facilitates  
88 mate recognition in nematodes. Since physical contact is prevalent in mate recognition

89 across the animal kingdom, our study opens an avenue to study the roles of mechanical  
90 cues in social behaviors and the underlying neural mechanisms.

91

## 92 **Results**

### 93 ***C. elegans* Males Utilize Contact-mediated Cues to Recognize Species, Sex, and** 94 **Developmental Stages**

95 To search for contact-mediated cues on hermaphrodites, we performed male retention  
96 assay that has been used to evaluate the sex drive of individual males with different ages,  
97 mating history, and nutritional status (Figure S1A)<sup>20,21,33</sup>. We placed day 1 (D1) adult  
98 males with or without hermaphrodites on a food source and measured the sex drive of  
99 males by calculating the percentage of leaving males (See STAR Methods)<sup>33</sup>. If alone,  
100 males had a strong tendency to leave the food source and explore the environment. In  
101 contrast, the presence of adult hermaphrodites fixed by paraformaldehyde for 30 min  
102 profoundly switched male locomotion patterns from exploration to local stay (Figure 1A  
103 and 1C). We used these behavioral changes as a readout to study mate recognition, as the  
104 presence of suitable mates retains males, while the loss of essential sensory information  
105 reduces male retention. Consistent with previous studies<sup>6,7,21,34,35</sup>, we found that  
106 hermaphrodites utilized distinct sets of molecules apart from pheromonal, germline, and  
107 vulval signaling to retain males (Figure 1A, S1B, and S1C).

108 We next wondered whether males are able to discriminate species, sex, and  
109 developmental stages of potential mates. In a previous study, Lipton et al. showed that  
110 males can discriminate the sexes through physical contact<sup>20</sup>. Indeed, while the presence  
111 of hermaphrodites completely suppressed male exploration, the exploration behavior of

112 males persisted in the presence of other males (Figure 1C) <sup>20</sup>. Notably, we still observed a  
113 marginal but significant decrease in leaving tendency with male-male interaction  
114 compared to solitary males, suggesting that males still respond to the presence of  
115 conspecific males (Figure 1A and 1C).

116 We further asked whether *C. elegans* males can distinguish different species (Figure  
117 S1D and S1E). *C. elegans* males displayed a strong leaving tendency to four  
118 evolutionarily distant species, *Steinernema carpocapsae*, *Rhabditis sp.*, *Aunema tufa*, and  
119 *Pristionchus pacificus*, but the leaving tendency of males toward sister species  
120 *Caenorhabditis briggsae* and *remanei* was also mildly increased without reaching  
121 statistical significance (Figure S1D and S1E). These data indicate that contact-mediated  
122 mate recognition of *C. elegans* is most prominent among different genera. Interestingly,  
123 we found that discrimination of sister strains was more discernable in *C. remanei*, a male-  
124 female species, suggesting that dioecious species have a stronger selection of different  
125 species (Figure S1F). These results demonstrate that *Caenorhabditis* males recognize  
126 different species through physical contact, and the degree of recognition is associated  
127 with the evolutionary distance (Figure 1A).

128 Lastly, we focused on the recognition of different developmental stages. *C. elegans*  
129 become reproductive adults after four larva stages in the well-fed condition. In  
130 unfavorable conditions, larvae switch off the reproductive cycle and enter the diapause  
131 stage called dauer. We found that the ability of hermaphrodites to retain males increased  
132 dramatically from the L3 larva stage and reached full capacity at the adult stage (Figure  
133 S1G). By contrast, dauer did not retain males (Figure S1G). We also showed that D1, D5,  
134 and D10 hermaphrodites were equally attractive, suggesting that age did not affect male



135 retention (Figure S1H). Thus, males can discriminate developmental stages but not the  
136 ages (Figure 1A). Altogether, we conclude that *C. elegans* hermaphrodites present cues  
137 for males to recognize conspecific and reproductive mates.

138

### 139 **Sexual State and Developmental Stages of Hypodermis Are Critical for** 140 **Hermaphrodites to Retain Males**

141 To gain insight into contact-mediated cues on hermaphrodites, we asked which tissue is  
142 essential for hermaphrodites to retain males. In *C. elegans*, the sex determination  
143 pathway is autonomously controlled by transcriptional programming. Masculinization of  
144 somatic tissues can be achieved by overexpressing the E3 ubiquitin ligase FEM-3, which  
145 degrades the transcription factor TRA-1 essential for hermaphroditic fate<sup>36</sup>. We  
146 expressed FEM-3 in the hermaphrodites driven by tissue-specific promoters and  
147 subjected these masculinized hermaphrodites to male retention assay. Hypodermis-  
148 masculinized hermaphrodites significantly lost male retention, whereas masculinization  
149 of neurons and intestines had no effect (Figure 1B and 1C). Masculinization of  
150 hypodermis also led to the protrusive vulva and ray-like tail in hermaphrodites,  
151 supporting that the sexual identity was altered (Figure S2). Furthermore, hypodermis-  
152 feminized males with hermaphrodite-like tails enhanced male retention compared with  
153 wild-type males (Figure 1B, 1C, and S2). These data indicate the sexual identity of the  
154 hypodermis is critical for mates to retain males.

155 Since the sexual identity of hypodermis is crucial for retaining males, we wondered  
156 whether genes involved in the maturity of hypodermis affect male retention in  
157 hermaphrodites. *lin-4* is a heterochronic gene involved in the maturation of neuronal and

158 hypodermal cells<sup>37</sup>. In the *lin-4* mutant hermaphrodites, the development of hypodermal  
159 cells is retarded and cannot form adult-specific hypodermis. Adult *lin-4* mutant  
160 hermaphrodites significantly lost the ability to retain males with immature hypodermis  
161 (Figure 1D). We also tested precocious mutant *lin-28*, whose adult-specific hypodermis  
162 are abnormally formed at L3 larva stage<sup>38</sup>. In contrast to the *lin-4* mutant, *lin-28* mutant  
163 hermaphrodites retained males precociously at L3 larva stage (Figure 1D). To further  
164 corroborate the role of hypodermis, we tested an evolutionarily conserved transcription  
165 factor *blmp-1* (B lymphocyte-induced maturation protein 1) that controls several aspects  
166 of the development of hypodermal cells<sup>39-41</sup>. Consistently, loss of *blmp-1* in  
167 hermaphrodites did not retain males effectively (Figure 1D). Altogether, these data  
168 suggest that the maturity of hypodermal cells is a determinant factor for mate recognition.

169

### 170 **Epicuticular Components Play Minimal Roles in Retaining Males**

171 To find the effectors in the hypodermis, we analyzed published RNA-seq data of *blmp-1*  
172 mutant hermaphrodites from a previous study<sup>40</sup>. GO analysis of 975 differentially  
173 expressed genes revealed an enrichment of cuticular constituents (Figure 1E; Table S1).  
174 Cuticular constituents are secreted by the hypodermis, and the apical layer is covered by  
175 a surface coat, composed of lipids and glycoproteins (Figure 2A)<sup>42,43</sup>. Several lines of  
176 evidence indicate that the surface coat is not required. First, adult hermaphrodites treated  
177 with organic solvents such as hexane or ethanol remained attractive to males, suggesting  
178 that lipids had a minimal effect (Figure S3A). Consistent with this, mutants of  
179 desaturases *fat-5*, *fat-6*, *fat-7*, or their double mutants did not alter male retention (Figure  
180 S3A). Furthermore, mutations in *srf-2*, *srf-3*, *srf-5*, *bus-1*, *bus-4*, *bus-13*, and *bus-17* have

181 been shown to reduce but do not completely abolish the contact response of males via  
182 regulating the expression of surface epitopes for defending pathogens<sup>25,44-46</sup>. However,  
183 these mutants effectively retained males compared with that in the otherwise wild-type  
184 animals (Figure S3B). Altogether, these data support that the surface coat of cuticles does  
185 not play a crucial role in contact-mediated mate recognition.

186

### 187 **Furrow Collagens Are Essential for Hermaphrodites to Retain Males**

188 The cortical layer of the cuticle is mainly composed of collagens. We hypothesized that  
189 collagens or related proteins are essential to retaining males. Consistent with this  
190 hypothesis, we found that the mutation in *dpy-18*, a crucial enzyme for processing  
191 collagens<sup>47</sup>, became less attractive to males (Figure 2A and 2B). To search for specific  
192 collagens involved in male retention, we focused on the downregulated genes encoding  
193 collagens or surface proteins in the *blmp-1* mutant and selected candidates with gene  
194 expression at L4 and adult stages (Table S1). When deletion mutant strains were  
195 unavailable, we constructed deleted mutants by introducing a STOP-IN cassette using the  
196 CRISPR-Cas9 method as previously described<sup>48</sup>. While the locomotion behaviors and  
197 morphology of these mutants were grossly normal, mutant hermaphrodites exhibited a  
198 marginal decrease in male retention but did not reach statistical significance (Figure  
199 S4A). These observations prompted us to examine other collagen mutants with more  
200 robust phenotypes in body shape<sup>41,49</sup>, including *dpy-2*, *dpy-3*, *dpy-5*, *dpy-7*, *dpy-8*, *dpy-9*,  
201 *dpy-10*, *dpy-13*, *lon-2*, *sqt-1*, *sqt-3*, and *rol-6* mutants. Strikingly, we found that males  
202 were barely retained by hermaphrodites without furrow collagens, including *dpy-2*, *dpy-*  
203 *3*, *dpy-7*, *dpy-8*, *dpy-9*, and *dpy-10* (Figure 2B, S4A, and S4B). Conversely, mutations in

204 the rest of the genes remained attractive to males. It is noteworthy that alterations of body  
205 length in the Dpy and Lon mutants were not associated with the degree of male retention,  
206 suggesting that body length is not a determinant factor for mate recognition (Figure 2B,  
207 S4A, and S4C).

208 Because the *dpy-7* mutant showed the most severe defects, we focused on *dpy-7* and  
209 studied the underlying molecular mechanisms. Feeding RNAi of *dpy-7* resulted in  
210 comparable defects of the *e88* allele, confirming that DPY-7 activity is required for  
211 hermaphrodites to retain males (Figure S4D). Moreover, restoration of *dpy-7* but not *dpy-*  
212 *10* driven from a hypodermis-specific promoter rescued male retention in the *dpy-7*  
213 mutant hermaphrodites, indicating that *dpy-7* functions in the hypodermis and is not  
214 interchangeable quantitatively with other collagens (Figure 2C). Because *dpy-7* is  
215 evolutionarily conserved among nematodes<sup>50,51</sup>, we wondered whether the sequence  
216 variations in *dpy-7* of different species confer the specificity of species recognition. To  
217 test this hypothesis, we cloned *C. briggsae* and *P. pacificus* *dpy-7* orthologs and  
218 expressed them under the control of *C. elegans* hypodermis-specific promoter. The  
219 expression of CBR-*dpy-7* significantly restored the male retention and body length in the  
220 *C. elegans* *dpy-7* mutant, whereas the expression of PPA-*dpy-7* had no discernible effects  
221 on both phenotypes (Figure 2C and S4E). These data indicate that the function of *dpy-7*  
222 for retaining males is conserved in *Caenorhabditis* nematodes. Consistent with it, we  
223 generated a CBR-*dpy-7* mutant by inserting a STOP-IN cassette in the *dpy-7* ortholog of  
224 *C. briggsae* and showed that mutant hermaphrodites were unable to retain males (Figure  
225 S4F).

226 We next investigated whether *dpy-7* is required to present sexual and reproductive  
227 signals on mates. *dpy-7* mutant animals exhibited the same male retention regardless of  
228 their sexual identity. Likewise, the defects of the *dpy-7* mutant were not further enhanced  
229 in juvenile larvae (L3) hermaphrodites (Figure 2D), indicating that *dpy-7* is necessary to  
230 present mate information of sexual identity and developmental stages. Moreover, we  
231 overexpressed *dpy-7* in *C. elegans* males and found that transgenic males became more  
232 attractive, suggesting that the expression level of *dpy-7* is crucial for mates to retain  
233 males (Figure 2E).

234

### 235 **Separable Functions of *dpy-7* for Body Shape and Male Retention**

236 Our previous data indicated that body size is not a determinant factor for retaining males  
237 (Figure S4C), so we speculated that collagen/*dpy-7* has two separate functions in male  
238 retention and body shape. To test this hypothesis, we analyzed two additional alleles of  
239 missense *dpy-7* mutations. Specifically, *sc27* and *e1324* are missense temperature-  
240 sensitive mutations in the collagen domain with severe Dpy and Rol phenotypes that  
241 shorten body length at restrictive temperatures<sup>52</sup>. At the permissive temperature of 15°C,  
242 both mutants retained males comparable to the otherwise wild-type hermaphrodites  
243 (Figure 2F). However, at the restrictive temperature of 25°C, the *sc27* mutant allele was  
244 unable to retain males, whereas the *e1324* mutant allele remained attractive (Figure 2F).  
245 By contrast, body length was drastically shortened in the *e1324* mutant allele but not in  
246 the *sc27* mutant allele (Figure 2G). Thus, the different expressivities of body shape and  
247 male retention in the *sc27* and *e1324* alleles support the notion that collagen/*dpy-7* has  
248 separable functions for male retention and body shape.

249

250 **The Purified Cuticles Are Not Sufficient for Male Retention**

251 How do collagens carry the mate information on cuticles? Because physical contact with  
252 suitable mates is essential for males to restrict locomotion in the male retention assay  
253 <sup>20,21</sup>, we first asked whether these collagens are required for the initial step by evoking the  
254 contact response of males. Adult *C. elegans* cuticles were then dissociated from interior  
255 organs by the SDS-containing buffer (See STAR Methods). Consistent with the prior  
256 studies <sup>44</sup>, we found that purified cuticles from some *Srf/Col/Bus* mutants evoked slightly  
257 lower contact response (Figure S5A), suggesting that purified cuticles preserved features  
258 in live animals. Surprisingly, purified cuticles from wild-type and the *dpy-7* mutant  
259 hermaphrodites evoked robust contact responses of males with reversals and turns  
260 (Figure 3A, 3B, and S5A), indicating that males can sense and respond to purified  
261 cuticles independent of *dpy-7* collagen. Notably, purified cuticles from the wild-type and  
262 the *dpy-7* mutant hermaphrodites evoked transient contact response but never sustained  
263 prolonged scanning essential for contact-mediated mate recognition, consistent with the  
264 observations that purified cuticles alone were unable to retain males regardless of the  
265 species, reproductive status, and sex (Figure 3C). Despite this partial effect, we found  
266 that such response to purified cuticles is species- and stage-specific because males rarely  
267 responded to *P. pacificus* and L3 larval cuticles, supporting the presence of conspecific  
268 cuticular cues in the adult stage for the recognition of species and developmental stages  
269 (Figure 3B).

270 We also asked whether the degree of contact response is associated with sexual  
271 discrimination. Sexual transformations of hypodermis did not significantly affect the

272 transient contact responses of males to purified cuticles (Figure S5A), indicating the  
273 presence of additional cues for sexual discrimination during the prolonged scanning.  
274 These data support the previous speculations that multiple sensory cues are required for  
275 mate recognition <sup>20,21</sup>. Together, we conclude that furrow collagens are likely involved in  
276 the subsequent step after male contact response.

277

### 278 **Furrow Structures Are Not Required for Male Retention**

279 *dpy-2*, *dpy-3*, *dpy-7*, *dpy-8*, *dpy-9* and *dpy-10* are necessary for forming circumferential  
280 furrow on cuticles <sup>29,53,54</sup>. Previous studies have found that the furrow functions as a  
281 signaling hub to control innate immunity and physiology in *C. elegans* <sup>49,55</sup>. We next  
282 asked whether the furrow structure provides a specific surface texture as a sensory cue.  
283 Using a scanning electron microscope (SEM) to visualize the ridges on cuticles, we  
284 confirmed that furrows were not formed in the *dpy-2*, *dpy-7*, and *dpy-10* mutants but  
285 remained intact in the *dpy-5* and *dpy-13* mutants <sup>29,32,49</sup> (Figure 3D). However, furrows  
286 were detectable in the low attractive *dpy-18* mutant, suggesting that the furrow might not  
287 contribute to the attractiveness of mates (Figure 3D-F).

288

### 289 **Collagens Maintain Body Stiffness of Mates for Male Retention**

290 In addition to the changes in surface texture, body stiffness measured by probing the  
291 cuticle with AFM (atomic force microscopy) is significantly reduced in the furrow  
292 mutants but not in other Dpy mutants like *dpy-13* <sup>32</sup>. This observation raised the  
293 possibility that body stiffness is a mechanical cue for contact-mediated mate recognition.  
294 Interestingly, we observed that *dpy-2*, *dpy-7*, and *dpy-10* furrow mutants but not *dpy-5*

295 and *dpy-13* mutants were often ruptured from the vulva, arguably due to the changes of  
296 body stiffness (Figure 4A and S6A). Furthermore, we found that body stiffness was  
297 significantly increased in males measured by AFM, supporting the role of body stiffness  
298 in mate discrimination (Figure 4B). To directly test the role of body stiffness, we poked  
299 wild-type worms with microinjection needles to release the internal pressure and fixed  
300 ruptured worms with paraformaldehyde for male retention assay (Figure 4C and 4D). The  
301 release of internal pressure of wild-type hermaphrodites resulted in low male retention  
302 comparable to the furrow mutants (Figure 2B and 4D). We reasoned that the leaving of  
303 males was not due to aversive substances from the ruptured body because supernatant  
304 from ruptured worms did not affect male retention with intact hermaphrodites (Figure  
305 S6B and S6C). In addition to physical intervention, male attraction was also reduced with  
306 the *gon-2* mutant hermaphrodites (Figure 4C and 4E), in which the body stiffness is  
307 significantly reduced due to the loss of internal pressure<sup>30</sup>. We also manipulated the  
308 stiffness of hermaphrodites by treating animals with SDS-containing lysis buffer. We  
309 found that 30 minutes of fixation by paraformaldehyde could not prevent body rupture by  
310 the lysis buffer leading to softer body stiffness (Figure S6D), and the corpses were unable  
311 to retain males (Figure 4F and 4G). Conversely, hermaphrodites fixed with  
312 paraformaldehyde for 24 hours were resistant to the lysis buffer and preserved male  
313 retention (Figure 4F and 4G). These combined results suggest that body stiffness is  
314 essential for male retention. We conclude that collagens promote mate recognition by  
315 maintaining body stiffness.

316 AFM analysis showed that *P. pacificus* hermaphrodites had a comparable range of  
317 stiffness to *C. elegans* hermaphrodites (Figure 4B), but *P. pacificus* was not able to retain



318 *C. elegans* males (Figure S1E), indicating that body stiffness is not sufficient for male  
319 retention similar to purified cuticles. This is consistent with the notion that males utilize  
320 multiple sensory cues to verify the presence of suitable mates. We thus propose a two-  
321 step mechanism in which the first sensory cue existing on the cuticle evokes the contact  
322 response, and then body stiffness serves as the second sensory cue to sustain or  
323 substantiate the sensation. The combination of two sensory cues ensures the alternation of  
324 male behaviors in male retention assay. To test this hypothesis, we purified cuticles from  
325 the *dpy-7* mutant to exclude the plausible mechanical stimulation by furrow and evoke  
326 the contact response of males (Figure 5A). Because we were only able to prepare 2-3  
327 animals at a time, we compared the pooled data collected from several experiments.  
328 Consistent with our prediction, *P. pacificus* coated with purified *dpy-7* cuticles evoked  
329 contact response and significantly restored male retention (Figure 5B and S5B),  
330 supporting a two-step sensory mechanism gated by cuticular cues and body stiffness for  
331 contact-mediated mate recognition.

332 To further understand whether mate recognition requires a specific range of body  
333 stiffness, we generated bionic worms made of resin through 3D printing that preserves  
334 the furrow structure on the surface, offering a much stiffer body shape (Figure 4B and  
335 5C; see STAR Methods). 3D-printed bionic worms alone did not evoke contact  
336 responses, nor did they attract males in male retention assay (Figure 5D and S5B). In  
337 addition, we found that fake worms coated with purified *dpy-7* cuticles evoked contact  
338 response but had a negligible effect on male retention, favoring that males likely sense a  
339 range of body stiffness for mate recognition (Figure 5D, 5E, and S5B). Together, our  
340 results suggest that cuticular cues evoke contact behaviors as the initial step, and then the

341 body stiffness provides additional information for males to complete contact-mediated  
342 mate recognition.

343

#### 344 **Contact-mediated Mate Recognition Is Associated with Reproductive Success**

345 Our results established a model whereby body stiffness controlled by furrow collagens is  
346 a mechanical property that retains males for contact-mediated mate recognition.

347 However, it is unclear whether male retention contributes to reproductive success. To  
348 examine this issue, we paired 10 males with three wild-type, *dpy-7*, or *dpy-13* mutant  
349 hermaphrodites, and counted the ratio of cross progeny after mating for 24 hours.

350 Reproductive success was significantly reduced with the *dpy-7* mutant hermaphrodite,  
351 compared to wild-type and *dpy-13* mutant hermaphrodites (Figure 6A). Expression of  
352 DPY-7 in hypodermis restored mating success in the *dpy-7* mutant hermaphrodites  
353 (Figure 6A). Male retention by contact-mediated mate recognition is thus important for  
354 reproductive success.

355

#### 356 **DISCUSSION**

357 Mate recognition is crucial to faithfully and efficiently exchange genetic materials within  
358 conspecific mates. In *C. elegans*, soluble ascaroside and as yet unknown volatile  
359 pheromones are secreted by hermaphrodites as long-range mate recognition cues to  
360 attract males<sup>2,6,7,35</sup>. Here, we find that body stiffness is a mechanical property that allows  
361 males to validate the existence of potential mates. In addition to body stiffness, our  
362 results indicate that an unidentified cue(s) on cuticles serve as the first gated mechanism  
363 to evoke contact responses in males. The first gating mechanism offers effective

364 discrimination between species and developmental stages, but not sexual identity. Unlike  
365 almost no contact response to different species and developmental stages, different sexual  
366 identities only slightly affected the transient contact response but had drastically different  
367 abilities to retain males. In addition, *Srf* mutant hermaphrodites evoked slightly lower  
368 contact responses but did not show discernible defects in male retention. These data  
369 suggest that male retention is sufficiently preserved with a certain level of contact  
370 responses, and the differences may not be revealed with the sensitivity of male retention  
371 assay. Once contact response is evoked, body stiffness of mates is universally required as  
372 a second step to sustain or amplify contact signals with the prolonged scanning that leads  
373 to long-term behavioral changes. Our data indicate that males were stiffer than  
374 hermaphrodites, but the contact responses were generally unchanged by sexual  
375 transformation, highlighting the role of body stiffness in sex discrimination. We note that  
376 our data do not completely exclude the role of sex-specific cuticular cues for sex  
377 discrimination with the sensitivity of our contact assay, because the sexual state of  
378 hypodermis is shown to be critical in mate-choice decisions through contact in a different  
379 experimental setting<sup>56</sup>. Based on these observations, we propose that contact-mediated  
380 mate recognition requires the integration of multiple sensations through a two-step  
381 mechanism that accesses cuticular cues and body stiffness, respectively, to construct the  
382 representations of suitable mates in *C. elegans* (Figure 6B), thereby establishing a  
383 paradigm to study the neural computation of decision-making with well-annotated neural  
384 circuits in *C. elegans* males<sup>57</sup>.

385

### 386 **Body Stiffness is A Mechanical Property Used for Mate Recognition**

387 In *Drosophila*, conspecific courtship behaviors require species-specific contact-  
388 dependent pheromones on cuticles for courtship behaviors<sup>8,16,17,58</sup>. Furthermore, males  
389 and females possess different surface pheromones to prevent homosexual courtship  
390 behaviors<sup>17,59</sup>. The specific distribution of surface-coated molecules, such as  
391 glycoproteins, could even convey spatial information that facilitates mating behaviors<sup>60</sup>.  
392 Our study showed that collagens provide a mechanical signal as a short-range sensory  
393 cue to validate the presence of suitable mates. How does body stiffness facilitate mate  
394 recognition? Our data suggest that surface texture is negligible as males could recognize  
395 *P. pacificus* coating cuticles without furrow structure, and the 3D-printed bionic worms  
396 did not retain males even with the furrow structure. In addition, body stiffness is  
397 insufficient for mate recognition because males do not display contact response to 3D-  
398 printed bionic worms and *P. pacificus*. Thus, our data favor the model that body stiffness  
399 provides a second checkpoint to confirm the physical presence of mates. Studying the  
400 precise range of the mechanical property is needed to comprehensively understand the  
401 neural basis of contact-mediated mate recognition, which is a future direction to uncover  
402 the complex sensory mechanisms at cellular and molecular levels.

403 We showed that the furrow collagen had a conserved function for mate recognition  
404 in *Caenorhabditis* species, and three mutant alleles of *dpy-7* differentially affected mate  
405 attractiveness and body length. *dpy-7* encodes 318 amino acids, and *e88*, *sc27*, and *e1324*  
406 alleles are missense mutations on residue 101 (G to R), 156 (G to R), and 189 (G to Y),  
407 respectively<sup>52</sup>. One speculation is that the N-terminal region of *dpy-7* is crucial for body  
408 stiffness, whereas the C-terminal region mainly controls body length. Although our data  
409 suggest that overexpression of *dpy-7* in males enhanced male retention, it is still unknown

410 whether *dpy-7* is sufficient to modulate proper body stiffness. Further studies are needed  
411 to address these unsolved questions.

412

### 413 **Unknown Cuticular Cues for Contact-mediated Mate Recognition**

414 Our study also supports the hypothesis that unknown surface cues on cuticles initiate the  
415 contact response of males. Our genetic analysis of lipid- and glycan-related surface genes  
416 revealed that purified cuticles from some surface mutants evoke less contact response of  
417 males, suggesting that, similar to *Drosophila* and *Rotifer*, lipid- and glycan-derivatives  
418 may have a minor role in the first step of mate recognition in *C. elegans*. In addition to  
419 lipid- and glycan-derivatives, *self-1*, a small peptide secreted from hypodermis, has been  
420 reported for kin recognition in *P. pacificus*<sup>61</sup>. Even though *self-1* has no apparent  
421 homolog in *C. elegans*, other peptides are known to be secreted by the epidermis<sup>27,62</sup>.  
422 Therefore, it is tempting to speculate that peptidergic signaling may be involved in mate  
423 recognition. Although our genetic analysis favors a model that collagens mainly  
424 contribute to body stiffness for mate attractiveness, we do not exclude the possibility that  
425 collagens are also components of cuticular cues for mate recognition, given that the *dpy-*  
426 *18* mutant is distinct from furrow mutants with a lower occurrence of body ruptured.  
427 Further studies are needed to identify the surface cues and clarify these missing gaps for  
428 contact-mediated mate recognition.

429

### 430 **What Is the Possible Sensory Mechanism in *C. elegans* Males?**

431 *C. elegans* males integrate the sensory information from multiple cues associated with  
432 collagens and decide appropriate behaviors to increase the chance of mating. What is the

433 neural mechanism in males? We reason that the role of body stiffness in mate recognition  
434 is to sustain the contact between males and hermaphrodites for decoding mate  
435 information. One possibility is that body stiffness provides specific mechanical signals  
436 (stiffness) sensed by males. In this model, sensory neurons such as ray neurons and post-  
437 cloacal sensilla may be directly activated by body stiffness or indirectly activated by the  
438 stretch with the opening and closing of rays in males<sup>63</sup>. Another possibility is that body  
439 stiffness is required to build the scaffold of the body shape where cuticular cues can be  
440 fully exposed to males for persistent scanning. Although our data with stiffer 3D-printed  
441 bionic worms favor the first model that proper body stiffness is a mechanical cue, further  
442 research is needed to identify the mechanosensory receptors and dissect neural  
443 mechanisms for contact-mediated mate recognition. Our understanding of contact-  
444 dependent cues on hermaphrodites is thus crucial to dissect the neural networks. Because  
445 the neural mechanisms of contact-mediated mate recognition are largely unknown, our  
446 study provides a genetic framework to understand the neural mechanisms and neural  
447 computation of decision-making in social behaviors.

448

#### 449 **Figure Legends**

450 **Figure 1. Sex and developmental stages of hypodermis are crucial for mate**  
451 **attractiveness. See also Figure S1, S2 and Table S1.**

452 (A) Summary of figure S1 and a schematic diagram of male retention assay with the  
453 equation for quantification. (B) Schematic diagram of masculinized hermaphrodites and  
454 feminized males by expressing FEM-3 and TRA-2(IC). (C-D) Percentage of male leavers  
455 in male retention assay with transgenic and mutant hermaphrodites. In the subsequent

456 figures, each dot represents the percentage of leavers in a population containing 15-20  
457 males as one replica, and n indicates the number of total animals tested in all replicas.  
458 Error bar = S.E.M. One-way ANOVA with Bonferroni correction and *p* values are  
459 indicated. (E) Gene Ontology (GO) analysis of differentially expressed genes comparing  
460 the *blmp-1* mutant and the wild-type.

461

462 **Figure 2. Collagens are required and sufficient to promote mate attractiveness. See**  
463 **also Figure S3 and S4.**

464 (A) (Top) A schematic diagram of *C. elegans* cuticles (blue) with its surface coat (green)  
465 and furrow (arrow). Mutant hermaphrodites tested for mate recognition are listed below.

466 (Bottom) The schematic diagram of the biosynthesis pathway of collagens and related  
467 mutants. (B-F) Percentage of male leavers in male retention assay with mutant  
468 hermaphrodites in indicated genotypes. (G) Quantification of body length in the *dpy-7*  
469 temperature-sensitive mutants.

470

471 **Figure 3. The surface properties of cuticles are likely not involved in mate**  
472 **attractiveness. See also Figure S5.**

473 (A) Time series of bright field images of one *C. elegans* male contacting purified cuticles.

474 Time in seconds. Scale bar = 0.25 mm. (B) Quantification of contact response with  
475 purified cuticles from indicated genotypes. The number of males tested with purified  
476 cuticles is indicated. Chi-square test with *p* values indicated. (C) Percentage of male  
477 leavers in male retention assay with purified cuticles from indicated species or genotypes.  
478 (D, E) Scanning electron microscope images of wild-type and indicated genotypes, and

479 yellow arrows indicate the furrows. Scale bar = 5  $\mu$ m. (F) Summary of results of male  
480 retention assay and SEM analysis in indicated genotypes.

481

482 **Figure 4. Collagens maintain body stiffness for mate attractiveness. See also Figure**  
483 **S6.**

484 (A) Bright-field images of wild type and bursting hermaphrodites, including *dpy-2*, *dpy-*  
485 *7*, and *dpy-10*. Scale bar = 1 mm. (B) Young's Modulus of *C. elegans* with indicated  
486 genotypes and treatments. Whisker and box plot with the median for stiffness  
487 measurement. Individual dots indicate the measurement of each trial. Whisker labels the  
488 range from maximum to minimum. (C) Schematic diagram of experimental procedures to  
489 release internal pressure by poking wild-type hermaphrodites or using the *gon-2* mutant.  
490 (D, E) Percentage of male leavers in male retention assay with the mutant or the wild-  
491 type hermaphrodites under indicated treatments. (F) Bright-field images of wild-type  
492 hermaphrodites in different conditions. Scale bar = 1 mm. (G) Percentage of male leavers  
493 in male retention assay with the mutant or the wild-type hermaphrodites under indicated  
494 treatments.

495

496 **Figure 5. A proper range of body stiffness of mates is likely essential to retain males.**

497 (A) Bright-field images of 3D-printed bionic worms and *P. pacificus* (Middle). Scale bar  
498 = 1mm. (B) Percentage of male leavers in male retention assay with the mutant or the  
499 wild-type hermaphrodites under indicated treatments. (C) AFM images of WT  
500 hermaphrodites and 3D-printed bionic worm. The scale bar is as indicated. (D)  
501 Percentage of male leavers in male retention assay with the 3D-printed bionic worm



502 under indicated treatments. Chi-squared test and  $p$  values are indicated. (E) Two models  
503 of how stiffness controls mate attractiveness. The red line indicates a model where body  
504 stiffness falls in a range in different species that confers specific mechanical properties  
505 for mate recognition (model 1). The blue line indicates another model that mates become  
506 attractive once the body stiffness surpasses the threshold (model 2).

507

508 **Figure 6. Loss of mate attractiveness is associated with lower mating efficiency.**

509 (A) Quantification of the ratio of crossed progeny with wild-type and indicated mutant  
510 hermaphrodites. (B) A proposed model for contact-mediated mate recognition in *C.*  
511 *elegans*. Surface-associated cues on cuticles evoke the contact response of *C. elegans*  
512 males as the first checkpoint. Mechanical signals conveyed by body stiffness then serve  
513 as the second signal for contact-mediated mate recognition.

514

515 **ACKNOWLEDGMENTS**

516 We thank members of the Chen and the Sternberg laboratories for insightful  
517 discussions. We also thank Ray Hong, Chun-Liang Pan, Yi-Chun Wu, and Shih-Peng  
518 Chan for sharing reagents and strains. We thank WormBase for providing organized  
519 genetic and genomic information. Some strains were provided by the *C. elegans*  
520 Genetics Center, funded by the NIH Office of Research Infrastructure Programs (P40  
521 OD010440). We are grateful to the technical assistance of Technology Commons,  
522 College of Life Science, National Taiwan University with scanning electron  
523 microscopy (SEM) and confocal microscopy (CLSM). We thank the *C. elegans* Core  
524 Facility of the National Core Facility for Biopharmaceuticals, Ministry of Science of

525 Technology in Taiwan. This study was funded by the Ministry of Science and  
526 Technology, Taiwan, to C.-H.C. (MOST 109-2636-B-002-016; MOST 110-2636-B-002-  
527 013, NSTC 111-2636-B-002-027) and N. I. H. to P.W.S. (R01NS113119 and  
528 R240D023041). The study is also funded by the French National Research Agency  
529 ANR-22-CE13-0037-01, by the “Investissements d’Avenir” French Government  
530 program (ANR-16-CONV-0001), and from Excellence Initiative of Aix-Marseille  
531 University -A\*MIDEX and institutional grants from CNRS, Aix Marseille University,  
532 National Institute of Health and Medical Research (Inserm) to the CIML. The  
533 NanoWizard III (JPK) was funded by the Ministry of Science, Research and the Arts of  
534 Baden-Württemberg (Az: 33-7532.20) and the University of Freiburg ('Strategiefonds'),  
535 granted to Winfried Römer.

536

537

### 538 **AUTHOR CONTRIBUTIONS**

539 Conceptualization, C.-H.C., J.-W. W., and P.W.S.; Methodology, J.-W. W, H. P., R.-T. C., C.  
540 V., N. P., C.-H.C.; Investigation, J.-W. W, H. P., R.-T. C., C. V., C. L. E., N. P., C.-H.C.;  
541 Writing-Original draft, C.-H.C., J.-W.W., and P.W.S.; Writing-Review & Editing, C.-H.C.,  
542 J.-W.W., and P.W.S.; Supervision, C.-H.C. and P.W.S.; Funding acquisition, C.-H.C. and  
543 P.W.S.

544

### 545 **DECLARATION OF INTERESTS**

546 The authors declare no conflict of interest.

547

548 **INCLUSION AND DIVERSITY**

549 We support inclusive, diverse, and equitable conduct of research.

550

551 **STAR METHODS**

552 **RESOURCE AVAILABILITY**

553 **Lead contact**

554 Further information and requests for resources and reagents should be directed to

555 and will be fulfilled by the lead contact, Chun-Hao Chen ([chunhaochen@ntu.edu.tw](mailto:chunhaochen@ntu.edu.tw))

556

557 **Material availability**

558 *C. elegans* strains and DNA constructs generated in this study are available from the

559 Lead Contact upon request.

560

561 **Data and Code Availability**

562 All the primary data used in this study have been deposited at Figshare and are

563 publicly available as of the date of publication. The DOI is listed in the key resources

564 table. Microscopy data reported in this study will be shared by the lead contact upon

565 request.

566 This study does not use original code for data analysis.

567 Any additional information required to reanalyze the data reported in this paper is

568 available from the lead contact upon request.

569

570 **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

571 **Animals**

572 *C. elegans* strains were cultured and maintained as previously described<sup>64</sup>. In brief,  
573 animals were cultivated on nematode growth medium (NGM) plates seeded with *E. coli*  
574 OP50 bacteria at 20°C, except for the temperature-sensitive strains. A list of mutant  
575 alleles and transgenic strains used in this study is available in the key resources table.

576

577 **Microbe strains**

578 The OP50 *E. coli* strain was used as the food for the worms.

579

580 **METHOD DETAILS**

581 **Germline Transformation**

582 Germline transformation was performed by microinjecting plasmids in gonads as  
583 described<sup>65</sup>. In brief, gonads of D1 adult worms were visualized by an inverted  
584 microscope (Zeiss, Axio Observer) at 40X objective, and the DNA mixture with co-  
585 injection markers was injected through a microinjector (Eppendorf FemtoJet 4X).

586

587 **Molecular Biology**

588 Molecular cloning was mostly conducted by recombination-based cloning method with  
589 GenBuilder DNA Assembly kit (GenScript, Catalog number: L00744). cDNA of *fem-3*  
590 and *tra-2(IC)* was amplified from the cDNA library prepared by SuperScript III First-  
591 Strand Synthesis System (ThermoFisher, Catalog number: 18080051) and cloned into  
592 pPD95.75 based plasmid containing either hypodermis (*dpy-7*-), pan-neuronal (*rab-3*-),  
593 or intestine (*elt-3*)-specific promoter and SL2::mCherry sequence. cDNA for *dpy-7*,

594 CBR-*dpy-7*, and PPA-*dpy-7* prepared as mentioned above were cloned into the plasmid  
595 with a hypodermis-specific promoter for the rescue experiments. For feeding RNAi  
596 experiments, ~0.8 kb of *dpy-7* genomic DNA was cloned using primers described below:  
597 *dpy-7-F* (5'-CCACGTGGCAAAAGCCACCG-3'), *dpy-7-R* (5'-  
598 CCACCACGTGCTGGCTTCTC-3'. Sequences were then cloned into L4440 and  
599 transformed with *E. coli* HT115.

600

### 601 **Male Retention Assay**

602 The male retention assay was conducted as previously described<sup>21</sup>. The assay was  
603 performed in a 9 cm plate filled with 10 ml nematode growth medium (NGM) agar, and  
604 each plate had 18  $\mu$ l OP50 bacteria (OD<sub>600</sub>=0.4-0.6) spotted (~1 cm<sup>2</sup>) on the center  
605 overnight. Before the assay, mates with indicated sex, developmental stages, or species  
606 were fixed by 8% paraformaldehyde for 30 min at room temperature unless noted  
607 specifically. Fixed worms were washed twice with M9 buffer before being placed on the  
608 bacteria patch. Next, one male was placed with two mates on the food patch of each assay  
609 plate, and a population of 15-20 virgin D1 males was assayed for one experimental set. A  
610 male is considered a leaver when the moving track reaches 0.5 cm away from the plate  
611 edge. Plates were kept at 20°C, and the proportion of males was scored at 24 hours.

612 To release the internal pressure of worms, animals were poked by a microinjection  
613 needle 3-5 times until the worm body was ruptured. Then, ruptured worms were  
614 immediately fixed with 8% paraformaldehyde for 30 minutes. Cuticles were coated by  
615 gently placing purified cuticles on fixed *P. pacificus* or 3D-printed bionic worms.

616

617 **Quantification of the percentage of leaving males in male retention assay**

618 We define the leaver as the locomotion track reaching 0.5 cm away from the plate edge in  
619 male retention assay at 24 hours<sup>33</sup>. The percentage of leaving is calculated by dividing  
620 the number of leavers by the number of total tested animals, as shown below.

621 
$$\% \text{ of leavers} = (\# \text{ of leavers} / \# \text{ of total animals}) \times 100$$

622 The value of the percentage of leaving males is pooled across replicas to estimate the  
623 standard error of mean (S.E.M.).

624

625 **Gene Ontology (GO) and Enrichment Analysis**

626 The N2 and *blmp-1* transcriptome comparison was performed using the published dataset  
627 from Gene Expression Omnibus (Access number: GSE179411)<sup>40</sup>. In brief, differential  
628 expression between wild-type and *blmp-1* mutant strains was determined with DESeq2  
629 (v1.22.0; padj<0.05 and FDR>2)<sup>66</sup>. 883 upregulated and 653 downregulated genes were  
630 subjected to gene ontology (GO) and enrichment analysis by Gene Enrichment Analysis  
631 in WormBase with *q* value threshold of 0.1 (Table S1)<sup>67,68</sup>. 975 genes were included in  
632 the GO analysis.

633

634 **RNA Interference**

635 Feeding RNAi experiments were conducted as described with the *E. coli* HT115 bacterial  
636 strain<sup>69</sup>. In brief, liquid RNAi bacterial cultures were diluted 1:100 and incubated at 37  
637 °C for 3-4 hours until the OD<sub>600</sub> reached 0.4-0.6, followed by RNAi production with 1  
638 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) for 1 hour at room temperature. 100  
639 μl bacteria culture was seeded on nematode growth medium (NGM) plates with 1 mM

640 IPTG and ampicillin. Synchronized L1 (P0) larvae were reared on RNAi plates, and adult  
641 F1 animals were transferred to a new RNAi plate. Quantification was done with F2  
642 animals in all feeding RNAi experiments.

643

#### 644 **CRISPR/Cas9 Mutagenesis**

645 CRISPR-Cas9 stop-in method is performed as described with modifications<sup>48</sup>. In brief,  
646 all crRNA and the universal tracrRNA were synthesized from IDT, Coralville, IA. The  
647 guide RNA sequences for all deletion mutants generated by the CRISPR stop-in method  
648 are listed in Table S2. crRNA and tracrRNA were dissolved in RNAase-free water to  
649 make 100  $\mu$ M working stocks. Purified Cas9 protein (IDT, Catalog number: 1081060)  
650 was used with the solution buffer (20 mM HEPES, pH 7.5, 150 mM KCl, 10% Glycerol,  
651 and 1 mM DTT). The CRISPR-Cas9 complex was made in sequential steps. First, 5  $\mu$ l of  
652 tracrRNA (0.4  $\mu$ g/ $\mu$ l) and 2.8  $\mu$ l of crRNA (0.4  $\mu$ g/ $\mu$ l) were mixed with 0.5  $\mu$ l of the  
653 Cas9 protein (10  $\mu$ g/ $\mu$ l) and then were incubated at 37 °C for 10 minutes followed by  
654 cooling down at room temperature. 2.2  $\mu$ l of single-strand DNA donor (1  $\mu$ g/ $\mu$ l) and  
655 pRF4(*rol-6(su1006)*) and RNase-free water were then added to the mixture to reach a  
656 final volume of 20  $\mu$ l. The CRISPR-Cas9 complex was used for microinjection with N2  
657 (*C. elegans*) or AF16 (*C. briggsae*) strain.

658

#### 659 **Scanning Electron Microscopy (SEM) of *C. elegans***

660 SEM was conducted as previously described<sup>70</sup>. Roughly a hundred synchronized  
661 animals in the adult stage were collected in a 1.5 mL Eppendorf and pre-fixed in  
662 0.1M phosphate buffer with 2.5% glutaraldehyde overnight. After fixation, animals

663 were washed with 0.1 M phosphate buffer three times and subsequently incubated  
664 with 1% osmium tetroxide in 0.12M phosphate buffer for 30 minutes at 4 °C. After  
665 washing animals with 0.1M phosphate buffer three times, samples were dehydrated  
666 through a sequential gradient of ethanol from 30%-100% and were completely  
667 dehydrated by 100% acetone. Then, animals were dried by critical point  
668 dehydration (CPD) by a critical point dryer (Hitachi HCP-2), mounted on stubs, and  
669 coated with gold using an ion coater (Eiko Engineering IB-2). Cuticle surface  
670 structures from at least 10 animals in indicated genotypes were observed by  
671 scanning electron microscope (FEI Inspect S, 15kV).

672

### 673 **Microscopy and Quantification of Images**

674 Images were taken by ZEN Blue 3.3 software using 10X objective in a Zeiss Imager  
675 Z2 microscope with an Axiocam 506 Mono camera. For imaging, worms were  
676 anesthetized in 1% sodium azide and mounted on 2 % agarose pads. In order to  
677 measure the body length, worms were anesthetized as mentioned above, and the  
678 DIC images under 10X objective were taken and analyzed by ZEN Blue 3.3 software.  
679 Bright-field images of ruptured worms, cuticles, and 3D-printed bionic worms were  
680 taken with a C-mount camera on a dissecting microscope.

681

### 682 **Purification of Nematode Cuticles**

683 The purification method is modified from the previous study to purify intact cuticles <sup>71</sup>.  
684 Synchronized animals at preferred stages were collected in a 1.5 ml Eppendorf tube and  
685 washed three times in M9 by centrifugation at 1000 rpm. We discarded the supernatant



686 and resuspended worm pellet with M9 buffer to reach the density of 50-100 worms/ $\mu$ l. 1  
687  $\mu$ l of worm was dropped on a glass slide and 50  $\mu$ l of SDS-DTT solution (0.25% SDS,  
688 200 mM DTT, 20mM HEPES, pH 8.0, 3% sucrose, stored at -20°C) was added for 30  
689 minutes at room temperature until tissues were transparent. A microinjection needle was  
690 used to transfer purified cuticles on an NGM plate, and purified cuticles were washed  
691 three times with M9 buffer. Two intact cuticles with indicated genotypes or species were  
692 used for male retention assay and 7-10 purified cuticles were used for contact response  
693 assay.

694

#### 695 **Quantification of Male Contact Response to Purified Cuticles**

696 All the experiments were done with day 1 (D1) adult virgin males. Purified cuticles with  
697 indicated genotypes, sex, or developmental stages were placed on NGM plates seeded  
698 with *E. coli* OP50. A single male was placed on the cuticle-containing NGM plate (7-10  
699 purified cuticles), and the behavioral response of first contact with purified cuticles was  
700 scored. Contact response was defined as backward movements for at least 0.5 body  
701 length with curling tail postures of males when moving across a purified cuticle. Each  
702 male was scored once and removed from the plate after scoring. Over 30 males were  
703 tested in each condition.

704

#### 705 **Fabrication of 3D-printed Bionic Worms**

706 3D-printed worms were fabricated by a projecting microstereolithography-based 3D  
707 printing technique with a customized acrylic photosensitive resin, HTL (BMF Nano  
708 Materials Technology Co., Ltd), with a tensile strength of around 71500 kPa. A 3D

709 model of bionic worms including furrows was generated by AutoCAD (AutoDesk, Inc)  
710 and was imported into the printer for 3D printing. 3D printing was conducted by curing  
711 the resin layer-by-layer exposed to 405 nm UV light (30 mW / cm<sup>2</sup>), and the resolution  
712 was 2-10 μm. The printed products were cleaned, vacuum-dried, and polished before use  
713 in experiments.

714

### 715 **Quantification of Mating Efficiency**

716 10 D1 adult males and 3 D1 hermaphrodites were placed on a NGM plate with a small  
717 food patch (~7 μL OP50 bacteria) on the center for 24 hours. Then, males and  
718 hermaphrodites were removed, and the fertilized eggs cultured for 3 days until the  
719 animals reached the L4 larval stage. The number of total animals and males (cross  
720 progeny) were counted, and the mating efficiency calculated as (# of males/ # of total  
721 animals).

722

### 723 **Atomic Force Microscopy**

724 AFM was conducted as previously described<sup>32,54</sup>. D1 young adult worms were fixed in  
725 4% paraformaldehyde (PFA) for 30 min and then rinsed several times in 50 mM NaCl or  
726 M9 buffer. The worms were then transferred as described before to a ~2 mm thick 4 %  
727 agarose bed in a Petri dish (30 mm). Heads and tails were fixed with the tissue glue  
728 (Dermabond, Ethicon), and the dish was filled with 50 mM NaCl or M9 buffer. AFM  
729 data of worms were obtained using a NanoWizard IV or III (JPK, Bruker, Santa Barbara,  
730 CA, USA) coupled with an inverted optical microscope Ti-Eclipse (Nikon, Japan).  
731 NSC12 tipless cantilevers (7.5 N/m; MikroMash) with a 10 μm diameter borosilicate

732 bead attached (produced by sQUBE www.sQUBE.de) were used. The cantilever  
 733 sensitivity and stiffness were calibrated without contact based on the thermal spectrum in  
 734 liquid. The tip was positioned above the worm, using the optical image to ensure to  
 735 measure glue-free area below the pharynx and/or in the tail. Force curves were generated  
 736 in force spectroscopy mode, applying a setpoint of 450 nN while the cantilever speed was  
 737 set at 0.5 or 1.0  $\mu\text{m/s}$ , reaching an indentation of approximately 0.5 to 2  $\mu\text{m}$  with at least  
 738 5 curves recorded in a few microns separated areas. AFM data were analyzed using the  
 739 JPK analysis software. All force curves were processed to set the baseline to determine  
 740 the tip-sample contact point and to subtract cantilever bending. Young's Modulus was  
 741 calculated within the software by fitting the Hertz/Sneddon model.

742

743 **QUANTIFICATION AND STATISTICAL ANALYSIS**

744 One-way ANOVA and two-way ANOVA with Bonferroni correction and Chi-squared  
 745 test are conducted by Prism (Version 9.3.1) as indicated in Figure Legends. Error bars in  
 746 bar graphs represent the standard error of means (S.E.M.).

747

748 **KEY RESOURCE TABLE**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial Strains		
<i>E. coli</i> : Strain OP50	Caenorhabditis Genetics Center (CGC)	WormBase: OP50
Experimental Models: <i>C. elegans</i> Strains		
<i>Caenorhabditis elegans</i> : Strain N2: wild isolate	CGC	WormBase: N2
<i>Caenorhabditis remanei</i> : Strain PB4641: wild isolate	CGC	WormBase: <i>C. remanei</i>
<i>Caenorhabditis briggsae</i> : Strain AF16: wild isolate	CGC	WormBase: <i>C. briggsae</i>

<i>Auanema. sp.</i> : Strain PS8402: wild isolate	Paul Sternberg Lab	WormBase: <i>Auanema. sp.</i>
<i>Rhabditis. n. sp.</i> : Strain PS1130: wild isolate	Paul Sternberg Lab	WormBase: <i>Rhabditis. n. sp.</i>
<i>Pristionchus pacificus</i> : Strain PS312: wild isolate	Ray Hong Lab	WormBase: <i>P. pacificus</i>
<i>Steinernema carpocapsae</i> : wild isolate	Paul Sternberg Lab	N/A
<i>C. elegans</i> : Strain MT4009: <i>lin-39(n1760)/ dpy-17(e164) unc-32(e189)</i>	CGC	WormBase: <i>lin-39</i>
<i>C. elegans</i> : Strain DR476: <i>daf-22(m130)</i>	Chun-Liang Pan Lab	WormBase: <i>daf-22</i>
<i>C. elegans</i> : Strain PS867: <i>unc-32(e189) glp-1(q231)</i>	Paul Sternberg Lab	WormBase: <i>glp-1</i>
<i>C. elegans</i> : Strain CB5414: <i>srd-1(eh1)</i>	CGC	WormBase: <i>srd-1</i>
<i>C. elegans</i> : Strain BC119: <i>blmp-1(s71)</i>	CGC	WormBase: <i>blmp-1</i>
<i>C. elegans</i> : Strain CT8: <i>lin-4 (ma104)</i>	Chih-Peng Chan Lab	WormBase: <i>lin-4</i>
<i>C. elegans</i> : Strain MT1524: <i>lin-28 (n719)</i>	Chih-Peng Chan Lab	WormBase: <i>lin-14</i>
<i>C. elegans</i> : Strain VC1463: <i>gon-2(ok465) I/hT2 [bli-4(e937) let-?(q782) qIs48] (I;III)</i>	CGC	WormBase: <i>gon-2</i>
<i>C. elegans</i> : Strain AT6: <i>srf-2(yj262)</i>	CGC	WormBase: <i>srf-2</i>
<i>C. elegans</i> : Strain CB6627: <i>srf-3(e2689)</i>	CGC	WormBase: <i>srf-3</i>
<i>C. elegans</i> : Strain CL261: <i>him-5(e1490); srf-5(ct115)</i>	CGC	WormBase: <i>srf-5</i>
<i>C. elegans</i> : Strain CB5609: <i>bus-1(e2678)</i>	CGC	WormBase: <i>bus-1</i>
<i>C. elegans</i> : Strain CB5443: <i>bus-4(e2693)</i>	CGC	WormBase: <i>bus-4</i>
<i>C. elegans</i> : Strain CB5635: <i>bus-13(e2710)</i>	CGC	WormBase: <i>bus-13</i>
<i>C. elegans</i> : Strain CB6081: <i>bus-17(e2800)</i>	CGC	WormBase: <i>bus-17</i>
<i>C. elegans</i> : Strain CB3584: <i>dpy-5(e61); him-5(e1490)</i>	Paul Sternberg Lab	WormBase: <i>dpy-5</i>
<i>C. elegans</i> : Strain BE93: <i>dpy-2 (e8)</i>	CGC	WormBase: <i>dpy-2</i>

<i>C. elegans</i> : Strain CB88: <i>dpy-7(e88)</i>	CGC	WormBase: <i>dpy-7</i>
<i>C. elegans</i> : Strain BE27: <i>dpy-7(sc27)</i>	CGC	WormBase: <i>dpy-7</i>
<i>C. elegans</i> : Strain CB1324: <i>dpy-7(e1324)</i>	CGC	WormBase: <i>dpy-7</i>
<i>C. elegans</i> : Strain CB128: <i>dpy-10(e128)</i>	CGC	WormBase: <i>dpy-10</i>
<i>C. elegans</i> : Strain PS424: <i>dpy-13(e184)</i>	Paul Sternberg Lab	WormBase: <i>dpy-13</i>
<i>C. elegans</i> : Strain IG1685: <i>frIs7(Pnlp-29::GFP, Pcol-19::DsRed); dpy-3(e27)</i>	Nathalie Pujol Lab	WormBase: <i>dpy-3</i>
<i>C. elegans</i> : Strain IG1699: <i>frIs7(Pnlp-29::GFP, Pcol-19::DsRed); dpy-8(e130)</i>	Nathalie Pujol Lab	WormBase: <i>dpy-8</i>
<i>C. elegans</i> : Strain IG466: <i>frIs7(Pnlp-29::GFP, Pcol-19::DsRed) dpy-9(e12)</i>	Nathalie Pujol Lab	WormBase: <i>dpy-9</i>
<i>C. elegans</i> : Strain NTU27: <i>dpy-18(e364)</i>	CGC	WormBase: <i>dpy-18</i>
<i>C. elegans</i> : Strain CB1350: <i>sqt-1(e1350)</i>	CGC	WormBase: <i>sqt-1</i>
<i>C. elegans</i> : Strain BE8: <i>sqt-3(sc8)</i>	CGC	WormBase: <i>sqt-3</i>
<i>C. elegans</i> : Strain HE1006: <i>rol-6(su1006)</i>	CGC	WormBase: <i>rol-6</i>
<i>C. elegans</i> : Strain CB769: <i>bli-1(e769)</i>	CGC	WormBase: <i>bli-1</i>
<i>C. elegans</i> : Strain CB768: <i>bli-2(e768)</i>	CGC	WormBase: <i>bli-2</i>
<i>C. elegans</i> : Strain CB678: <i>lon-2(e678)</i>	CGC	WormBase: <i>lon-2</i>
<i>C. elegans</i> : Strain PS8825: <i>clec-47(sy1532)</i>	In this paper	WormBase: <i>clec-47</i>
<i>C. elegans</i> : Strain PS8544: <i>clec-87(sy1396)</i>	In this paper	WormBase: <i>clec-87</i>
<i>C. elegans</i> : Strain PS8578: <i>clec-88(sy1409)</i>	In this paper	WormBase: <i>clec-88</i>
<i>C. elegans</i> : Strain PS8584: <i>clec-91(sy1415)</i>	In this paper	WormBase: <i>clec-91</i>
<i>C. elegans</i> : Strain PS8787: <i>col-81(sy1520)</i>	In this paper	WormBase: <i>col-81</i>
<i>C. elegans</i> : Strain PS8780: <i>col-88(sy1513)</i>	In this paper	WormBase: <i>col-88</i>

<i>C. elegans</i> : Strain PS8819: <i>col-120(sy1526)</i>	In this paper	WormBase: <i>col-120</i>
<i>C. elegans</i> : Strain PS8821: <i>col-129(sy1528)</i>	In this paper	WormBase: <i>col-129</i>
<i>C. elegans</i> : Strain PS8634: <i>col-135(sy1435)</i>	In this paper	WormBase: <i>col-135</i>
<i>C. elegans</i> : Strain PS8822: <i>col-137(sy1529)</i>	In this paper	WormBase: <i>col-137</i>
<i>C. elegans</i> : Strain PS8827: <i>col-139(sy1534)</i>	In this paper	WormBase: <i>col-139</i>
<i>C. elegans</i> : Strain PS8829: <i>col-156(sy1536)</i>	In this paper	WormBase: <i>col-156</i>
<i>C. briggsae</i> : Strain PS8520: <i>dpy-10(sy1387)</i>	In this paper	N/A
<i>C. briggsae</i> : Strain: NTU6: <i>dpy-7(chc1)</i>	In this paper	N/A
<i>C. elegans</i> : Strain NTU95: <i>chcEx045 Pelt-2::FEM-3::SL2::mCherry</i>	In this paper	N/A
<i>C. elegans</i> : Strain NTU125: <i>Pdpy-7::FEM-3::SL2::mCherry</i>	In this paper	N/A
<i>C. elegans</i> : Strain NTU94: <i>chcEx044(Pdpy-7::TRA-2(IC)::SL2::mCherry)</i>	In this paper	N/A
<i>C. elegans</i> : Strain NTU183: <i>dpy-7(e88); chcEx99(Pdpy-7::Ppa-dpy-7)</i>	In this paper	N/A
<i>C. elegans</i> : Strain NTU177: <i>dpy-7(e88); chcEx96(Pdpy-7::dpy-10)</i>	In this paper	N/A
<i>C. elegans</i> : Strain NTU3: <i>dpy-7(e88); Pdpy-7::chcEx1(Pdpy-7::cbr-dpy-7)</i>	In this paper	N/A
<i>C. elegans</i> : Strain NTU7: <i>chcEx2(Pdpy-7::dpy-7, Pblmp-1::GFP)</i>	In this paper	N/A
<i>C. elegans</i> : Strain NTU5: <i>dpy-7(e88); chcEx2(Pdpy-7::dpy-7, Pblmp-1::GFP)</i>	In this paper	N/A
<i>C. elegans</i> : Strain NTU90: <i>chcEx043(Prab-3::fem-3::SL2::mcherry)</i>	In this paper	N/A

<i>C. elegans</i> : Strain NTU89: <i>chcEx042 (Prab-3::tra-2(IC)::SL2::mcherry)</i>	In this paper	N/A
<i>C. elegans</i> : Strain BX156: <i>fat-6(tm331) ; fat-7(wa36)</i>	CGC	WormBase: <i>fat-6; fat-7</i>
<i>C. elegans</i> : Strain BX160: <i>fat-7(wa36) fat-5(tm420)</i>	CGC	WormBase: <i>fat-7; fat-5</i>
<i>C. elegans</i> : Strain BX52: <i>fat-4(wa14) fat-1(wa9)</i>	CGC	WormBase: <i>fat-4; fat-1</i>
<i>C. elegans</i> : Strain BX107: <i>fat-5(tm420)</i>	CGC	WormBase: <i>fat-5</i>
<i>C. elegans</i> : Strain BX153: <i>fat-7(wa36)</i>	CGC	WormBase: <i>fat-7</i>
Chemicals		
Hexane	Merck	Catalog number: 139386
Ethanol	Merck	Catalog number: 51976
Paraformaldehyde	Agar Scientific	Catalog number: AGR1026
Software and algorithms		
Primary datasets	Figshare	DOI:10.6084/m9.figshare.23641011
Others		
3D-printed worms	BMF Nano Materials Technology Co., Ltd	N/A

749

## 750 REFERENCES

- 751 1. Asaba, A., Hattori, T., Mogi, K., and Kikusui, T. (2014). Sexual attractiveness of  
752 male chemicals and vocalizations in mice. *Front Neurosci* 8, 231.  
753 10.3389/fnins.2014.00231.
- 754 2. Leighton, D.H., and Sternberg, P.W. (2016). Mating pheromones of Nematoda:  
755 olfactory signaling with physiological consequences. *Curr Opin Neurobiol* 38,  
756 119-124. 10.1016/j.conb.2016.04.008.
- 757 3. Yamamoto, D., and Koganezawa, M. (2013). Genes and circuits of courtship  
758 behaviour in *Drosophila* males. *Nature reviews. Neuroscience* 14, 681-692.  
759 10.1038/nrn3567.
- 760 4. Ellingsen, D.M., Leknes, S., Loseth, G., Wessberg, J., and Olausson, H. (2015).  
761 The Neurobiology Shaping Affective Touch: Expectation, Motivation, and  
762 Meaning in the Multisensory Context. *Front Psychol* 6, 1986.  
763 10.3389/fpsyg.2015.01986.
- 764 5. Ferrero, D.M., Moeller, L.M., Osakada, T., Horio, N., Li, Q., Roy, D.S., Cichy, A.,  
765 Spehr, M., Touhara, K., and Liberles, S.D. (2013). A juvenile mouse pheromone  
766 inhibits sexual behaviour through the vomeronasal system. *Nature* 502, 368-  
767 371. 10.1038/nature12579.
- 768 6. Srinivasan, J., Kaplan, F., Ajredini, R., Zachariah, C., Alborn, H.T., Teal, P.E.,

- 769 Malik, R.U., Edison, A.S., Sternberg, P.W., and Schroeder, F.C. (2008). A blend of  
770 small molecules regulates both mating and development in *Caenorhabditis*  
771 *elegans*. *Nature* 454, 1115-1118. 10.1038/nature07168.
- 772 7. Chasnov, J.R., So, W.K., Chan, C.M., and Chow, K.L. (2007). The species, sex, and  
773 stage specificity of a *Caenorhabditis* sex pheromone. *Proceedings of the*  
774 *National Academy of Sciences of the United States of America* 104, 6730-  
775 6735. 10.1073/pnas.0608050104.
- 776 8. Billeter, J.C., Atallah, J., Krupp, J.J., Millar, J.G., and Levine, J.D. (2009).  
777 Specialized cells tag sexual and species identity in *Drosophila melanogaster*.  
778 *Nature* 461, 987-991. 10.1038/nature08495.
- 779 9. Hsueh, Y.P., Gronquist, M.R., Schwarz, E.M., Nath, R.D., Lee, C.H., Gharib, S.,  
780 Schroeder, F.C., and Sternberg, P.W. (2017). Nematophagous fungus  
781 *Arthrobotrys oligospora* mimics olfactory cues of sex and food to lure its  
782 nematode prey. *eLife* 6. 10.7554/eLife.20023.
- 783 10. Yang, Y., Richards-Zawacki, C.L., Devar, A., and Dugas, M.B. (2016). Poison frog  
784 color morphs express assortative mate preferences in allopatry but not  
785 sympatry. *Evolution* 70, 2778-2788. 10.1111/evo.13079.
- 786 11. Baker, C.A., Clemens, J., and Murthy, M. (2019). Acoustic Pattern Recognition  
787 and Courtship Songs: Insights from Insects. *Annual review of neuroscience*  
788 42, 129-147. 10.1146/annurev-neuro-080317-061839.
- 789 12. Conte, G.L., and Schluter, D. (2013). Experimental confirmation that body size  
790 determines mate preference via phenotype matching in a stickleback species  
791 pair. *Evolution* 67, 1477-1484. 10.1111/evo.12041.
- 792 13. Connolly, K., Burnet, B., and Sewell, D. (1969). Selective Mating and Eye  
793 Pigmentation: An Analysis of the Visual Component in the Courtship  
794 Behavior of *Drosophila Melanogaster*. *Evolution* 23, 548-559.  
795 10.1111/j.1558-5646.1969.tb03540.x.
- 796 14. Kopp, A., Duncan, I., Godt, D., and Carroll, S.B. (2000). Genetic control and  
797 evolution of sexually dimorphic characters in *Drosophila*. *Nature* 408, 553-  
798 559. 10.1038/35046017.
- 799 15. Sato, K., and Yamamoto, D. (2020). Contact-Chemosensory Evolution  
800 Underlying Reproductive Isolation in *Drosophila* Species. *Front Behav*  
801 *Neurosci* 14, 597428. 10.3389/fnbeh.2020.597428.
- 802 16. Kohatsu, S., Koganezawa, M., and Yamamoto, D. (2011). Female contact  
803 activates male-specific interneurons that trigger stereotypic courtship  
804 behavior in *Drosophila*. *Neuron* 69, 498-508. 10.1016/j.neuron.2010.12.017.
- 805 17. Thistle, R., Cameron, P., Ghorayshi, A., Dennison, L., and Scott, K. (2012).  
806 Contact chemoreceptors mediate male-male repulsion and male-female  
807 attraction during *Drosophila* courtship. *Cell* 149, 1140-1151.  
808 10.1016/j.cell.2012.03.045.
- 809 18. Simon, J.M., and Sternberg, P.W. (2002). Evidence of a mate-finding cue in the  
810 hermaphrodite nematode *Caenorhabditis elegans*. *Proceedings of the*  
811 *National Academy of Sciences of the United States of America* 99, 1598-1603.  
812 10.1073/pnas.032225799.
- 813 19. White, J.Q., Nicholas, T.J., Gritton, J., Truong, L., Davidson, E.R., and Jorgensen,  
814 E.M. (2007). The sensory circuitry for sexual attraction in *C. elegans* males.



- 815 Curr Biol 17, 1847-1857. 10.1016/j.cub.2007.09.011.
- 816 20. Lipton, J., Kleemann, G., Ghosh, R., Lints, R., and Emmons, S.W. (2004). Mate  
817 searching in *Caenorhabditis elegans*: a genetic model for sex drive in a simple  
818 invertebrate. *J Neurosci* 24, 7427-7434. 10.1523/JNEUROSCI.1746-04.2004.
- 819 21. Barrios, A., Nurrish, S., and Emmons, S.W. (2008). Sensory regulation of *C.*  
820 *elegans* male mate-searching behavior. *Current biology : CB* 18, 1865-1871.  
821 10.1016/j.cub.2008.10.050.
- 822 22. Sakai, N., Iwata, R., Yokoi, S., Butcher, R.A., Clardy, J., Tomioka, M., and Iino, Y.  
823 (2013). A sexually conditioned switch of chemosensory behavior in *C.*  
824 *elegans*. *PLoS One* 8, e68676. 10.1371/journal.pone.0068676.
- 825 23. Zhang, H., Yue, X., Cheng, H., Zhang, X., Cai, Y., Zou, W., Huang, G., Cheng, L., Ye,  
826 F., and Kang, L. (2018). OSM-9 and an amiloride-sensitive channel, but not  
827 PKD-2, are involved in mechanosensation in *C. elegans* male ray neurons. *Sci*  
828 *Rep* 8, 7192. 10.1038/s41598-018-25542-1.
- 829 24. Brugman, K.I., Susoy, V., Whittaker, A.J., Palma, W., Nava, S., Samuel, A.D.T., and  
830 Sternberg, P.W. (2022). PEZO-1 and TRP-4 mechanosensors are involved in  
831 mating behavior in *Caenorhabditis elegans*. *PNAS Nexus* 1, pgac213.  
832 10.1093/pnasnexus/pgac213.
- 833 25. Rouger, V., Bordet, G., Couillault, C., Monneret, S., Mailfert, S., Ewbank, J.J.,  
834 Pujol, N., and Marguet, D. (2014). Independent synchronized control and  
835 visualization of interactions between living cells and organisms. *Biophys J*  
836 106, 2096-2104. 10.1016/j.bpj.2014.03.044.
- 837 26. Martineau, C.N., Kirienko, N.V., and Pujol, N. (2021). Innate immunity in *C.*  
838 *elegans*. *Curr Top Dev Biol* 144, 309-351. 10.1016/bs.ctdb.2020.12.007.
- 839 27. Pujol, N., Zugasti, O., Wong, D., Couillault, C., Kurz, C.L., Schulenburg, H., and  
840 Ewbank, J.J. (2008). Anti-fungal innate immunity in *C. elegans* is enhanced by  
841 evolutionary diversification of antimicrobial peptides. *PLoS Pathog* 4,  
842 e1000105. 10.1371/journal.ppat.1000105.
- 843 28. Park, S.J., Goodman, M.B., and Pruitt, B.L. (2007). Analysis of nematode  
844 mechanics by piezoresistive displacement clamp. *Proceedings of the National*  
845 *Academy of Sciences of the United States of America* 104, 17376-17381.  
846 10.1073/pnas.0702138104.
- 847 29. McMahon, L., Muriel, J.M., Roberts, B., Quinn, M., and Johnstone, I.L. (2003).  
848 Two sets of interacting collagens form functionally distinct substructures  
849 within a *Caenorhabditis elegans* extracellular matrix. *Mol Biol Cell* 14, 1366-  
850 1378. 10.1091/mbc.e02-08-0479.
- 851 30. Gilpin, W., Uppaluri, S., and Brangwynne, C.P. (2015). Worms under Pressure:  
852 Bulk Mechanical Properties of *C. elegans* Are Independent of the Cuticle.  
853 *Biophys J* 108, 1887-1898. 10.1016/j.bpj.2015.03.020.
- 854 31. Petzold, B.C., Park, S.J., Ponce, P., Roozeboom, C., Powell, C., Goodman, M.B.,  
855 and Pruitt, B.L. (2011). *Caenorhabditis elegans* body mechanics are regulated  
856 by body wall muscle tone. *Biophys J* 100, 1977-1985.  
857 10.1016/j.bpj.2011.02.035.
- 858 32. Aggad, D., Brouilly, N., Omi, S., Essmann, C.L., Dehapiot, B., Savage-Dunn, C.,  
859 Richard, F., Cazevielle, C., Politi, K.A., Hall, D.H., et al. (2023). Meisosomes,  
860 folded membrane microdomains between the apical extracellular matrix and

- 861 epidermis. *eLife* 12. 10.7554/eLife.75906.
- 862 33. Wexler, L.R., Miller, R.M., and Portman, D.S. (2020). *C. elegans* Males Integrate  
863 Food Signals and Biological Sex to Modulate State-Dependent  
864 Chemosensation and Behavioral Prioritization. *Current biology : CB* 30, 2695-  
865 2706 e2694. 10.1016/j.cub.2020.05.006.
- 866 34. Leighton, D.H., Choe, A., Wu, S.Y., and Sternberg, P.W. (2014). Communication  
867 between oocytes and somatic cells regulates volatile pheromone production  
868 in *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences*  
869 *of the United States of America* 111, 17905-17910.  
870 10.1073/pnas.1420439111.
- 871 35. Wan, X., Zhou, Y., Chan, C.M., Yang, H., Yeung, C., and Chow, K.L. (2019). SRD-1  
872 in AWA neurons is the receptor for female volatile sex pheromones in *C.*  
873 *elegans* males. *EMBO Rep* 20. 10.15252/embr.201846288.
- 874 36. Barton, M.K., Schedl, T.B., and Kimble, J. (1987). Gain-of-function mutations of  
875 *fem-3*, a sex-determination gene in *Caenorhabditis elegans*. *Genetics* 115,  
876 107-119.
- 877 37. Lee, R.C., Feinbaum, R.L., and Ambros, V. (1993). The *C. elegans* heterochronic  
878 gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell*  
879 75, 843-854.
- 880 38. Ambros, V. (1989). A hierarchy of regulatory genes controls a larva-to-adult  
881 developmental switch in *C. elegans*. *Cell* 57, 49-57. 10.1016/0092-  
882 8674(89)90171-2.
- 883 39. Kaletsky, R., Yao, V., Williams, A., Runnels, A.M., Tadych, A., Zhou, S.,  
884 Troyanskaya, O.G., and Murphy, C.T. (2018). Transcriptome analysis of adult  
885 *Caenorhabditis elegans* cells reveals tissue-specific gene and isoform  
886 expression. *PLoS genetics* 14, e1007559. 10.1371/journal.pgen.1007559.
- 887 40. Wu, Y.Z., Jiang, H.S., Han, H.F., Li, P.H., Lu, M.R., Tsai, I.J., and Wu, Y.C. (2022). *C.*  
888 *elegans* BLMP-1 controls apical epidermal cell morphology by repressing  
889 expression of mannosyltransferase *bus-8* and molting signal *mlt-8*.  
890 *Developmental biology*. 10.1016/j.ydbio.2022.03.011.
- 891 41. Sandhu, A., Badal, D., Sheokand, R., Tyagi, S., and Singh, V. (2021). Specific  
892 collagens maintain the cuticle permeability barrier in *Caenorhabditis*  
893 *elegans*. *Genetics* 217. 10.1093/genetics/iyaa047.
- 894 42. Blaxter, M.L. (1993). Cuticle surface proteins of wild type and mutant  
895 *Caenorhabditis elegans*. *The Journal of biological chemistry* 268, 6600-6609.
- 896 43. Page, A.P., and Johnstone, I.L. (2007). The cuticle. *WormBook*, 1-15.  
897 10.1895/wormbook.1.138.1.
- 898 44. Gravato-Nobre, M.J., Stroud, D., O'Rourke, D., Darby, C., and Hodgkin, J. (2011).  
899 Glycosylation genes expressed in seam cells determine complex surface  
900 properties and bacterial adhesion to the cuticle of *Caenorhabditis elegans*.  
901 *Genetics* 187, 141-155. 10.1534/genetics.110.122002.
- 902 45. Blaxter, M.L., Page, A.P., Rudin, W., and Maizels, R.M. (1992). Nematode  
903 surface coats: actively evading immunity. *Parasitol Today* 8, 243-247.  
904 10.1016/0169-4758(92)90126-m.
- 905 46. Darby, C., Chakraborti, A., Politz, S.M., Daniels, C.C., Tan, L., and Drace, K.  
906 (2007). *Caenorhabditis elegans* mutants resistant to attachment of *Yersinia*

- 907 biofilms. *Genetics* 176, 221-230. 10.1534/genetics.106.067496.
- 908 47. Hill, K.L., Harfe, B.D., Dobbins, C.A., and L'Hernault, S.W. (2000). dpy-18  
909 encodes an alpha-subunit of prolyl-4-hydroxylase in *Caenorhabditis elegans*.  
910 *Genetics* 155, 1139-1148.
- 911 48. Wang, H., Park, H., Liu, J., and Sternberg, P.W. (2018). An Efficient Genome  
912 Editing Strategy To Generate Putative Null Mutants in *Caenorhabditis elegans*  
913 Using CRISPR/Cas9. *G3* 8, 3607-3616. 10.1534/g3.118.200662.
- 914 49. Dodd, W., Tang, L., Lone, J.C., Wimberly, K., Wu, C.W., Consalvo, C., Wright, J.E.,  
915 Pujol, N., and Choe, K.P. (2018). A Damage Sensor Associated with the Cuticle  
916 Coordinates Three Core Environmental Stress Responses in *Caenorhabditis*  
917 *elegans*. *Genetics* 208, 1467-1482. 10.1534/genetics.118.300827.
- 918 50. Ratnappan, R., Vadnal, J., Keaney, M., Eleftherianos, I., O'Halloran, D., and  
919 Hawdon, J.M. (2016). RNAi-mediated gene knockdown by microinjection in  
920 the model entomopathogenic nematode *Heterorhabditis bacteriophora*.  
921 *Parasit Vectors* 9, 160. 10.1186/s13071-016-1442-4.
- 922 51. Gilleard, J.S., Barry, J.D., and Johnstone, I.L. (1997). cis regulatory  
923 requirements for hypodermal cell-specific expression of the *Caenorhabditis*  
924 *elegans* cuticle collagen gene dpy-7. *Molecular and cellular biology* 17, 2301-  
925 2311. 10.1128/MCB.17.4.2301.
- 926 52. Johnstone, I.L., Shafi, Y., and Barry, J.D. (1992). Molecular analysis of  
927 mutations in the *Caenorhabditis elegans* collagen gene dpy-7. *The EMBO*  
928 *journal* 11, 3857-3863. 10.1002/j.1460-2075.1992.tb05478.x.
- 929 53. Thein, M.C., McCormack, G., Winter, A.D., Johnstone, I.L., Shoemaker, C.B., and  
930 Page, A.P. (2003). *Caenorhabditis elegans* exoskeleton collagen COL-19: an  
931 adult-specific marker for collagen modification and assembly, and the  
932 analysis of organismal morphology. *Developmental dynamics : an official*  
933 *publication of the American Association of Anatomists* 226, 523-539.  
934 10.1002/dvdy.10259.
- 935 54. Essmann, C.L., Elmi, M., Shaw, M., Anand, G.M., Pawar, V.M., and Srinivasan,  
936 M.A. (2017). In-vivo high resolution AFM topographic imaging of  
937 *Caenorhabditis elegans* reveals previously unreported surface structures of  
938 cuticle mutants. *Nanomedicine* 13, 183-189. 10.1016/j.nano.2016.09.006.
- 939 55. Taffoni, C., and Pujol, N. (2015). Mechanisms of innate immunity in *C. elegans*  
940 epidermis. *Tissue Barriers* 3, e1078432. 10.1080/21688370.2015.1078432.
- 941 56. Luo, J., Barrios, A., and Portman, D.S. (2023). *C. elegans* males optimize mate-  
942 choice decisions via sex-specific responses to multimodal sensory cues.  
943 *bioRxiv*. 10.1101/2023.04.08.536021.
- 944 57. Cook, S.J., Jarrell, T.A., Brittin, C.A., Wang, Y., Bloniarz, A.E., Yakovlev, M.A.,  
945 Nguyen, K.C.Q., Tang, L.T., Bayer, E.A., Duerr, J.S., et al. (2019). Whole-animal  
946 connectomes of both *Caenorhabditis elegans* sexes. *Nature* 571, 63-71.  
947 10.1038/s41586-019-1352-7.
- 948 58. Billeter, J.C., and Levine, J.D. (2013). Who is he and what is he to you?  
949 Recognition in *Drosophila melanogaster*. *Curr Opin Neurobiol* 23, 17-23.  
950 10.1016/j.conb.2012.08.009.
- 951 59. Moon, S.J., Lee, Y., Jiao, Y., and Montell, C. (2009). A *Drosophila* gustatory  
952 receptor essential for aversive taste and inhibiting male-to-male courtship.

953 Current biology : CB 19, 1623-1627. 10.1016/j.cub.2009.07.061.

954 60. Snell, T.W., Shearer, T.L., Smith, H.A., Kubanek, J., Gribble, K.E., and Welch, D.B.

955 (2009). Genetic determinants of mate recognition in *Brachionus manjavacas*

956 (Rotifera). *BMC Biol* 7, 60. 10.1186/1741-7007-7-60.

957 61. Lightfoot, J.W., Wilecki, M., Rodelsperger, C., Moreno, E., Susoy, V., Witte, H.,

958 and Sommer, R.J. (2019). Small peptide-mediated self-recognition prevents

959 cannibalism in predatory nematodes. *Science* 364, 86-89.

960 10.1126/science.aav9856.

961 62. Lee, S.H., Omi, S., Thakur, N., Taffoni, C., Belougne, J., Engelmann, I., Ewbank,

962 J.J., and Pujol, N. (2018). Modulatory upregulation of an insulin peptide gene

963 by different pathogens in *C. elegans*. *Virulence* 9, 648-658.

964 10.1080/21505594.2018.1433969.

965 63. Liu, K.S., and Sternberg, P.W. (1995). Sensory regulation of male mating

966 behavior in *Caenorhabditis elegans*. *Neuron* 14, 79-89.

967 64. Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics* 77, 71-

968 94.

969 65. Mello, C.C., Kramer, J.M., Stinchcomb, D., and Ambros, V. (1991). Efficient gene

970 transfer in *C.elegans*: extrachromosomal maintenance and integration of

971 transforming sequences. *EMBO J* 10, 3959-3970.

972 66. Love, M.I., Huber, W., and Anders, S. (2014). Moderated estimation of fold

973 change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 15, 550.

974 10.1186/s13059-014-0550-8.

975 67. Angeles-Albores, D., Lee, R., Chan, J., and Sternberg, P. (2018). Two new

976 functions in the WormBase Enrichment Suite. *MicroPubl Biol* 2018.

977 10.17912/W25Q2N.

978 68. Davis, P., Zarowiecki, M., Arnaboldi, V., Becerra, A., Cain, S., Chan, J., Chen, W.J.,

979 Cho, J., da Veiga Beltrame, E., Diamantakis, S., et al. (2022). WormBase in

980 2022-data, processes, and tools for analyzing *Caenorhabditis elegans*.

981 *Genetics* 220. 10.1093/genetics/iyac003.

982 69. Kamath, R.S., Martinez-Campos, M., Zipperlen, P., Fraser, A.G., and Ahringer, J.

983 (2001). Effectiveness of specific RNA-mediated interference through ingested

984 double-stranded RNA in *Caenorhabditis elegans*. *Genome Biol* 2,

985 RESEARCH0002. 10.1186/gb-2000-2-1-research0002.

986 70. Peixoto, C.A., de Melo, J.V., Kramer, J.M., and de Souza, W. (1998).

987 Ultrastructural analyses of the *Caenorhabditis elegans* rol-6 (su1006)

988 mutant, which produces abnormal cuticle collagen. *J Parasitol* 84, 45-49.

989 71. Zhang, S., Banerjee, D., and Kuhn, J.R. (2011). Isolation and culture of larval

990 cells from *C. elegans*. *PLoS One* 6, e19505. 10.1371/journal.pone.0019505.

991