Males Shorten the Life Span of *C. elegans* Hermaphrodites via Secreted Compounds

Travis J. Maures *et al.*

Science **343**, 541 (2014);
DOI: 10.1126/science.1244160

Males Shorten the Life Span of *C. elegans* Hermaphrodites via Secreted Compounds

Travis J. Maures,[1] Lauren N. Booth,[1] Bérénice A. Benayoun,[1] Yevgeniy Izrayelit,[2] Frank C. Schroeder,[2] Anne Brunet[1,3]*

How an individual's longevity is affected by the opposite sex is still largely unclear. In the nematode *Caenorhabditis elegans*, the presence of males accelerated aging and shortened the life span of individuals of the opposite sex (hermaphrodites), including long-lived or sterile hermaphrodites. The male-induced demise could occur without mating and required only exposure of hermaphrodites to medium in which males were once present. Such communication through pheromones or other diffusible substances points to a nonindividual autonomous mode of aging regulation. The male-induced demise also occurred in other species of nematodes, suggesting an evolutionary conserved process whereby males may induce the disposal of the opposite sex to save resources for the next generation or to prevent competition from other males.

In species ranging from worms to nonhuman primates, the life span of individuals is nearly always assessed in conditions where males and females are kept separate. Yet in the wild, the opposite sexes coexist, at least during attraction and mating. In flies and worms, the presence of males decreases longevity in the opposite sex (*1*, *2*). In *Drosophila*, males shorten the life span of females after mating through peptides present in seminal fluid (*2*). In *Caenorhabditis elegans*, male-induced life-span shortening of the opposite sex (hermaphrodites) has been proposed to result from physical damage caused by copulation (*1*). Whether additional mechanisms of male-induced killing exist in *C. elegans*, and the extent to which such mechanisms may be evolutionarily conserved, is largely unknown.

The continuous presence of young males significantly shortened the life span of hermaphrodites (>20% decrease) (Fig. 1A and table S1). The male-induced shortening of life span was seen whether males were placed with hermaphrodites at the beginning of their life (day 1) or at sexual maturity (day 4) (Fig. 1A). This life-span shortening was not a result of crowding, because the total numbers of worms were the same in all conditions. Males also induced behavioral and morphological phenotypes characteristic of an advanced age in these hermaphrodites: Movement was slowed, paralysis increased, and a general decrepitude was observed, as exemplified by increased incidence of vacuole-like structures and structural decline within the cuticle, muscle, pharynx, and intestine (Fig. 1B; fig. S1, A to I; and movies S1 to S3) (*3*–*6*). We termed this phenomenon male-induced demise (MID).

Long-lived hermaphrodites, such as insulin receptor mutants *daf-2*, germline-deficient mutants *glp-1*, and wild-type worms subjected to dietary deprivation (DD), also exhibited a shortened life span (Fig. 1, C and D, and fig. S1J). *daf-2* mutant hermaphrodites displayed a large (>60%) reduction in median life span in the presence of wild-type males (Fig. 1C). *glp-1* mutant hermaphrodites exhibited shortening of life span in response to males, even though they are sterile (Fig. 1D), in agreement with observations that sterile hermaphrodites are equally susceptible to male-induced life-span shortening (*1*). Thus, the deterioration of hermaphrodites in the presence of males is not a simple result of increased progeny production from sexual reproduction (*7*), and extension of life span through several well-known longevity pathways is not sufficient to alleviate this form of demise.

To understand how males restrict the life span of the opposite sex, we assessed genome-wide changes in hermaphrodite gene expression triggered by males. To avoid expression changes due to fertilized embryos in the mother, we used sterile hermaphrodites (*glp-1*). We placed *glp-1* young adult hermaphrodites with wild-type young males for 8 days, then removed the males and collected the hermaphrodites' RNA for microarray analysis (Fig. 2A). As a control, we collected RNA from *glp-1* hermaphrodites that were not placed in the presence of males but were grown at the same density with other hermaphrodites (Fig. 2A). Unbiased clustering of the microarray data revealed that the presence of males induced large changes in gene expression

[1]Department of Genetics, Stanford University, Stanford, CA 94305, USA. [2]Boyce Thompson Institute and Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853, USA. [3]Glenn Laboratories for the Biology of Aging at Stanford, Stanford, CA 94305, USA.

*Corresponding author. E-mail: abrunet1@stanford.edu

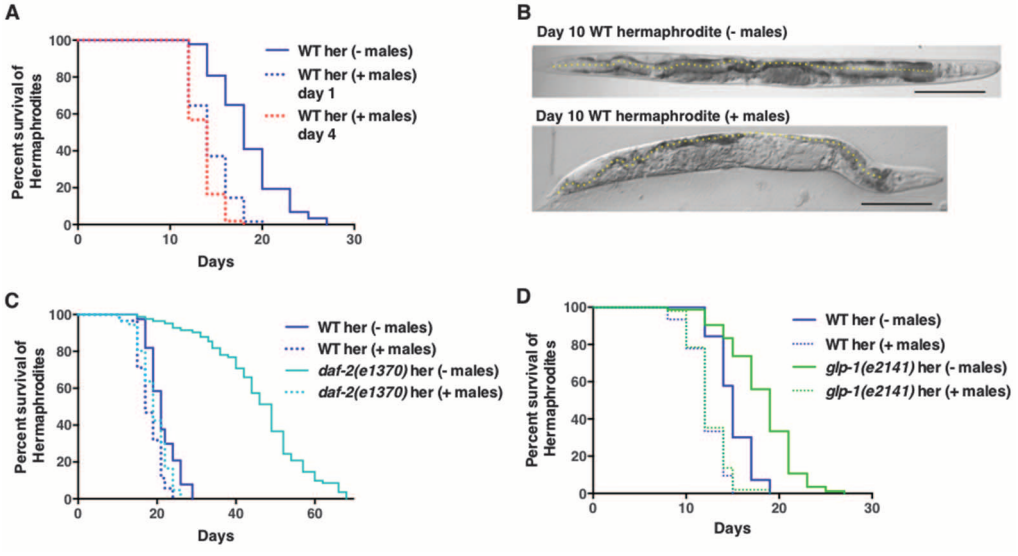

Fig. 1. Reduced life span and increased hallmarks of aging in hermaphrodites exposed to males. (**A**) Shortened life span of wild-type hermaphrodites when kept in the constant presence of young mating-competent males added at the beginning of the hermaphrodite's life (day 1, log rank $P < 0.0001$) or when hermaphrodites reached young adulthood (day 4, log rank $P < 0.0001$). (**B**) Representative images of the male-induced deterioration of hermaphrodites at day 10. The dotted yellow line traces the center of the intestine, which is irregular and often appears discontinuous in hermaphrodites undergoing MID. Scale bar, 200 μm. (**C**) Shortened life span of long-lived *daf-2(e1370)* mutant hermaphrodites (log rank $P < 0.0001$) kept in the constant presence of young males. As a control, wild-type males also shorten the life span of wild-type hermaphrodites (log rank $P < 0.0001$). Note the difference in scale with Fig. 1A. (**D**) Shortened life span of long-lived and sterile *glp-1(e2141)* mutant hermaphrodites (log rank $P < 0.0001$) in the constant presence of young males. As a control, wild-type males also shorten the life span of wild-type hermaphrodites (log rank $P < 0.0001$). Statistics are included in table S1.

in hermaphrodites (Fig. 2B, fig. S2A, and table S2). Genes whose expression was increased in response to males were enriched for insulin signaling ($P = 4.3 \times 10^{-3}$) [e.g., insulin peptides (*ins-4*, *ins-11*, *ins-23*, and *ins-31*), which are expressed in neurons], transthyretin-related family members ($P = 4.3 \times 10^{-3}$) [which are involved in neurodegenerative diseases in mammals (*8*)], and G protein–coupled chemoreceptors ($P = 1.9 \times 10^{-3}$) (which are expressed in sensory neurons) (fig. S2B). In contrast, genes whose expression was decreased in response to males were

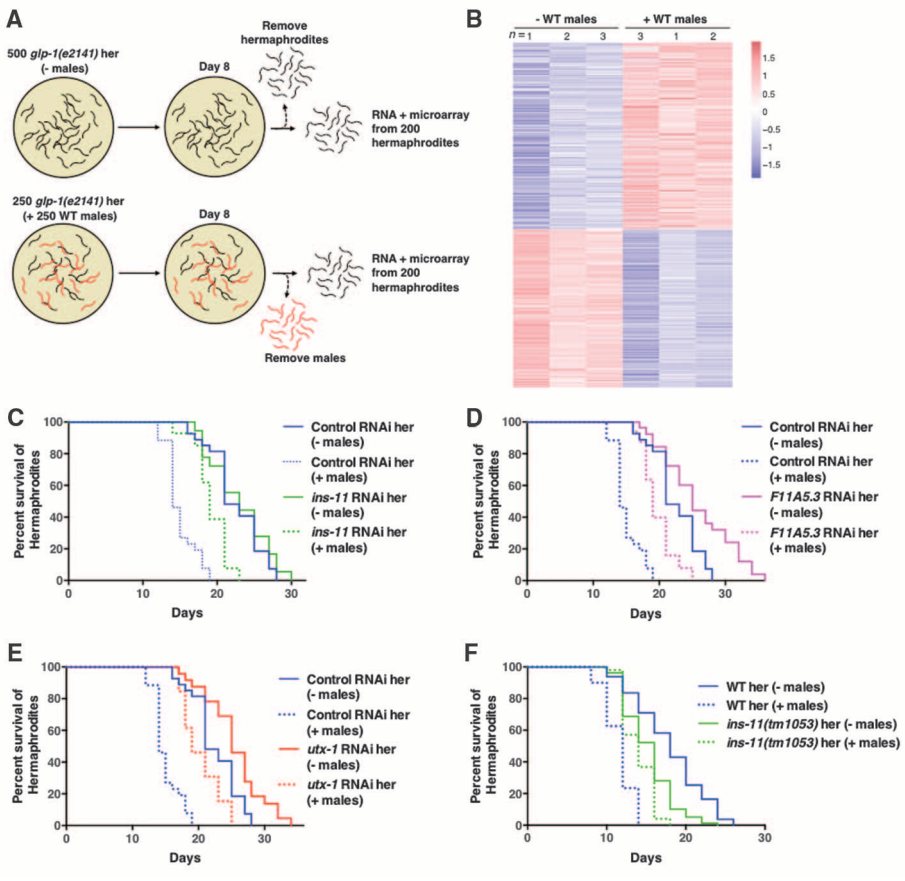

Fig. 2. Male-induced changes in gene expression in hermaphrodites and attenuation of male-induced demise in *ins-11* mutants. (**A**) Schematic of the microarray design. (**B**) Unbiased clustering of the genes whose mRNAs show increased (red) (341) or decreased (blue) (289) abundance in hermaphrodites in response to males (three independent experiments). (**C**) RNAi to *ins-11* specifically ameliorates the male-induced shortening of life span in hermaphrodites from a strain that is sensitized for neuronal RNAi (log rank $P < 0.0001$). (**D**) RNAi to *F11A5.3* reduces the male-induced shortening of life span in hermaphrodites from a strain that is sensitized for neuronal RNAi (log rank $P < 0.0001$). (**E**) RNAi to *utx-1* reduces the male-induced shortening of life span in hermaphrodites from a strain that is sensitized for neuronal RNAi (log rank $P < 0.0001$). (**F**) *ins-11(tm1053)* hermaphrodite mutants exhibit partial protection from the demise induced by wild-type males (log rank $P < 0.0001$), although they are able to successfully mate. Statistics are included in table S1.

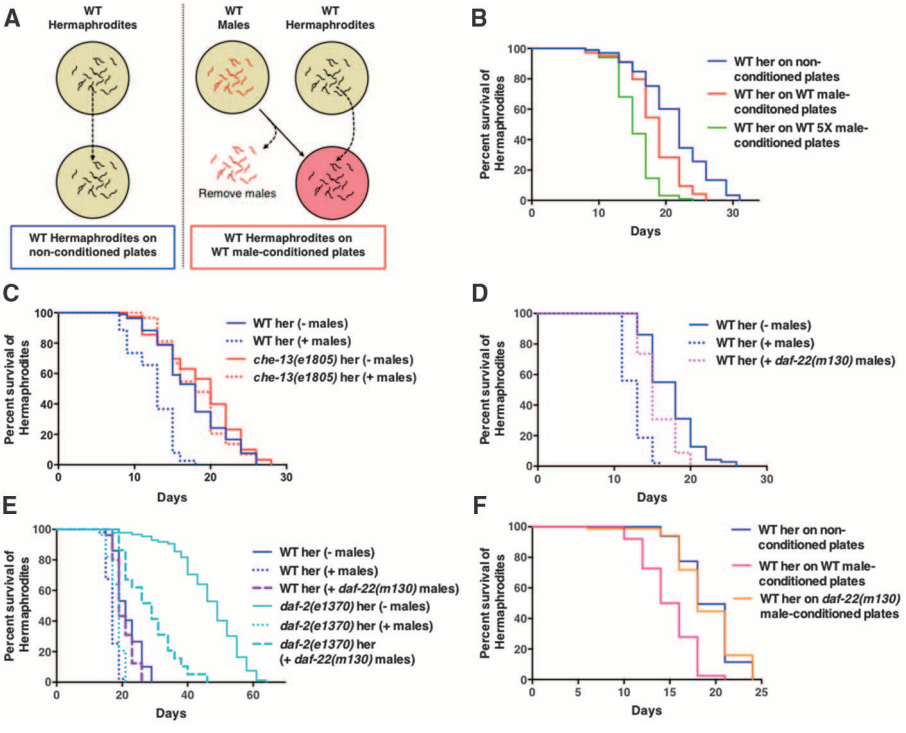

Fig. 3. Role of diffusible substances secreted by males in shortening the life span of hermaphrodites. (**A**) Schematic for male-conditioned plates. (**B**) Shortened life span of wild-type hermaphrodites exposed to secreted substances from males [log rank $P < 0.0001$ when plates were conditioned with 30 males/plate or 150 males/plate (5X)]. (**C**) *che-13(e1805)* hermaphrodites are resistant to male-induced shortening of life span (fig. S3, A and B) (log rank $P < 0.5038$). (**D**) *daf-22(m130)* males do not shorten the life span of wild-type hermaphrodites, as well as wild-type males (fig. S3, A and B) (statistically significant difference by log rank, $P < 0.0001$). (**E**) *daf-22(m130)* males do not shorten the life span of *daf-2(e1370)* hermaphrodites, as well as wild-type males (statistically significant difference by log rank, $P < 0.0001$). (**F**) Medium conditioned by *daf-22(m130)* males does not significantly shorten the life span of wild-type hermaphrodites (log rank $P = 0.9177$). Statistics are included in table S1.

enriched for C-type lectins and the cuticle (fig. S2B). That the presence of males triggered changes in the expression of neuronally expressed genes suggests that mechanisms in addition to structural damage resulting from copulation also contribute to MID.

We next tested whether modulating the expression of genes whose expression was increased in hermaphrodites in response to males and expressed in neurons could rescue MID. We used RNA interference (RNAi) to decrease expression of 10 hand-picked genes that are expressed in neurons and either belong to a significant functional annotation enrichment category or undergo large changes in message abundance in response to males. We used a strain of *C. elegans* that is sensitized for RNAi in neurons *Punc-119*::*sid-1*. Depletion of mRNA from three of these genes (*ins-11*, *F11A5.3*, and *utx-1*) partially rescued MID (Fig. 2, C to E, and fig. S2, C to L). *ins-11* encodes an insulin-like peptide that is expressed primarily in sensory neurons (*9*). *F11A5.3* encodes a conserved small guanosine triphosphatase (GTPase) of the Rab family of little-known function in worms but whose human ortholog (*RAB2A*) functions in vesicular trafficking (*10*). *utx-1* encodes a histone H3 demethylase (H3K27me3 demethylase), depletion of which increases longevity in *C. elegans* (*11*, *12*). Whereas decreased expression of *F11A5.3* and *utx-1* also extended the life span of hermaphrodites without males, decreased expression of *ins-11* specifically ameliorated MID without affecting the life span of hermaphrodites kept in the absence of males. The specific rescue of MID after depletion of *ins-11* likely results from the action of this gene in the hermaphrodites themselves and not in the males because *ins-11* mutant hermaphrodites were also partially resistant to demise induced by wild-type males (Fig. 2F). Thus, the shortening of life span induced by males can be ameliorated by depletion of the insulin peptide INS-11.

Because MID could be rescued by manipulating a single gene in the hermaphrodites, the phenomenon seems unlikely to result solely from structural damage caused by copulation. To test more directly whether males could shorten the life span of hermaphrodites without being in physical contact with them, we placed males on plates for 2 days, removed these males, and then added hermaphrodites to the male-conditioned plates (Fig. 3A). Conditioning the plates with males shortened the life span of wild-type hermaphrodites, in a manner that depended on the number of males used to make the conditioned medium (Fig. 3B). Hermaphrodites placed on male-conditioned plates underwent signs of MID (movies S4, to S6). Although there may be a physical component to MID, one or more diffusible substances secreted or released by males on the plate is sufficient to decrease life span of hermaphrodites.

C. elegans secrete small molecules called ascarosides, which act as pheromones to regulate various processes, including development, behavior, and life span (*13*–*18*). Ascaroside production has been primarily studied in the context of hermaphrodites, but males also excrete a sex-specific blend of ascarosides (*19*). We therefore tested whether pheromone sensing by hermaphrodites and pheromone production by males were required for MID. Hermaphrodites deficient for processing a range of sensory signals, including those from pheromones (*20*, *21*) (*che-13*), were not susceptible to MID (Fig. 3C and table S1), even though they mated normally with wild-type males (fig. S3, A and B). Males deficient in ascaroside pheromone biogenesis (*daf-22*) triggered MID less effectively in both wild-type or long-lived hermaphrodites (Fig. 3, D and E), although these *daf-22* males mated normally with wild-type hermaphrodites (fig. S3, A and B). Conditioned medium from males that are defective in pheromone production (*daf-22*) did not trigger MID in wild-type hermaphrodites (Fig. 3F). Thus, the ability to secrete and sense pheromones appears to be necessary for males to induce shortening of life span in hermaphrodites.

Is the male-induced demise a more general, conserved phenomenon? The genus *Caenorhabditis* includes several distantly related species, each of them with different strains (fig. S4A). Similar to what we observed in the long-domesticated strain of *C. elegans* (N2), males from a wild *C. elegans* strain, AB1, also shortened the life span of hermaphrodites of that strain (fig. S4B). Males from the species *C. briggsae*, which diverged from *C. elegans* about 20 to 30 million years ago (*22*), decreased the life span of hermaphrodites of two different *C. briggsae* isolates (Fig. 4A). Males from the species *C. remanei*, which has obligate males and females, led to life-span shortening of females (Fig. 4B). Together, these results indicate that MID is conserved at least over 20 to 30 million years of evolution and is not linked to hermaphroditism. The evolutionary conservation of MID raises the possibility that this phenomenon has adaptive value and may be caused by conserved mechanisms.

We find that continuous presence of mating-competent males shortens the life span of the opposite sex and triggers a phenotype that resembles progeria. In our study, *C. elegans* hermaphrodites were exposed to an unnaturally high percentage of males for the duration of their life spans. The percentage of male *C. elegans* is usually low (0.01 to 0.1%), although this percentage can be increased in response to stress stimuli. However, MID does not appear to be solely due to an artificially high percentage of males, as *C. remanei* females, which naturally exist in a 1:1 ratio with males, also displayed MID in our experimental setting. Males shorten the life span of the opposite sex in part by releasing one or more diffusible substances, possibly a pheromone. Thus, while male secretions promote reproduction under normal circumstances, they might also accelerate demise, especially when these secretions are concentrated. In addition to male pheromones, the male-conditioned plates are also likely to contain sperm and seminal fluid from male-to-male copulation attempts (*23*). Copulation may help male secretions to be produced or act efficiently. Although male sperm has been previously ruled out as a cause of the male-induced shortening of life span in *C. elegans* (*1*), seminal fluid, which may itself contain pheromones, cannot be excluded as a causal agent.

In both worms and flies, sensory deficiencies in olfactory and gustatory neurons extend life span (*24*–*26*). Although the exact sensory neurons and specific chemoreceptors responsible for pheromone perception are just beginning to be identified (*16*, *18*, *27*, *28*), pheromones activate conserved downstream signaling pathways, including those activated by transforming growth factor (TGF)–β and insulin (*29*). Male-induced demise could be influenced by similar neuronal circuits and signaling pathways. Indeed, interfering with the gene encoding the insulin peptide INS-11, which is expressed in sensory neurons, specifically rescued the male-induced demise of hermaphrodites. INS-11 and other genes identified in our microarray analysis could provide a handle on the dissection of sensory or intersexual interactions. If males in wild worm populations shortened life span of the opposite sex after reproduction occurred, this might have the

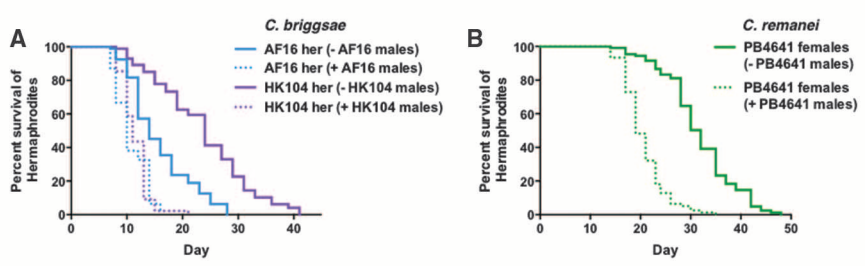

Fig. 4. Males also shorten the life span of individuals of the opposite sex in other species of nematode. (A) Males from the *C. briggsae* strains AF16 and HK104 shorten the life span of hermaphrodites from the respective strains (log rank $P < 0.0001$). **(B)** Males from the *C. remanei* strain PB4641 shorten the life span of PB4641 females (log rank $P < 0.0001$). Statistics are included in table S1.

evolutionary advantage of preserving limited resources for the offspring (*30*) or preventing competition from other males.

References and Notes

1. D. Gems, D. L. Riddle, *Nature* **379**, 723–725 (1996).
2. T. Chapman, L. F. Liddle, J. M. Kalb, M. F. Wolfner, L. Partridge, *Nature* **373**, 241–244 (1995).
3. L. A. Herndon *et al.*, *Nature* **419**, 808–814 (2002).
4. C. F. Glenn *et al.*, *J. Gerontol. A Biol. Sci. Med. Sci.* **59**, 1251–1260 (2004).
5. D. K. Chow, C. F. Glenn, J. L. Johnston, I. G. Goldberg, C. A. Wolkow, *Exp. Gerontol.* **41**, 252–260 (2006).
6. M. D. McGee *et al.*, *Aging Cell* **10**, 699–710 (2011).
7. J. Hodgkin, T. M. Barnes, *Proc. Biol. Sci.* **246**, 19–24 (1991).
8. K. C. Kiontke *et al.*, *BMC Evol. Biol.* **11**, 339 (2011).
9. S. B. Pierce *et al.*, *Genes Dev.* **15**, 672–686 (2001).
10. E. J. Tisdale, J. R. Bourne, R. Khosravi-Far, C. J. Der, W. E. Balch, *J. Cell Biol.* **119**, 749–761 (1992).
11. T. J. Maures, E. L. Greer, A. G. Hauswirth, A. Brunet, *Aging Cell* **10**, 980–990 (2011).
12. C. Jin *et al.*, *Cell Metab.* **14**, 161–172 (2011).
13. R. A. Butcher, M. Fujita, F. C. Schroeder, J. Clardy, *Nat. Chem. Biol.* **3**, 420–422 (2007).
14. P. Y. Jeong *et al.*, *Nature* **433**, 541–545 (2005).
15. J. Srinivasan *et al.*, *Nature* **454**, 1115–1118 (2008).
16. P. T. McGrath *et al.*, *Nature* **477**, 321–325 (2011).
17. E. Z. Macosko *et al.*, *Nature* **458**, 1171–1175 (2009).
18. A. H. Ludewig *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **110**, 5522–5527 (2013).
19. Y. Izrayelit *et al.*, *ACS Chem. Biol.* **7**, 1321–1325 (2012).
20. L. A. Perkins, E. M. Hedgecock, J. N. Thomson, J. G. Culotti, *Dev. Biol.* **117**, 456–487 (1986).
21. B. van Swinderen, L. B. Metz, L. D. Shebester, C. M. Crowder, *Genetics* **161**, 109–119 (2002).
22. A. D. Cutter, *Mol. Biol. Evol.* **25**, 778–786 (2008).
23. D. Gems, D. L. Riddle, *Genetics* **154**, 1597–1610 (2000).
24. J. Apfeld, C. Kenyon, *Nature* **402**, 804–809 (1999).
25. J. Alcedo, C. Kenyon, *Neuron* **41**, 45–55 (2004).
26. S. Libert *et al.*, *Science* **315**, 1133–1137 (2007).
27. C. I. Bargmann, H. R. Horvitz, *Science* **251**, 1243–1246 (1991).
28. A. H. Ludewig, F. C. Schroeder, *WormBook* **2013**, 1–22 (2013).
29. P. Ren *et al.*, *Science* **274**, 1389–1391 (1996).
30. T. B. Kirkwood, *Nature* **270**, 301–304 (1977).

Acknowledgments: We thank members of the Brunet laboratory, S. Kim, A. Fire, and A. Villeneuve for helpful suggestions and J. Lim and S. Zimmerman for critical reading of the manuscript. We thank N. Kosovilka and the Protein and Nucleic Acid Facility facility for the microarray experiments and T. Stiernagle from the Caenorhabditis Genetics Center. Supported by R01AG031198, DP1AG044848, the Glenn Foundation for Medical Research (A.B.), postdoctoral fellowship F32AG37254 (T.J.M.), T32HG000044 and the Helen Hay Whitney Foundation (L.N.B.), Stanford Dean's Fellowship (BAB), R01GM088290 (F.C.S.), and T32GM008500 (Y.I.).

Supplementary Materials

www.sciencemag.org/content/343/6170/541/suppl/DC1
Materials and Methods
Figs. S1 to S3
Tables S1 and S2
Movies S1 to S6
References (*31*–*37*)

2 August 2013; accepted 11 November 2013
Published online 28 November 2013;
10.1126/science.1244160

Drosophila Life Span and Physiology Are Modulated by Sexual Perception and Reward

Christi M. Gendron,[1]* Tsung-Han Kuo,[2]* Zachary M. Harvanek,[1,3] Brian Y. Chung,[1] Joanne Y. Yew,[4,5] Herman A. Dierick,[2] Scott D. Pletcher[1]†

Sensory perception can modulate aging and physiology across taxa. We found that perception of female sexual pheromones through a specific gustatory receptor expressed in a subset of foreleg neurons in male fruit flies, *Drosophila melanogaster*, rapidly and reversibly decreases fat stores, reduces resistance to starvation, and limits life span. Neurons that express the reward-mediating neuropeptide F are also required for pheromone effects. High-throughput whole-genome RNA sequencing experiments revealed a set of molecular processes that were affected by the activity of the longevity circuit, thereby identifying new candidate cell-nonautonomous aging mechanisms. Mating reversed the effects of pheromone perception; therefore, life span may be modulated through the integrated action of sensory and reward circuits, and healthy aging may be compromised when the expectations defined by sensory perception are discordant with ensuing experience.

Sensory perception can modulate aging and physiology in multiple species (*1*–*6*). In *Drosophila*, exposure to food-based odorants partially reverses the anti-aging effect of dietary restriction, whereas broad reduction in olfactory function promotes longevity and alters fat metabolism (*2*, *4*). Even the well-known relation between body temperature and life span may have a sensory component (*7*, *8*).

To identify sensory cues and neuronal circuitry that underlie the effects of sensory perception on aging, we focused on the perception of potential mates. Social interactions are prevalent throughout nature, and the influence of social context on health and longevity is well known in several species, including humans (*9*). Such influences include behavioral interactions with mates and broader physiological "costs of reproduction," which often form the basis for evolutionary models of aging (*10*, *11*).

In *Drosophila*, the presence of potential mates is perceived largely through nonvolatile cuticular hydrocarbons, which are produced by cells called oenocytes and are secreted to the cuticular surface, where they function as pheromones (*12*, *13*). To test whether differential pheromone exposure influenced life span or physiology, we housed "experimental" flies of the same genotype with "donor" animals of the same sex that either expressed normal pheromone profiles or were genetically engineered to express pheromone profiles characteristic of the opposite sex (Fig. 1A). Donor males with feminized pheromone profiles were generated by targeting expression of the sex determination gene, *transformer*, to the oenocytes [via *OK72-GAL4* or *Prom-E800-GAL4* (*14*) (fig. S1)], whereas masculinization of female flies was accomplished by expressing *tra*-RNAi in a similar way (*15*). This design allowed manipulation of the experimental animals' perceived sexual environment without introducing complications associated with mating itself.

In *Drosophila*, sensory manipulations can affect life span, fat storage [as determined by baseline measures of triacylglyceride (TAG)], and certain aspects of stress resistance (*2*, *4*). We found that flies exposed to pheromones of the opposite sex showed differences in these phenotypes. Experimental male flies exposed to male donor pheromone had higher amounts of TAG, were substantially more resistant to starvation, and exhibited a significantly longer life span than genetically identical male siblings exposed to female donor pheromone (Fig. 1, B to D). Females exhibited similar phenotypes in response to male donor pheromone, but the magnitude of the effects was smaller (fig. S2). Subsequent experiments were therefore focused on males.

The characteristics of pheromone exposure were indicative of a mechanism involving sensory perception. Effects were similar in several genetic backgrounds, including a strain recently collected in the wild (fig. S3), and were largely unaffected by cohort composition (fig. S4). Pheromone-induced phenotypes were detected after as little as 2 days' exposure to donor animals (Fig. 1, B and C), persisted with longer manipulations (Fig. 1D), and were progressively

[1]Department of Molecular and Integrative Physiology and Geriatrics Center, Biomedical Sciences and Research Building, University of Michigan, Ann Arbor, MI 48109, USA. [2]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030, USA. [3]Medical Scientist Training Program, Taubman Medical Library, University of Michigan, Ann Arbor, MI 48109, USA. [4]Temasek Life Sciences Laboratory, National University of Singapore, Singapore 117604. [5]Department of Biological Sciences, National University of Singapore, Singapore 117543.

*These authors contributed equally to this work.
†Corresponding author. E-mail: spletch@umich.edu