Report

Current Biology

Sexually Antagonistic Male Signals Manipulate Germline and Soma of *C. elegans* Hermaphrodites

Highlights

- *C. elegans* males produce multiple signals that affect hermaphrodite physiology
- Male ascaroside pheromones delay the loss of hermaphrodite germline precursor cells
- Male ascarosides promote somatic aging even in hermaphrodites lacking the germline
- An unknown signal accelerates larval development, specifically the onset of puberty

Authors

Erin Z. Aprison, Ilya Ruvinsky

Correspondence

ilya.ruvinsky@northwestern.edu

In Brief

Aprison and Ruvinsky report on male-produced signals that affect hermaphrodites in *C. elegans*. Ascaroside pheromones delay the loss of germline precursor cells, but promote somatic aging independent of the germline. Another signal accelerates the onset of puberty. Male signals alter germline-soma balance, inadvertently harming hermaphrodites.

Aprison & Ruvinsky, 2016, Current Biology *26*, 2827–2833
October 24, 2016
http://dx.doi.org/10.1016/j.cub.2016.08.024

CellPress

Current Biology
Report

Sexually Antagonistic Male Signals Manipulate Germline and Soma of *C. elegans* Hermaphrodites

Erin Z. Aprison[1,3] and Ilya Ruvinsky[1,2,3,4,*]
[1]Department of Ecology and Evolution, The University of Chicago, Chicago, IL 60637, USA
[2]Department of Organismal Biology and Anatomy, The University of Chicago, Chicago, IL 60637, USA
[3]Present address: Department of Molecular Biosciences, Northwestern University, Evanston, IL 60208, USA
[4]Lead Contact
*Correspondence: ilya.ruvinsky@northwestern.edu
http://dx.doi.org/10.1016/j.cub.2016.08.024

SUMMARY

Males and females pursue different reproductive strategies, which often bring them into conflict—many traits exist that benefit one sex at a cost to another [1]. Decreased female survival following mating dramatically demonstrates one aspect of this phenomenon [2–5]. Particularly intriguing is the evidence that secreted compounds can shorten lifespan of members of the opposite sex in *Drosophila* [6] and Caenorhabditid nematodes [7] even without copulation taking place. The purpose of such signals is not clear, however. While it is possible that they could limit subsequent mating with competitors or hasten post-reproductive demise, thus decreasing competition for resources, they are also likely to harm unmated individuals. Why would a system exist that reduces the vigor of potential mates prior to mating? Addressing this question could provide insights into mechanisms and evolution of sexual conflict and reveal sensory inputs that regulate aging. Here, we describe two distinct ways in which *Caenorhabditis elegans* males cause faster somatic aging of hermaphrodites but also manipulate different aspects of their reproductive physiology. The first, mediated by conserved ascaroside pheromones, delays the loss of germline progenitor cells. The second accelerates development, resulting in faster sexual maturation. These signals promote male reproductive strategy and the effects harmful to hermaphrodites appear to be collateral damage rather than the goal.

RESULTS AND DISCUSSION

Male-Specific Ascaroside Pheromones Contribute to Delayed Reproductive Senescence

To explore the effects of male signals, we examined *Caenorhabditis elegans* hermaphrodites growing on male-scented plates. Males dwelt on these plates for 24 hr, but were removed prior to the start of experiments (Figure 1A). In agreement with a published report [7], hermaphrodites exposed to male secretions die younger and accumulate somatic defects indicative of faster aging, even though no live males were present (Figure S1). To assess the reproductive function of aging hermaphrodites, we mated them to males after the supply of self-sperm had been exhausted. Unexpectedly, we found that, although the numbers of cross progeny were variable, animals that were aged on male-scented plates on average produced more offspring upon mating (Figures 1B and S1). We concluded that although hermaphrodites exposed to male scent aged faster, their reproductive ability declined slower.

As other organ systems, the germline deteriorates with age eventually rendering animals sterile [9, 10]. One hallmark of this reproductive senescence is progressive loss of germline precursor cells (GPCs) [10, 11]. These germline progenitors are located in the distal portion of the gonad and contain stem cells and their mitotic progeny that have not yet entered meiosis [12]. This cell population dynamically responds to several environmental stimuli [13]. We therefore compared the number of GPCs in hermaphrodites that were aged on male-scented versus control plates and found that the former had significantly more nuclei in this compartment of the gonad (Figures 1C, 1D, and S1D). Because the number of GPCs was highly reproducible between trials and their loss during aging is correlated with reproductive senescence [10, 11], we focused our experiments on this cell population.

The natural candidates to mediate male-to-hermaphrodite signaling are ascarosides, a family of glycolipid molecules, many of which serve as pheromones [14]. Consistent with this idea, hermaphrodites aging on plates that previously housed *daf-22* males, which are defective in the production of short-chain ascarosides [15, 16], produced as many cross progeny upon mating (E.Z.A., unpublished data) and had as many GPCs as hermaphrodites on unscented plates (Figure 1E). No pheromones unique to either sex have been described in *C. elegans*, but males and hermaphrodites produce two molecules, ascr#3 and ascr#10, in sex-specific ratios [17], which have different physiological effects [17, 18]. Hermaphrodites that were aged in the presence of male-specific (ascr#10/ascr#3, 7.2 fmol/1.9 fmol), but not hermaphrodite-specific (ascr#3/ascr#10, 6 fmol/1.7 fmol) physiological concentrations of these two molecules, the amounts equivalent to those secreted by single animals over 24 hr [17], had significantly more GPCs (Figure 1F). We tested whether hermaphrodites that were aged on ascaroside blends produced larger numbers of cross progeny upon mating compared to animals aged on control plates, but observed no discernable difference

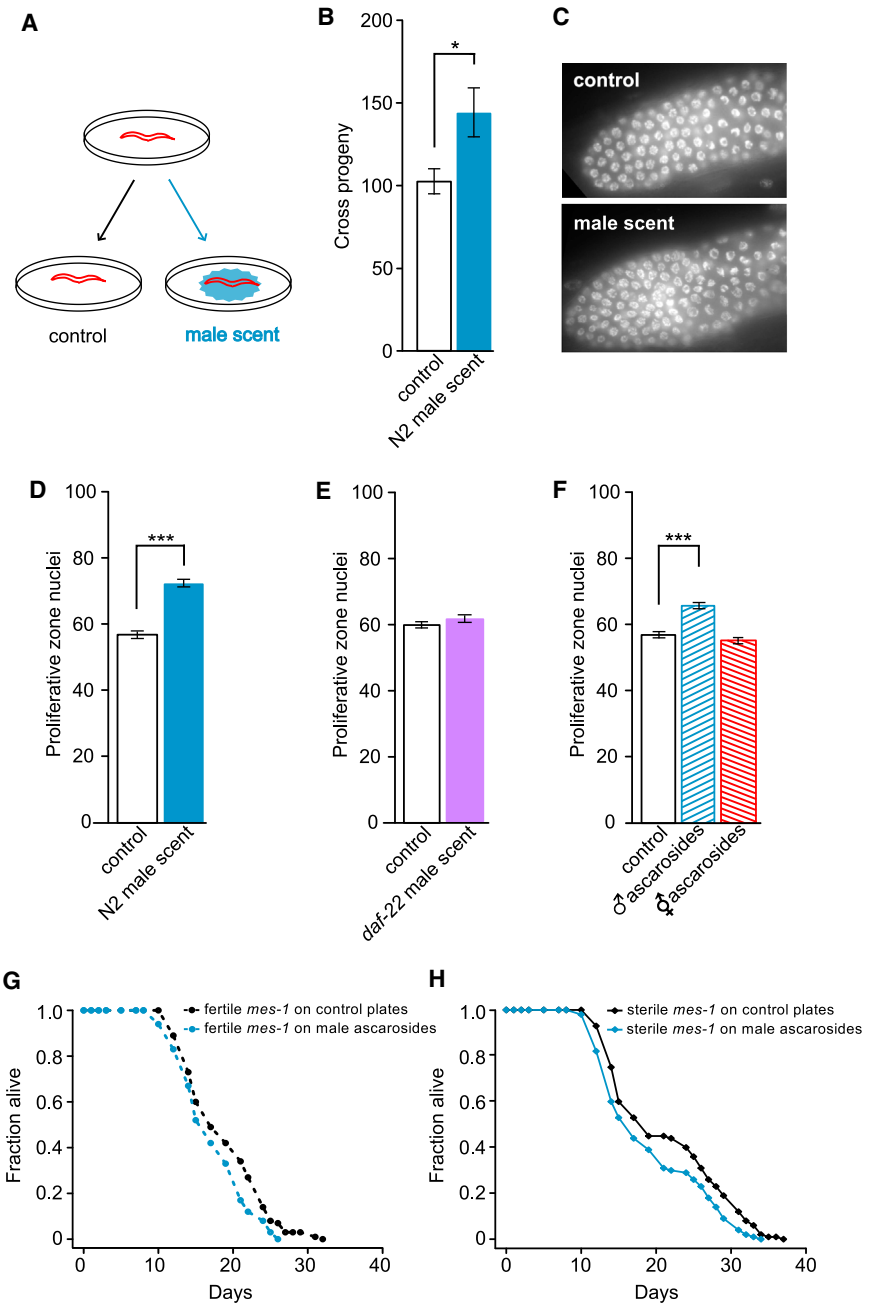

Figure 1. Ascaroside Pheromones of *C. elegans* Males Delay Reproductive Senescence of Hermaphrodites but Promote Somatic Aging

(A) Schematic of experimental design.

(B) Number of cross progeny produced by mating of N2 hermaphrodites aged for 5 days after L4/adult molt (after self-sperm have been exhausted) on male-scented versus control plates ($p = 0.03$, Kolmogorov-Smirnov [K-S] test). Control plates were essentially hermaphrodite-scented because live animals dwelt on them.

(C) Representative images of DAPI-stained distal gonads of hermaphrodites aged on control versus male-scented plates. The boundary of the transition zone is defined as the first row of two crescent-shaped nuclei [8].

(D) Hermaphrodites aging on male-scented plates had significantly more GPCs ($p = 1.9 \times 10^{-13}$, K-S test).

(E) The scent of *daf-22*(*m130*) males did not increase the number of GPCs in aging hermaphrodites.

(F) Hermaphrodites aging on male-specific, but not hermaphrodite-specific, physiological concentrations of ascr#10 and ascr#3 had significantly more GPCs ($p = 1.8 \times 10^{-4}$, K-S test).

(G) Lifespans of fertile *mes-1*(*bn7*) hermaphrodites on control plates or plates supplemented with ascr#10 and ascr#3 in male-specific concentrations. Control: n = 100 (27 censored), mean adult lifespan = 18.8 days; ascaroside-containing plates: n = 100 (34 censored), mean adult lifespan = 17.3 days (log rank $p = 0.014$, log rank test).

(H) Lifespans of sterile *mes-1*(*bn7*) hermaphrodites on control plates or plates supplemented with ascr#10 and ascr#3 in male-specific concentrations. Control: n = 100 (11 censored), mean adult lifespan = 20.5 days; ascaroside-containing plates: n = 100 (7 censored), mean adult lifespan = 19.1 days (log rank $p = 4.7 \times 10^{-3}$, log rank test).

$^*p < 0.05$, $^{***}p < 0.001$. Error bars represent ± SEM. See also Table S1 for raw experimental data and Figure S1.

(E.Z.A., unpublished data). This suggests that while a blend of ascr#10 and ascr#3 can slow down the loss of GPCs, it may not be sufficient to delay reproductive senescence, particularly in an assay that relies on mating. The relationship between slower loss of GPCs and delayed reproductive senescence is not currently clear. It is clear, however, that male ascarosides could affect the female germline in several ways including mitotic precursors and producing attractive signals for sperm guidance [18]. Finally, the slower loss of GPCs seen on plates with the male-specific cocktail of ascr#10 and ascr#3 was not simply due to increased retention of gametes, because these ascarosides did not alter the rate or duration of egg-laying (Figure S1).

Male-Specific Ascaroside Pheromones Promote Faster Somatic Aging

The finding that male ascaroside pheromones increase the number of GPCs in aging hermaphrodites is intriguing. Complete male scent promotes somatic aging [7] and ascarosides are a prominent class of secreted molecules. Importantly, germline signals [19, 20], in particular those from germline stem cells [21], are known to promote aging. It is therefore possible that the slower loss of germline precursor cells in hermaphrodites exposed to male ascarosides may be directly causing faster somatic demise seen in the presence of male-secreted factors.

To test this idea, we examined the lifespan of *mes-1*(*bn7*) hermaphrodites, approximately half of which lack the germline. Sterile animals of this strain are long-lived [21]. We exposed the tested animals to low, physiologically relevant amounts of ascarosides (ascr#10/ascr#3, 7.2 fmol/1.9 fmol) corresponding

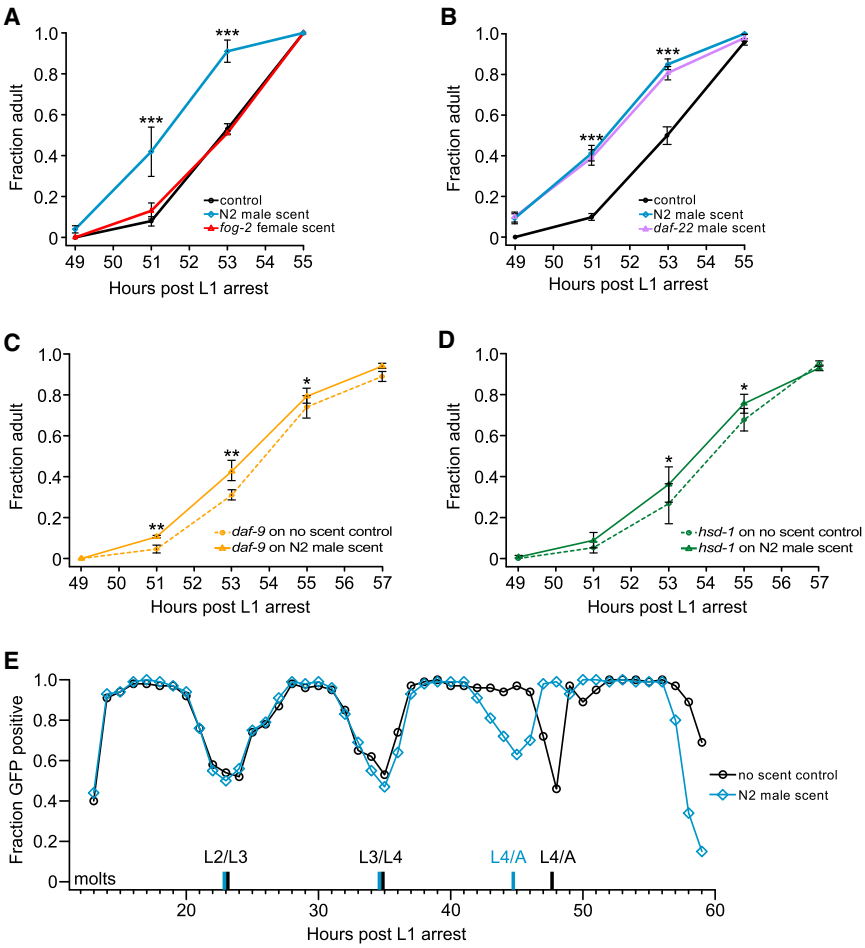

Figure 2. Male Signal Accelerates Hermaphrodite Development

(A) Singled N2 hermaphrodites reach adulthood faster on plates scented by N2 males than on plates scented by *fog-2*(*q71*) females or on unscented control plates (comparing plates scented by N2 males to control, binomial test).

(B) Singled N2 hermaphrodites on plates scented by *daf-22*(*m130*) males reach adulthood as fast as on plates scented by N2 males (comparing plates scented by *daf-22* males to control, binomial test).

(C and D) *daf-9*(*dh6*);*din-1*(*dh127*) (C) and *hsd-1*(*mg433*) (D) hermaphrodites develop marginally faster in the presence of N2 male scent (binomial test).

(E) Fractions of GFP-positive *mlt-10*::GFP animals do not differ between male-scented and control plates until the L4, demonstrating that developmental acceleration occurs specifically at this larval stage (n ≈ 90). Boundaries between larval stages were judged based on levels of GFP expression.

*p < 0.05, **p < 0.01, ***p < 0.001. Error bars represent ± SEM. See also Table S1 for raw experimental data and Figure S2.

to those secreted by a single male. Although these are not the only ascarosides secreted by males [17], and whereas males produce a non-ascaroside signal that reduces lifespan (see below), we observed faster aging of fertile hermaphrodites on ascaroside-supplemented plates (Figure 1G). Importantly, we found that the lifespan of sterile *mes-1* hermaphrodites was also shorter on ascaroside-scented plates (Figure 1H).

Larval germline stem cells signal [21] to shorten lifespan (Figure S1). Because a male-specific mixture of ascr#10 and ascr#3 can shorten lifespan independently of germline precursors, our results imply the existence of additional mechanisms that hasten hermaphrodite somatic aging in response to ascarosides. The role of these molecules in lifespan regulation is most likely complex. Previous work demonstrated that ascr#3 alone, although at considerably higher concentration (>40 pM), extended lifespan [22], suggesting an antagonistic relationship between ascr#10 and ascr#3 and possibly other components of the complete male scent.

Male Signal Causes Developmental Acceleration

In the course of these experiments, we noticed that hermaphrodites developed more rapidly on male-scented plates. Young L1 larvae, both singled (Figure 2A) and in small populations (Figure S2), raised on male- but not hermaphrodite-scented plates, reached adulthood several hours faster than animals on control plates (regardless of the method of synchronization; Figure S2). Surprisingly, however, two lines of evidence indicate that the signal promoting this developmental acceleration is distinct from the one that increases the number of GPCs in aging hermaphrodites. First, development on plates scented by *daf-22* males, which do not produce short-chain ascarosides [15, 16], was as fast as on plates scented by wild-type males (Figure 2B). Second, a male-specific cocktail of ascr#10 and ascr#3 failed to accelerate development, even when applied at much higher concentrations than those that increased the number of GPCs (Figure S2). We concluded that males can manipulate hermaphrodite physiology via two distinct signaling systems—one that relies on ascr#10 and ascr#3 to slow down the loss of germline precursors and another that hastens the rate of development. Both signals also contribute to faster somatic aging of hermaphrodites. This view is consistent with the observation that *daf-22* males, which do not secrete short-chain ascarosides, promote faster hermaphrodite aging, although not to the same extent as wild-type males [7].

In search of a mechanism that mediates faster maturation of hermaphrodites, we considered steroid hormones that are known to regulate metabolism and promote reproductive development in a variety of species [23, 24]. Hermaphrodites carrying mutations in *daf-9* and *hsd-1*, two enzymes involved in steroid hormone synthesis [25], developed only marginally faster on male-scented plates (Figures 2C and 2D). However, hermaphrodites deficient for another steroidogenic enzyme, DAF-36 [25], accelerated as much as the wild-type animals (Figure S2), possibly revealing distinct roles of different steroid derivatives in controlling developmental rate. The nuclear hormone receptor DAF-12 is one likely output of steroid signaling [26], but because

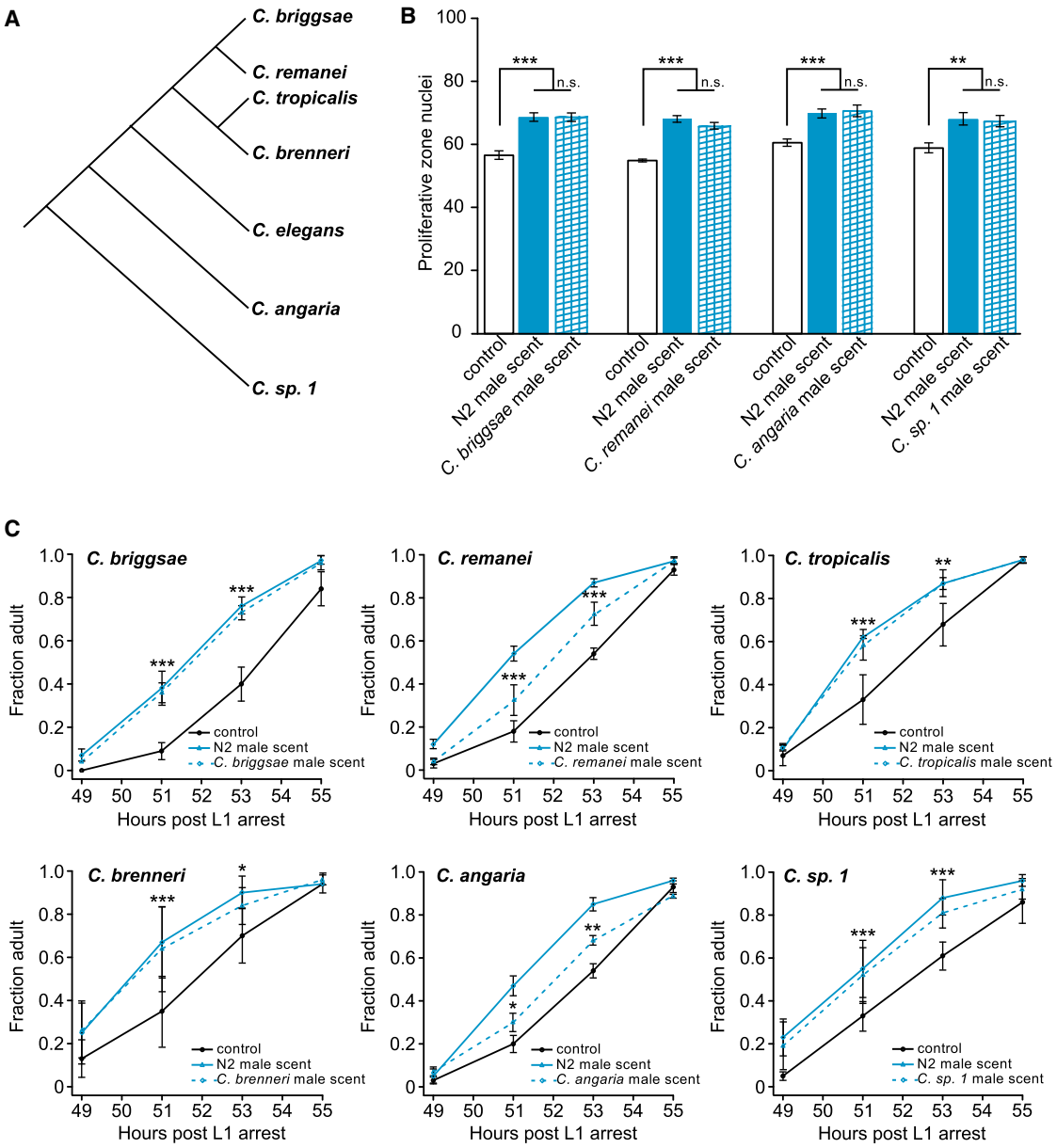

Figure 3. Evolutionary Conservation of Male Signals

(A) Phylogenetic tree of the tested species.

(B) Significantly more GPCs are found in *C. elegans* hermaphrodites aged for five days (after L4/adult molt) on plates scented by *C. briggsae* ($p = 2 \times 10^{-6}$, K-S test), *C. remanei* ($p = 2.4 \times 10^{-14}$, K-S test), *C. angaria* ($p = 1.3 \times 10^{-4}$, K-S test), and *C. sp. 1* ($p = 6.3 \times 10^{-3}$, K-S test) males.

(C) Singled *C. elegans* N2 hermaphrodites reached adulthood faster on plates scented by *C. briggsae*, *C. remanei*, *C. tropicalis*, *C. brenneri*, *C. angaria*, and *C. sp. 1* males.

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Error bars represent ± SEM. See also Table S1 for raw experimental data.

loss of acceleration was less severe in *daf-12* than in *daf*-9 and *hsd-1* mutants (Figure S2), other outputs may exist as well. *C. elegans* most likely produce complex mixtures of steroids, the biosynthesis and functions of which are still being explored [25], and the complement of male steroids is yet to be described. While these results argue that steroid hormones play a role in executing developmental acceleration, the nature of the signal produced by males remains to be elucidated.

To determine whether the observed acceleration was uniformly distributed across all larval stages or restricted to a particular stage, we examined the temporal profile of expression of a *mlt-10::GFP* transgene. The *mlt-10* gene is dynamically expressed during larval stages, reaching the highest levels several hours prior to molts [27]. We found that the temporal profiles of *mlt-10::GFP* expression were remarkably similar between animals reared on male-scented and control plates until after the

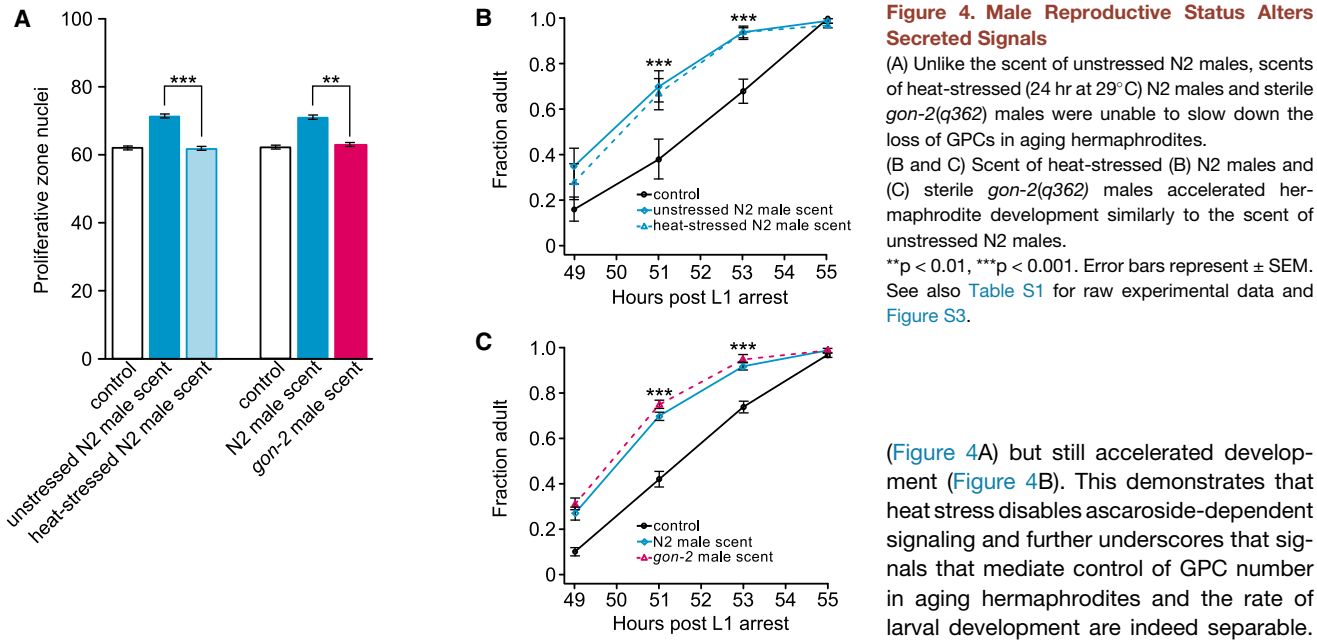

Figure 4. Male Reproductive Status Alters Secreted Signals

(A) Unlike the scent of unstressed N2 males, scents of heat-stressed (24 hr at 29°C) N2 males and sterile *gon-2(q362)* males were unable to slow down the loss of GPCs in aging hermaphrodites.

(B and C) Scent of heat-stressed (B) N2 males and (C) sterile *gon-2(q362)* males accelerated hermaphrodite development similarly to the scent of unstressed N2 males.

**p < 0.01, ***p < 0.001. Error bars represent ± SEM. See also Table S1 for raw experimental data and Figure S3.

L3/L4 molt (Figure 2E). However, animals on male-scented plates initiated and completed the L4/adult molt earlier, suggesting that the effect of this male signal was focused on hastening sexual maturation of hermaphrodites.

Male Signals Are Conserved

In the wild, *C. elegans* has been found to share habitats with other nematodes of the same genus, in particular *C. briggsae* [28] and *C. remanei* [29]. To test whether the two male signals we identified could play a role in interspecific communication, we tested whether they were conserved in other nematodes of the genus Caenorhabditis (Figure 3A). We found that substances secreted by males of all tested species were as potent in delaying the decrease in the number of GPCs in aging *C. elegans* hermaphrodites as the scent of conspecific males (Figure 3B), suggesting broad conservation of ascaroside functions [18, 30]. Thus, while ascarosides act like pheromones in some respects, their extensive conservation contravenes the classical view of pheromones as chemical factors that mediate strictly intraspecific communication [31]. Whereas *C. elegans* hermaphrodites developed faster on plates conditioned by males of several species, the effects on plates scented by *C. remanei* and *C. angaria* were less potent and more variable (Figure 3C). Therefore, both signals could convey information between members of different species, although the specifics most likely depend on ecological conditions in natural habitats.

Ascarosides Communicate Reproductive Status

To serve as effective means of communication, male-to-hermaphrodite signals should faithfully reflect the reproductive status of sender males. We therefore tested whether males with severely compromised reproductive function could signal to hermaphrodites. Prolonged heat stress reduces male fertility drastically and irrecoverably [32]. The scent of males treated in this way no longer altered the number of GPCs in aging hermaphrodites (Figure 4A) but still accelerated development (Figure 4B). This demonstrates that heat stress disables ascaroside-dependent signaling and further underscores that signals that mediate control of GPC number in aging hermaphrodites and the rate of larval development are indeed separable. Males carrying a *gon-2* mutation are sterile because development of their somatic gonad, and consequently the germline, is severely impaired [33]. Similarly to heat-stressed males, the scent of *gon-2* males did not slow down the loss of GPCs in hermaphrodites, whereas it still sped up larval development (Figures 4A, 4C, and S3). Thus, while control of developmental rate appears to be a constitutive property of male-to-hermaphrodite communication, ascaroside signaling that delays the loss of germline progenitors depends on the status of the male reproductive system.

Conclusions

Our results reveal at least two different ways in which *C. elegans* males manipulate reproductive biology of their mates. The first, mediated by ascaroside pheromones, acts to delay the loss of germline cells in aging hermaphrodites and, consequently, forestalls reproductive senescence. This role complements other reproduction-related functions of male pheromones in attracting and holding mates [17, 34] as well as stimulating their reproductive system [18]. The second male signal, the nature of which is not currently known, speeds up hermaphrodite development by specifically shortening the last larval stage preceding adulthood, a period that may be functionally equivalent to puberty. The ability of males to stimulate faster sexual maturation in females has been documented in mammals [35] and thus may be common in animals, although the mechanisms remain to be understood. Combinatorial encoding of social information that relies on different types of messengers to influence different aspects of organismal physiology is found in animals [36, 37] and bacteria [38] and may serve to reliably convey complex messages in variable environments.

Discovering how and why males and females exchange signals that can harm recipients is important for understanding animal communication. We demonstrated that *C. elegans* males use ascarosides and an unknown signal to delay reproductive senescence and promote sexual maturation of potential mates. Collectively, these signals advance the male reproductive

strategy. The negative effects that shorten hermaphrodite lifespan most likely represent "collateral harm" [1], because they are not the goal, but rather inadvertent consequences of an effort to manipulate physiology. Moreover, although the consequences of male signals appear to be harmful to hermaphrodites, it is not certain that they are detrimental to fitness, particularly under natural conditions. What is clear is that the male-produced signals alter hermaphrodite/female physiology in favor of the germline at the expense of the soma. How this shift impacts reproductive success of hermaphrodites in complex native environments remains to be investigated. Our findings underscore the elaborate nature of social communication even in a superficially simple animal and suggest that other signaling modalities influencing important physiological processes remain to be discovered in *C. elegans* and other species.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, three figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2016.08.024.

AUTHOR CONTRIBUTIONS

Conceptualization, E.Z.A. and I.R.; Methodology, E.Z.A. and I.R.; Validation, E.Z.A. and I.R.; Formal Analysis, E.Z.A. and I.R.; Investigation, E.Z.A.; Writing – Original Draft, E.Z.A. and I.R.; Writing – Review & Editing, E.Z.A. and I.R.; Funding Acquisition, I.R.; Supervision, I.R.

ACKNOWLEDGMENTS

We are grateful to Frank Schroeder (Cornell) for the generous gift of ascr#3 and ascr#10 and David Mangelsdorf (UT Southwestern) for sharing strains. We particularly thank Sana Hira for excellent technical help and Lynne Necke and Grace Hirzel for assistance. We thank Chip Ferguson (UChicago) and the three reviewers who offered valuable comments that improved the manuscript. This work was funded in part by the Chicago Biomedical Consortium with support from the Searle Funds at The Chicago Community Trust (to I.R.). Some strains were provided by the Caenorhabditis Genetics Center, which is funded by the NIH Office of Research Infrastructure Programs (P40 OD010440).

Received: June 9, 2016
Revised: July 20, 2016
Accepted: August 9, 2016
Published: September 8, 2016

REFERENCES

1. Parker, G.A. (2006). Sexual conflict over mating and fertilization: an overview. Philos. Trans. R. Soc. Lond. B Biol. Sci. *361*, 235–259.
2. Rice, W.R. (1996). Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. Nature *381*, 232–234.
3. Chapman, T., Liddle, L.F., Kalb, J.M., Wolfner, M.F., and Partridge, L. (1995). Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. Nature *373*, 241–244.
4. Gems, D., and Riddle, D.L. (1996). Longevity in *Caenorhabditis elegans* reduced by mating but not gamete production. Nature *379*, 723–725.
5. Shi, C., and Murphy, C.T. (2014). Mating induces shrinking and death in Caenorhabditis mothers. Science *343*, 536–540.
6. Gendron, C.M., Kuo, T.H., Harvanek, Z.M., Chung, B.Y., Yew, J.Y., Dierick, H.A., and Pletcher, S.D. (2014). Drosophila life span and physiology are modulated by sexual perception and reward. Science *343*, 544–548.
7. Maures, T.J., Booth, L.N., Benayoun, B.A., Izrayelit, Y., Schroeder, F.C., and Brunet, A. (2014). Males shorten the life span of *C. elegans* hermaphrodites via secreted compounds. Science *343*, 541–544.
8. Crittenden, S.L., Leonhard, K.A., Byrd, D.T., and Kimble, J. (2006). Cellular analyses of the mitotic region in the *Caenorhabditis elegans* adult germ line. Mol. Biol. Cell *17*, 3051–3061.
9. Hughes, S.E., Evason, K., Xiong, C., and Kornfeld, K. (2007). Genetic and pharmacological factors that influence reproductive aging in nematodes. PLoS Genet. *3*, e25.
10. Luo, S., Kleemann, G.A., Ashraf, J.M., Shaw, W.M., and Murphy, C.T. (2010). TGF-β and insulin signaling regulate reproductive aging via oocyte and germline quality maintenance. Cell *143*, 299–312.
11. Qin, Z., and Hubbard, E.J. (2015). Non-autonomous DAF-16/FOXO activity antagonizes age-related loss of *C. elegans* germline stem/progenitor cells. Nat. Commun. *6*, 7107.
12. Kimble, J., and Crittenden, S.L. (2007). Controls of germline stem cells, entry into meiosis, and the sperm/oocyte decision in *Caenorhabditis elegans*. Annu. Rev. Cell Dev. Biol. *23*, 405–433.
13. Cinquin, A., Chiang, M., Paz, A., Hallman, S., Yuan, O., Vysniauskaite, I., Fowlkes, C.C., and Cinquin, O. (2016). Intermittent stem cell cycling balances self-renewal and senescence of the *C. elegans* germ line. PLoS Genet. *12*, e1005985.
14. Srinivasan, J., Kaplan, F., Ajredini, R., Zachariah, C., Alborn, H.T., Teal, P.E., Malik, R.U., Edison, A.S., Sternberg, P.W., and Schroeder, F.C. (2008). A blend of small molecules regulates both mating and development in *Caenorhabditis elegans*. Nature *454*, 1115–1118.
15. Butcher, R.A., Ragains, J.R., Li, W., Ruvkun, G., Clardy, J., and Mak, H.Y. (2009). Biosynthesis of the *Caenorhabditis elegans* dauer pheromone. Proc. Natl. Acad. Sci. USA *106*, 1875–1879.
16. von Reuss, S.H., Bose, N., Srinivasan, J., Yim, J.J., Judkins, J.C., Sternberg, P.W., and Schroeder, F.C. (2012). Comparative metabolomics reveals biogenesis of ascarosides, a modular library of small-molecule signals in *C. elegans*. J. Am. Chem. Soc. *134*, 1817–1824.
17. Izrayelit, Y., Srinivasan, J., Campbell, S.L., Jo, Y., von Reuss, S.H., Genoff, M.C., Sternberg, P.W., and Schroeder, F.C. (2012). Targeted metabolomics reveals a male pheromone and sex-specific ascaroside biosynthesis in *Caenorhabditis elegans*. ACS Chem. Biol. *7*, 1321–1325.
18. Aprison, E.Z., and Ruvinsky, I. (2015). Sex pheromones of *C. elegans* males prime the female reproductive system and ameliorate the effects of heat stress. PLoS Genet. *11*, e1005729.
19. Hsin, H., and Kenyon, C. (1999). Signals from the reproductive system regulate the lifespan of *C. elegans*. Nature *399*, 362–366.
20. Patel, M.N., Knight, C.G., Karageorgi, C., and Leroi, A.M. (2002). Evolution of germ-line signals that regulate growth and aging in nematodes. Proc. Natl. Acad. Sci. USA *99*, 769–774.
21. Arantes-Oliveira, N., Apfeld, J., Dillin, A., and Kenyon, C. (2002). Regulation of life-span by germ-line stem cells in *Caenorhabditis elegans*. Science *295*, 502–505.
22. Ludewig, A.H., Izrayelit, Y., Park, D., Malik, R.U., Zimmermann, A., Mahanti, P., Fox, B.W., Bethke, A., Doering, F., Riddle, D.L., and Schroeder, F.C. (2013). Pheromone sensing regulates *Caenorhabditis elegans* lifespan and stress resistance via the deacetylase SIR-2.1. Proc. Natl. Acad. Sci. USA *110*, 5522–5527.
23. Wollam, J., and Antebi, A. (2011). Sterol regulation of metabolism, homeostasis, and development. Annu. Rev. Biochem. *80*, 885–916.
24. Wang, Z., Stoltzfus, J., You, Y.J., Ranjit, N., Tang, H., Xie, Y., Lok, J.B., Mangelsdorf, D.J., and Kliewer, S.A. (2015). The nuclear receptor DAF-12 regulates nutrient metabolism and reproductive growth in nematodes. PLoS Genet. *11*, e1005027.
25. Mahanti, P., Bose, N., Bethke, A., Judkins, J.C., Wollam, J., Dumas, K.J., Zimmerman, A.M., Campbell, S.L., Hu, P.J., Antebi, A., and Schroeder, F.C. (2014). Comparative metabolomics reveals endogenous ligands of DAF-12, a nuclear hormone receptor, regulating *C. elegans* development and lifespan. Cell Metab. *19*, 73–83.

26. Antebi, A., Culotti, J.G., and Hedgecock, E.M. (1998). *daf-12* regulates developmental age and the dauer alternative in *Caenorhabditis elegans*. Development *125*, 1191–1205.

27. Frand, A.R., Russel, S., and Ruvkun, G. (2005). Functional genomic analysis of *C. elegans* molting. PLoS Biol. *3*, e312.

28. Félix, M.A., and Duveau, F. (2012). Population dynamics and habitat sharing of natural populations of *Caenorhabditis elegans* and *C. briggsae*. BMC Biol. *10*, 59.

29. Petersen, C., Dirksen, P., Prahl, S., Strathmann, E.A., and Schulenburg, H. (2014). The prevalence of *Caenorhabditis elegans* across 1.5 years in selected North German locations: the importance of substrate type, abiotic parameters, and Caenorhabditis competitors. BMC Ecol. *14*, 4.

30. Choe, A., von Reuss, S.H., Kogan, D., Gasser, R.B., Platzer, E.G., Schroeder, F.C., and Sternberg, P.W. (2012). Ascaroside signaling is widely conserved among nematodes. Curr. Biol. *22*, 772–780.

31. Karlson, P., and Lüscher, M. (1959). Pheromones': a new term for a class of biologically active substances. Nature *183*, 55–56.

32. Petrella, L.N. (2014). Natural variants of *C. elegans* demonstrate defects in both sperm function and oogenesis at elevated temperatures. PLoS ONE *9*, e112377.

33. Sun, A.Y., and Lambie, E.J. (1997). *gon-2*, a gene required for gonadogenesis in *Caenorhabditis elegans*. Genetics *147*, 1077–1089.

34. Choe, A., Chuman, T., von Reuss, S.H., Dossey, A.T., Yim, J.J., Ajredini, R., Kolawa, A.A., Kaplan, F., Alborn, H.T., Teal, P.E., et al. (2012). Sex-specific mating pheromones in the nematode *Panagrellus redivivus*. Proc. Natl. Acad. Sci. USA *109*, 20949–20954.

35. Vandenbergh, J.G. (1969). Male odor accelerates female sexual maturation in mice. Endocrinology *84*, 658–660.

36. Chin, J.S., Ellis, S.R., Pham, H.T., Blanksby, S.J., Mori, K., Koh, Q.L., Etges, W.J., and Yew, J.Y. (2014). Sex-specific triacylglycerides are widely conserved in Drosophila and mediate mating behavior. eLife *3*, e01751.

37. Brennan, P.A., and Zufall, F. (2006). Pheromonal communication in vertebrates. Nature *444*, 308–315.

38. Waters, C.M., and Bassler, B.L. (2005). Quorum sensing: cell-to-cell communication in bacteria. Annu. Rev. Cell Dev. Biol. *21*, 319–346.