Supporting Information

Targeted Metabolomics Reveals a Male Pheromone and Sex-Specific Ascaroside Biosynthesis in *Caenorhabditis elegans*

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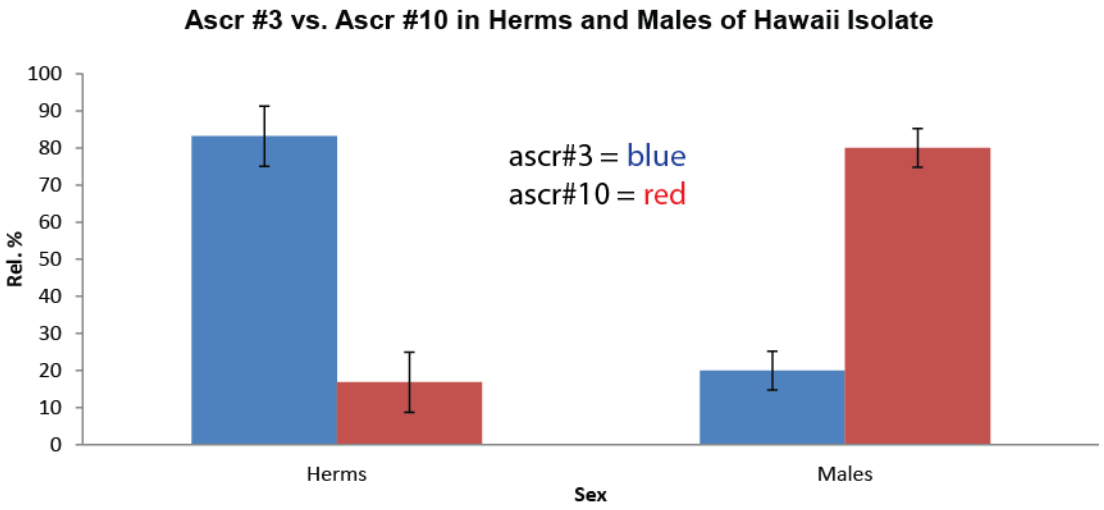

Figure S1. Relative abundances of ascr#10 and ascr#3 in males and hermaphrodites of *C. elegans* wild-type strain CB4856 (isolated at Hawaii). Error bars: SD.

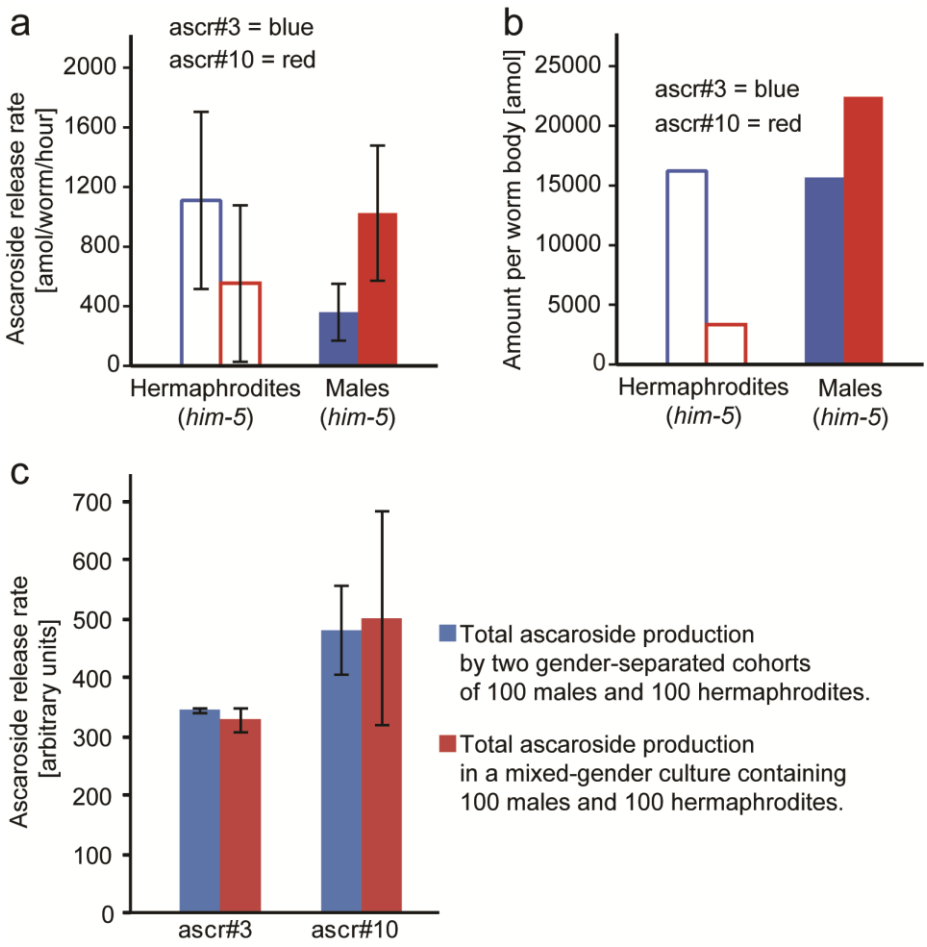

Figure S2. Ascaroside production in males and hermaphrodites (error bars: SD). a) Ascaroside release rates of *him-5* males and hermaphrodites. b) Ascaroside content of worm whole-body extracts (*him-5* males and hermaphrodites). c) Comparison of ascaroside production by separate and mixed cohorts of wild-type males and hermaphrodites. ascr#3 and ascr#10 production of a mixed cohort of males and hermaphrodites (red) is very similar to gender-averaged ascaroside production in separately incubated males and hermaphrodites (see Methods for details).

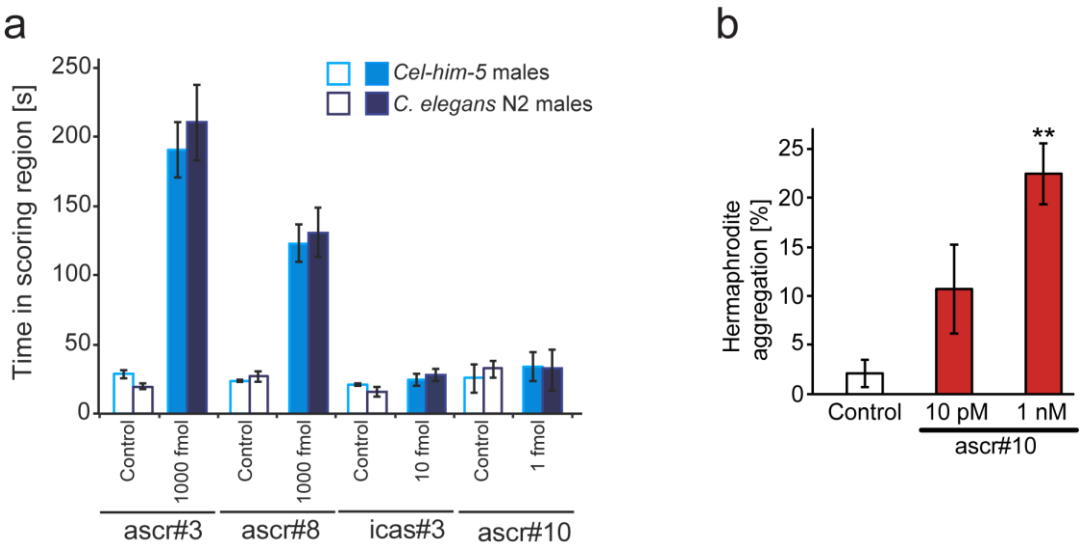

Figure S3. a) Comparison of ascaroside responses of *him-5* and wild-type males. Wild-type male responses to previously described male-specific attractants ascr#3 and ascr#8 and to the hermaphrodite-specific attractants icas#3 and ascr#10 are indistinguishable from those of *him-5* males.(*1*) icas#3 and ascr#10 were assayed at concentrations that elicit strong attraction or retention in hermaphrodites. b) Ascr#10 increases aggregation behavior of normally solitary N2 hermaphrodites;(*1*) one-factor ANOVA followed by Dunnett's post-test, *P < 0.05, **P < 0.01.

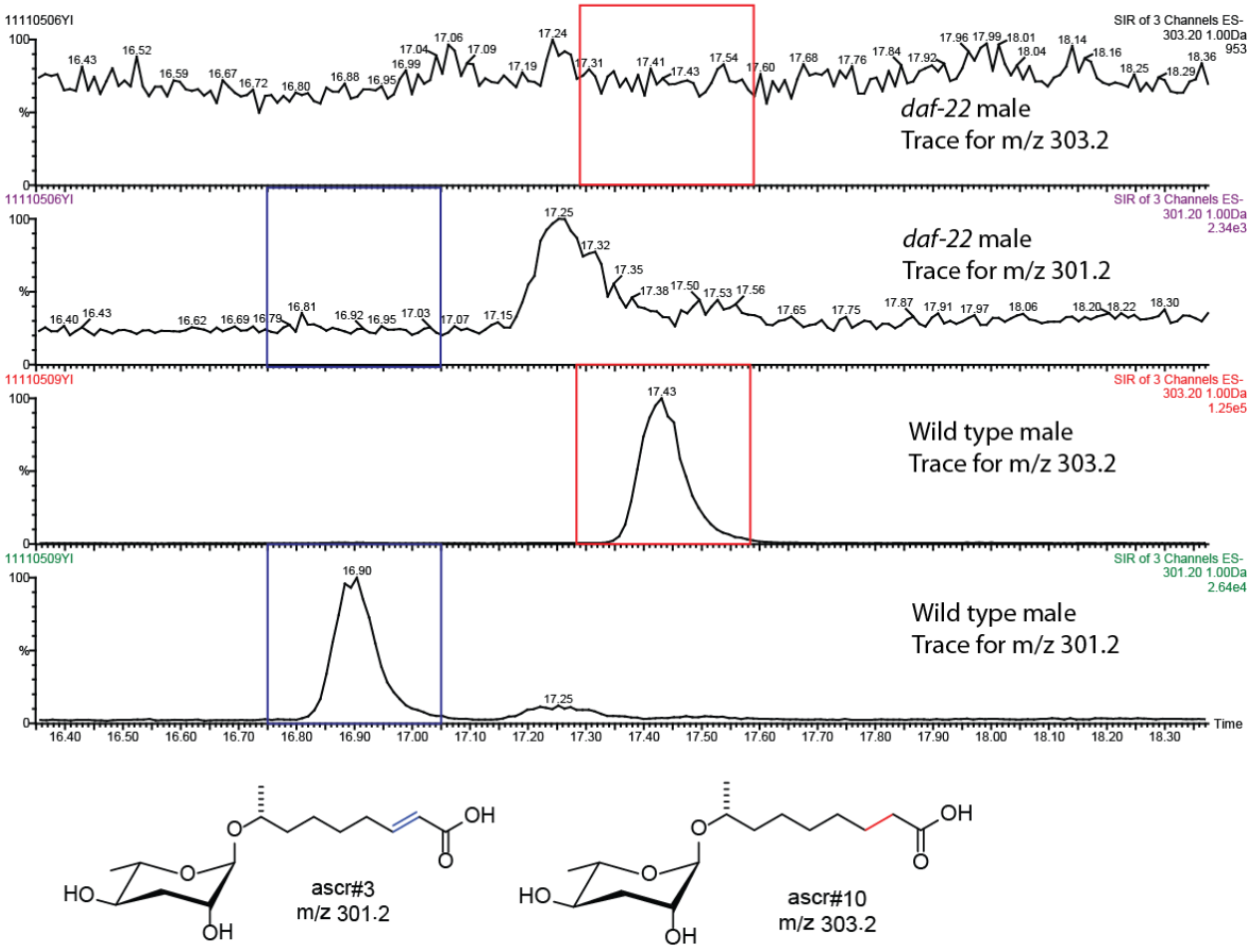

Figure S4. HPLC-MS analysis of *daf-22* male exometabolome. Shown are ion traces for *m/z* of 303.2 (ascr#10) and m/z of 301.2 (ascr#3) obtained via HPLC-MS analysis (ESI^-) of samples from 100 *daf-22* mutant males and 100 wild-type (N2 Bristol) males. Blue boxes highlight the retention time window for ascr#3 (16.90±0.02 min) and red boxes highlight the retention time window for ascr#10 (17.44±0.02 min).(*2*)

References

1. Srinivasan, J., von Reuss, S. H., Bose, N., Zaslaver, A., Mahanti, P., Ho, M. C., O'Doherty, O. G., Edison, A. S., Sternberg, P. W., and Schroeder, F. C. (2012) A Modular Library of Small Molecule Signals Regulates Social Behaviors in *Caenorhabditis elegans*, *PLoS Biol. 10*, e1001237.

2. 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.