This study used N2, the laboratory reference strain of C. elegans, which was obtained from the Caenorhabditis Genetics Center (CGC). We followed the general protocol published previously for obtaining liquid cultures of synchronized worms(1,2). This defines our biological replicate: A single L1 animal from a synchronized culture was placed onto an agar plate seeded with E. coli MG1655. This plate was grown until there were a large number of young gravid adult hermaphrodites (about 48 h at 24 °C. The plate was then washed into a 15 mL tube with M9 buffer, rinsed 3x with M9, and lysed with an alkaline hypochlorite solution until about 50% of the worms were dissolved (no more than 5 min). Then, M9 buffer was added to dilute the lysing solution, and the liquid was removed after gentle centrifugation at 580 g for 2 min to pellet the eggs without breaking them. This step was repeated 3x to completely remove the lysis solution. After the final rinse, eggs were resuspended in sterile water before a sucrose gradient to remove cellular debris and bacteria. An equal volume (5 mL) of 60% sucrose was added to the eggs in water and centrifuged at 350 g for 4 min. The eggs were rinsed to remove residual sucrose and once they hatched, approximately 200,000 animals were transferred to 20 mL of Scomplete with 2 mL of 50% MG1655. This material was grown to the desired developmental stage and prepared as described below.

The C. elegans cultures were synchronized, but they gradually lost synchrony over time. We collected samples at 5 different time points (T1-T5) in development. We report results using these time points rather than developmental larval stages, since they are not all pure stage cultures. The first time, T1, was collected immediately after hatching and was perfectly synchronized L1 animals, but as time progressed the cultures became more mixed. The other samples were collected at 22, 36, 49, and 90 hours (T2, T3, T4, and T5, respectively) after feeding the cultures. T5 was a mixture of adults, gravid adults, and offspring. Each of the five time points were replicated seven times. Stage-specific information can be recovered, even with samples that have lost synchrony. Our strategy was to obtain Biosorter data on each individual sample before homogenization. This information provides a population distribution and count for each sample, because the location of individual data points in a Biosorter dataset is related to the size and optical density of each worm.

- J. Srinivasan, F. Kaplan, R. Ajredini, C. Zachariah, H. T. Alborn, P. E. Teal, R. U. Malik, A.S. Edison, P. W. Sternberg and F. C. Schroeder: A blend of small molecules regulates both mating and development in Caenorhabditis elegans. Nature, 454(7208), 1115-8 (2008) doi:10.1038/nature07168
- F. Kaplan, D. V. Badri, C. Zachariah, R. Ajredini, F. J. Sandoval, S. Roje, L. H. Levine, F. Zhang, S. L. Robinette, H. T. Alborn, W. Zhao, M. Stadler, R. Nimalendran, A. T. Dossey, R. Bruschweiler, J. M. Vivanco and A. S. Edison: Bacterial attraction and quorum sensing inhibition in Caenorhabditis elegans exudates. J Chem Ecol, 35(8), 878-92 (2009) doi:10.1007/s10886-009-9670-0