

Electronic supplementary material (ESM)

Supplementary Figure Legends

Supplementary Figure 1. Vital dye staining of *Pristionchus pacificus*.

(a) Control *P. pacificus* imaged with Cy3, FITC, and DAPI filters, and a merge with Differential Interference Contrast (DIC). Histogram on the right represents quantification of intensity with each filter. (b) Same as (a) but stained with 0.005% Neutral Red, c, 50 μ M CellTracker Green Bodipy (Thermo Fischer), or (d) 50 μ M CellTracker Blue CMAC Dye (Thermo Fischer). J2s were stained (see Materials and Methods), and ensuing adult animals were imaged 3 days later on a Zeiss Axio Imager 2 with an Axiocam 506 mono, and processed using Zen2 pro software. Image brightness and contrast were enhanced in ImageJ for display, with a minimum displayed value of 10 and maximum of 100 for all images. Note that while Neutral Red and CellTracker Green staining are bright and specific to their respective channels, CellTracker Blue is indistinguishable from background fluorescence.

Supplementary Figure 2. Vital dye staining of *Caenorhabditis elegans*.

(a-d) Same as Supplementary Figure 1, but with *C. elegans*.

Supplementary Figure 3. Vital dye staining of *Pristionchus pacificus* dauers.

(a) Control *P. pacificus* dauer imaged with DIC, Cy3, and FITC filters. (b) Dauers stained

with either 0.005% Neutral Red or 50 μ M CellTracker Green Bodipy and imaged immediately after staining with DIC, Cy3, and FITC filters and merged with DIC. Images were taken using Zeiss Axio Imager 2 with an Axiocam 506 mono, processed using Zen2pro software, and adjusted in ImageJ, with a display value minimum of 21 and maximum of 117.

Supplementary Figure 4. Vital dye staining lasts several days through development.

(a) 50 μ M Cell Tracker Green Bodipy (GB) and (b) 0.005% Neutral Red (NR)-stained J2s were imaged every day for five days. Percent of individuals retaining the dyes are shown in panels (c-g) for each day. Both stains are seen in all organisms for three days; NR persists for at least five, while the number of stained GB drops on day four. All images are merged with DIC, n=31 GB, 63 NR day 1, 68 GB, 56 NR day 2, 50 GB, 50 NR day 3, 50 GB, 50 NR day 4, 50 GB, 50 NR day 5.

Supplementary Figure 5. Vital dye staining does not affect *P. pacificus* mouth form or development.

(a) Neutral Red and CellTracker Green Bodipy-stained J2s reach adulthood at the same rate as unstained J2s (3 days). (b) All of the J2s stained retain the dye in adulthood in the intestine. (c) Neither dye affects mouth form; both unstained and stained worms remain 100% St (n=30). (d-f) Same as for (a-c) except with dauers instead of J2s, and only with Neutral Red.

Supplementary Figure 6. Table of binomial regression *p* values for vital-dye method and excess crowding.

(a) p values from binomial regression of vital-dye method for age and number added. (b) p values from binomial regression of number reaching adult and Eu counts for each number of individuals added relative to 1,000 individuals added.

Supplementary Figure 7. Pheromone profiling quality control.

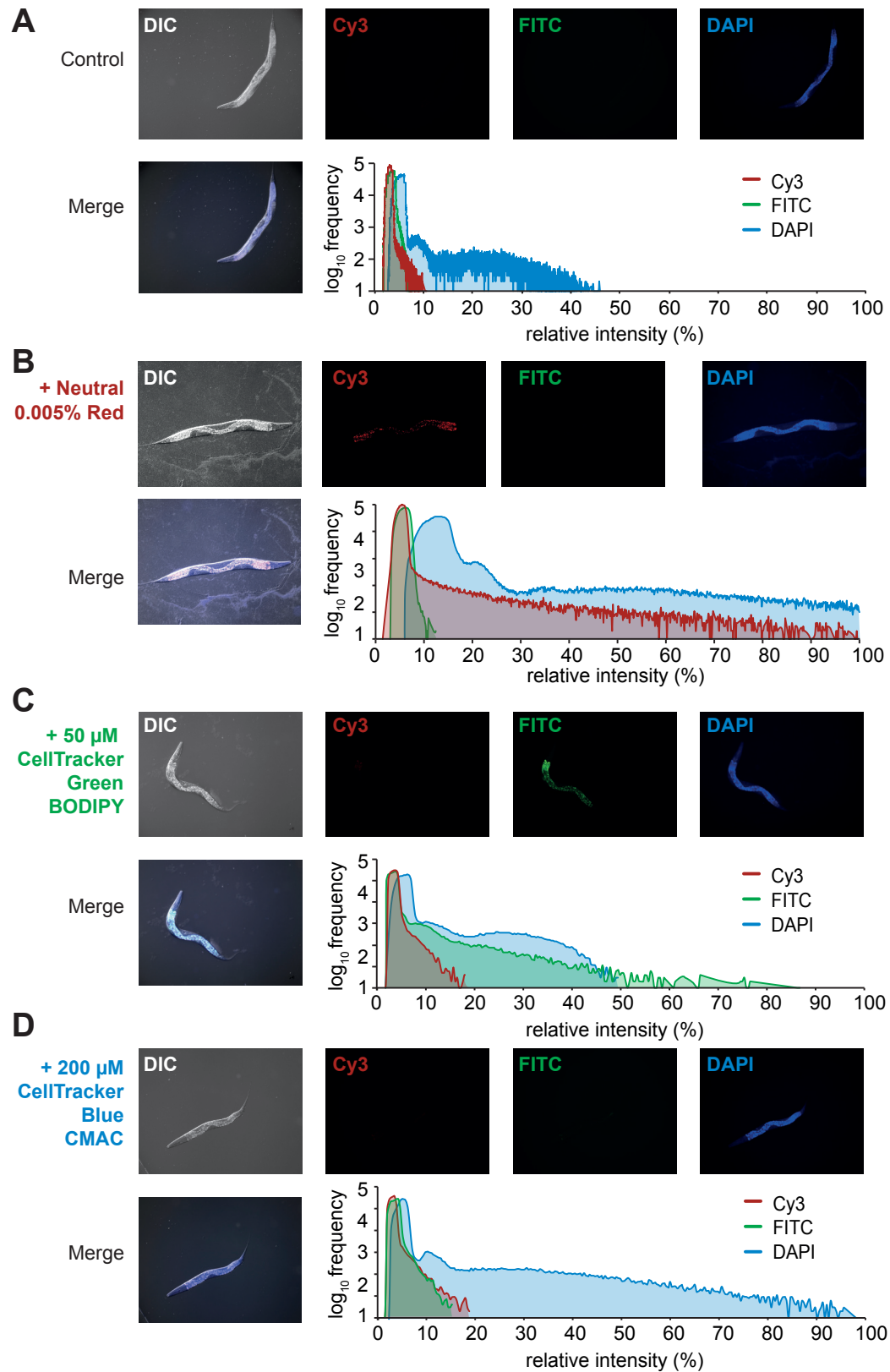
(a) Extracted ion traces (width 0.1 m/z) of 11 of the 12 NDMMs used in this publication from a seven-day mixed-stage sample, double peak of 247.12 m/z indicate isomeric structures (Part#9/Ascr#9). (b) Example of an averaged spectrum over a calibration segment, sodium-formiat cluster building solution has been used to ensure high mass accuracy in each run. (c) Comparison of an endometabolome sample from a seven day mixed-stage cultured compared to the endometabolome of eggs, produced by using bleached eggs from 80 x 60 mm plates.

Supplementary Figure 8. Table of linear regression p values with FDR corrections for strain and stage comparison of NDMM levels. (a) FDR-corrected and uncorrected p values from linear regression. Red values indicate $FDR < 0.05$. (b) Pairwise comparison of dasc#1, npar#1, and ascr#9 using a two-tailed student's t -test.

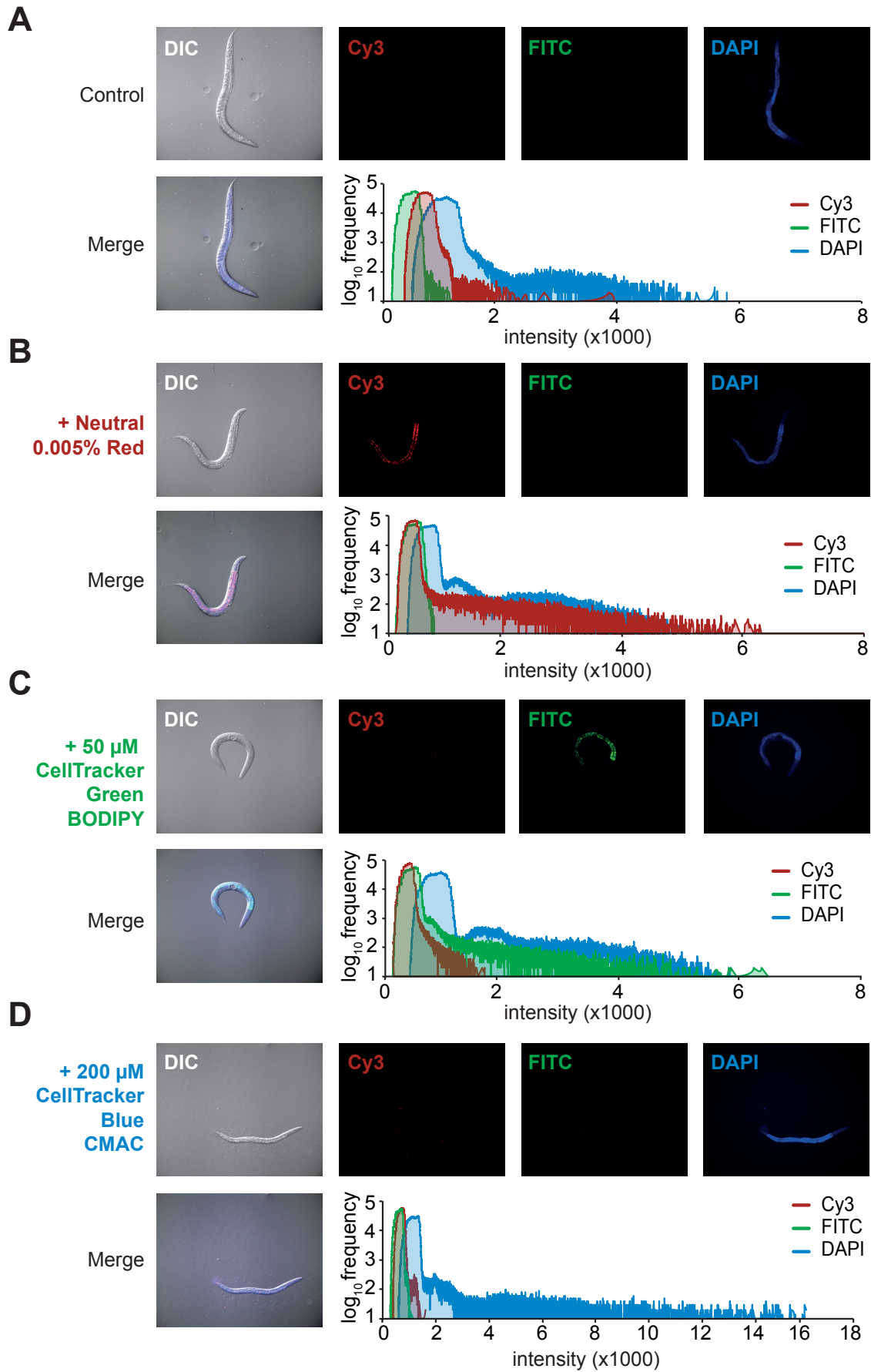
Supplementary Figure 9. Enzyme that synthesize NDMMs is transcriptionally regulated during development. Comparison of *daf-22.1* and *daf-22.2* expression (FPKM) by RNA-seq through different stages of development, data from Baskaran et al., 2015 [45].

Supplementary Figure 10. Ecological rationale for age-specific influence on mouth form.

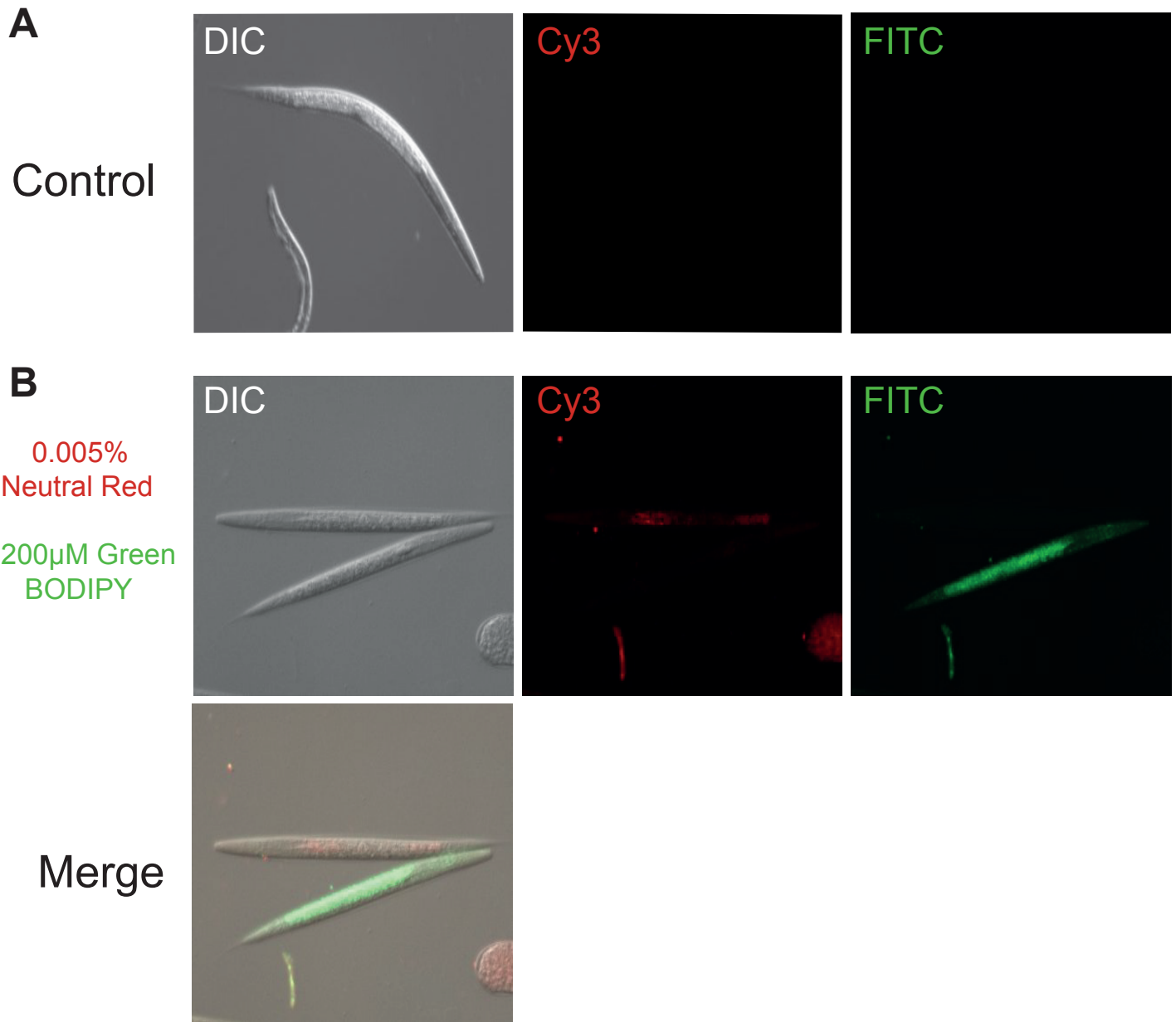
(a-b) Conceptual life cycle models of (a) monomorphic or (b) dimorphic mouth form nematodes. At some point microbial food supplies will run out, leading to a Malthusian catastrophe. Nematodes temporarily escape this trap by entering the dauer state, hitchhiking to a new insect carrier, and re-starting the cycle. Dimorphic nematodes can sense the impending catastrophe earlier by recognizing an abundance of adults in the population, switching to the Eu morph, and exploiting new resources. By analogy to economic models, dauer and mouth form are technological innovations to escape resource traps.



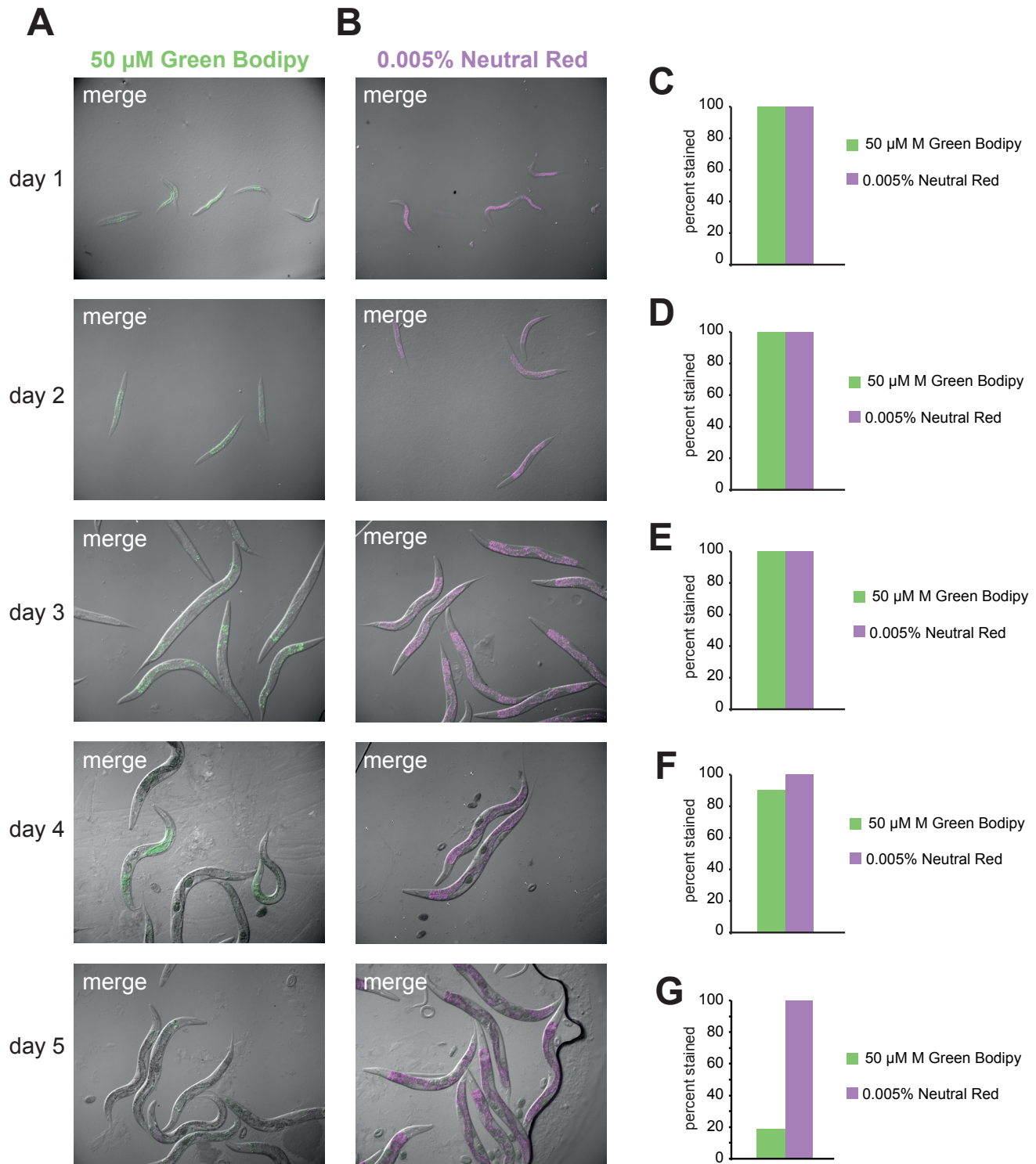
Supplementary figure 1. Vital dye staining of *Pristionchus pacificus*.



Supplementary figure 2. Vital dye staining of *Caenorhabditis elegans*.



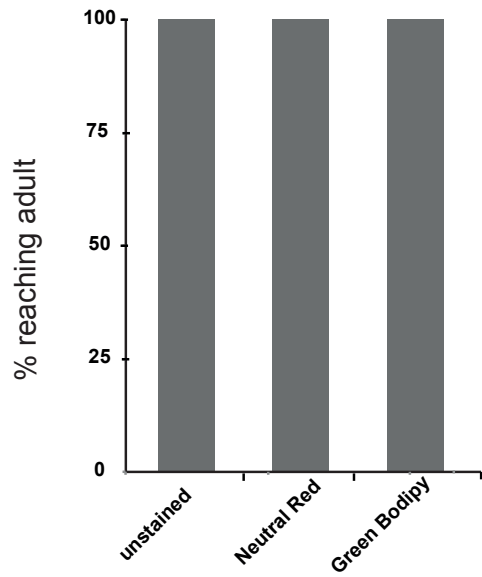
Supplementary figure 3. Vital dye staining of *Pristionchus pacificus* dauers.



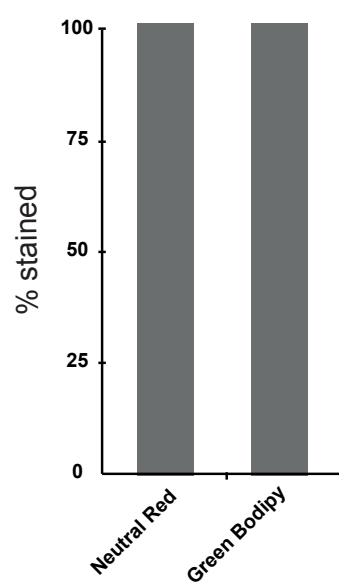
Supplementary figure 4. Vital dye staining lasts several days through development.

J2

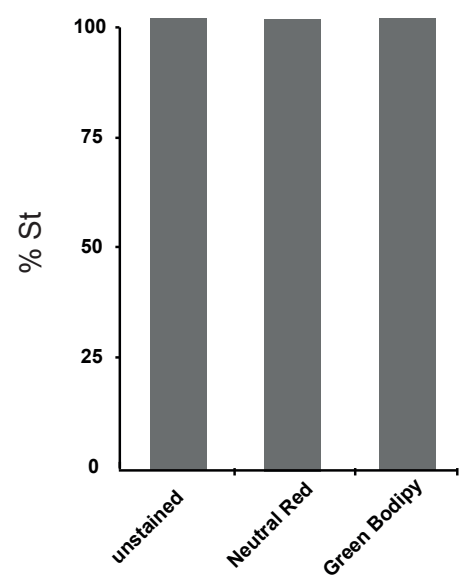
A



B

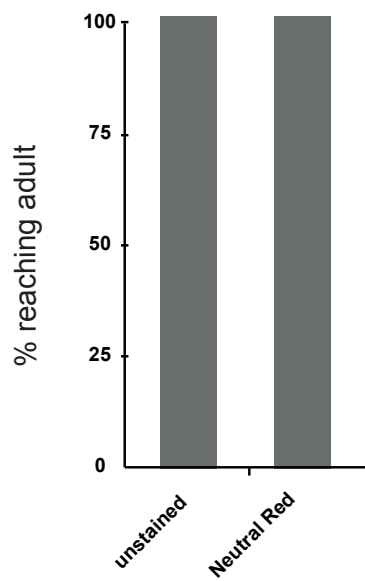


C

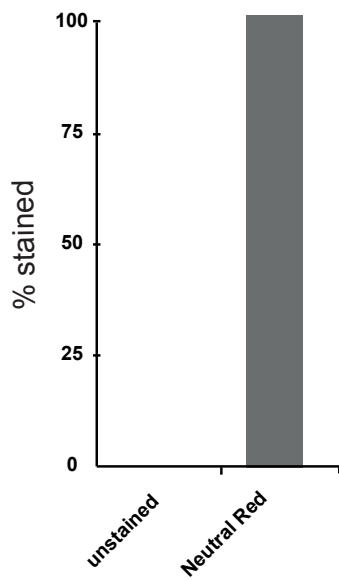


Dauer

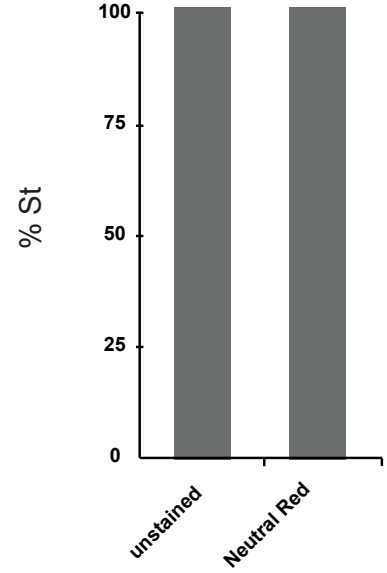
D



E



F



Supplementary figure 5. Vital dye staining does not affect *P. pacificus* mouth form or development.

A

binomial regression	<i>p</i> value J2s	<i>p</i> value dauers
age added	0.0259	0.002955
number added	4.28e-13	0.000404

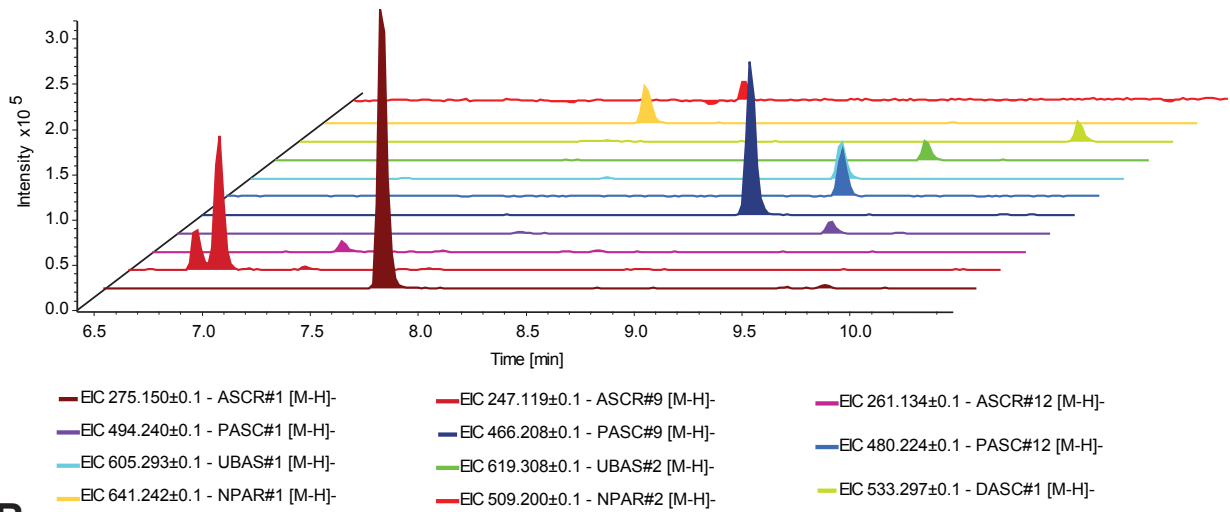
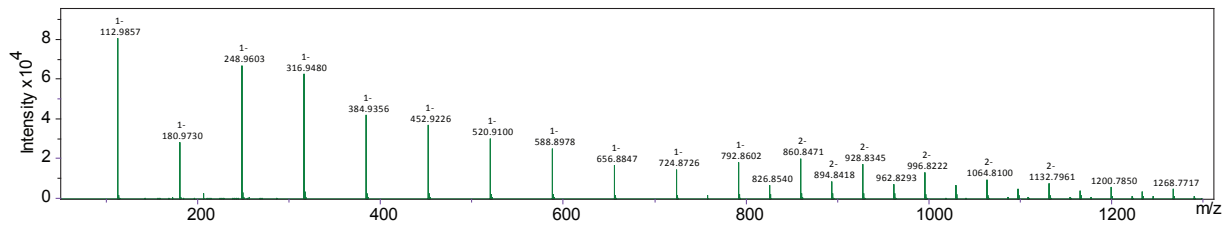
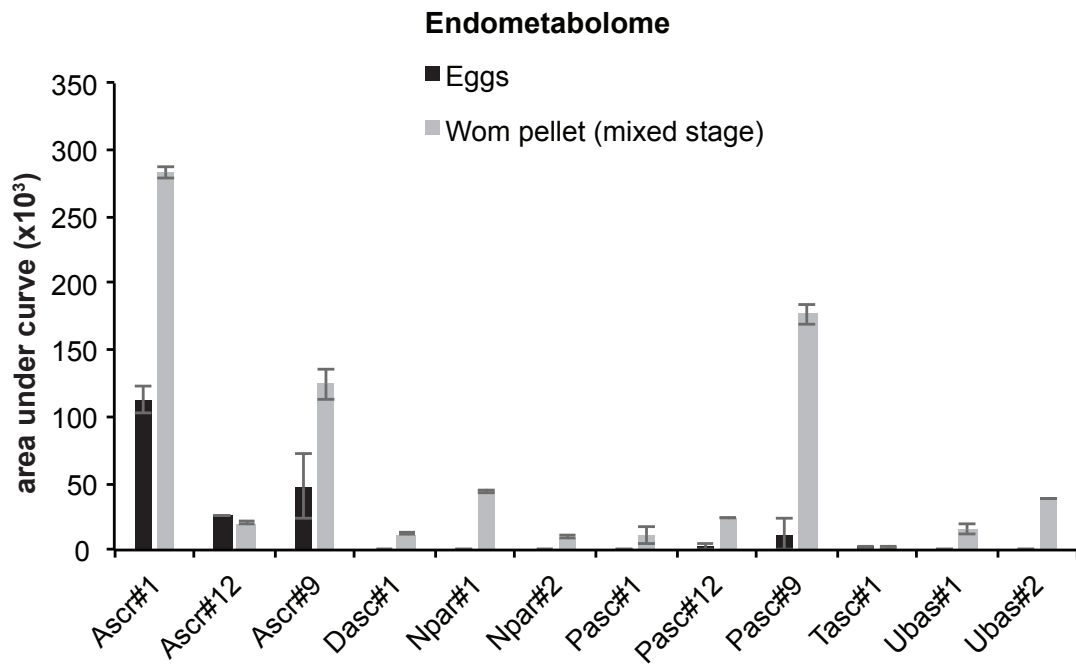
affect of population age on mouth form of developing juveniles

B

binomial regression	<i>p</i> value for development (relative to 1,000)	<i>p</i> value for Eu (relative to 1,000)
3,000 J2s added	0.3408	1.0
4,000 J2s added	0.0424	1.0
5,000 J2s added	6.06E-14	0.99
10,000 J2s added	4.09E-14	0.99

affect of number of peers on development and mouth form (proxy for potential starvation effects on mouth form)

Supplementary figure 6. Table of binomial regression *p* values for vital-dye method and excess crowding.

A**B****C**

Supplementary Figure 7. Pheromone profiling quality control.

A

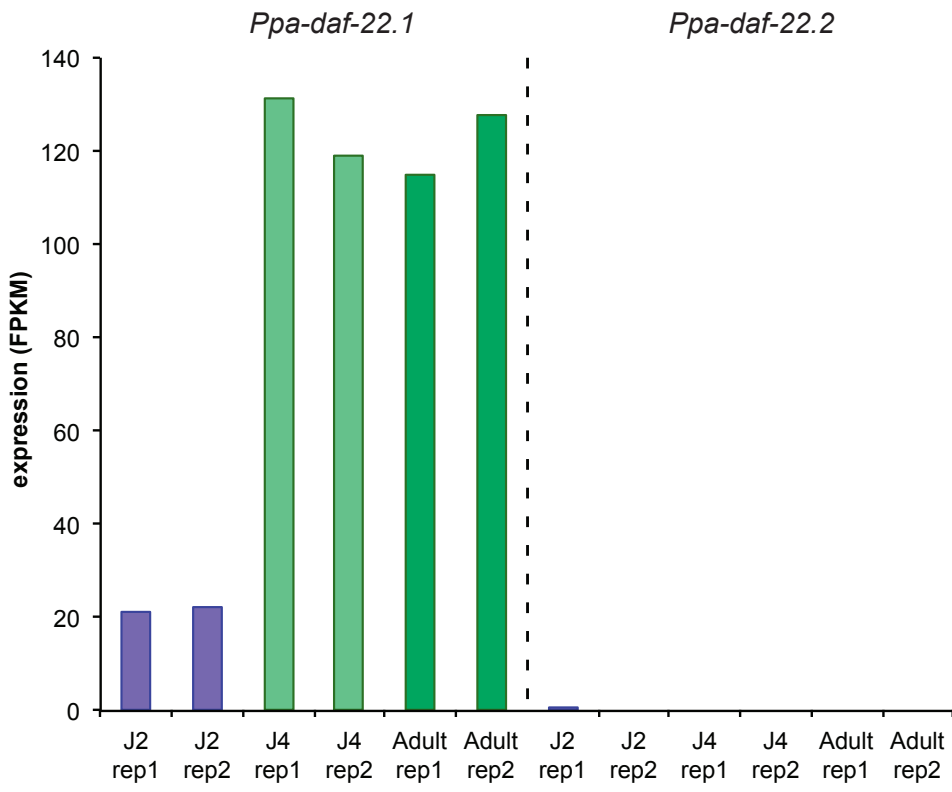
NDMM comparison	pvalue	fdr corrected
ascr1_stage	0.4733	0.774490909
ascr1_strain	0.0429	0.110314286
ascr1_stage:strain	0.031	0.085846154
ascr9_stage	3.79E-05	0.0002274
ascr9_strain	0.651	0.778064516
ascr9_stage:strain	0.272	0.50148
ascr12_stage	0.0029	0.01404
ascr12_strain	0.0897	0.201825
ascr12_stage:strain	0.0302	0.085846154
dasc1_stage	9.62E-08	8.66E-07
dasc1_strain	0.11363	0.240628235
dasc1_stage:strain	0.00351	0.01404
npar1_stage	0.0033	0.01404
npar1_strain	0.9426	0.984
npar1_stage:strain	0.6355	0.778064516
npar2_stage	0.0516	0.12384
npar_2strain	0.984	0.984
npar2_stage:strain	0.9716	0.984
pasc1_stage	0.449	0.769714286
pasc1_strain	0.753	0.847125
pasc1_stage:strain	0.564	0.778064516
pasc9_stage	0.616	0.778064516
pasc9_strain	0.267	0.50148
pasc9_stage:strain	0.523	0.778064516
pasc12_stage	0.6122	0.778064516
pasc12_strain	0.2786	0.50148
pasc12_stage:strain	0.67	0.778064516
tasc1_stage	0.522	0.778064516
tasc1_strain	0.862	0.940363636
tasc1_stage:strain	0.57	0.778064516
ubas1_stage	3.13E-12	1.13E-10
ubas1_strain	0.00538	0.019368
ubas1_stage:strain	6.69E-08	8.03E-07
ubas2_stage	1.34E-11	2.41E-10
ubas2_strain	0.00711	0.023269091
ubas2_stage:strain	6.18E-07	4.45E-06

B

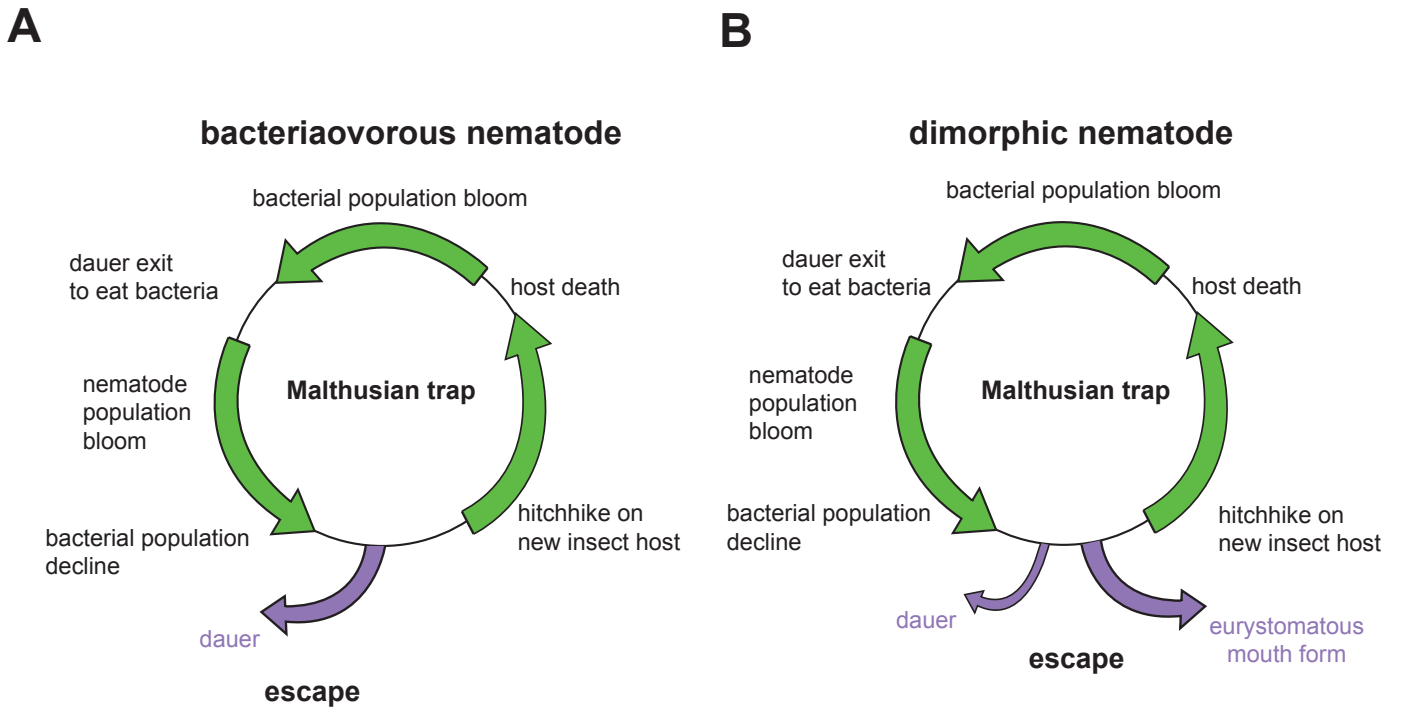
RS2333	dasc#1	npar#1	ascr#9
72 hrs compared to 24 hrs	5.7511E-07	3.4672E-05	0.00010345
72 hrs compared to 48 hrs	0.00571003	0.17579174	0.19705545
RSC017	dasc#1	npar#1	ascr#9
72 hrs compared to 24 hrs	0.02548973	0.00365597	0.02028689
72 hrs compared to 48 hrs	0.21178072	0.36578067	0.10414329

Supplementary Figure 8. Table of linear regression p values with FDR correction for strain and stage comparison of NDMM levels.

A



Supplementary Figure 9. Enzyme that synthesizes NDMMs is transcriptionally regulated during development.



Supplementary Figure 10. Ecological rationale for age-specific influence on mouth form.