Appendixes

Modifications to synchronized worm hatch

All worms were handled using standard growth and cultivation techniques (Lewis, 1995). Worms were synchronized at the hatch as described by (Baugh et al., 2009) with changes as follows. Embryos of the second bleach were seeded onto 10 cm NGM plates and topped with a 1:1 (v/v) mixture of OP50 and S complete medium (Sulston and Brenner, 1974) and incubated in a 20°C incubator. This drying step helps keeping un-hatched eggs from re-suspending after the wash. Sixteen hours after the bleach, plates were inspected for embryos only and plates containing hatched worms were discarded. Plates were incubated for a further hour and then washed with S basal to suspend all hatched worms. Less than 1% of eggs were found after this wash. Pooled worms were washed once in S complete and used for subsequent experiments.

Purification of RNA

100 µl of acid washed (1M HCl) sand was aliquoted into eppendorf tubes. Frozen worms in TRIzol were thawed (Start volume= TRIzol + worms = 1 ml; SV), transferred to sand containing tubes and vortexed vigorously for 10 min. Tubes were allowed to settle at room temperature for 5 min. 20% SV of $ChCl_3$, and TRIzol solution were aliquoted into fresh tubes mixed thoroughly for 15 sec and centrifuged at 12000 RCF for 15 minutes 2-8°c. Aqueous phase was transferred to new tubes mixed with 50% SV of 100% Isopropanol and let precipitate at -20°c overnight. Samples were centrifuged at 12000 RCF for 10

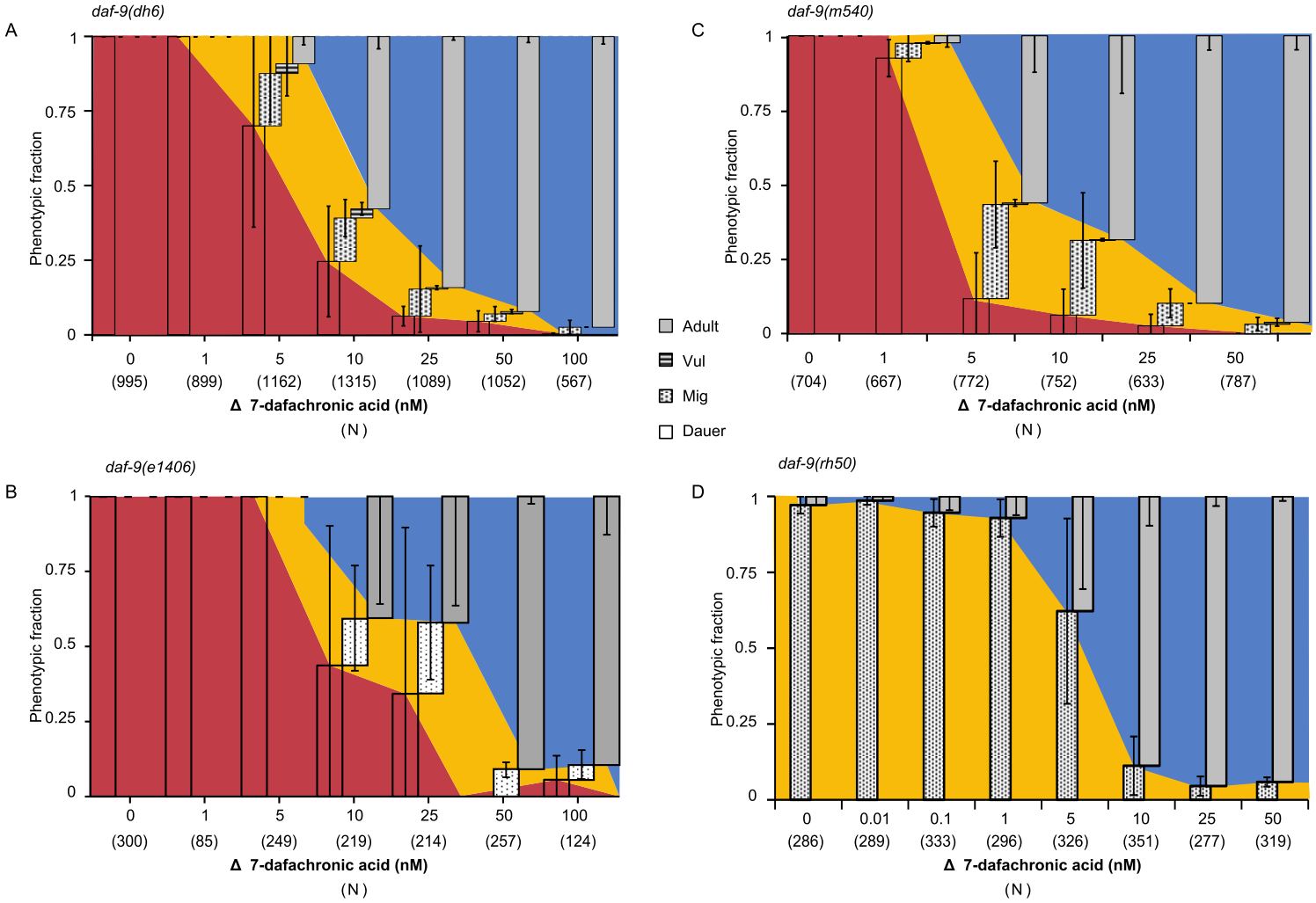
min 4°c, pellet was washed with 1x SV of 75% (v/v) Ethanol (in DEPC-H_2O) and EtOH remainders were aspirated. Pellets were resuspended in 10% SV of DEPC treated DDW.

Computational worm straightening

Each worm was outlined on the DIC image and a central axis was drawn through all bent segments. Pixels on the perpendicular line between the central axis and the worm boundary were recorded and added to the previous section until the entire worm was rendered in a straight alignment. Since each worm had a representative image in DIC and in fluorescence imaging, the same coordinate system per central axis and worm boundary was applied to both images and the same computation was performed to retrieve fluorescence data. Each worm renders an array of coordinate pixels which were transformed into mean grey value (ranging between 0, black to 255, white).

Supplementary Figures

Figure S2.1

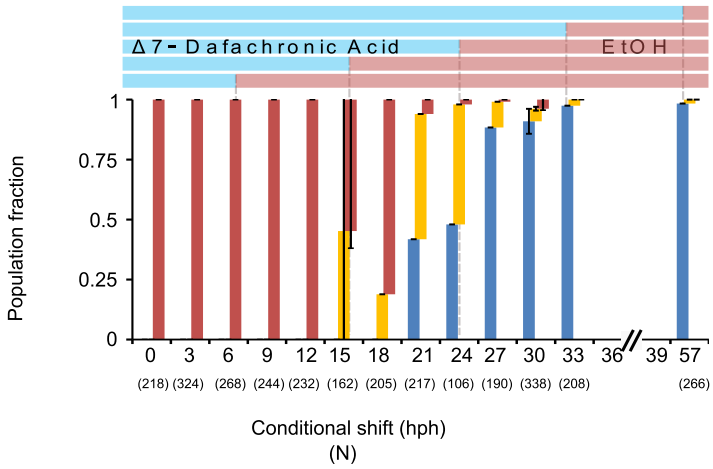

A
daf-9(dh6)
Phenotypic fraction
1
0.75
0.5
0.25
0
0 1 5 10 25 50 100
(995) (899) (1162) (1315) (1089) (1052) (567)
Δ 7-dafachronic acid (nM)
(N)
B
daf-9(e1406)
Phenotypic fraction
0 1 5 10 25 50 100
(300) (85) (249) (219) (214) (257) (124)
Δ 7-dafachronic acid (nM)
(N)
C
daf-9(m540)
Phenotypic fraction
0 1 5 10 25 50
(704) (667) (772) (752) (633) (787)
Δ 7-dafachronic acid (nM)
(N)
Adult
Vul
Mig
Dauer
D
daf-9(rh50)
Phenotypic fraction
0 0.01 0.1 1 5 10 25 50
(286) (289) (333) (296) (326) (351) (277) (319)
Δ 7-dafachronic acid (nM)
(N)

S1.1: High amounts of DA are required for complete adult development

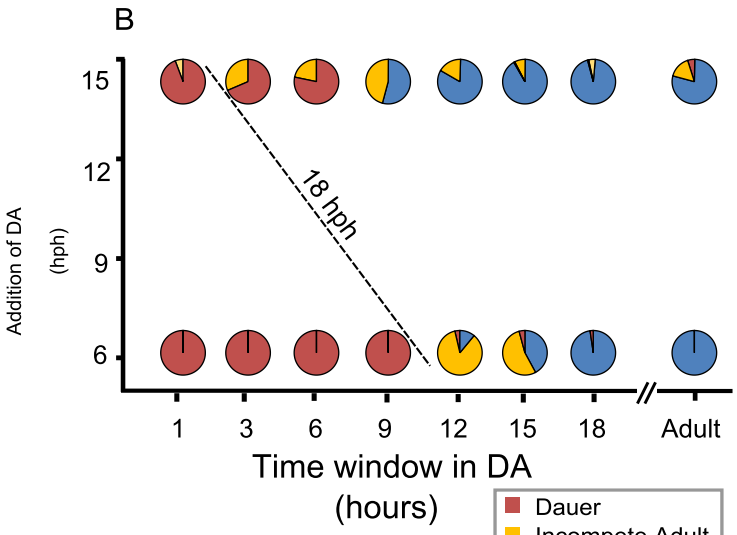
Distributions of dauer (transparent), Mig (dotted), Vul (straight lines) cut (hatched) and wild type adult (grey) phenotypes when mutants were hatched after a non-synchronous bleach (worms typically hatch over a 15 hour window at these growth conditions). (A) *daf-9(dh6)*, (B) *e1406 daf-9* (C)*(m540)* and (D) *daf-9(rh50)* strains. Red represents all dauers, yellow represents all incomplete adult phenotypes and blue represents all complete adults. Bars represent means ± standard deviations across three biological experiments. Numbers in parentheses indicate total worms counted per time point.

Figure S1.2

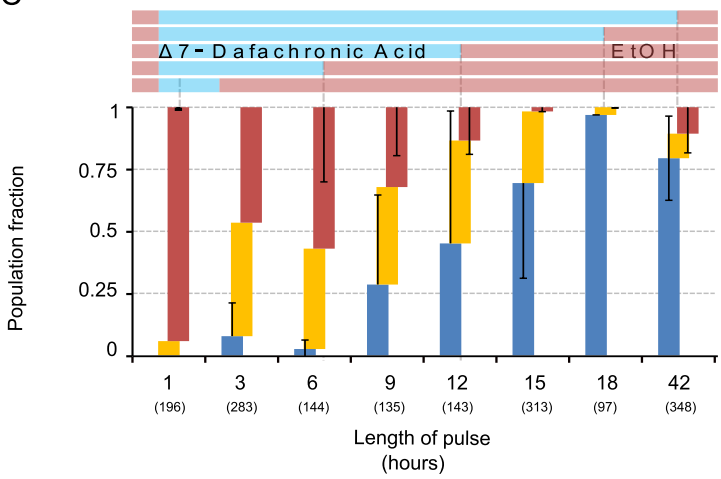
A



B



C



D

	Addition of DA (hph)					
	daf-9(e1406)		*daf-9(dh6)*			
	6	**15**	**6**	**9**	**12**	**15**
1	293	196	205	248	173	265
3	290	283	119	268	91	335
6	276	144	129	185	59	331
9	216	135	62	133	54	369
12	64	143	135	164	108	383
15	82	313	40	147	142	454
18	95	97	117	242	175	192
adult	280	348	143	237	235	360

S1.2: Temporal activity of Δ7-DA.

(A) *daf-9(e1406)* worms start responding to ∆7-DA at 15 hph and require an additional 12-15 hours of ∆7-DA for complete adult development. Top; representative colored bars indicating the shift experiment: red bars indicate EtOH carrier and blue bars indicate Δ7-DA. Bottom; histograms indicate proportions of phenotype frequencies between biological replicates ± standard deviations. (B) Pie charts indicate proportions of dauers (red), incomplete adults (yellow) and complete adults (blue) as a function of total amount of time exposed to Δ7-DA (x-axis) when exposed to Δ7-DA at different hours post hatch (y-axis). (C) Pulse experiments indicate minimal times necessary for complete development. Top; diagram of pulses used per experiment. Bottom, bar graphs indicate proportions of phenotype frequencies between biological replicates ± standard deviations. (D) Number of worms scored for initiation of response to DA. d*af-9(e1406)* indicates the number of worms scored for Figure S4B and *daf-9(dh6)* indicates the number of worms scored for Figure 4B.

Supplementary Tables

Supplementary Table 1: Values of q statistic calculated by a Tukey type multiple comparison test for differences among variances.

Variances are arranged in ascending order from left to right and from top to bottom. This test takes the difference in natural logarithms of variance values of each time point and normalizes it to a standard error of $\sqrt{\frac{2}{k-1}}$ where k are the number of biological replicates at each time point. Significant differences in pair-wise comparisons of variances which are larger than $q_{,0.05,\infty,k}$ are marked in red and non-significant differences are marked in green. Numbers indicate the computed q statistic. Table 1 shows the computed Tukey type multiple comparison test for differences among variances for shift to growth experiment (Figure 2. 2B), Table 2 shows comparisons for Shift to stringent experiment (Figure 2.2C) and Table 3 shows comparisons of *daf-9(dh6)* shift from EtOH to Δ7-Dafachroninc acid (Figure 3.2C).

Table 1A

	C	**27**	**D**	**30**	**39**	**36**	**33**
24	1.2183	2.9516	4.0405	4.7329	5.4368	8.5794	10.1991
C		1.7333	2.8222	3.5146	4.2185	7.3611	8.9808
27		`	1.0888	1.7812	2.4851	5.6277	7.2474
D				0.6923	1.3963	4.5389	1.3963
30					0.70392	3.8465	5.4661
39						3.1425	4.7622
36							1.6196

Table 1B

	W24+9	W24+1	W24+6	W24+3
W24	0.4254	1.2938	1.3975	4.0812
W24+9		0.8999	1.1340	3.6558
W24+1			0.1499	2.4846
W24+6				2.5217

	39	27	30	0	24	21	36	33
None	-4.1058	-2.83804	-2.33764	-0.5823	0.8135	2.2849	4.2021	5.3723
39		1.267766	1.768167	3.5235	4.9193	6.3907	8.3079	9.4781
27			0.500401	2.2557	3.6515	5.1229	7.0401	8.2104
30				1.7553	3.1511	4.6225	6.5397	7.71
0					1.3958	2.8672	4.7844	5.9546
24						1.4714	3.3886	4.5588
21							1.9172	3.0874
36								1.1702

Table 1C

Table 2: Oligonuclotides used in quantification of daf-9 transcripts**.**

Gene	Expression	Spans exons	Forward Primer	Reverse Primer	Amplicon length
daf-9	XXXL/R, Hypodermis	9-10	tcagacgccgtatgtgagag	gctgggataatctcggtgtt	140
daf-9.1a	XXXL/R, Hypodermis	2,4	aaacgaatgtccagtttggtg	ttcgaagtcagggaccactt	183
daf-9.1b	XXXL/R, Hypodermis	3,4	tgagagagcctccatttggt	ttcgaagtcagggaccactt	157
pmp-3	Intestine	3,4	gttcccgtgttcatcactcat	acaccgtcgagaagctgtaga	115
Y45F10D.4	Unknown	1,2	gtcgcttcaaatcagttcagc	gttcttgtcaagtgatccgaca	139
ver-2	ADLL/R	7,8	tgtgacattcgccacaaaat	aaaaactcggcgtttgtttg	178