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Supplemental Information

Transgenerational Sterility of Piwi Mutants

Represents a Dynamic Form

of Adult Reproductive Diapause

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SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Strains used include Bristol N2 wild type, *dpy-5(e61) I*, *unc-13(e450) I*, *prg-1(n4357) I*, *prg-1(tm876) I*, *cplIs36[Pmex-5::mNeonGreen::3xFlag::AraD::tbb-2 3'UTR + unc-119(+)] II*; *unc-119(ed3) III*, *unc-29(e193) I*, *daf-16(mg50) I*, *daf-16(mu86) I*, *hsf-1 (sy441) I*, *nhr-49 (nr2041) I*, *nhr-49 (ok2165) I*, *cep-1 (w40)*, *cep-1 (gk130) I*, *daf-2 (e1368) III*, *daf-2(e1370) III*, *daf-2(m41) III*, *ced-4(n1162) III*, *dpy-17(e164) III*, *daf-18(e1375)*, *daf-18(ok480) IV*, *dpy-9(e12) IV*, *unc-24(e120) IV*, *ced-3(n717) IV*, *crt-1 (jh101)*, *mut-7 (pk204) III*, *Pced-1::ced-1::gfp*.

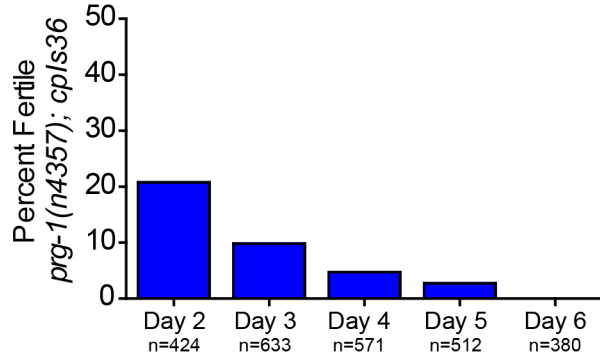
Note that we previously found that sterile generation *prg-1* mutant adults that were sterile on the second day of adulthood remained sterile when grown on standard NGM plates seeded with OP50 bacteria. Fertility could be restored for a fraction of these animals that were placed specifically on RNAi plates expressing dsRNA targeting *daf-2* or the downstream PI3 kinase subunit *age-1*, but fertility did not occur on vector control RNAi plates (Simon et al., 2014). These results contrast with our current results that show that complete sterility does not occur for sterile-generation *prg-1* adults until Day 5 of adulthood, and that fertility can be restored to a small fraction of Day 5 sterile adults that are placed on RNAi plates containing any type of RNAi bacteria. We do not have an explanation for these differences, but note that our present results have been independently repeated using numerous *prg-1* mutants lines and high n values per experiment, suggesting that the observed differences could be due to changes in the metabolic environment in the lab (Schulenburg and Felix, 2017).

SUPPLEMENTAL REFERENCES

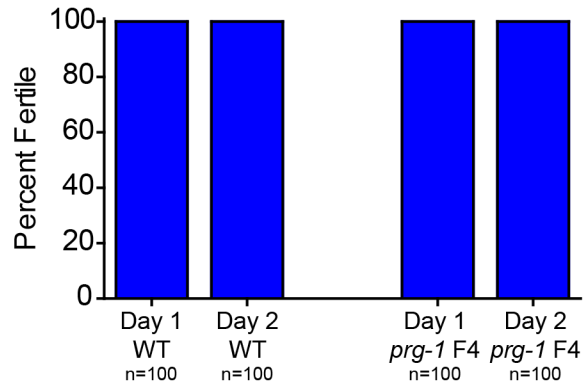
Schulenburg, H., and Felix, M.A. (2017). The Natural Biotic Environment of *Caenorhabditis elegans*. *Genetics* 206, 55-86.

Supplemental Figure 1

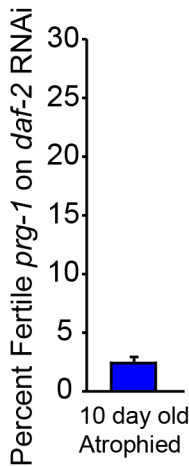
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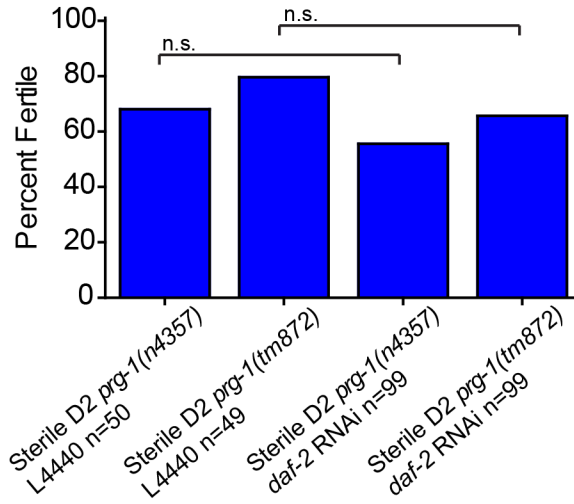
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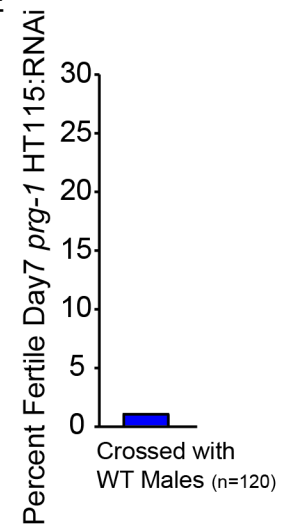
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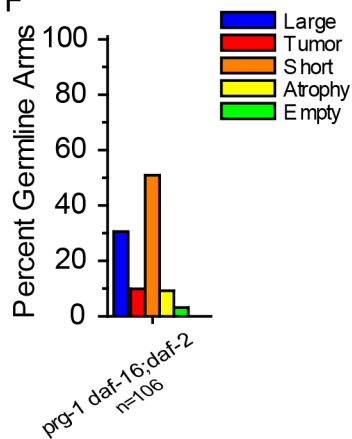
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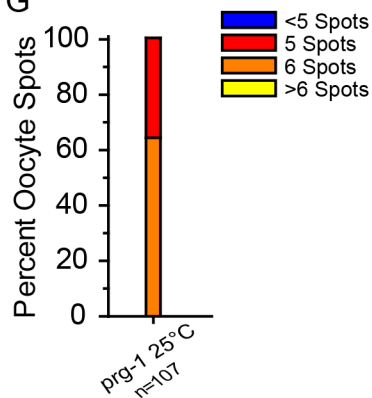
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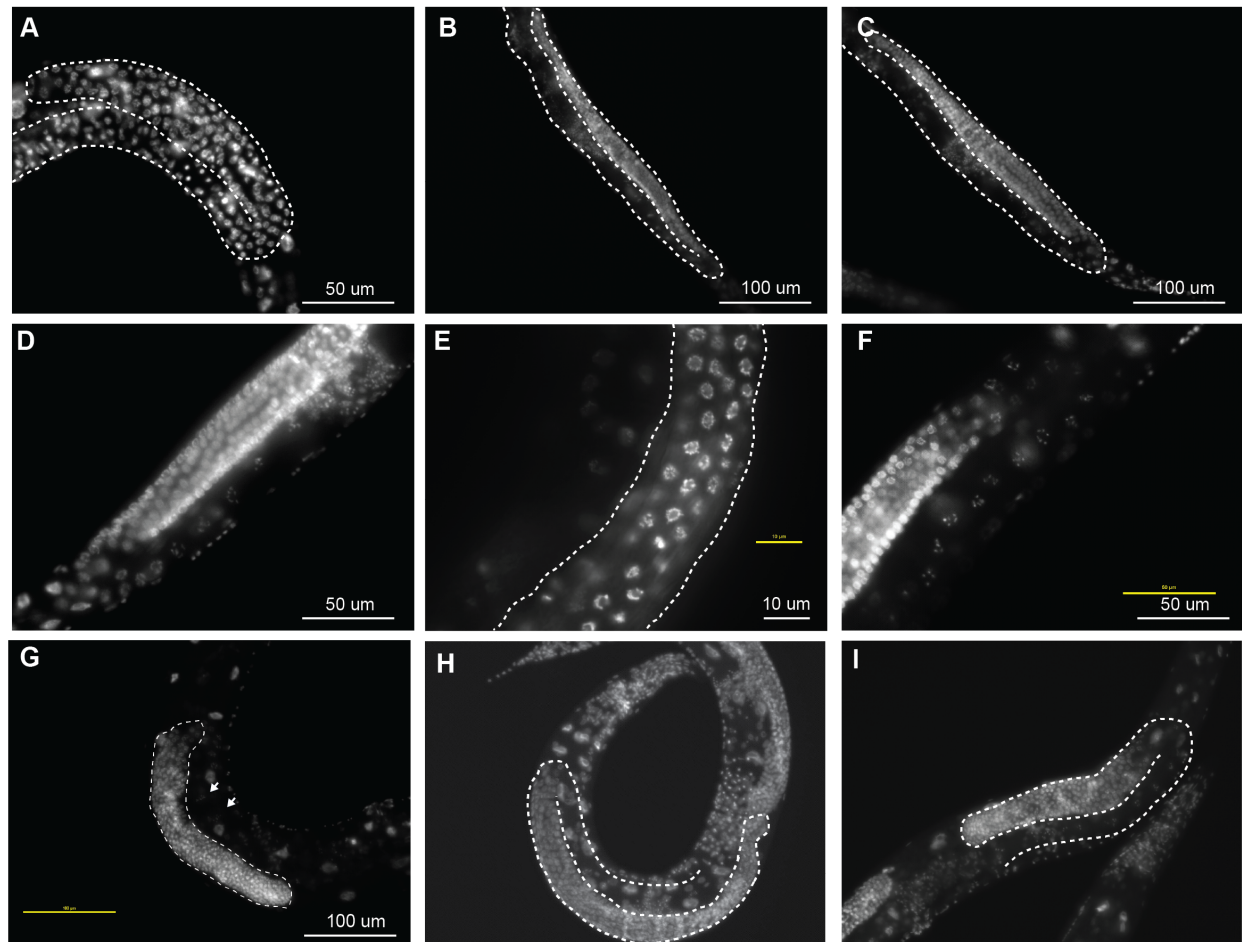
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Supplemental Figure S1. Related to Figures 1 and 5. Fertility of *prg-1* mutants. (A) Sterile Day one *prg-1*; *cpls36* mutants reverse sterility through the first five days of adulthood. (B) Early generation *prg-1* mutants (F4) are completely fertile. (C) *prg-1* mutants who had been sterile for ten days become fertile on RNAi bacteria targeting

daf-2. Error bars are S.E.M. (D) Percent fertile of 2 alleles of Day 2 sterile *prg-1*. n=total worms scored. *daf-2* RNAi had no significant effect on percent fertile (p=n.s., Chi-square test with Bonferroni correction). (E) Crossing with wild-type males did not enhance reversal of sterility of sterile Day 7 *prg-1* mutants in the HT115 RNAi condition. (F) Germline profile of *prg-1 daf-16; daf-2* Day 1 sterile mutants. (G) Number of DAPI spots in sterile Day 1 *prg-1* mutant worms at 25°C.

Supplemental Figure 2



Supplemental Figure S2. Related to Figure 2. Germline atrophy in sterile *prg-1* animals. DAPI-stained photos of (A) a *prg-1* early generation L4, (B) *prg-1* early generation fertile adult, (C) a N2 wild-type adult, (D) a *prg-1* early generation adult with mitotic-meiotic transition and normal oocyte production, (E) mitotic cells in a likely sterile *prg-1* L4, (F) a rare sterile *prg-1* animal with intact germline lacking embryo production, (G) a *prg-1 daf-16* sterile adult with oocytes indicated by arrows, (H) N2 wild-type germline showing full bend, (I) sterile *prg-1* large germline showing early bend.

p values determined using Chi-squared with Bonferroni correction

	N2 (n=87)	arg-1 (n=483)	arg-1, ccd-3 (n=310)	arg-1, ccd-4 (n=552)	arg-1, csp-1 (n=228)	arg-1, nhr-49 (n=194)	arg-1, hsf-1 (n=168)	arg-1, daf-16 (n=169)	arg-1, daf-18 (n=377)	arg-1, daf-16, ccd-4 (n=252)	arg-1, nhr-49, ccd-4 (n=165)	arg-1, csp-1, daf-18 (n=180)
N2 (n=87)	1.04743E-73	3.21872E-26	8.62893E-30	1.18329E-13	4.24406E-36	9.34429E-32	9.08427E-32	4.37486E-51	2.39832E-52	6.07129E-30	1.28655E-08	
arg-1 (n=483)	1.04743E-73	9.75882E-20	1.91748E-07	1.09518E-44	2.23703E-28	2.60034E-37	3.25497E-49	3.00024E-11	7.0185E-40	2.29325E-18	3.41602E-53	
arg-1, ccd-3 (n=310)	3.21872E-26	9.75882E-20	0.032842055	6.91215E-05	6.26089E-07	3.18186E-12	5.43015E-18	8.74289E-13	6.89607E-18	0.137269472	6.61844E-10	
arg-1, ccd-4 (n=552)	8.62893E-30	1.91748E-07	0.032842055	6.30262E-05	0.00260344	1.31003E-07	9.47228E-13	2.05693E-11	1.11005E-16	0.085338857	1.53227E-07	
arg-1, csp-1 (n=228)	1.18329E-13	1.09518E-44	6.91215E-05	6.30262E-05	7.37602E-15	9.5472E-13	5.92393E-18	1.0188E-22	1.9949E-27	0.152759114	0.152759114	
arg-1, nhr-49 (n=194)	4.24406E-36	2.23703E-28	6.26089E-07	0.00260344	7.37602E-15		0.0285444	0.005614851	1	0.472862318	3.18579E-14	
arg-1, hsf-1 (n=168)	9.34429E-32	2.60034E-37	3.18186E-12	1.31003E-07	9.5472E-13	0.0285444		1	0.022315438	0.060957694	1.20143E-12	
arg-1, daf-16 (n=169)	9.08427E-32	3.25497E-49	5.43015E-18	9.47228E-13	5.92393E-18	0.005614851		1	0.183721705	0.33676E-05	4.30191E-13	
arg-1, daf-18 (n=377)	4.37486E-51	3.00024E-11	8.74289E-13	2.05693E-11	1.0188E-22	1	1	0.183721705	1	0.46885813	5.16183E-23	
arg-1, daf-16, ccd-4 (n=252)	2.39832E-52	7.0185E-40	8.86031E-18	1.11005E-16	1.9949E-27	0.472862318	0.022315438	0.021762663	1	0.00020915	1.2435E-26	
arg-1, nhr-49, ccd-4 (n=165)	6.07129E-30	2.29325E-18	0.137269472	0.085338857	8.7906E-07	0.571308717	0.060957694	4.33676E-05	0.46885813	0.00020915	8.45661E-11	
arg-1, csp-1, daf-18 (n=180)	1.28655E-08	7.41602E-53	6.61844E-10	1.53227E-07	0.12579811	3.18579E-14	1.20143E-12	4.36181E-13	5.16183E-23	1.2435E-26	6.45661E-11	

	N2	arg-1	arg-1, csp-1	arg-1, csp-1, crt-1	arg-1, csp-1, crt-1	arg-1, csp-1, ccd-3	arg-1, csp-1, ccd-4
N2		3.32272E-74	3.765E-14	3.99493E-12	7.37115E-39	0.000770994	2.97502E-59
arg-1	3.32272E-74		3.48466E-45	3.01222E-46	9.06566E-72	9.88018E-76	3.82783E-40
arg-1, csp-1	3.765E-14	3.48466E-45		0.002462578	4.00033E-21	1.28993E-07	1.53182E-19
arg-1, csp-1, crt-1	3.99493E-12	3.01222E-46	0.002462578		2.78165E-07	0.000129617	1.09351E-16
arg-1, csp-1, crt-1	7.37115E-39	9.06566E-72	4.00033E-21	2.78165E-07		7.58376E-35	4.42727E-18
arg-1, csp-1, ccd-3	0.000770994	0.88018E-76	1.28993E-07	0.000129617	7.58376E-35		2.7317E-34
arg-1, csp-1, ccd-4	2.97502E-59	3.82783E-40	1.53182E-19	1.09351E-16	4.42727E-18	2.7317E-34	

Supplemental Table S1. Related to Figures 1 and 5. Significance values for germline profile comparisons. Related to Figure 1D and 5C. P values for germline arm profiles were determined using pairwise Chi-square tests with Bonferroni correction.