

## **SUPPLEMENT**

### **Energy demand and the context-dependent effects of genetic interactions**

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**Supplemental Tables S1-S8**

**Supplemental Figures S1-S2**

**Supplemental Table S1.** Temperature-dependent effects of mito-nuclear interactions on development time are modified by the developmental photoperiod.

Phenotype	Factor <sup>1</sup>	numDF	denDf	<i>F</i> -value	<i>P</i> -value
16°C Development time ( <i>N</i> =5261, 167 vials)	Photoperiod	1	159	94.04	<0.0001
	mtDNA	1	159	47.29	<0.0001
	Nuclear	1	159	16.49	0.0001
	Photoperiod x mtDNA	1	159	0.10	0.7535
	Photoperiod x Nuclear	1	159	2.59	0.1092
	mtDNA x Nuclear	1	159	19.46	<0.0001
	Photoperiod x mtDNA x Nuclear	1	159	25.43	<0.0001
22°C Development time ( <i>N</i> =6191, 161 vials)	Photoperiod	1	153	688.29	<0.0001
	mtDNA	1	153	1302.74	<0.0001
	Nuclear	1	153	885.38	<0.0001
	Photoperiod x mtDNA	1	153	332.88	<0.0001
	Photoperiod x Nuclear	1	153	193.61	<0.0001
	mtDNA x Nuclear	1	153	797.40	<0.0001
	Photoperiod x mtDNA x Nuclear	1	153	299.07	<0.0001

<sup>1</sup> Mixed-model ANOVA fit using restricted maximum likelihood and including culture vial as a random factor. Accompanies data presented in Figure 1. There was no evidence for a 4-way interaction between  $T_{DEV}$ , photoperiod, mtDNA and nuclear genome ( $F_{1,312} = 0.41$ ,  $P = 0.5244$ )

**Supplemental Table S2.** Developmental temperature and photoperiod can both independently modulate mito-nuclear genetic effects on development time.

Phenotype	Factor <sup>1</sup>	numDF	denDf	<i>F</i> -value	<i>P</i> -value
16°C, 12:12h Development time ( <i>N</i> =2300, 84 vials)	mtDNA	1	80	51.57	<0.0001
	Nuclear	1	80	5.91	0.0173
	mtDNA x Nuclear	1	80	0.38	0.5413
16°C, 24:0h Development time ( <i>N</i> =2961, 83 vials)	mtDNA	1	79	14.20	0.0003
	Nuclear	1	79	10.83	0.0015
	mtDNA x Nuclear	1	79	30.13	<0.0001
22°C, 12:12h Development time ( <i>N</i> =3105, 80 vials)	mtDNA	1	76	263.6	<0.0001
	Nuclear	1	76	213.2	<0.0001
	mtDNA x Nuclear	1	76	104.0	<0.0001
22°C, 24:0h Development time ( <i>N</i> =3086, 81 vials)	mtDNA	1	77	1059.82	<0.0001
	Nuclear	1	77	663.62	<0.0001
	mtDNA x Nuclear	1	77	724.88	<0.0001

<sup>1</sup>Mixed-model ANOVA fit using restricted maximum likelihood and including culture vial as a random factor. Accompanies data presented in Figure 1.

**Supplemental Table S3.** Gene-environment interactions affect adult body mass.

Phenotype	Factor	numDF	denDf	<i>F</i> -value	<i>P</i> -value
Adult mass <sup>1</sup> ( <i>N</i> =333)	T <sub>DEV</sub>	1	317	486.085	< 0.0001
	Sex	1	317	807.744	< 0.0001
	mtDNA	1	317	1.549	0.2142
	Nuclear	1	317	10.184	0.0016
	T <sub>DEV</sub> x sex	1	317	12.141	0.0006
	T <sub>DEV</sub> x mtDNA	1	317	1.351	0.2460
	Sex x mtDNA	1	317	0.028	0.8674
	T <sub>DEV</sub> x nuclear	1	317	216.574	<0.0001
	Sex x nuclear	1	317	0.016	0.9001
	mtDNA x Nuclear	1	317	6.078	0.0142
	T <sub>DEV</sub> x sex x mtDNA	1	317	0.152	0.6968
	T <sub>DEV</sub> x sex x nuclear	1	317	0.546	0.4604
	T <sub>DEV</sub> x mtDNA x nuclear	1	317	3.444	0.0644
	Sex x mtDNA x nuclear	1	317	0.001	0.9734
	T <sub>DEV</sub> x sex x mtDNA x nuclear	1	317	0.016	0.1979

<sup>1</sup> The dependent variable is the  $\ln(\text{mass})$  of groups of 10 adult flies developed at either 16°C or 22°C.

**Supplemental Table S4.** Mito-nuclear genetic effects on adult body mass are specific to females developed at 16°C.

Phenotype	Factor	numDF	denDf	<i>F</i> -value	<i>P</i> -value
16°C ♂ mass <sup>1</sup> ( <i>N</i> =86)	mtDNA	1	82	0.059	0.809
	Nuclear	1	82	30.29	<0.0001
	mtDNA x Nuclear	1	82	2.692	0.105
16°C ♀ mass <sup>1</sup> ( <i>N</i> =84)	mtDNA	1	80	0.001	0.9777
	Nuclear	1	80	37.06	<0.0001
	mtDNA x Nuclear	1	80	8.033	0.0058
25°C ♂ mass <sup>1</sup> ( <i>N</i> = 81)	mtDNA	1	77	1.090	0.300
	Nuclear	1	77	96.79	<0.0001
	mtDNA x Nuclear	1	77	1.187	0.279
25°C ♀ mass <sup>1</sup> ( <i>N</i> = 82)	mtDNA	1	78	1.931	0.169
	Nuclear	1	78	66.00	<0.0001
	mtDNA x Nuclear	1	78	0.101	0.752

<sup>1</sup> The dependent variable is the  $\ln(\text{mass})$  of groups of 10 adult flies developed at either 16°C or 25°C.

**Supplemental Table S5.** Mito-nuclear genetic effects on adult metabolic rate are specific to females developed at 16°C.

Phenotype <sup>1</sup>	Genotype	Common slope (95%CI) <sup>2</sup>	Common slope y-axis intercept (CI) <sup>3</sup>	Shift along x- axis (mass)
16°C ♂ Metabolic Rate		0.885 (0.710,1.104)		
	<i>(ore);OreR</i>		0.3281 <sup>a</sup> (0.1518, 0.5045)	no
	<i>(simw<sup>501</sup>);OreR</i>		0.3269 <sup>a</sup> (0.1482, 0.5056)	no
	<i>(ore);Aut</i>		0.3201 <sup>a</sup> (0.1410, 0.4991)	no
16°C ♀ Metabolic Rate	<i>(simw<sup>501</sup>);Aut</i>		0.3283 <sup>a</sup> (0.1568, 0.5000)	no
		1.098 (0.896,1.345)		
	<i>(ore);OreR</i>		0.1569 <sup>a</sup> (-0.0733, 0.3872)	no
	<i>(simw<sup>501</sup>);OreR</i>		<b>0.1328<sup>b</sup></b> (-0.0957, 0.3613)	no
25°C ♂ Metabolic Rate	<i>(ore);Aut</i>		0.1764 <sup>a</sup> (-0.0553, 0.4082)	no
	<i>(simw<sup>501</sup>);Aut</i>		0.1908 <sup>a</sup> (-0.0351, 0.4166)	no
		1.134 (0.876,1.469)		
	<i>(ore);OreR</i>		0.4480 <sup>a</sup> (0.2110, 0.6850)	no
25°C ♀ Metabolic Rate	<i>(simw<sup>501</sup>);OreR</i>		0.4687 <sup>a</sup> (0.2308, 0.7065)	no
	<i>(ore);Aut</i>		0.4516 <sup>a</sup> (0.2107, 0.6925)	no
	<i>(simw<sup>501</sup>);Aut</i>		0.4650 <sup>a</sup> (0.2249, 0.7051)	no
		1.077 (0.884,1.316)		
	<i>(ore);OreR</i>		<b>0.4622<sup>a</sup></b> (0.2620, 0.6625)	no
	<i>(simw<sup>501</sup>);OreR</i>		<b>0.4492<sup>a</sup></b> (0.2462, 0.6522)	no
	<i>(ore);Aut</i>		0.5355 <sup>b</sup> (0.3332, 0.7378)	no
	<i>(simw<sup>501</sup>);Aut</i>		0.5314 <sup>b</sup> (0.3289, 0.7339)	no

<sup>1</sup> Routine metabolic rate of adults of each sex measured at either 16°C or 25°C (data from the two development temperatures are pooled within measurement temperature). Accompanies data presented in Figure 2A and Supplemental Figure S1.

<sup>2</sup> Common slope from a Type II model regression analysis of ln(metabolic rate) on ln(adult mass).

<sup>3</sup> Different letters within a common slope denote significant differences in the y-intercept (i.e. the mass-specific metabolic rate) ( $P < 0.05$ ).

**Supplemental Table S6.** Mito-nuclear interactions do not affect adult mass-corrected metabolic rate.

Phenotype	Factor	numDF	denDf	<i>F</i> -value	<i>P</i> -value
16°C ♀ MCMR <sup>1</sup> ( <i>N</i> =81)	T <sub>DEV</sub>	1	73	1.789	0.1852
	mtDNA	1	73	0.115	0.7356
	Nuclear	1	73	6.929	0.0103
	T <sub>DEV</sub> x mtDNA	1	73	1.080	0.3022
	T <sub>DEV</sub> x Nuclear	1	73	0.714	0.4010
	mtDNA x Nuclear	1	73	1.186	0.2796
	T <sub>DEV</sub> x mtDNA x Nuclear	1	73	0.541	0.4646
16°C ♂ MCMR <sup>1</sup> ( <i>N</i> =82)	T <sub>DEV</sub>	1	74	0.176	0.6759
	mtDNA	1	74	0.814	0.3698
	Nuclear	1	74	0.083	0.7735
	T <sub>DEV</sub> x mtDNA	1	74	0.004	0.9476
	T <sub>DEV</sub> x Nuclear	1	74	0.244	0.6226
	mtDNA x Nuclear	1	74	0.576	0.4502
	T <sub>DEV</sub> x mtDNA x Nuclear	1	74	1.251	0.2670
25°C ♀ MCMR <sup>1</sup> ( <i>N</i> = 87)	T <sub>DEV</sub>	1	79	0.678	0.4127
	mtDNA	1	79	0.060	0.8064
	Nuclear	1	79	34.678	<0.0001
	T <sub>DEV</sub> x mtDNA	1	79	1.233	0.2703
	T <sub>DEV</sub> x Nuclear	1	79	0.358	0.5516
	mtDNA x Nuclear	1	79	0.001	0.9787
	T <sub>DEV</sub> x mtDNA x Nuclear	1	79	0.023	0.8810
25°C ♂ MCMR <sup>1</sup> ( <i>N</i> = 86)	T <sub>DEV</sub>	1	78	5.949	0.0170
	mtDNA	1	78	1.754	0.1892
	Nuclear	1	78	0.187	0.6666
	T <sub>DEV</sub> x mtDNA	1	78	0.0003	0.9860
	T <sub>DEV</sub> x Nuclear	1	78	6.676	0.0589
	mtDNA x Nuclear	1	78	0.061	0.8060
	T <sub>DEV</sub> x mtDNA x Nuclear	1	78	0.082	0.7751

<sup>1</sup> The dependent variable is the mass-corrected metabolic rate at each measurement temperature of groups of 10 adult flies developed at either 16°C or 22°C. See main text for calculation of mass-corrected metabolic rates. Accompanies data in Figure 2 and Supplemental Figure S2.

**Supplemental Table S7.** Mito-nuclear interactions do not affect metabolic plasticity (i.e., the  $Q_{10}$  for metabolic rate) in adult females or males.

Phenotype	Factor	numDF	denDf	<i>F</i> -value	<i>P</i> -value
♀ MCMR <sup>1</sup> ( <i>N</i> =168)	T <sub>MEASURE</sub>	1	152	945.888	<.0001
	T <sub>DEV</sub>	1	152	0.035	0.8514
	mtDNA	1	152	0.100	0.7519
	Nuclear	1	152	41.32	<.0001
	T <sub>MEASURE</sub> x T <sub>DEV</sub>	1	152	1.947	0.1649
	T <sub>MEASURE</sub> x mtDNA	1	152	0.001	0.9817
	T <sub>DEV</sub> x mtDNA	1	152	0.397	0.5295
	T <sub>MEASURE</sub> x nuclear	1	152	16.015	0.0001
	T <sub>DEV</sub> x nuclear	1	152	0.044	0.8347
	mtDNA x Nuclear	1	152	0.179	0.6731
	T <sub>MEASURE</sub> x T <sub>DEV</sub> x mtDNA	1	152	2.030	0.1562
	T <sub>MEASURE</sub> x T <sub>DEV</sub> x nuclear	1	152	0.823	0.3659
	T <sub>MEASURE</sub> x mtDNA x nuclear	1	152	0.202	0.6537
	T <sub>DEV</sub> x mtDNA x nuclear	1	152	0.029	0.8651
	T <sub>MEASURE</sub> x T <sub>DEV</sub> x mtDNA x nuclear	1	152	0.203	0.6532
♂ MCMR <sup>1</sup> ( <i>N</i> =168)	T <sub>MEASURE</sub>	1	152	751.276	<.0001
	T <sub>DEV</sub>	1	152	5.861	0.0167
	mtDNA	1	152	0.769	0.3818
	Nuclear	1	152	0.231	0.6313
	T <sub>MEASURE</sub> x T <sub>DEV</sub>	1	152	4.322	0.0393
	T <sub>MEASURE</sub> x mtDNA	1	152	2.348	0.1275
	T <sub>DEV</sub> x mtDNA	1	152	0	0.997
	T <sub>MEASURE</sub> x nuclear	1	152	0.080	0.7772
	T <sub>DEV</sub> x nuclear	1	152	3.860	0.0513
	mtDNA x Nuclear	1	152	0.236	0.6278
	T <sub>MEASURE</sub> x T <sub>DEV</sub> x mtDNA	1	152	0.003	0.9558
	T <sub>MEASURE</sub> x T <sub>DEV</sub> x nuclear	1	152	2.430	0.1211
	T <sub>MEASURE</sub> x mtDNA x nuclear	1	152	0.004	0.9501
	T <sub>DEV</sub> x mtDNA x nuclear	1	152	0.020	0.8875
	T <sub>MEASURE</sub> x T <sub>DEV</sub> x mtDNA x nuclear	1	152	0.463	0.4973

<sup>1</sup> The dependent variable is the mass-corrected routine metabolic rate (MCMR) at each measurement temperature of groups of 10 adult flies developed at either 16°C or 22°C. See main text for calculation of MCMR. Accompanies data in Figure 2 and Supplemental Figure S2.

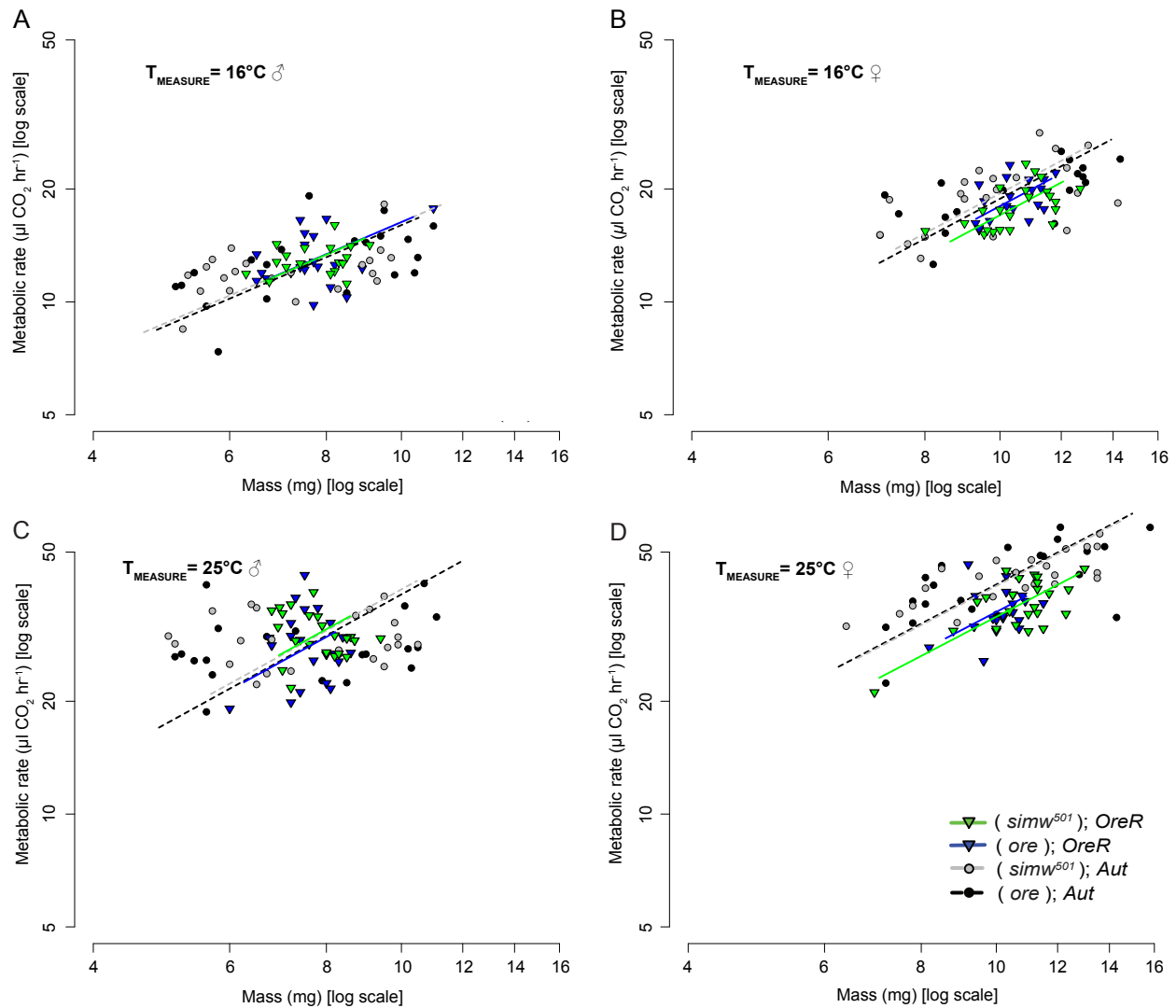


**Supplemental Table S8.** Mito-nuclear interactions affect female, but not male, reproductive fitness.

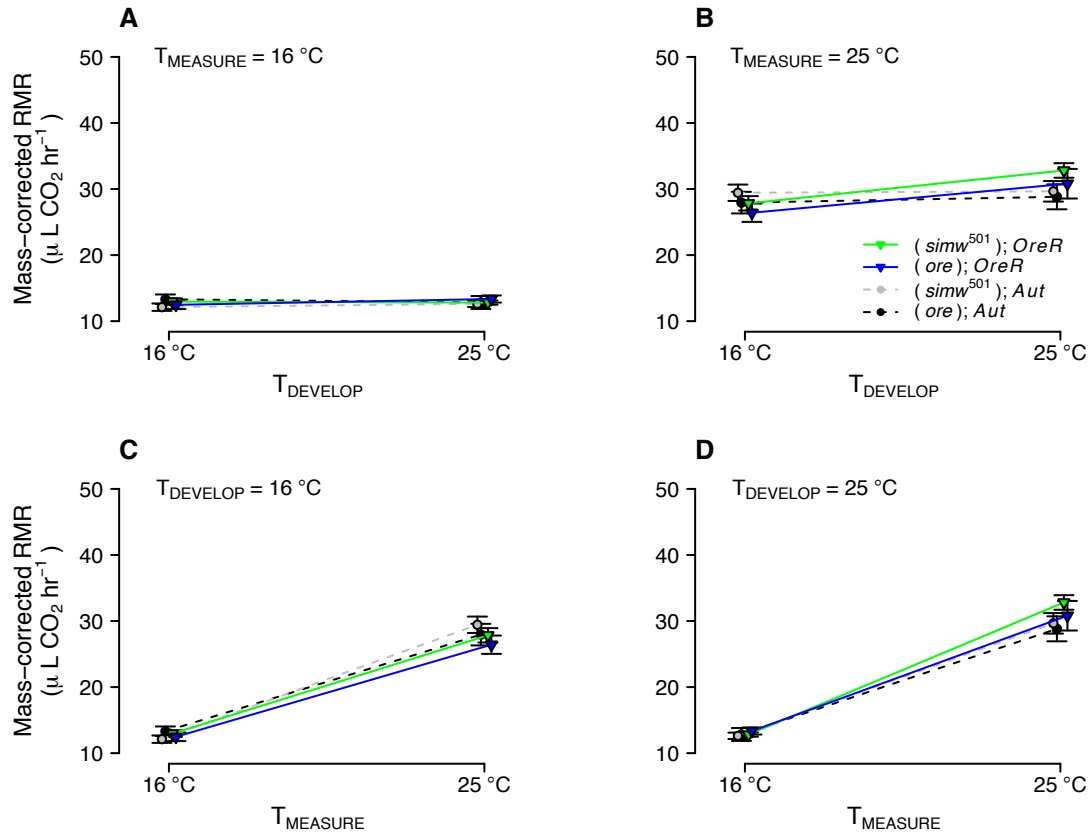
Phenotype	Factor	numDF	denDf	<i>F</i> -value	<i>P</i> -value
Male fertility <sup>1</sup> ( <i>N</i> =115)	mtDNA	1	107	4.236	0.042
	Nuclear	1	107	15.21	0.0002
	Block	1	107	1.541	0.217
	mtDNA x nuclear	1	107	0.185	0.668
	mtDNA x block	1	107	0.889	0.348
	Nuclear x block	1	107	4.128	0.045
	mtDNA x nuclear x block	1	107	1.435	0.234
Female fecundity <sup>2</sup> ( <i>N</i> = 35)	mtDNA	1	31	13.57	0.0009
	Nuclear	1	31	120.8	<0.0001
	mtDNA x nuclear	1	31	16.98	0.0003

<sup>1</sup> Male fertility was scored as the number of offspring sired by individual males of each genotype per female mated.

<sup>2</sup> Female fecundity was scored as the total number of eggs produced by individual females of each genotype over the course of 10 days. Data are from Meiklejohn *et al.* (2013).



**Supplemental Figure S1.** Weak effects of mito-nuclear genotype on adult metabolic rate depend upon sex and measurement temperature. Plots show routine metabolic rate as a function of mass on a log-log scale for pools of 10 male (A,C) or female (B,D) flies measured at 16°C (A,B) and at 25°C (C,D). Panel B reveals that the only evidence that mito-nuclear genotype affects adult metabolic rate is a modest, but significant, decrease in metabolic rate across masses specifically in females of the incompatible (*simw*<sup>501</sup>);*OreR* genotype when measured at 16°C ( $P < 0.05$ , Supplemental Table S5). For all other sex- $T_{\text{MEASURE}}$  combinations, the (*simw*<sup>501</sup>);*OreR* genotype has a metabolic rate statistically similar to the (*ore*);*OreR* nuclear control genotype.



**Supplemental Figure S2.** Adult male metabolic plasticity is not affected by mito-nuclear genetic effects. A,B. Developmental reaction norms show robust thermal acclimation of male mass-corrected metabolic rate (MCMR) within measurement temperatures, although there were weakly significant effects of development temperature on male metabolic rate when measured at 25°C that depended weakly on nuclear genotype ( $T_{DEV}$ ,  $F_{1,78} = 5.949$ ,  $P = 0.0170$ ,  $T_{DEV} \times \text{Nuclear}$ ,  $F_{1,78} = 6.676$ ,  $P = 0.0589$ ; Supplemental Table S7). C, D. Thermal reaction norms show that the  $Q_{10}$  for male MCMR is similar under both developmental temperatures. The mtDNA x nuclear interaction did not affect male MCMR at either measurement temperature ( $P > 0.45$ ; Supplemental Table S6). Error bars are  $\pm 1$  SEM.