

Table S1. Mitochondrial haplotype significantly impacted climbing performance. ANOVA of repeated measured for (A-B) exercise conditioning over an 18-day conditioning period showed a significant first order effect for mitochondrial haplotype in both the (A) velocity and (B) normalized climbing index (velocity for a time point/average velocity of the first time point, different for each unique vial). There was no significant first order effect for exercise conditioning, but there was a significant second order effect for mitochondrial haplotype x exercise conditioning in the normalized climbing index. (C-D) Resistance to endurance fatigue had significant first order effects for both mitochondrial haplotype and flies' resistance to endurance fatigue for both the (C) velocity and (D) normalized climbing index, but not a second order effect for mitotype x resistance to fatigue.

Interaction term significance key: $P \leq 0.05$ (*); $P \leq 0.005$ (**); $P \leq 0.0005$ (***)

A				
Exercise conditioning – velocity				
Interaction Terms	F Value	DF	Den DF	Pr > F
Mitochondrial haplotype	66.9734	3	39	0.0000 ***
Exercise conditioning	0.0453	1	13	0.8348
Mitochondrial haplotype x Exercise conditioning	1.4007	3	39	0.2571
B				
Exercise conditioning – normalized climbing index				
Interaction Terms	F Value	DF	Den DF	Pr > F
Mitochondrial haplotype	23.4887	3	39	0.0000 ***
Exercise conditioning	0.8365	1	13	0.3771
Mitochondrial haplotype x Exercise conditioning	21.7142	3	39	0.0000 ***
C				
Resistance to endurance fatigue – velocity				
Interaction Terms	F Value	DF	Den DF	Pr > F
Mitochondrial haplotype	17.5597	4	24	0.0000 ***
Resistance to fatigue	21.8684	1	6	0.0034 **
Mitochondrial haplotype x Resistance to fatigue	4.087	4	24	0.0115 *
D				
Resistance to endurance fatigue – normalized climbing index				
Interaction Terms	F Value	DF	Den DF	Pr > F
Mitochondrial haplotype	1.6059	4	24	0.2052
Resistance to fatigue	26.2571	1	6	0.0022 **
Mitochondrial haplotype x Resistance to fatigue	2.1012	4	24	0.112

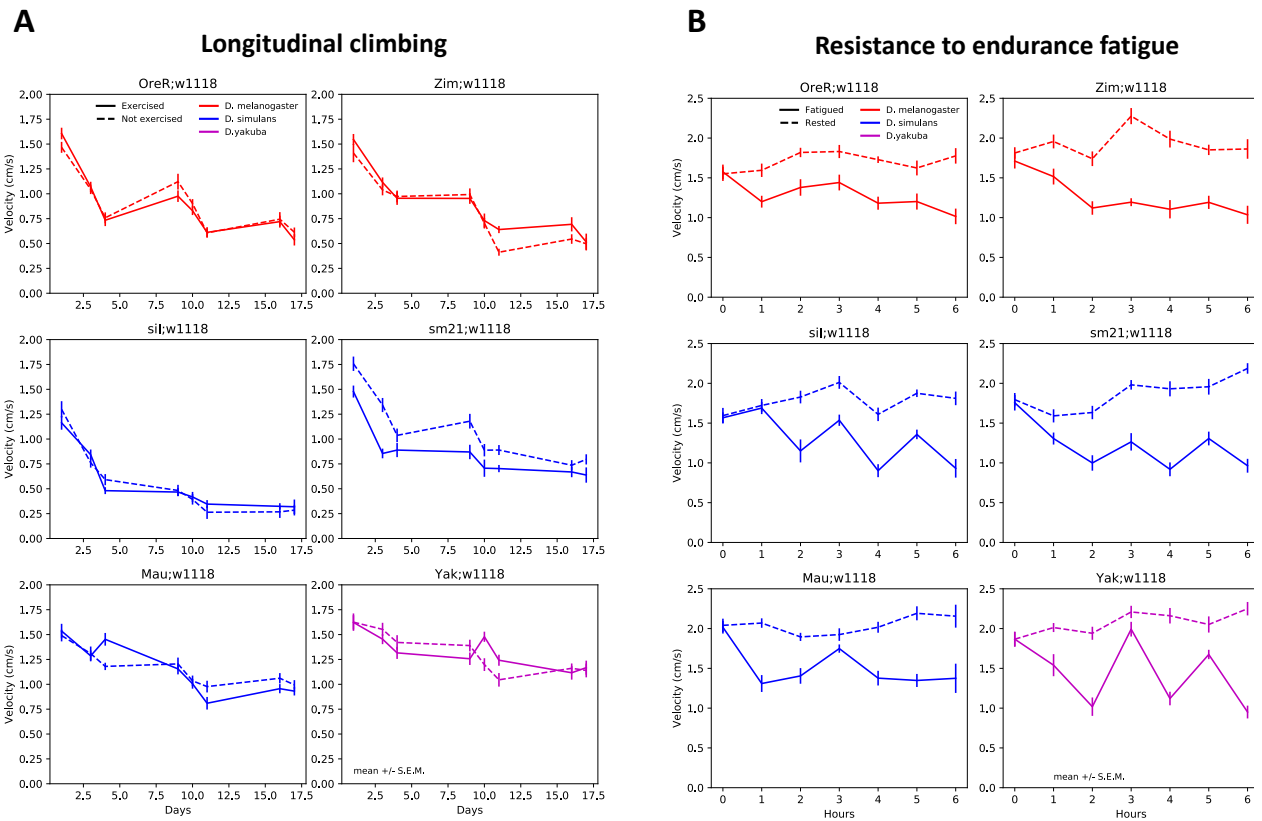


Figure S2. Individual mitotype performance vs. time curves. Exercised (trained or fatigued, solid line) flies and unexercised flies (untrained or rested, dashed line) had different effects across mitochondrial haplotypes (colored by species: *D. melanogaster*, red; *D. simulans*, blue; *D. yakuba*, purple). (A) Longitudinal climbing performance had a significant mitochondrial haplotype effect ($F = 67.0$, $P < 0.0001$) over time, but no significant effect for exercise conditioning ($F = 4.5E-2$, $P = 0.83$) or mitotype x exercise-conditioning effect ($F = 1.40$, $P = 0.26$). $n = 1007$ videos analyzed. (B) Resistance to endurance fatigue assay, measuring the progressive decline over hours of repeated climbing, had significant mitochondrial haplotype ($F = 17.6$, $P < 0.0001$) and exercise effects ($F = 21.9$, $P < 0.005$), but no two-way interaction between the two ($F = 4.1$, $P < 0.05$). $n = 297$ videos analyzed. Separate sets of flies were used between the two experiments, with points representing the mean \pm S.E.M.