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: $P \le 0.05$ (*); $P \le 0.005$ (**); $P \le 0.0005$ (***)

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Α				
				nditioning – 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
В				
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

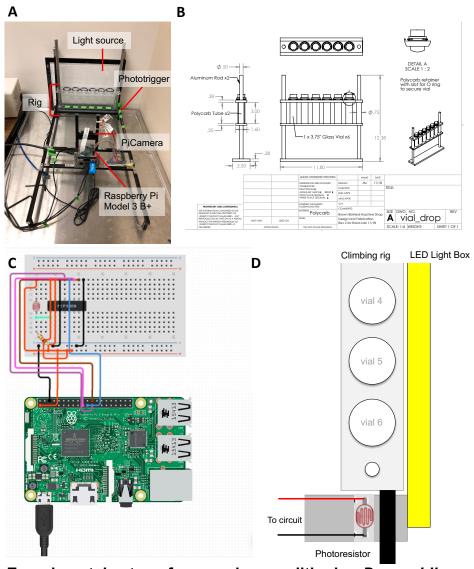


Figure S1. Experimental setups for exercise conditioning *Drosophila* and assaying climbing performance, and Raspberry Pi circuit diagram and phototrigger assembly. (A) Exercised flies were assayed for climbing performance using a custom setup to standardize conditions for this manuscript. Here, a MakerBeam frame held a Raspberry Pi Model 3 B+ and PiCamera V2 a fixed distance away from the stage. An LED light source was placed behind the climbing rig backlit flies as they climbed. A phototrigger, constructed from a photoresistor and analog-to-digital converter, was used to begin recording videos precisely when the assay began. The rig (B) was constructed from polycarbonate materials and slid along aluminum rod tracks. Rubber O-rings along the top of each vial slot held vials in place during the assay. (C) A circuit diagram for wiring a phototrigger connected a photoresistor through an MCP3008 analog-digital converter to the Raspberry Pi General Processing Input Output (GPIO) pins. (D) The photoresistor was placed on the MakerBeam frame (gray rectangle) near the LED light box (yellow rectangle). An opaque tab (black rectangle) on the climbing rig disrupted the light path from the LED light box to the photoresistor when the climbing rig chassis was lowered, but did not disrupt the path when it was raised.

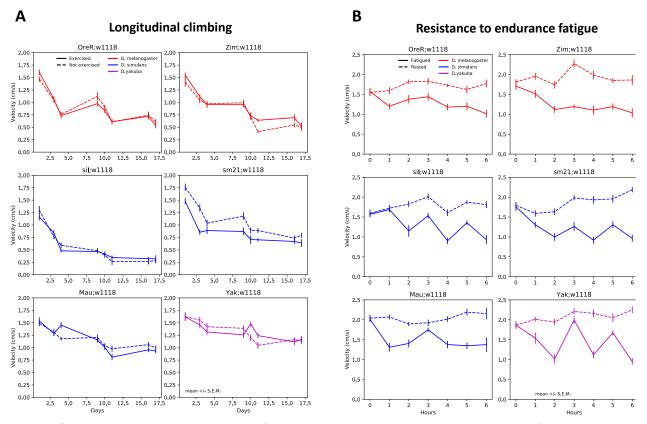


Figure S2. Individual mitotype performance vs. time curves. Exercised (trained or fatigued, solid line) flies and unexercised flies (untrained or rested, dashed line) had different effects across mitochondrial haplotypes (colored by species: D. melanogaster, red; D. simulans, blue; D. yakuba, purple). (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 $\underline{+}$ S.E.M.