Supplementary Tables for

The adaptive benefit of evolved increases in hemoglobin-O_2 affinity is contingent on tissue O_2 diffusing capacity in high-altitude deer mice

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Table S1. Effects of inspired *P*O_2 and acclimation to hypoxia on cardiorespiratory physiology of F_2 inter-population hybrid deer mice at $\dot{V}O_2$max, without accounting for effects of genotype.

Trait	Animal mass	Acclimation	PO_2	Interaction[1]
$\dot{V}O_2$max[2]	$F_{1,98}$ = 37.9184 P < 0.0001*	$F_{1,98}$ = 16.8561 P = 0.0001*	$F_{1,98}$ = 94.4737 P < 0.0001*	NS
Arterial O_2 saturation	$F_{1,98}$ = 3.0602 P = 0.0909	$F_{1,98}$ = 14.5282 P = 0.0003*	$F_{1,98}$ = 181.5338 P < 0.0001*	$F_{1,98}$ = 13.0275 P = 0.0006*
Heart rate[2]	$F_{1,97}$ = 4.3086 P = 0.0457*	$F_{1,97}$ = 4.6517 P = 0.0342*	$F_{1,97}$ = 27.5056 P < 0.0001*	$F_{1,97}$ = 7.6175 P = 0.0073*
Total ventilation	NS	$F_{1,99}$ = 49.9311 P < 0.0001*	$F_{1,99}$ = 3.5126 P = 0.0648	$F_{1,99}$ = 5.5837 P = 0.0207*
Tidal volume	NS	$F_{1,99}$ = 22.2705 P < 0.0001*	$F_{1,99}$ = 3.3406 P = 0.0716	$F_{1,99}$ = 5.1393 P = 0.0263*
Breathing frequency	NS	$F_{1,100}$ = 24.9573 P < 0.0001*	$F_{1,100}$ < 0.0001 P = 0.9936	NS

*P < 0.05. [1]Interaction between acclimation and inspired *P*O_2. NS denotes no significant effect of the factor, which was removed from the final statistical model. [2]Mouse family was also included as a random factor in the mixed model as 0.05 < P < 0.1.

Table S2. Effects of acclimation to hypoxia and globin genotype on cardiorespiratory physiology in hypoxia of F_2 inter-population hybrid deer mice.

Trait	Animal mass	Acclimation	Hb genotype	Interaction[1]
Hypoxic $\dot{V}O_2$max	$F_{1,41} = 28.0073$ $P < 0.0001$*	$F_{1,41} = 13.0967$ $P = 0.0013$*	$F_{4,41} = 1.7576$ $P = 0.1764$	NS
Arterial O_2 saturation	NS	$F_{1,42} = 15.8951$ $P = 0.0005$*	$F_{4,42} = 3.0259$ $P = 0.0407$*	NS
Heart rate[2]	NS	$F_{1,41} = 10.7106$ $P = 0.0031$*	$F_{4,41} = 0.7020$ $P = 0.5999$	NS
Total ventilation	NS	$F_{1,42} = 34.0654$ $P < 0.0001$*	$F_{4,42} = 0.3396$ $P = 0.8481$	NS
Tidal volume	NS	$F_{1,42} = 15.9684$ $P = 0.0005$*	$F_{4,42} = 0.6548$ $P = 0.6300$	NS
Breathing frequency	NS	$F_{1,42} = 23.7780$ $P < 0.0001$*	$F_{4,42} = 0.5053$ $P = 0.7323$	NS
Hematocrit	NS	$F_{1,42} = 222.7637$ $P < 0.0001$*	$F_{4,42} = 0.2031$ $P = 0.9338$	NS
Blood Hb concentration	NS	$F_{1,42} = 59.4601$ $P < 0.0001$*	$F_{4,42} = 1.3807$ $P = 0.2745$	NS
Red blood cell P_{50}	NS	$F_{1,42} = 18.8344$ $P = 0.0002$*	$F_{4,42} = 3.9622$ $P = 0.0150$*	NS
Hill coefficient	NS	$F_{1,42} = 0.0315$ $P = 0.8599$	$F_{4,42} = 0.2514$ $P = 0.9073$	NS

*$P < 0.05$. [1]Interaction between acclimation and globin genotype. NS denotes no significant effect of the factor, which was removed from the final statistical model. [2]Mouse family was also included as a random factor in the mixed model, for which $0.05 < P < 0.1$.

Table S3. Effects of inspired PO_2 and hemoglobin genotype on cardiorespiratory physiology of F_2 inter-population hybrid deer mice acclimated to normoxia.

Trait	Animal mass	PO_2	Hb genotype	Interaction[1]
$\dot{V}O_2max$	$F_{1,41}$ = 31.2238 P < 0.0001*	$F_{1,41}$ = 61.0119 P < 0.0001*	$F_{4,41}$ = 3.0346 P = 0.0416*	NS
Arterial O_2 saturation	NS	$F_{1,41}$ = 122.1012 P < 0.0001*	$F_{4,41}$ = 3.7422 P = 0.0189*	$F_{4,41}$ = 3.0674 P = 0.0389*
Heart rate[2]	NS	$F_{1,41}$ = 32.4464 P < 0.0001*	$F_{4,41}$ = 2.8000 P = 0.0545	NS
Total ventilation	NS	$F_{1,42}$ = 0.3248 P = 0.5738	$F_{4,42}$ = 0.6799 P = 0.6136	NS
Tidal volume	NS	$F_{1,42}$ = 0.1428 P = 0.7087	$F_{4,42}$ = 1.4425 P = 0.2551	NS
Breathing frequency	$F_{1,41}$ = 5.9183 P = 0.0242*	$F_{1,41}$ = 0.8443 P = 0.3669	$F_{4,41}$ = 1.0643 P = 0.3999	NS
Hematocrit	NS	NA	$F_{4,21}$ = 0.3604 P = 0.8339	NA
Blood Hb concentration	NS	NA	$F_{4,21}$ = 0.2008 P = 0.9351	NA
Red blood cell P_{50}	NS	NA	$F_{4,21}$ = 5.1298 P = 0.0048*	NA
Hill coefficient	NS	NA	$F_{4,21}$ = 0.4016 P = 0.8053	NA

*P < 0.05. [1]Interaction between inspired PO_2 and globin genotype. NS denotes no significant effect of the factor, which was removed from the final statistical model. NA, not applicable.

[2]Mouse family was also included as a random factor in the mixed model, for which P < 0.05.

Table S4. Effects of acclimation to hypoxia and globin genotype on cardiorespiratory physiology in normoxia of F_2 inter-population hybrid deer mice.

Trait	Animal mass	Acclimation	Hb genotype	Interaction[1]
Normoxic $\dot{V}O_2max$[2]	$F_{1,40}$ = 23.2664 P < 0.0001*	$F_{1,40}$ = 4.2325 P = 0.0502	$F_{4,40}$ = 1.6212 P = 0.2115	NS
Arterial O_2 saturation	$F_{1,41}$ = 15.3290 P = 0.0003*	$F_{1,41}$ = 0.4014 P = 0.5296	$F_{4,41}$ = 2.9739 P = 0.0291*	NS
Heart rate[2]	$F_{1,40}$ = 4.8558 P = 0.0381*	$F_{1,40}$ = 0.1977 P = 0.6605	$F_{4,40}$ = 2.6116 P = 0.0698	NS
Total ventilation	NS	$F_{1,42}$ = 22.9660 P < 0.0001*	$F_{4,42}$ = 0.3536 P = 0.8386	NS
Tidal volume	$F_{1,41}$ = 5.1639 P = 0.0317*	$F_{1,41}$ = 3.1282 P = 0.0892	$F_{4,41}$ = 1.5707 P = 0.2199	NS
Breathing frequency	NS	$F_{1,42}$ = 8.0995 P = 0.0087*	$F_{4,42}$ = 0.6049 P = 0.6634	NS

*P < 0.05. [1]Interaction between acclimation and globin genotype. NS denotes no significant effect of the factor, which was removed from the final statistical model. [2]Mouse family was also included as a random factor in the mixed model as P < 0.05.

Table S5. Parameters used to generate the initial solution in the model of the oxygen transport pathway representing the 'ancestral condition' with the most lowland P_{50}.

Variable	Value
Measured input parameters	
P_B (kPa)	101
F_IO_2	0.123
$\dot{V}$ (ml min^{-1} g^{-1})	4.96
V_T (μl g^{-1})	13.0
[Hb] (g dl^{-1})	14.2
P_{50} (kPa)	4.84
n	2.85
T_b (˚C)	31.4
Estimated input parameters	
V_D (μl g^{-1})*	6.40
$\dot{Q}$ (ml min^{-1} g^{-1}) †	1.06
Calculated input parameters	
D_LO_2 (ml kPa^{-1} min^{-1})	0.0661
D_TO_2 (ml kPa^{-1} min^{-1})	0.0322
Output parameters (ancestral values shown)	
$\boldsymbol{P_AO_2}$ (kPa)	7.18
$\boldsymbol{P_aO_2}$ (kPa) †	6.85
$\boldsymbol{P_vO_2}$ (kPa)†	2.29
$\boldsymbol{\dot{V}O_2}$**max** (ml min^{-1} g^{-1})	0.131

P_B, barometric pressure; F_IO_2, inspired oxygen fraction; $\dot{V}O_2$max, maximal oxygen consumption rate measured during acute cold exposure; $\dot{V}$, total ventilation; V_T, tidal volume; [Hb], blood hemoglobin concentration; P_{50}, PO_2 at 50% O_2 saturation; n, Hill coefficient; P_aO_2, arterial O_2 tension; T_b, body temperature; V_D, dead space volume; $\dot{Q}$, cardiac ouput; P_vO_2, mixed venous O_2 tension; P_AO_2, alveolar O_2 tension; D_LO_2, O_2 diffusing capacity of the lungs; D_TO_2, O_2 diffusing capacity of the tissues. *Indicates value was taken from Fallica *et al* 2011 (1). †Indicates value was taken from, or calculated using, data in Tate *et al* 2020 (2). Variables in bold were then calculated by the model in our sensitivity analysis in response to changes in D_TO_2 and/or P_{50}.

References

1. J. Fallica, S. Das, M. Horton, W. Mitzner, Application of carbon monoxide diffusing capacity in the mouse lung. *J. Appl. Physiol.* **110**, 1455-1459 (2011).
2. K. B. Tate *et al.*, Coordinated changes across the O2 transport pathway underlie adaptive increases in thermogenic capacity in high-altitude deer mice. *Proc. R. Soc. London., B, Biol. Sci.* **287**, 20192750 (2020).