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Coordinated changes across the O_2 transport pathway underlie adaptive increases in thermogenic capacity in high-altitude deer mice

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Animals native to the hypoxic and cold environment at high altitude provide an excellent opportunity to elucidate the integrative mechanisms underlying the adaptive evolution and plasticity of complex traits. The capacity for aerobic thermogenesis can be a critical determinant of survival for small mammals at high altitude, but the physiological mechanisms underlying the evolution of this performance trait remain unresolved. We examined this issue by comparing high-altitude deer mice (*Peromyscus maniculatus*) with low-altitude deer mice and white-footed mice (*P. leucopus*). Mice were bred in captivity and adults were acclimated to each of four treatments: warm (25°C) normoxia, warm hypoxia (12 kPa O_2), cold (5°C) normoxia or cold hypoxia. Acclimation to hypoxia and/or cold increased thermogenic capacity in deer mice, but hypoxia acclimation led to much greater increases in thermogenic capacity in highlanders than in lowlanders. The high thermogenic capacity of highlanders was associated with increases in pulmonary O_2 extraction, arterial O_2 saturation, cardiac output and arterial–venous O_2 difference. Mechanisms underlying the evolution of enhanced thermogenic capacity in highlanders were partially distinct from those underlying the ancestral acclimation responses of lowlanders. Environmental adaptation has thus enhanced phenotypic plasticity and expanded the physiological toolkit for coping with the challenges at high altitude.

1. Introduction

Explaining the mechanistic basis for adaptive variation in organismal performance is a central and long-standing goal of evolutionary physiology [1,2]. Organismal performance traits are complex phenotypes supported by the coordinated function of various organ systems. The capacity for sustained aerobic exercise, for example, is supported by systems for partitioning O_2 and metabolic fuels to the mitochondria in active locomotory muscles [3,4]. Studies of the O_2 transport pathway, the conceptual steps involved in O_2 transport from the environment to O_2 utilization by mitochondria (ventilation, pulmonary diffusion, circulation, tissue diffusion and mitochondrial O_2 utilization), have been particularly useful for understanding the systems-level mechanisms underlying the evolution of aerobic exercise performance. Some comparative studies have suggested that variation in aerobic exercise capacity—reflected by the maximal rate of O_2 consumption ($\dot{V}_{O_2\mathrm{max}}$) that can be achieved during exercise—is underpinned by matching variation in the capacity of multiple steps in the O_2 pathway [5,6]. Such observations led to the concept of symmorphosis, which proposed that structural design is optimized to match but not exceed functional demands, such that each step in the O_2 pathway has an equivalent capacity to support O_2 flux [5,6]. However, results from artificial selection experiments [7–9] and theoretical modelling of the respiratory system [10,11] have suggested that evolved changes in exercise $\dot{V}_{O_2max}$ do not require matched changes across the O_2 pathway and can arise from changes in just a single step. The truth may lie somewhere between these two extremes, but there have been relatively few comparative studies of the evolution of $\dot{V}_{O_2max}$ that have fully investigated the function of the O_2 transport pathway.

Thermogenic capacity is another vital organismal performance trait that can push the limits of aerobic metabolism. Often measured as the $\dot{V}_{O_2max}$ during acute cold exposure, values of thermogenic $\dot{V}_{O_2max}$ can equal or exceed values of $\dot{V}_{O_2max}$ during exercise in small mammals [12–14]. As a result, thermogenic capacity requires high rates of O_2 flux through the O_2 transport pathway, but unlike aerobic exercise, aerobic thermogenesis involves the activity of both muscles (shivering thermogenesis) and adipose tissues (non-shivering thermogenesis). Thermogenic capacity can influence survival, fitness and the ability of small mammals to stay active in the cold [15,16]. However, the systems-level mechanisms underlying the evolution of thermogenic capacity are poorly understood.

Thermogenic capacity is also a plastic trait that can increase in response to chronic cold exposure via metabolic adjustments in thermogenic tissues [17–21], so studies of this performance trait can provide general insight into the plasticity of complex phenotypes. Theory and empirical evidence show that phenotypic plasticity can facilitate the survival and reproductive success of initial colonizers of a novel environment, and plasticity can then evolve by various forms of genetic accommodation and move the colonizing population closer to the fitness optimum [22–25]. However, most previous studies of the evolution of plasticity in complex traits have not considered the tight functional integration that can exist between the component mechanisms that underlie such traits [26]. Studies of thermogenic capacity and its underlying physiological mechanisms have the potential to shed light on this issue and to better understand the role of phenotypic plasticity in environmental adaptation.

Animals native to high-altitude environments provide an excellent opportunity to elucidate the mechanisms underlying the plasticity and adaptive evolution of thermogenic capacity. The cold and oxygen-depleted (hypoxic; low partial pressure of O_2, P_{O_2}) environment at high altitude requires that endotherms maintain high rates of O_2 transport and utilization for thermogenesis while facing a diminished O_2 availability. Growing evidence suggests that high-altitude natives overcome this challenge through evolved changes in various cardiorespiratory and metabolic phenotypes [4,27,28]. However, with the exception of high-altitude humans, who do not suffer the same thermoregulatory challenges as do small mammals in the cold, there have been few comparative studies that have fully investigated the function of the O_2 pathway at $\dot{V}_{O_2max}$ in high-altitude natives [28,29].

Deer mice (*Peromyscus maniculatus*) native to high elevation are a powerful model for understanding the physiological bases of phenotypic plasticity and local adaptation. This species can be found across a wide altitudinal range, from below sea level in Death Valley, CA, USA to over 4300 m above sea level in the Rocky Mountains [30,31]. At high altitude, free-ranging deer mice sustain high metabolic rates [32], and there is evidence that increased thermogenic $\dot{V}_{O_2max}$ improves survival [15], presumably as a result of the high demands for heat generation in cold alpine environments. Various studies suggest that high-altitude deer mice have responded to this strong selection pressure by evolving increases in $\dot{V}_{O_2max}$ in hypoxia, based on comparisons to low-altitude populations of deer mice and white-footed mice (*P. leucopus*; a congener that is restricted to low altitudes) [33–35]. Highland deer mice have a particularly strong advantage in hypoxic environments, because thermogenic $\dot{V}_{O_2max}$ (measured in hypoxia) increases by a much greater magnitude after hypoxia acclimation in highlanders than in lowlanders [36]. The functional changes in the O_2 transport pathway that underlie this evolved increase in thermogenic $\dot{V}_{O_2max}$ have yet to be fully explained. Furthermore, cold acclimation is known to increase thermogenic $\dot{V}_{O_2max}$ and cardiopulmonary organ sizes in deer mice [12,20,37], but it is unknown whether the acclimation response to cold or to the combination of cold and hypoxia has evolved in high-altitude populations. This study therefore aims to examine how acclimation to hypoxia and cold—alone or in combination—affects thermogenic $\dot{V}_{O_2max}$ in hypoxia, to determine whether high-altitude deer mice have evolved heightened acclimation responses, and to elucidate the functional changes in the O_2 pathway that contribute to enhancements of thermogenic $\dot{V}_{O_2max}$.

2. Methods

(a) Acclimation treatments

Captive breeding populations were established from wild deer mouse populations native to high altitude (near the summit of Mount Evans, CO, USA; 4350 m above sea level) and from wild populations of both deer mouse and white-footed mouse from low altitude (Nine Mile Prairie, Lancaster County, NE, USA; 430 m above sea level), as described previously [36,38] and detailed in the electronic supplementary material, Methods. First generation adult mice from each population were acclimated to each of four acclimation environments: (i) warm (25°C) normobaric normoxia (barometric pressure of 100 kPa, P_{O_2} of 20 kPa); (ii) warm (25°C) hypobaric hypoxia (barometric pressure of 60 kPa, P_{O_2} of 12.5 kPa); (iii) cold (5°C) normobaric normoxia; and (iv) cold (5°C) hypobaric hypoxia. Routine husbandry and the use of hypobaric chambers to create hypoxia have been described previously [35,39], and cold conditions were maintained in large environmental chambers with temperature control.

(b) Cardiorespiratory measurements at thermogenic $\dot{V}_{O_2max}$ in hypoxia

Thermogenic $\dot{V}_{O_2max}$ was measured after six to eight weeks of acclimation using open-flow respirometry during exposure to a cold (−5°C) hypoxic heliox gas mixture (12% O_2, 88% He). We employed methods we have described previously [36,38] and are detailed in the electronic supplementary material, Methods. Concurrent measurements of breathing were made by plethysmography, and measurements of arterial O_2 saturation and heart rate were made using a MouseOx Plus pulse oximeter (Starr Life Sciences, PA, USA).

We subsequently cannulated a subset of these mice to sample and measure the O_2 content of mixed venous blood at thermogenic $\dot{V}_{O_2max}$. These measurements were made on highland deer mice and lowland white-footed mice across all four acclimation environments but could only be made on lowland deer mice in warm normoxia and cold hypoxia. After at least 3 days recovery in the appropriate acclimation environment from the initial $\dot{V}_{O_2\max}$ measurement, mice were surgically implanted with a central venous cannula using standard surgical procedures under sterile conditions (see electronic supplementary material, Methods). This was achieved by occlusively cannulating the jugular vein using a microrenathane catheter, advancing the catheter into the central venous cavity and externalizing it through the skin at the nape of the neck. Mice were recovered at room temperature for 4–6 h and then at their appropriate acclimation environment for at least 3 days. A second thermogenic $\dot{V}_{O_2\max}$ trial was then conducted under the same exposure conditions as the first, and a 50 µl sample of central venous blood was collected at $\dot{V}_{O_2\max}$ and immediately analysed for total O_2 content at 37°C using the Tucker method [40]. A second venous blood sample was then collected for measurement of haemoglobin content using Drabkin's Reagent (Sigma-Aldrich, Oakville, ON, Canada). The $\dot{V}_{O_2\max}$ values from the second $\dot{V}_{O_2\max}$ trial did not differ statistically from those measured in the first, as described in the electronic supplementary material, Methods, where the standard equations used to calculate venous O_2 saturation, cardiac output, stroke volume and pulmonary O_2 extraction can also be found.

(c) Statistical analyses

Linear mixed effects models were used to test for effects of mouse population, acclimation P_{O_2} and acclimation temperature and were performed using the lme4 package [41] in R [42]. We first included data from across all four acclimation environments to test for the fixed effects of population, P_{O_2} and temperature, as well as all possible interactions between these three factors, which allowed us to evaluate whether populations differed in their interactive response to hypoxia and cold. However, this approach did not allow us to examine the potential population differences in the acclimation responses to hypoxia or cold on their own, which required two additional series of tests. We tested for the fixed effects of population, acclimation P_{O_2}, and the interaction between them by only including data for the warm acclimation environments, which allowed us to evaluate potential population differences in the response to chronic hypoxia in the absence of cold. We tested for the fixed effects of population, acclimation temperature, and the interaction between them by only including data among the normoxic acclimation environments, which allowed us to evaluate potential population differences in the response to chronic cold in the absence of hypoxia. Each series of tests was initially run including all of the potential interactions between the fixed factors, including body mass as a covariate and the random effects of sex and family. If any of the interactions or if the effects of body mass, sex or family did not approach significance ($p \geq 0.1$), we carried out a second final test in which these particular effects were removed. Only highlanders and lowland white-footed mice were included in models for the subset of cardiovascular measurements that were not also made in lowland deer mice. The full results of the linear mixed effects models are included in the electronic supplementary material (tables S1–S6), and the salient findings are reported in the Results. Tukey post-hoc tests were performed using the multcomp package in R [43]. These statistical analyses were carried out on absolute values of traits that were not corrected for body mass (because effects of body mass were accounted for in statistical models), but some data presented here are expressed relative to body mass as is conventional in the literature ($\dot{V}_{O_2\max}$, ventilatory and cardiac volumes). We also calculated Pearson correlations between $\dot{V}_{O_2\max}$ and its potential determinants using GraphPad Prism software (version 8.4; La Jolla, CA, USA), for which the *p*-values are reported in the Results and additional details are in electronic supplementary material table S7. $p < 0.05$ was considered statistically significant.

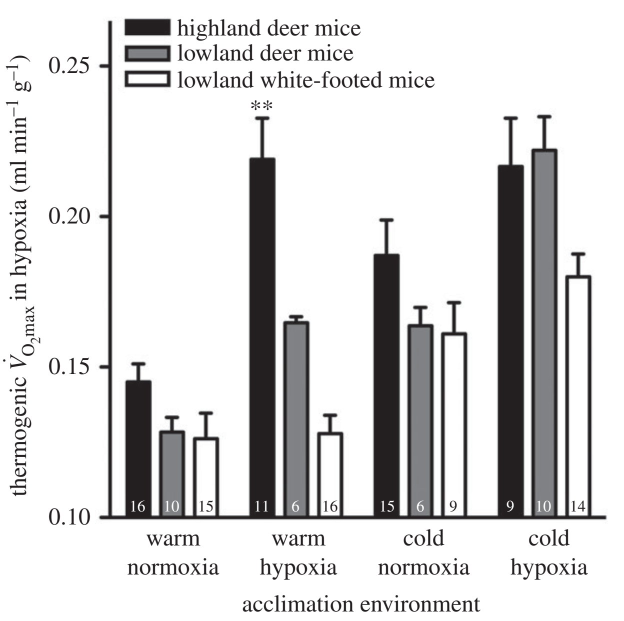

Figure 1. Thermogenic capacity, measured in hypoxia as the maximal rate of O_2 consumption ($\dot{V}_{O_2\max}$) during acute cold exposure, was found to vary across populations and acclimation treatments in statistical tests using linear mixed effects models (table 1). Asterisks indicate significant pairwise difference in highland deer mice compared with *lowland white-footed mice or **both lowland populations within an acclimation environment ($p < 0.05$). Data are means ± s.e.m., with *n* for each group indicated within each bar.

3. Results

(a) Thermogenic $\dot{V}_{O_2\max}$ in hypoxia

Thermogenic $\dot{V}_{O_2\max}$ in hypoxia was strongly affected by acclimation environment (figure 1), as reflected by highly significant effects of acclimation P_{O_2} and temperature in linear mixed effects models (table 1), but highland deer mice exhibited an especially pronounced acclimation response to hypoxia. As expected, body mass differed between species, as white-footed mice (26.7 ± 1.4 g in warm normoxia) were generally larger than both highland (19.6 ± 0.8 g) and lowland (22.2 ± 1.1 g) deer mice ($p < 0.001$) (electronic supplementary material, table S8). Body mass had statistically significant effects on $\dot{V}_{O_2\max}$ in linear mixed effects models and it varied across treatment groups (electronic supplementary material, tables S1–S6, S8), driven primarily by modest declines after acclimation to warm hypoxia in the lowland populations ($p = 0.009$ for the main effect of acclimation P_{O_2}). After taking the effects of body mass into account, hypoxia acclimation at warm temperature had a strong main effect on $\dot{V}_{O_2\max}$ ($p < 0.001$) that was driven entirely by deer mice ($\dot{V}_{O_2\max}$ did not differ between warm hypoxia and warm normoxia for white-footed mice). However, the effects of hypoxia acclimation were much greater in highlanders compared with lowlanders ($p < 0.001$ for the interaction between population and acclimation P_{O_2}), and $\dot{V}_{O_2\max}$ was 1.3- to 1.7-fold higher in highlanders than in lowlanders of both species in comparisons between populations acclimated to warm hypoxia. By contrast, cold acclimation had a strong main effect on $\dot{V}_{O_2\max}$ ($p < 0.001$) that was similar in magnitude across populations (non-significant population × temperature interaction). Acclimation to the combination of hypoxia and cold increased $\dot{V}_{O_2\max}$ in all populations (1.4- to 1.7-fold compared with warm normoxic mice), but the relative importance of each

Table 1. Summary of the results of linear mixed effects models that were used to test for effects of population and acclimation environment. $\dot{V}_{O_2max}$, maximal rate of O_2 consumption; $\dot{V}_I$, total ventilation; V_T, tidal volume; f_R, breathing frequency; EI_{O_2}, pulmonary O_2 extraction; Sa_{O_2}, arterial O_2 saturation; $\dot{Q}$, cardiac output; V_S, stroke volume; f_H, heart rate; [Hb], blood haemoglobin content; $S\bar{v}_{O_2}$, mixed venous O_2 saturation; P_{O_2}, partial pressure of O_2. Significant main effects and interactions between population (Pop) and acclimation P_{O_2} and/or temperature (*T*) are shown for models including data from all acclimation environments, only data from warm environments and only data from normoxic environments. See Methods for a full description and electronic supplementary material (tables S1–S6) for full results.

trait	only warm environments	only normoxic environments	all environments
$\dot{V}_{O_2max}$	P_{O_2}, Pop × P_{O_2}	*T*	P_{O_2}, *T*, Pop × P_{O_2}, Pop × P_{O_2} × *T*
$\dot{V}_I$	Pop, Pop × P_{O_2}	—	*T*, Pop × P_{O_2}
V_T	Pop, P_{O_2}, Pop × P_{O_2}	—	Pop, P_{O_2}, Pop × P_{O_2}
f_R	Pop, P_{O_2}, Pop × P_{O_2}	Pop, *T*	Pop, P_{O_2}, *T*
EI_{O_2}	Pop, P_{O_2}	*T*	Pop, P_{O_2}, *T*
Sa_{O_2}	Pop	Pop	Pop, P_{O_2}
$\dot{Q}$	P_{O_2}	Pop, *T*	Pop, P_{O_2}, *T*
V_S	—	Pop, *T*	*T*, Pop × P_{O_2}
f_H	—	—	P_{O_2}, Pop × *T*, P_{O_2} × *T*
[Hb]	Pop, P_{O_2}	Pop	Pop, P_{O_2}
$S\bar{v}_{O_2}$	—	*T*	P_{O_2}, *T*

environmental parameter on the acclimation response differed between populations, as reflected by a significant population × P_{O_2} × temperature interaction ($p = 0.045$). In lowlanders of both species, the response to cold hypoxia appeared to be slightly greater than the sum of the individual responses to hypoxia or cold alone. For example, in lowland deer mice, the sum of the magnitude of the responses to warm hypoxia and cold normoxia (0.04 ml min^{-1} g^{-1} in each case) was slightly less than the magnitude of the response to cold hypoxia (0.09 ml min^{-1} g^{-1}), when each response magnitude was calculated as the average absolute difference from warm normoxia. Similarly, in white-footed mice, the sum of the responses to warm hypoxia and cold normoxia (0 and 0.04, respectively) was slightly less than the response to cold hypoxia (0.05 ml min^{-1} g^{-1}). This was not the case in highlanders, however, in which the response to warm hypoxia was just as large as the response to cold hypoxia.

(b) Breathing and pulmonary O_2 uptake at $\dot{V}_{O_2max}$

Breathing was measured by plethysmography to examine whether it contributed to some of the variation in $\dot{V}_{O_2max}$ across treatment groups (figure 2, table 1). Breathing frequency at $\dot{V}_{O_2max}$ appeared to vary across groups, largely because highlanders had higher breathing frequencies than lowlanders after acclimation to warm hypoxia ($p = 0.007$ for population × P_{O_2} interaction) or cold normoxia ($p = 0.015$ for population effect). Tidal volumes at $\dot{V}_{O_2max}$ varied little across acclimation environments in both populations of deer mice, such that the patterns of variation in total ventilation appeared to be very similar to the variation in breathing frequency for these populations. However, hypoxia acclimation tended to reduce tidal volume at $\dot{V}_{O_2max}$ in white-footed mice, which is likely responsible for the significant population × P_{O_2} interaction for this trait ($p = 0.014$), and there was also a significant population × P_{O_2} interaction for total ventilation ($p = 0.001$). Nevertheless, total ventilation was significantly correlated with $\dot{V}_{O_2max}$ across all groups ($p = 0.004$).

Pulmonary O_2 extraction at $\dot{V}_{O_2max}$ also appeared to vary across treatment groups (figure 3, table 1). Pulmonary O_2 extraction tended to increase in cold and/or hypoxic acclimation environments compared with warm normoxic controls, as reflected by significant main effects of acclimation P_{O_2} ($p = 0.024$) and temperature ($p = 0.040$). There was a significant main effect of population overall ($p = 0.002$), driven largely by higher values of pulmonary O_2 extraction in highlanders that were greatest after hypoxia acclimation. Pulmonary O_2 uptake was strongly correlated with $\dot{V}_{O_2max}$ across all groups ($p < 0.0001$).

(c) Circulatory O_2 transport at $\dot{V}_{O_2max}$

We observed significant variation in arterial O_2 saturation (measured by pulse oximetry) and the content of haemoglobin in the blood across treatment groups (table 1). Arterial O_2 saturation varied little in response to acclimation environment but saturation was consistently 7% to 12% higher in highlanders than in lowlanders of both species ($p < 0.001$ for population effect) (figure 4*a*), and there was a significant correlation between arterial O_2 saturation and $\dot{V}_{O_2max}$ across all groups ($p = 0.0004$). Blood haemoglobin content increased in response to hypoxic but not cold acclimation environments, as reflected by main effects of acclimation P_{O_2} ($p = 0.024$) but not temperature ($p = 0.574$) (table 2). However, blood haemoglobin content tended to be highest in white-footed mice ($p < 0.001$ for population effect), and the response to hypoxia acclimation was generally similar between highland and lowland deer mice (non-significant population × P_{O_2} interactions). As a result, blood haemoglobin content was not correlated to $\dot{V}_{O_2max}$ across groups ($p = 0.465$).

There was also some variation across treatment groups in venous O_2 saturation at $\dot{V}_{O_2max}$ (figure 4*b*, table 1), which was calculated from measurements of blood haemoglobin content and the O_2 content of mixed venous blood sampled from cannulated mice (electronic supplementary material, table S9). In contrast with arterial O_2 saturation, linear mixed effects

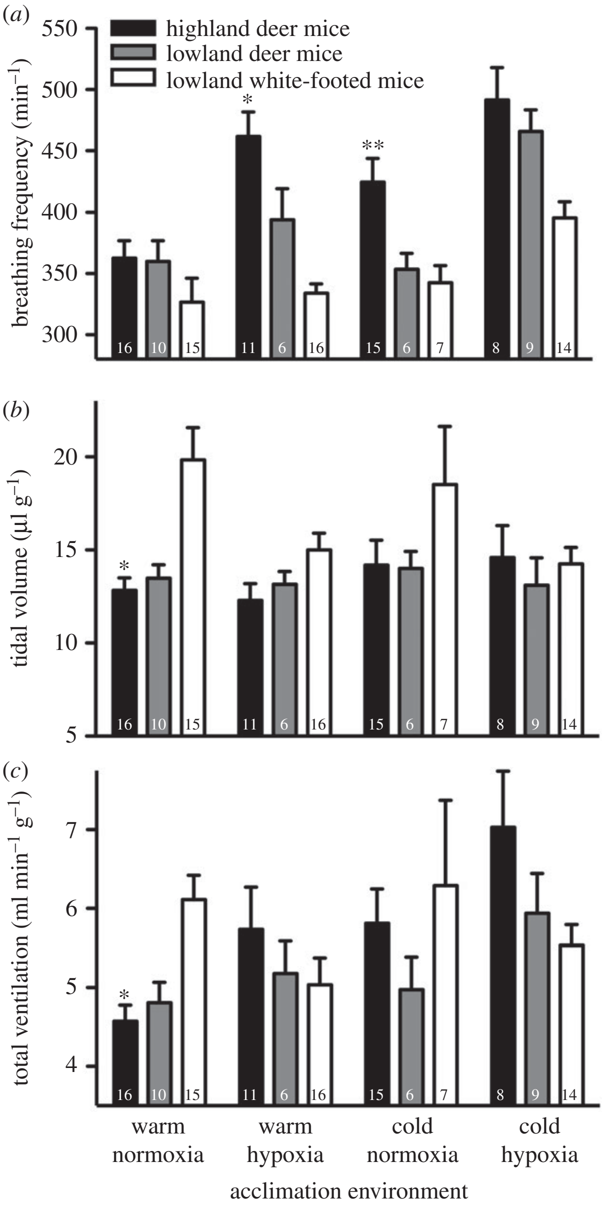

Figure 2. Breathing at $\dot{V}_{O_2max}$ was found to vary across populations and acclimation treatments. See figure 1 for symbol definitions and statistical details.

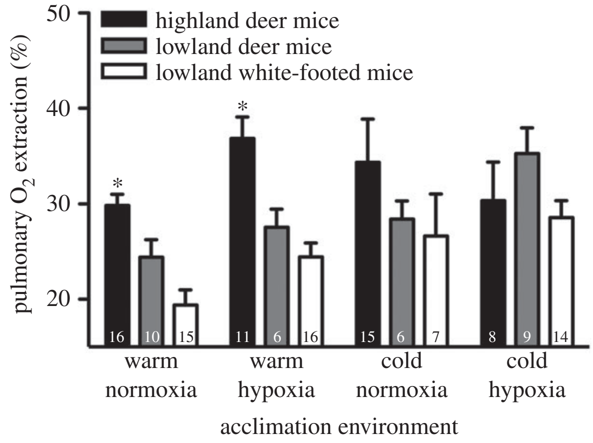

Figure 3. Pulmonary O_2 extraction at $\dot{V}_{O_2max}$ was found to vary across populations and acclimation treatments. See figure 1 for symbol definitions and statistical details.

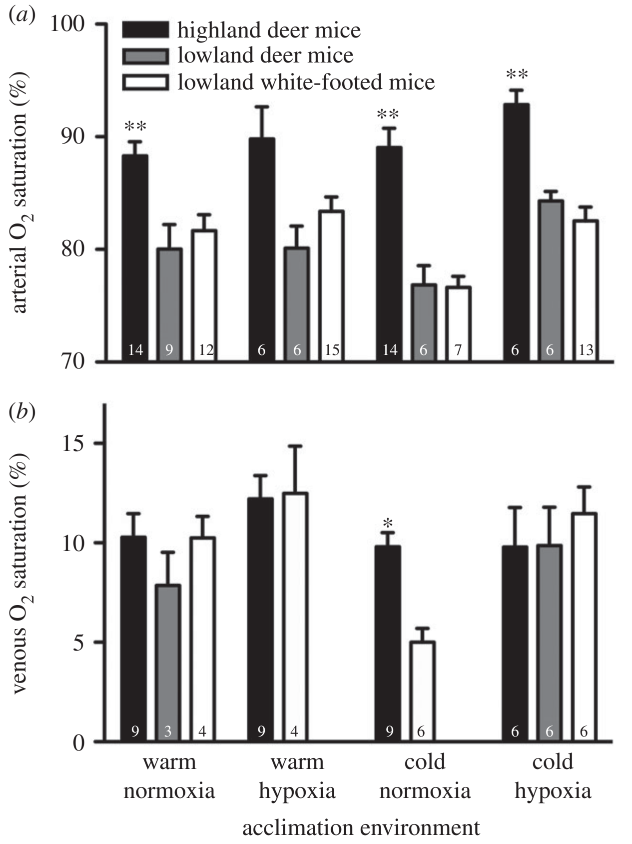

Figure 4. Arterial O_2 saturation at $\dot{V}_{O_2max}$ was augmented in high-altitude mice. See figure 1 for symbol definitions and statistical details.

models comparing highland deer mice and lowland white-footed mice did not detect any significant population effects on venous O_2 saturation. However, there was a significant main effect of cold acclimation on venous O_2 saturation ($p = 0.004$), driven largely by a lower value in white-footed mice compared with highland deer mice in cold normoxia. Tissue O_2 extraction—calculated as the difference between average arterial and venous O_2 saturations—was generally higher in highlanders (77–83%) than in lowlanders (71–74%), due primarily to the higher arterial O_2 saturations in highlanders (figure 4*a*). The apparent drop in venous O_2 saturation in cold-acclimated white-footed mice did not appear to increase tissue O_2 extraction (72% in this group), because it was associated with a non-significant decline in arterial O_2 saturation compared with other acclimation environments. There were some differences across acclimation groups in blood acid–base status and lactate concentration at $\dot{V}_{O_2max}$—cold acclimation groups appeared to have increased venous pH and bicarbonate concentration and decreased plasma lactate compared with warm acclimation groups—but there were no appreciable or consistent differences between populations (electronic supplementary material, figures S1 and S2).

Differences in cardiac output (calculated using the Fick equation) appeared to contribute to the variation in $\dot{V}_{O_2max}$ across treatment groups (figure 5, table 1), based on the strong correlation between cardiac output and $\dot{V}_{O_2max}$ across all groups ($p < 0.0001$). Highland mice generally had higher cardiac output at $\dot{V}_{O_2max}$ than lowland white-footed mice, as reflected by a significant population effect across all environments ($p = 0.029$) that appeared to be largely due to variation in stroke volume that neared significance ($p = 0.067$ for population effect across all environments). The significant main effects of both acclimation P_{O_2} and temperature ($p < 0.001$ each) suggested that cardiac output at $\dot{V}_{O_2max}$ increased in mice from cold and/or hypoxic acclimation environments

Table 2. Blood haemoglobin content (g dl^{-1}) of mice in each acclimation environment. Data are means ± s.e.m. (*n*).

acclimation environment	highland deer mice	lowland deer mice	lowland white-footed mice
warm (25°C) normoxia	15.7 ± 0.5 (13)	16.9 ± 0.9 (9)	17.5 ± 0.7 (14)
warm (25°C) hypoxia	17.9 ± 1.0 (10)	18.1 ± 0.4 (6)	20.5 ± 0.6 (14)
cold (5°C) normoxia	16.2 ± 0.5 (24)	14.7 ± 1.2 (6)	18.2 ± 0.5 (17)
cold (5°C) hypoxia	17.2 ± 1.5 (11)	19.9 ± 1.0 (9)	21.3 ± 1.0 (12)

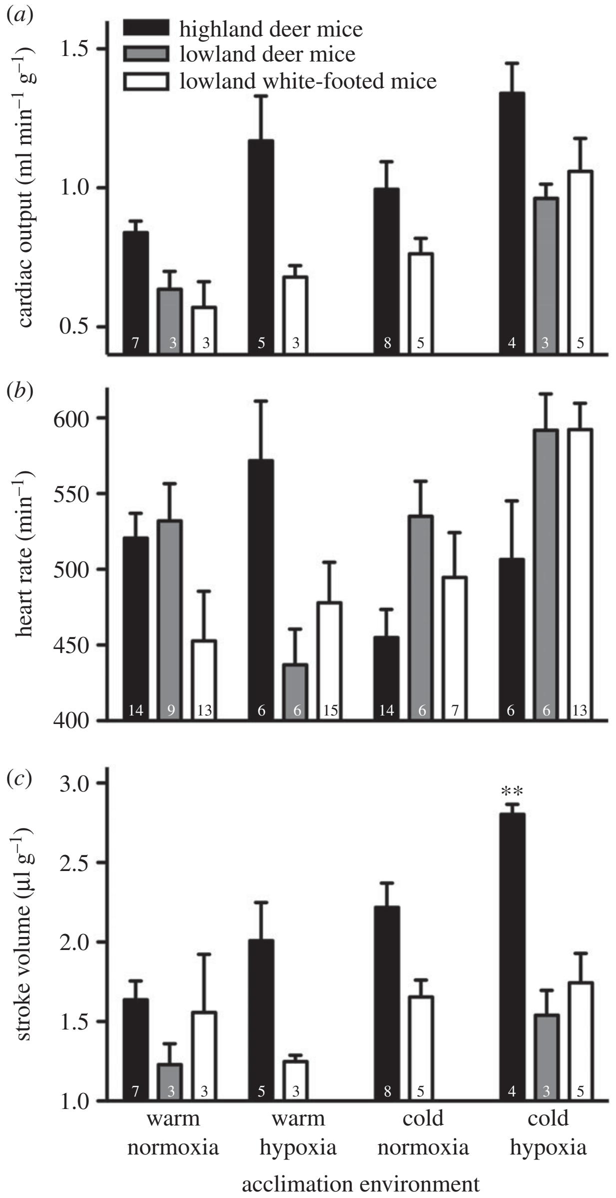

Figure 5. Cardiac output at $\dot{V}_{O_2\max}$ was found to vary across populations and acclimation treatments. See figure 1 for symbol definitions and statistical details.

compared with warm normoxic controls, but the magnitude of the changes suggested that these effects were driven much more by highland deer mice than lowland white-footed mice. The relative influence of changes in stroke volume versus heart rate on cardiac output between acclimation environments also differed across populations. For stroke volume, there was a significant population × P_{O_2} interaction across all environments ($p = 0.020$), and there appeared to be greater increases in stroke volume in highlanders in response to hypoxic and/or cold acclimation environments, reaching values that were 1.6- to 1.8-fold greater on average than lowlanders of both species in cold hypoxia. For heart rate, there was a significant population × temperature interaction ($p < 0.001$), in large part because cold acclimation environments tended to reduce heart rates at $\dot{V}_{O_2\max}$ in highlanders but not in lowlanders (as compared with heart rates of mice from the warm normoxic environment). As such, the increase in cardiac output at $\dot{V}_{O_2\max}$ in response to cold hypoxia acclimation was driven primarily by increases in heart rate in lowland white-footed mice, but increases in stroke volume were a larger contributor in highland deer mice.

4. Discussion

Deer mice at high altitudes sustain high metabolic rates to support thermogenesis [32] and appear to be subject to strong directional selection for increased thermogenic capacity in hypoxia [15]. Here, we show that adaptive increases in thermogenic $\dot{V}_{O_2\max}$ in hypoxia arise from evolved changes and plasticity in response to the major stressors at high altitude, hypoxia and cold. High-altitude mice exhibited an exaggerated increase in $\dot{V}_{O_2\max}$ in response to chronic hypoxia, which appeared to completely dominate the response to concurrent hypoxia and cold. The variation in thermogenic $\dot{V}_{O_2\max}$ appeared to be explained by evolved and environmentally induced variation across the O_2 transport pathway, including breathing, pulmonary O_2 extraction, arterial O_2 saturation, cardiac output and tissue O_2 extraction. Therefore, both evolutionary adaptation and phenotypic plasticity contribute to coordinated changes in the function of the O_2 pathway that lead to adaptive increases in thermogenic capacity in deer mice at high altitudes.

(a) Combined effects of hypoxia and cold on thermogenic capacity

The effects of chronic hypoxia and/or cold on thermogenic capacity suggest that phenotypic plasticity can improve the ability of small mammals to cope with the cold environment at high altitude. Cold acclimation is well known to increase thermogenic $\dot{V}_{O_2\max}$ as well as the capacity for non-shivering thermogenesis (NST) in deer mice and other small mammals [17,21,44–48]. Hypoxia acclimation does not generally increase $\dot{V}_{O_2\max}$ in humans [49]; however, it has been shown to increase $\dot{V}_{O_2\max}$ in rodents during thermogenesis or exercise when measured in hypoxia, but not necessarily when measured in normoxia, suggesting that the responses of rodents to chronic hypoxia act primarily to reduce the depressive effects of hypoxia on $\dot{V}_{O_2\max}$ [35,36,50]. Little was previously known about how $\dot{V}_{O_2\max}$ changes after chronic exposure to hypoxia and cold in combination, but prior studies in house mice suggest that these stressors have opposing (but additive) effects on the capacity for NST in normoxia, such that cold hypoxic mice have similar NST capacity to warm normoxic mice [17]. Our results in lowland deer mice suggest that hypoxia and cold as acclimation treatments have additive or more than additive effects that increase thermogenic $\dot{V}_{O_2max}$ in hypoxia when they occur in combination, possibly because cold acclimation tends to increase $\dot{V}_{O_2max}$ in normoxia while hypoxia acclimation makes $\dot{V}_{O_2max}$ less sensitive to reductions in environmental P_{O_2}.

Plastic changes across the O_2 transport pathway appeared to underlie the increases in thermogenic capacity in response to chronic hypoxia and/or cold. Previous studies in rats also found that cold acclimation increased O_2 consumption at cold temperatures via increases in cardiac output, with no change in the arterial–venous difference in O_2 saturation or O_2 content, and the increased cardiac output largely served to increase blood flow to multiple depots of brown adipose tissue throughout the body [46]. Hypoxia acclimation has also been found to increase exercise $\dot{V}_{O_2max}$ in hypoxia (but not in normoxia) in rats, in association with decreases in arterial CO_2 tension (which could reflect an increase in alveolar ventilation) and with increases in arterial P_{O_2} and O_2 saturation, blood haemoglobin content and tissue O_2 extraction [50]. However, cardiac output and heart rate at $\dot{V}_{O_2max}$ were lower after hypoxia acclimation in this particular study [50], and subsequent arterial pacing studies suggested that these reductions in cardiac output constrained the plastic increases in $\dot{V}_{O_2max}$ [51]. Similarly, heart rate at $\dot{V}_{O_2max}$ was reduced after acclimation to warm hypoxia in lowland deer mice, but this was not observed in other populations, and cardiac output was highest in the cold hypoxic groups of all populations. Therefore, responses to chronic cold may over-ride the effects of chronic hypoxia that could otherwise constrain cardiac output and $\dot{V}_{O_2max}$ in lowland mice during acclimation to high-altitude conditions.

(b) High-altitude deer mice have evolved an enhanced hypoxia acclimation response

Our findings here suggest that directional selection for high thermogenic capacity at high altitude [15] has increased $\dot{V}_{O_2max}$ in highland mice by amplifying the plastic response to chronic hypoxia, consistent with our previous findings [36]. These findings are consistent with a scenario where, upon colonization of the high-altitude environment, directional selection on $\dot{V}_{O_2max}$ increased the magnitude of adaptive phenotypic plasticity in this trait, and thus shifted the population mean closer to the fitness optimum [22]. Our results contribute to growing evidence suggesting that high-altitude natives of various taxa have evolved to become more resistant to the depressive effects of hypoxia on $\dot{V}_{O_2max}$ than their low-altitude counterparts [52].

It is intriguing to consider why highland and lowland deer mouse populations exhibited similar plasticity of $\dot{V}_{O_2max}$ in response to the combination of cold and hypoxia. Although this may call into question the adaptive significance of the enhanced plasticity in response to warm hypoxia in highlanders, the population difference in the interaction between cold and hypoxia (i.e. significant population × P_{O_2} × temperature interaction) suggests that it may still have adaptive significance. The strong response of highlanders to hypoxia alone appeared to dominate the acclimation response to hypoxia and cold in combination, in stark contrast with the responses of lowland mice. We speculate that this strong hypoxia response of highlanders may allow them to respond more strongly than lowlanders if they are exposed to colder temperatures than those used for cold acclimations here (5°C). Indeed, 5°C may underestimate the intensity of cold exposure at high altitude in the wild, because the high peaks of the Rocky Mountains are snow covered for much of the year. Future studies of plasticity in response to hypoxia at colder temperatures are needed to explore this possibility.

Strong increases in some of the systems-level determinants of O_2 transport probably contributed to the evolved increase in $\dot{V}_{O_2max}$ in response to hypoxia acclimation in high-altitude deer mice. Increases in breathing frequency at $\dot{V}_{O_2max}$ after acclimation to warm hypoxia were much greater in highlanders than in lowlanders, which would be expected to help increase thermogenic $\dot{V}_{O_2max}$ if it augmented alveolar ventilation. However, highlanders tended to have relatively low tidal volumes at $\dot{V}_{O_2max}$, and as a result, variation in total ventilation was not clearly associated with the increased thermogenic $\dot{V}_{O_2max}$ in highlanders after acclimation to warm hypoxia. Highlanders may have also relied upon more pronounced increases in pulmonary O_2 extraction after acclimation to warm hypoxia to augment O_2 uptake into the blood. Cardiac output exhibited a particularly strong increase of 1.4-fold in highlanders after acclimation to warm hypoxia compared with warm normoxic controls. This is in stark contrast with low-altitude mice, in which cardiac output at $\dot{V}_{O_2max}$ changed very little as a result of hypoxia acclimation, and to previous studies in rats, in which cardiac output at $\dot{V}_{O_2max}$ decreased after exposure to chronic hypoxia [50].

Recent theoretical evidence suggests that the evolution of plasticity in complex traits depends upon the level of functional integration between the multiple component mechanisms underlying those traits [26]. For thermogenic capacity, the integration between its underlying component mechanisms (i.e. steps in the O_2 transport pathway) is extensive. For example, increases in arterial O_2 saturation may be of little benefit to aerobic capacity if they are not combined with increases in tissue O_2 extraction [53]. Our findings here suggest that this integration may contribute to the enhanced plasticity of thermogenic $\dot{V}_{O_2max}$ in chronic hypoxia in highlanders. The effect of hypoxia acclimation on $\dot{V}_{O_2max}$ (which increased 1.7-fold in warm hypoxia compared with warm normoxia) was greater in magnitude than the effects of hypoxia acclimation on any of its systems-level determinants from across the O_2 transport pathway. Therefore, no single component can explain the evolved increase in the plasticity of $\dot{V}_{O_2max}$ in highlanders, but it is instead explained by the interactive effects of changes in plasticity and/or mean trait value for each of these components. For example, the effects of increased plasticity in cardiac output combined with the increased mean values of arterial O_2 saturation and tissue O_2 extraction (neither of which were plastic themselves) could together be responsible for amplifying the increase in O_2 transport to thermogenic tissues and $\dot{V}_{O_2max}$ after hypoxia acclimation.

(c) Coordinated changes across the O_2 transport pathway augment thermogenic capacity in high-altitude deer mice

Our results suggest that high-altitude deer mice have evolved functional changes across the O_2 pathway to support thermogenic performance in hypoxia. Several plastic physiological processes—breathing frequency, pulmonary O_2 extraction, cardiac output and stroke volume—were often higher in highlanders than in lowlanders, particularly after hypoxia acclimation. Some other physiological processes exhibited very little plasticity—namely, arterial O_2 saturation and the arterial-venous difference in O_2 saturation—but were consistently greater in highlanders than in lowlanders. These latter changes may be at least partly explained by the evolved increases in haemoglobin-O_2 affinity [31,54,55] and in the capillarity and oxidative capacity of skeletal muscle [35,56–59] in high-altitude deer mice. Therefore, evolutionary adaptation to high altitude has amplified some of the mechanisms that contribute to plasticity in lowlanders, but it has also expanded the physiological toolkit for increasing thermogenic $\dot{V}_{O_2\mathrm{max}}$ under hypoxic conditions. Our results also suggest that the evolution of thermogenic $\dot{V}_{O_2\mathrm{max}}$ in high-altitude deer mice has occurred through similar mechanisms to the increases in exercise $\dot{V}_{O_2\mathrm{max}}$ in some human populations native to high altitude. For example, the augmented exercise $\dot{V}_{O_2\mathrm{max}}$ in high-altitude humans in hypoxia is associated with higher pulmonary O_2 diffusing capacity and cardiac output [4,28,29,52]. However, in many human studies, it has been difficult to disentangle the genetic and environmental components of variation in high-altitude phenotypes [52,60]. Our results here suggest that both plastic and evolved changes in the O_2 pathway of high-altitude deer mice have contributed to the success of these animals in harsh alpine environments.

Ethics. All procedures followed guidelines set out by the Canadian Council on Animal Care and were approved by the McMaster University Animal Research Ethics Board (Animal Use Protocol 16-01-02).

Data accessibility. Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.rjdfn2z7d [61].

Authors' contributions. Z.A.C., J.F.S., G.B.M. and G.R.S. designed the study. K.B.T., O.H.W. and C.M.I. ran the experiments and analysed the data. K.B.T., O.H.W. and G.R.S. wrote the manuscript, and all authors edited the manuscript.

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References

1. Dalziel AC, Rogers SM, Schulte PM. 2009 Linking genotypes to phenotypes and fitness: how mechanistic biology can inform molecular ecology. *Mol. Ecol.* **18**, 4997–5017. (doi:10.1111/j.1365-294X.2009.04427.x)
2. Garland TJ, Carter PA. 1994 Evolutionary physiology. *Annu. Rev. Physiol.* **56**, 579–621. (doi:10.1146/annurev.ph.56.030194.003051)
3. Weibel ER, Taylor CR, Weber JM, Vock R, Roberts TJ, Hoppeler H. 1996 Design of the oxygen and substrate pathways. VII. Different structural limits for oxygen and substrate supply to muscle mitochondria. *J. Exp. Biol.* **199**, 1699–1709.
4. McClelland GB, Scott GR. 2019 Evolved mechanisms of aerobic performance and hypoxia resistance in high-altitude natives. *Annu. Rev. Physiol.* **81**, 561–583. (doi:10.1146/annurev-physiol-021317-121527)
5. Weibel ER, Taylor CR, Hoppeler H. 1991 The concept of symmorphosis: a testable hypothesis of structure–function relationship. *Proc. Natl Acad. Sci. USA* **88**, 10 357–10 361. (doi:10.1073/pnas.88.22.10357)
6. Weibel ER, Taylor CR, Gehr P, Hoppeler H, Mathieu O, Maloiy GMO. 1981 Design of the mammalian respiratory system. IX. Functional and structural limits for oxygen flow. *Respir. Physiol.* **44**, 151–164. (doi:10.1016/0034-5687(81)90081-5)
7. Gonzalez NC, Kirkton SD, Howlett RA, Britton SL, Koch LG, Wagner HE, Wagner PD. 2006 Continued divergence in VO2max of rats artificially selected for running endurance is mediated by greater convective blood O2 delivery. *J. Appl. Physiol.* **101**, 1288–1296. (doi:10.1152/japplphysiol.01527.2005)
8. Kirkton SD, Howlett RA, Gonzalez NC, Giuliano PG, Britton SL, Koch LG, Wagner HE, Wagner PD. 2009 Continued artificial selection for running endurance in rats is associated with improved lung function. *J. Appl. Physiol.* **106**, 1810–1818. (doi:10.1152/japplphysiol.90419.2008)
9. Henderson KK, Wagner H, Favret F, Britton SL, Koch LG, Wagner PD, Gonzalez NC. 2002 Determinants of maximal O2 uptake in rats selectively bred for endurance running capacity. *J. Appl. Physiol.* **93**, 1265–1274. (doi:10.1152/japplphysiol.00809.2001)
10. Scott GR, Milsom WK. 2006 Flying high: a theoretical analysis of the factors limiting exercise performance in birds at altitude. *Respir. Physiol. Neurobiol.* **154**, 284–301. (doi:10.1016/j.resp.2006.02.012)
11. Wagner PD. 1996 A theoretical analysis of factors determining Vo2max at sea level and altitude. *Respir. Physiol.* **106**, 329–343. (doi:10.1016/S0034-5687(96)00086-2)
12. Chappell MA, Hammond KA. 2004 Maximal aerobic performance of deer mice in combined cold and exercise challenges. *J. Comp. Physiol. B* **174**, 41–48. (doi:10.1007/s00360-003-0387-z)
13. Rosenmann M, Morrison P. 1974 Maximum oxygen consumption and heat loss facilitation in small homeotherms by He-O_2. *Am. J. Physiol.* **226**, 490–495. (doi:10.1152/ajplegacy.1974.226.3.490)
14. Storz JF, Cheviron ZA, McClelland GB, Scott GR. 2019 Evolution of physiological performance capacities and environmental adaptation: insights from high-elevation deer mice (*Peromyscus maniculatus*). *J. Mamm.* **100**, 910–922. (doi:10.1093/jmammal/gyy173)
15. Hayes JP, O'Connor CS. 1999 Natural selection on thermogenic capacity of high-altitude deer mice. *Evolution* **53**, 1280–1287. (doi:10.1111/j.1558-5646.1999.tb04540.x)
16. Sears MW, Hayes JP, O'Connor CS, Geluso K, Sedinger JS. 2006 Individual variation in thermogenic capacity affects above-ground activity of high-altitude deer mice. *Funct. Ecol.* **20**, 97–104. (doi:10.1111/j.1365-2435.2006.01067.x).
17. Beaudry JL, McClelland GB. 2010 Thermogenesis in CD-1 mice after combined chronic hypoxia and cold acclimation. *Comp. Biochem. Physiol. B. Biochem. Mol. Biol.* **157**, 301–309. (doi:10.1016/j.cbpb.2010.07.004)
18. Chi QS, Wang DH. 2011 Thermal physiology and energetics in male desert hamsters (*Phodopus roborovskii*) during cold acclimation. *J. Comp. Physiol. B* **181**, 91–103. (doi:10.1007/s00360-010-0506-6)
19. Mineo PM, Cassell EA, Roberts ME, Schaeffer PJ. 2012 Chronic cold acclimation increases thermogenic capacity, non-shivering thermogenesis and muscle citrate synthase activity in both wild-type and brown adipose tissue deficient mice. *Comp. Biochem. Physiol. A* **161**, 395–400. (doi:10.1016/j.cbpa.2011.12.012)
20. Rezende EL, Chappell MA, Hammond KA. 2004 Cold-acclimation in *Peromyscus*: temporal effects and individual variation in maximum metabolism and ventilatory traits. *J. Exp. Biol.* **207**, 295–305. (doi:10.1242/jeb.00760)

21. Van Sant MJ, Hammond KA. 2008 Contribution of shivering and nonshivering thermogenesis to thermogenic capacity for the deer mouse (*Peromyscus maniculatus*). *Physiol. Biochem. Zool.* **81**, 605–611. (doi:10.1086/588175)
22. Crispo E. 2007 The Baldwin effect and genetic assimilation: revisiting two mechanisms of evolutionary change mediated by phenotypic plasticity. *Evolution* **61**, 2469–2479. (doi:10.1111/j.1558-5646.2007.00203.x)
23. Pigliucci M, Murren CJ, Schlichting CD. 2006 Phenotypic plasticity and evolution by genetic assimilation. *J. Exp. Biol.* **209**, 2362–2367. (doi:10.1242/jeb.02070)
24. Price TD, Qvarnström A, Irwin DE. 2003 The role of phenotypic plasticity in driving genetic evolution. *Proc. R. Soc. Lond. Ser. B* **270**, 1433–1440. (doi:10.1098/rspb.2003.2372)
25. Lande R. 2015 Evolution of phenotypic plasticity in colonizing species. *Mol. Ecol.* **24**, 2038–2045. (doi:10.1111/mec.13037)
26. Lande R. 2019 Developmental integration and evolution of labile plasticity in a complex quantitative character in a multiperiodic environment. *Proc. Natl Acad. Sci. USA* **116**, 11 361–11 369. (doi:10.1073/pnas.1900528116)
27. Monge C, León-Velarde F. 1991 Physiological adaptation to high altitude: oxygen transport in mammals and birds. *Physiol. Rev.* **71**, 1135–1172. (doi:10.1152/physrev.1991.71.4.1135)
28. Storz JF, Scott GR. 2019 Life ascending: mechanism and process in physiological adaptation to high-altitude hypoxia. *Annu. Rev. Ecol. Evol. Syst.* **50**, 503–526. (doi:10.1146/annurev-ecolsys-110218-025014)
29. Gilbert-Kawai ET, Milledge JS, Grocott MP, Martin DS. 2014 King of the mountains: Tibetan and Sherpa physiological adaptations for life at high altitude. *Physiology* **29**, 388–402. (doi:10.1152/physiol.00018.2014)
30. Natarajan C, Hoffmann FG, Lanier HC, Wolf CJ, Cheviron ZA, Spangler ML, Weber RE, Fago A, Storz JF. 2015 Intraspecific polymorphism, interspecific divergence, and the origins of function-altering mutations in deer mouse hemoglobin. *Mol. Biol. Evol.* **32**, 978–997. (doi:10.1093/molbev/msu403)
31. Snyder LRG, Born S, Lechner AJ. 1982 Blood oxygen affinity in high- and low-altitude populations of the deer mouse. *Respir. Physiol.* **48**, 89–105. (doi:10.1016/0034-5687(82)90052-4)
32. Hayes JP. 1989 Field and maximal metabolic rates of deer mice (*Peromyscus maniculatus*) at low and high altitudes. *Physiol. Zool.* **62**, 732–744. (doi:10.1086/638202).
33. Cheviron ZA, Bachman GC, Connaty AD, McClelland GB, Storz JF. 2012 Regulatory changes contribute to the adaptive enhancement of thermogenic capacity in high-altitude deer mice. *Proc. Natl Acad. Sci. USA* **109**, 8635–8640. (doi:10.1073/pnas.1120523109)
34. Cheviron ZA, Bachman GC, Storz JF. 2013 Contributions of phenotypic plasticity to differences in thermogenic performance between highland and lowland deer mice. *J. Exp. Biol.* **216**, 1160–1166. (doi:10.1242/jeb.075598)
35. Lui MA, Mahalingam S, Patel P, Connaty AD, Ivy CM, Cheviron ZA, Storz JF, McClelland GB, Scott GR. 2015 High-altitude ancestry and hypoxia acclimation have distinct effects on exercise capacity and muscle phenotype in deer mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **308**, R779-R791. (doi:10.1152/ajpregu.00362.2014)
36. Tate KB, Ivy CM, Velotta JP, Storz JF, McClelland GB, Cheviron ZA, Scott GR. 2017 Circulatory mechanisms underlying adaptive increases in thermogenic capacity in high-altitude deer mice. *J. Exp. Biol.* **220**, 3616–3620. (doi:10.1242/jeb.164491)
37. Hammond KA, Szewczak J, Krol E. 2001 Effects of altitude and temperature on organ phenotypic plasticity along an altitudinal gradient. *J. Exp. Biol.* **204**, 1991–2000.
38. Ivy CM, Scott GR. 2017 Control of breathing and ventilatory acclimatization to hypoxia in deer mice native to high altitudes. *Acta Physiol.* **221**, 266–282. (doi:10.1111/apha.12912)
39. Ivy CM, Scott GR. 2017 Ventilatory acclimatization to hypoxia in mice: methodological considerations. *Respir. Physiol. Neurobiol.* **235**, 95–103. (doi:10.1016/j.resp.2016.10.012)
40. Tucker VA. 1967 Method for oxygen content and dissociation curves on microliter blood samples. *J. Appl. Physiol.* **23**, 410–414. (doi:10.1152/jappl.1967.23.3.410)
41. Bates D, Machler M, Bolker BM, Walker SC. 2015 Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 1–48. (doi:10.18637/jss.v067.i01)
42. R Core Team. 2013 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing, http://www.R-project.org/.
43. Hothorn T, Bretz F, Westfall P. 2008 Simultaneous inference in general parametric models. *Biom. J.* **50**, 346–363. (doi:10.1002/bimj.200810425)
44. Heimer W, Morrison P. 1978 Effects of chronic and intermittent cold exposure on metabolic capacity of *Peromyscus* and *Microtus*. *Int. J. Biometeorol.* **22**, 129–134. (doi:10.1007/BF01552893)
45. Rezende EL, Bozinovic F, Garland TJ. 2004 Climatic adaptation and the evolution of basal and maximum rates of metabolism in rodents. *Evolution* **58**, 1361–1374. (doi:10.1111/j.0014-3820.2004.tb01714.x)
46. Foster DO, Frydman ML. 1979 Tissue distribution of cold-induced thermogenesis in conscious warm- or cold-acclimated rats reevaluated from changes in tissue blood flow: the dominant role of brown adipose tissue in the replacement of shivering by nonshivering thermogenesis. *Can. J. Physiol. Pharmacol.* **57**, 257–270. (doi:10.1139/y79-039)
47. Hayes JP, Chappell MA. 1986 Effects of cold acclimation on maximum oxygen consumption during cold exposure and treadmill exercise in deer mice, *Peromyscus maniculatus*. *Physiol. Zool.* **59**, 473–481. (doi:10.1086/physzool.59.4.30158600)
48. Davis TRA, Johnston DR, Bell FC, Cremer BJ. 1960 Regulation of shivering and nonshivering heat production during acclimation of rats. *Am. J. Physiol.* **198**, 471–475. (doi:10.1152/ajplegacy.1960.198.3.471).
49. Cerretelli P. 1980 Gas exchange at high altitude. In *Pulmonary gas exchange* (ed. JB West), pp. 97–147. New York, NY: Academic Press.
50. Gonzalez NC, Clancy RL, Wagner PD. 1993 Determinants of maximal oxygen uptake in rats acclimated to simulated altitude. *J. Appl. Physiol.* **75**, 1608–1614. (doi:10.1152/jappl.1993.75.4.1608)
51. Gonzalez NC, Clancy RL, Moue Y, Richalet J-P. 1998 Increasing maximal heart rate increases maximal O_2 uptake in rats acclimatized to simulated altitude. *J. Appl. Physiol.* **84**, 164–168. (doi:10.1152/jappl.1998.84.1.164)
52. Brutsaert T. 2016 Why are high altitude natives so strong at high altitude? Nature vs. nurture: genetic factors vs. growth and development. *Adv. Exp. Med. Biol.* **903**, 101–112. (doi:10.1007/978-1-4899-7678-9_7)
53. Wagner PD. 1997 Insensitivity of VO_{2max} to hemoglobin-P50 at sea level and altitude. *Respir. Physiol.* **107**, 205–212. (doi:10.1016/s0034-5687(96)02512-1)
54. Chappell MA, Snyder LRG. 1984 Biochemical and physiological correlates of deer mouse α-chain hemoglobin polymorphisms. *Proc. Natl Acad. Sci. USA* **81**, 5484–5488. (doi:10.1073/pnas.81.17.5484)
55. Storz JF, Runck AM, Moriyama H, Weber RE, Fago A. 2010 Genetic differences in hemoglobin function between highland and lowland deer mice. *J. Exp. Biol.* **213**, 2565–2574. (doi:10.1242/jeb.042598)
56. Mahalingam S, McClelland GB, Scott GR. 2017 Evolved changes in the intracellular distribution and physiology of muscle mitochondria in high-altitude native deer mice. *J. Physiol.* **595**, 4785–4801. (doi:10.1113/jp274130).
57. Scott GR, Elogio TS, Lui MA, Storz JF, Cheviron ZA. 2015 Adaptive modifications of muscle phenotype in high-altitude deer mice are associated with evolved changes in gene regulation. *Mol. Biol. Evol.* **32**, 1962–1976. (doi:10.1093/molbev/msv076).
58. Lau DS, Connaty AD, Mahalingam S, Wall N, Cheviron ZA, Storz JF, Scott GR, McClelland GB. 2017 Acclimation to hypoxia increases carbohydrate use during exercise in high-altitude deer mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **312**, R400-R411. (doi:10.1152/ajpregu.00365.2016)
59. Scott GR, Guo KH, Dawson NJ. 2018 The mitochondrial basis for adaptive variation in aerobic performance in high-altitude deer mice. *Integr. Comp. Biol.* **58**, 506–518. (doi:10.1093/icb/icy056)
60. Moore LG. 2017 Measuring high-altitude adaptation. *J. Appl. Physiol.* **123**, 1371–1385. (doi:10.1152/japplphysiol.00321.2017)
61. Tate KB, Wearing OH, Ivy CM, Cheviron ZA, Storz JF, McClelland GB, Scott GR. 2020 Data from: Coordinated changes across the O_2 transport pathway underlie adaptive increases in thermogenic capacity in high-altitude deer mice. Dryad Digital Repository. (doi:10.5061/dryad.rjdfn2z7d)