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RESEARCH ARTICLE

Phenotypic plasticity in blood–oxygen transport in highland and lowland deer mice

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SUMMARY

In vertebrates living at high altitude, arterial hypoxemia may be ameliorated by reversible changes in the oxygen-carrying capacity of the blood (regulated by erythropoiesis) and/or changes in blood–oxygen affinity (regulated by allosteric effectors of hemoglobin function). These hematological traits often differ between taxa that are native to different elevational zones, but it is often unknown whether the observed physiological differences reflect fixed, genetically based differences or environmentally induced acclimatization responses (phenotypic plasticity). Here, we report measurements of hematological traits related to blood–O_2 transport in populations of deer mice (*Peromyscus maniculatus*) that are native to high- and low-altitude environments. We conducted a common-garden breeding experiment to assess whether altitude-related physiological differences were attributable to developmental plasticity and/or physiological plasticity during adulthood. Under conditions prevailing in their native habitats, high-altitude deer mice from the Rocky Mountains exhibited a number of pronounced hematological differences relative to low-altitude conspecifics from the Great Plains: higher hemoglobin concentrations, higher hematocrits, higher erythrocytic concentrations of 2,3-diphosphoglycerate (an allosteric regulator of hemoglobin–oxygen affinity), lower mean corpuscular hemoglobin concentrations and smaller red blood cells. However, these differences disappeared after 6 weeks of acclimation to normoxia at low altitude. The measured traits were also indistinguishable between the F_1 progeny of highland and lowland mice, indicating that there were no persistent differences in phenotype that could be attributed to developmental plasticity. These results indicate that the naturally occurring hematological differences between highland and lowland mice are environmentally induced and are largely attributable to physiological plasticity during adulthood.

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Key words: physiological plasticity, high altitude, hemoglobin, hematocrit, hypoxia, *Peromyscus maniculatus*, red blood cell.

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INTRODUCTION

In air-breathing vertebrates living at high altitude, the reduced O_2 tension of inspired air requires compensatory physiological adjustments to ensure an adequate tissue O_2 supply. Some of these adjustments involve reversible changes in O_2 transport by red blood cells (RBCs), which is a function of the O_2-carrying capacity of the blood (regulated by erythropoiesis) and blood–O_2 affinity [regulated by allosteric effectors of hemoglobin (Hb) function]. Mammals that are native to high-altitude environments are typically characterized by a suite of hematological and vascular traits that includes an elevated blood–O_2 affinity, a normal or slightly increased hematocrit (Hct), and increased muscle capillary density (Hall et al., 1936; Chiodi, 1962; Bullard et al., 1966; Bullard, 1972; Monge and León-Velarde, 1991; Weber, 1995; Weber, 2007; Storz et al., 2010b; Mairbäurl and Weber, 2012). In comparisons between species or conspecific populations that are native to different elevational zones, common-garden and/or reciprocal-transplant experiments are required to assess whether observed physiological differences reflect fixed, genetically based differences or environmentally induced acclimatization responses (phenotypic plasticity). In the latter case, the acclimatization response may involve irreversible changes in phenotype (reflecting developmental plasticity) and/or reversible changes during adulthood (physiological plasticity or 'phenotypic flexibility') (Piersma and Drent, 2003). Regardless of the ontogenetic stage at which the plasticity is manifest, it is generally an open question as to whether a given change in phenotype represents an adaptive acclimatization response that has evolved under the influence of natural selection (Kingsolver and Huey, 1998; Huey et al., 1999; Woods and Harrison, 2002; Ghalambor et al., 2007).

If physiological differences between highland and lowland natives are attributable to adaptive phenotypic plasticity, then it prompts the question of why traits that are characteristic of hypoxia-tolerant high-altitude species are often not congruent with the typical acclimatization response to hypoxia in lowland species (Monge and León-Velarde, 1991; Storz et al., 2010b). Lowland mammals that are not genetically adapted to environmental hypoxia typically respond to chronic O_2 deprivation with an increased erythropoietic activity (resulting in correlated increases in Hb concentration and Hct) and a decreased blood–O_2 affinity [mediated by an increased RBC concentration of 2,3-diphosphoglycerate (DPG), an allosteric effector that reduces Hb–O_2 affinity) (Lenfant et al., 1968; Baumann et al., 1971; Duhm and Gerlach, 1971; Mairbäurl et al., 1993; Quatrini et al., 1993). DPG directly reduces Hb–O_2 affinity by preferentially binding and stabilizing deoxygenated Hb, thereby shifting the allosteric equilibrium in favor of the low-affinity 'T-

state' quaternary structure. The hypoxia-induced increase in the molar DPG/Hb ratio also indirectly reduces Hb–O_2 affinity by altering the Donnan equilibrium across the RBC membrane, as the increased intracellular concentration of non-diffusible anions leads to an associated influx of hydrogen ions, thereby enhancing the Bohr effect (Duhm, 1971; Samaja and Winslow, 1979).

Within limits, an increased Hb concentration may enhance tissue O_2 delivery under hypoxia because the associated increase in arterial O_2 content can compensate for a reduced O_2 saturation. However, results of several empirical and theoretical studies suggest that increasing the Hb concentration of partially saturated blood is not an ideal long-term solution to the problem of chronic hypoxemia because the associated increase in blood viscosity produces an elevated peripheral vascular resistance that can compromise cardiac output (Guyton and Richardson, 1961; Bullard, 1972; McGrath and Weil, 1978; Winslow and Monge, 1987; Monge and León-Velarde, 1991; Connes et al., 2006; Schuler et al., 2010; Storz, 2010). Studies of humans at high altitude have suggested that the optimal Hb concentration at rest and at exercise may actually be quite close to the typical sea level value (Winslow, 1988; Villafuerte et al., 2004), or perhaps only slightly higher (Reeves and León-Velarde, 2004), and it is well documented that excessive polycythemia is a causal factor in the development of chronic mountain sickness (Winslow et al., 1985; Winslow and Monge, 1987; Monge and León-Velarde, 1991; Rivera-Ch et al., 2007).

The adaptive significance of hypoxia-induced reductions in blood–O_2 affinity depends on the severity of hypoxia as well as the pulmonary O_2 diffusion capacity and numerous other taxon-specific physiological attributes. Under conditions of severe hypoxia, theoretical and experimental results indicate that an increase in RBC DPG concentration and the concomitant decrease in Hb–O_2 affinity will generally have detrimental effects on tissue oxygenation because the reduced arterial O_2 saturation more than offsets any benefit of increased O_2 unloading in the peripheral circulation (Turek et al., 1973; Eaton et al., 1974; Bencowitz et al., 1982; Willford et al., 1982).

Some of the best opportunities for examining plasticity in traits associated with hypoxia tolerance are provided by studies of population-level variation in species that are distributed across steep altitudinal gradients. One such species is the deer mouse (*Peromyscus maniculatus*), which has the broadest altitudinal distribution of any North American mammal (Hock, 1964). As deer mice are continuously distributed from sea level to elevations above 4300 m, this species has proven to be an exemplary subject for research on mechanisms of adaptation and acclimatization to high-altitude hypoxia. An extensive body of work has documented that deer mice native to high altitude have elevated blood–O_2 affinities relative to lowland conspecifics, and these differences are largely attributable to allelic differences in Hb–O_2 affinity (Snyder, 1982; Snyder et al., 1982; Snyder, 1985; Chappell and Snyder, 1984; Chappell et al., 1988; Storz, 2007; Storz et al., 2009; Storz et al., 2010a). Additional evidence that allelic variation in Hb function contributes to local adaptation is provided by tests of whole-organism physiological performance involving wild-derived strains that express different Hb variants (Chappell and Snyder, 1984; Chappell et al., 1988; Hayes and Chappell, 1990) and population genetic analyses of variation in the underlying globin genes (Snyder et al., 1988; Storz, 2007; Storz et al., 2007; Storz and Kelly, 2008; Storz et al., 2007; Storz et al., 2009; Storz et al., 2012a). In light of this evidence for adaptive, genetically based differences in Hb–O_2 affinity between deer mouse populations that are native to different elevational zones, it is also of interest to assess the role of phenotypic plasticity in modulating aspects of blood–O_2 transport. Here, we report measurements of hematological traits related to blood–O_2 transport capacity in populations of deer mice that are native to high- and low-altitude environments. The main objectives were (i) to characterize altitude-related differences in hematological traits, and (ii) to assess what fraction of the observed trait differences are attributable to phenotypic plasticity.

MATERIALS AND METHODS

Animals

In July–August of 2010 and 2011, we collected a total of 118 deer mice, *P. maniculatus* (Wagner 1845), from a high-altitude locality in the Southern Rocky Mountains and a low-altitude locality in the Great Plains, 770 km to the East. We collected 58 highland mice from the summit of Mount Evans (Clear Creek County, CO, USA; 39°35′18″N, 105°38′38″W, elevation 4350 m above sea level) and 60 lowland mice from the prairie grassland of eastern Nebraska (Nine Mile Prairie, Lancaster County, NE, USA; 40°52′12″N, 96°48′20.3″W, elevation 430 m above sea level). All mice were captured using Sherman live traps baited with peanut butter and oats. We drew approximately 200 µl of blood from the maxillary vein of each mouse using a 5 mm Goldenrod lancet (MEDIpoint Inc., Mineola, NY, USA).

A subset of 46 mice (24 highland and 22 lowland) were transferred to a common-garden environment at the Animal Research Facility at the University of Nebraska (elevation 300 m) where they were allowed to acclimate for 6 weeks. All mice were maintained at a constant temperature (25°C) and on a standard light:dark cycle (12 h:12 h) for the duration of the experiment. During the 6 week acclimation period all mice were offered a standard diet *ad libitum* (Harlan Teklad Rodent Chow, Indianapolis, IN, USA). After the acclimation period, we again drew blood from each mouse as described above, for repeat measurements of the same hematological traits. We also conducted crosses between wild-caught mice from the same collection locality and we reared the resultant F_1 progeny at the University of Nebraska and the University of Illinois under the same common-garden conditions as described above. A total of 71 F_1 mice (N=48 and 23 descendants of highland and lowland natives, respectively) were phenotyped after they reached a minimum age of 66 days. All experimental protocols were approved by the Institutional Animal Care and Use Committees at the University of Nebraska (IACUC no. 522) and the University of Illinois (IACUC no. 10244).

Measurement of hematological traits

We measured Hb concentration in whole blood using a HemoCue Hb 201+ analyzer, following the manufacturer's protocol (HemoCue AB, Ängelholm, Sweden). We measured Hct as the volume of packed RBCs relative to total blood volume in a heparinized capillary tube that was spun at 13,600 ***g*** for 5 min in a ZIPocrit centrifuge (LW Scientific Inc., Lawrenceville, GA, USA). We also calculated mean cell Hb concentration, MCHC (=Hb concentration×100/Hct). To measure RBC size, we used a Zeiss Axioplan 2 imaging microscope (Carl Zeiss, Gottingen, Germany) to measure the diameter of 10 RBCs per sample.

RBC DPG concentrations were determined spectrophotometrically using a DPG kit, following the manufacturer's protocol (Roche Applied Science, Indianapolis, IN, USA), with the exception that we used sample volumes of 100 µl whole blood rather than 1 ml. We drew blood from a subset of 21 mice (N=10 and 11 highland and lowland mice, respectively). Blood was collected in chilled heparinized capillary tubes and was

immediately deproteinized with 500 µl of cold 0.6 mol l^{-1} perchloric acid ($HClO_4$). Samples were centrifuged at 5000 r.p.m. for 10 min, and 400 µl of clear supernatant was then removed and neutralized with 50 µl of 2.5 mol l^{-1} potassium carbonate (K_2CO_3). Samples were kept on ice for at least 60 min and were then centrifuged again at 5000 r.p.m. for 10 min. The supernatant was removed and immediately frozen in liquid nitrogen prior to the spectrophotometric analysis. The concentration of DPG in whole blood was estimated from the coupled reduction of NADH to NAD^+ in the reaction assay at 340 nm (UVIKON 923 B Double Beam UV/VIS Spectrophotometer, Kontron Instruments, Milan, Italy). The reaction was completed after 25 min and absorbance did not change noticeably in later readings. Negative controls were run with each analysis and positive controls were performed with human blood.

Statistical analysis

For measures of Hb concentration in wild-caught mice (phenotyped at the site of capture) and for measures of all hematological traits in the F_1 progeny of wild-caught/lab-acclimated mice, we tested for altitude-related differences in phenotype using standard *t*-tests. For the subset of mice that were included in the common-garden acclimation experiment, we compared pre- and post-acclimation trait values using a repeated measures two-way ANOVA with native altitude (high *versus* low) and time (pre- *versus* post-acclimation) included as independent variables. In the analysis of trait variation in the common-garden F_1 mice, we controlled for variation in individual age (66–454 days) by performing an ANCOVA with native altitude as an independent variable and age as a covariate. We did not detect any significant differences between the sexes for any of the hematological traits, so data from males and females were pooled in all analyses. Similarly, we did not detect significant trait variation among sibships within each set of F_1 mice from high- and low-altitude, so data from all families were pooled. Kolmogorov–Smirnov (K–S) tests did not reveal any significant deviations from normality in any of the trait-specific data sets. All statistical analyses were conducted using the SAS software package (SAS Institute Inc., 2009) or VassarStats online statistical calculator (vassarstats.net).

RESULTS

Under the conditions prevailing in their natural habitats, the highland *P. maniculatus* exhibited a significantly higher Hb concentration than the lowland *P. maniculatus* [mean ± 1 s.d., 2.83±0.28 *versus* 2.33±0.32 mmol l^{-1}, respectively (Hb molecular mass 64.45 kDa); t_{116}=9.079, P<0.0001; Fig. 1). However, after 6 weeks of acclimation to normoxia in the common-garden laboratory environment, the mean Hb concentration of the highland mice dropped by 8.8% to 2.54±0.21 mmol l^{-1} and was statistically indistinguishable from that of the lab-acclimated lowland mice (2.55±0.20 mmol l^{-1}; Table 1, Fig. 2A). The F_1 progeny of highland and lowland *P. maniculatus* also had Hb concentrations that were statistically indistinguishable (2.33±0.21 *versus* 2.29±0.24 mmol l^{-1}, respectively; t_{69}=0.739, P=0.489). Similar to the repeated-measures analysis of Hb concentration (Fig. 2A), an analysis of pre- and post-acclimation measures of Hct, MCHC, RBC size and RBC DPG concentration for the same subset of highland and lowland mice revealed similar degrees of plasticity (Fig. 2B–E). Highland deer mice exhibited a significantly higher Hct relative to lowland mice (59.67±4.83 *versus* 48.94±4.82%, respectively; Table 1, Fig. 2A), but after 6 weeks of acclimation to normoxia, the average Hct of the highland mice dropped by 17% such that post-acclimation values of the highland and lowland mice were statistically indistinguishable (49.77±3.35 *versus* 49.96±3.78%, respectively; Fig. 2B). Relative to the lowland mice, the highland mice had a lower MCHC (4.74±0.28 *versus* 5.05±0.45 mmol l^{-1}, respectively) and a smaller average RBC size (5.38±0.12 *versus* 5.72±0.41 µm, respectively), but these differences disappeared after acclimation to normoxia (Fig. 2C,D). The highland mice exhibited a significantly higher RBC DPG concentration (2.16±0.44 *versus* 1.47±0.39 mmol l^{-1}; Table 1, Fig. 2E), but as with MCHC and RBC size, this difference disappeared after acclimation to normoxia. For each of the hematological traits, the '*in situ*' measurements of wild-caught mice were consistent with data from previous studies of *Peromyscus* mice (Fig. 3).

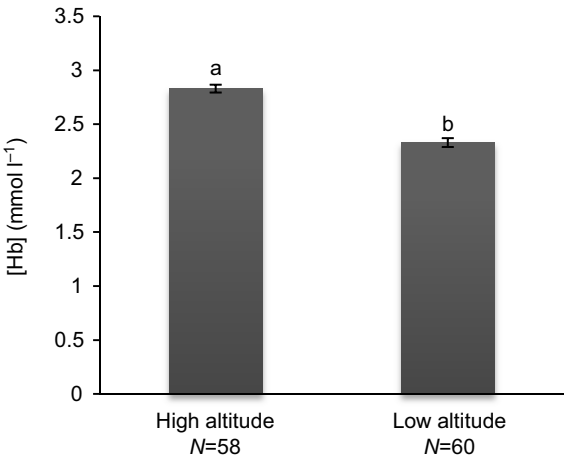

Fig. 1. Hemoglobin (Hb) concentrations (mean ± 1 s.e.m.) of highland and lowland *Peromyscus maniculatus* measured at their native altitudes. Significant differences in mean values (P<0.05) are denoted by different letters.

Similar to the case with Hb concentration, the F_1 progeny of highland and lowland parents did not exhibit significant differences in Hct (49.20±3.21 *versus* 50.00±4.26%; t_{50}=0.733, P=0.528), MCHC (4.81±0.34 *versus* 4.84±0.44 mmol l^{-1}; t_{50}=–0.228, P=0.841),

Table 1. Differences in hematological traits between groups of deer mice (high- *versus* low-altitude) and between pre- and post-acclimation time points

Trait	Factor	d.f.	F	P
[Hb]	Altitude	1,43	14.58	0.0004
	Time	1,44	2.33	0.1344
	Altitude × time	1,44	15.54	0.0003
Hct	Altitude	1,43	29.99	<0.0001
	Time	1,44	29.21	<0.0001
	Altitude × time	1,44	44.24	<0.0001
MCHC	Altitude	1,43	2.17	0.1483
	Time	1,44	8.43	0.0058
	Altitude × time	1,44	3.17	0.0821
RBC size	Altitude	1,18	0.37	0.549
	Time	1,19	22.83	0.0001
	Altitude × time	1,19	9.82	0.0055
DPG/Hb_4	Altitude	1,18	7.29	0.0147
	Time	1,19	27.6	<0.0001
	Altitude × time	1,19	16.69	0.0006

Tests were carried out using a repeated-measures, two-way ANOVA.
Hb, hemoglobin; Hct, hematocrit; MCHC, mean corpuscular Hb concentration; RBC, red blood cell; DPG, 2,3-diphosphoglycerate.

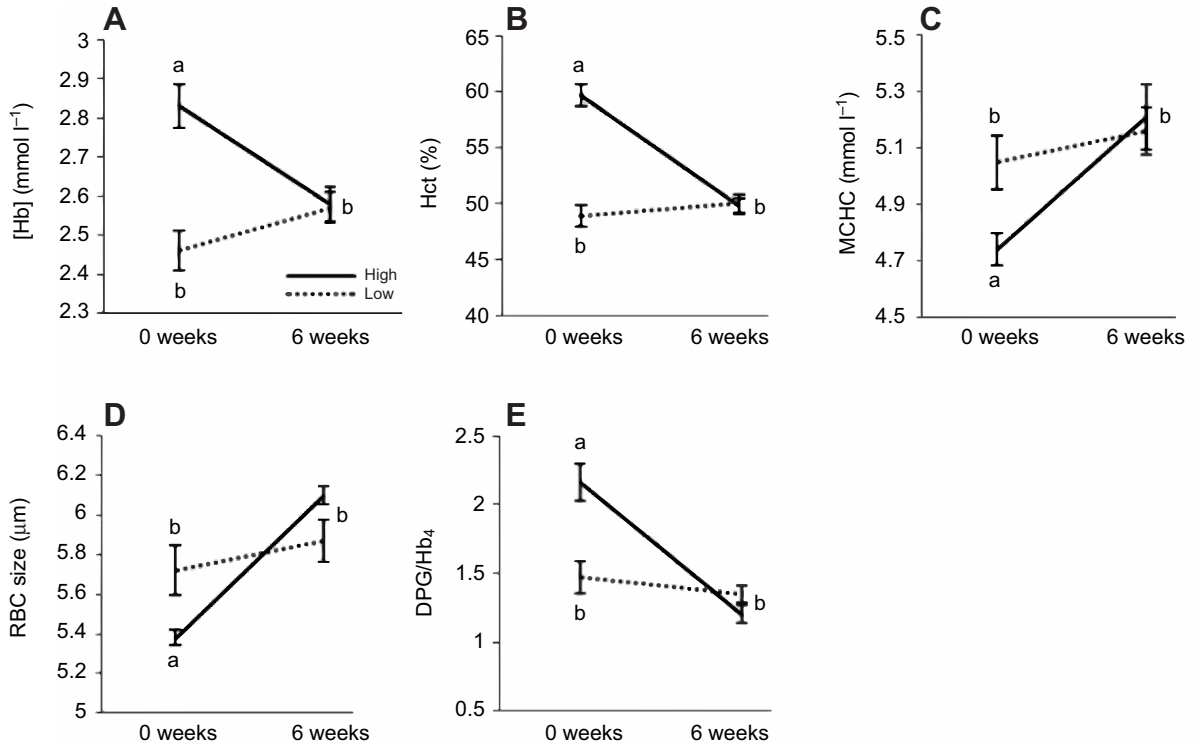

Fig. 2. Changes in hematological traits (mean ± 1 s.e.m.) in highland and lowland *P. maniculatus* induced by acclimation to normoxia over a 6 week period. (A) Hb concentration, (B) hematocrit (Hct), (C) mean corpuscular Hb concentration (MCHC), (D) red blood cell (RBC) size and (E) 2,3-diphosphoglycerate (DPG)/Hb_4 ratio. Measures of Hb concentration, Hct and MCHC are based on 24 highland mice and 22 lowland mice, whereas measures of RBC size and DPG/Hb_4 ratio were based on a subset of 10 highland mice and 11 lowland mice. A repeated-measures ANOVA was used to test for differences in mean values between treatment groups and between pre- and post-acclimation time points. Significant differences in mean values (P<0.05) are denoted by different letters.

RBC size (5.57±0.28 *versus* 5.45±0.21 μm; t_{42}=1.380, P=0.126) or DPG/Hb ratio (2.45±0.51 *versus* 2.24±0.61; t_{23}=0.858, P=0.458). These results indicate that the naturally occurring hematological differences between highland and lowland mice are environmentally induced and are largely attributable to physiological plasticity during adulthood.

DISCUSSION

Under conditions prevailing in their native habitats, highland deer mice from the summit of Mount Evans exhibited a number of pronounced hematological differences relative to lowland conspecifics from the Great Plains. In comparison with lowland mice, the highland mice exhibited higher Hb concentrations, higher Hcts, higher DPG/Hb ratios, lower MCHC values and smaller RBCs. However, these differences disappeared after 6 weeks of acclimation to normoxia at low altitude (Fig. 2). The measured traits were also indistinguishable between the F_1 progeny of highland and lowland mice, indicating that there were no persistent, irreversible differences in phenotype that could be attributed to developmental plasticity or fixed, genetically based differences. Studies of other physiological traits in deer mice have documented similar degrees of plasticity in response to cold and/or hypoxic stress (Hammond et al., 1999; Hammond et al., 2001; Hammond et al., 2002; Rezende et al., 2004; Chappell et al., 2007; Russell et al., 2008; Rezende et al., 2009; Cheviron et al., 2013).

In the case of Hb concentration and Hct, the native character states of the highland mice were largely congruent with the typical acclimatization response to hypoxia exhibited by lowland mammals that have no known evolutionary history of residence at high altitude (Monge and León-Velarde, 1991). The elevated Hb concentrations and Hcts observed in the highland deer mice are consistent with results of previous studies of *P. maniculatus* (Gough and Kilgore, 1964; Hock, 1964; Sealander, 1964; Dunaway and Lewis, 1965; Thompson et al., 1966; Sawin, 1970; Snyder, 1982; Snyder et al., 1982; Wyckoff and Frase, 1990; Hammond et al., 1999; Hammond et al., 2001) (supplementary material Table S1; Fig. 3). By contrast, most rodent species that are native to high altitudes appear to have Hb concentrations and Hcts that are substantially lower than those of hypoxia-acclimated laboratory rats or house mice (Hall et al., 1936; Chiodi, 1962; Morrison et al., 1963a; Morrison et al., 1963b; Bullard et al., 1966) and hematological traits in the majority of high-altitude Andean rodents studied by Morrison and colleagues remained unaltered after acclimation to normoxic conditions at sea level (Morrison et al., 1963a; Morrison et al., 1963b).

In humans, highlanders that are indigenous to the Tibetan and Ethiopian plateaus exhibit low Hb concentrations relative to Andean highlanders that are resident at comparable altitudes (Beall and Reischsman, 1984; Winslow et al., 1989; Beall et al., 1990; Beall et al., 1998; Beall et al., 2002; Garruto et al., 2003; Wu et al., 2005; Beall, 2006; Beall, 2007; Beall et al., 2010; Simonson et al., 2010). The high Hb concentrations of Andean highlanders mirror the expected acclimatization response to hypoxia in lowland natives, whereas the Tibetans and Ethiopians appear to have evolved a blunted erythropoietic response to environmental hypoxia. Andeans also suffer a much higher incidence of chronic mountain sickness (Winslow and Monge,

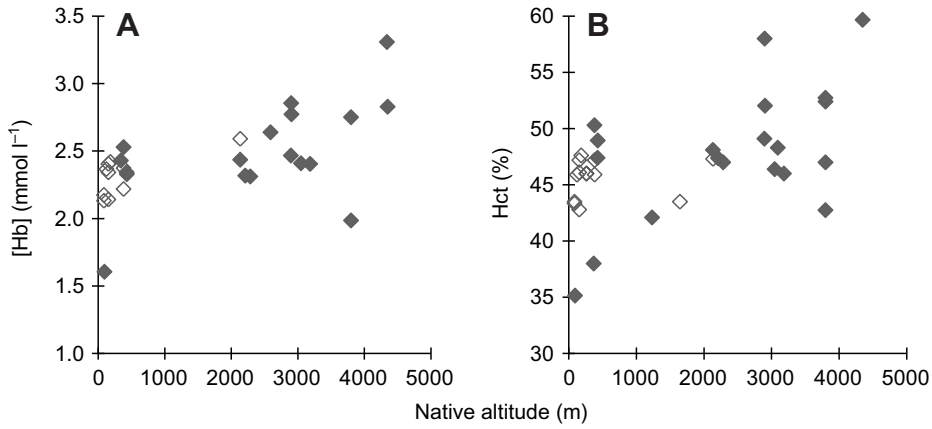

Fig. 3. Scatterplot of mean Hb concentration (A) and mean Hct (B) against native altitude for population samples of *P. maniculatus* and other *Peromyscus* species from this and other studies. Filled symbols denote data points for population samples of *P. maniculatus* and open symbols denote data points for other species of *Peromyscus* (*P. boylii*, *P. crinitus*, *P. leucopus*, *P. nuttalli* and *P. polionotus*). Raw data are provided in supplementary material Table S1.

1987). The elevated Hb concentrations of highland deer mice mirror the acclimatization response to hypoxia in lowland species (e.g. Baumann et al., 1971; Duhm and Gerlach, 1971), suggesting that the highland deer mice have a hematological profile that is more similar to that of Andean humans than to that of Tibetans or Ethiopians. However, additional experiments are required to assess whether highland and lowland mice differ in their acclimation responses to hypoxia, and it is an open question as to whether the elevated Hb concentration of high-altitude deer mice represents an example of adaptive or maladaptive plasticity.

In addition to the potentially adverse effects of increased blood viscosity on cardiac output, an increased Hct can also diminish plasma transport of free fatty acids and other metabolic fuels (McClelland, 2004). This is because the fuel transport capacity of blood plasma is determined by the product of plasma flow and fuel concentration, and plasma flow is an inverse function of Hct. In deer mice living at high altitude, the effects of elevated Hct on plasma fuel transport may be especially significant because deer mice rely heavily on fatty acid oxidation to fuel shivering thermogenesis (Cheviron et al., 2012).

The elevated RBC DPG concentrations observed in highland deer mice are mostly consistent with previous reports. Snyder measured RBC DPG concentrations in deer mice sampled from localities ranging from 2590 to 4340 m in the White Mountains of eastern California (Snyder, 1982). Although the DPG/Hb ratio was somewhat reduced in the high-altitude sample of mice from 4340 m, there was no monotonic altitudinal trend, as DPG/Hb ratios were highest in mice from intermediate elevations. Similar to the results reported by Snyder (Snyder, 1982), our common-garden experiment revealed a reversal in relative DPG concentrations such that the highland mice actually exhibited a slightly lower baseline DPG/Hb ratio after acclimation to normoxia (Fig. 2E). If the reduced baseline DPG/Hb ratio and elevated Hb–O_2 affinity of highland deer mice are construed as adaptive mechanisms for maintaining an elevated blood–O_2 affinity under hypoxia, then the hypoxia-induced increase in RBC DPG would seem to be counterproductive (Storz et al., 2010b). In considering this seemingly maladaptive acclimatization response, Snyder suggested that physiological constraints of RBC energy metabolism may prevent evolutionary modifications of intracellular DPG levels as the formation of DPG is stimulated by overall glycolytic activity (Snyder, 1982). However, given that the optimal blood–O_2 affinity is a non-linear function of ambient P_{O_2} (Turek et al., 1973; Eaton et al., 1974; Bencowitz et al., 1982; Willford et al., 1982), empirical performance curves are needed to evaluate whether hypoxia-induced reductions in the DPG/Hb ratio are advantageous or disadvantageous at a given altitude. As changes in the DPG/Hb ratio also affect intracellular pH and Cl^- concentration, and as the adult Hbs of deer mice and other muroid rodents have ligand affinities that are more strongly modulated by Cl^- ions than by DPG (Storz et al., 2009; Storz et al., 2010a; Storz et al., 2012b; Runck et al., 2010), the net effects on blood–O_2 affinity are difficult to predict. Moreover, if deer mice have a hyperventilatory response to hypoxia, as in other mammals, the resultant increase in plasma pH (respiratory hypocapnia) may effectively counterbalance the inhibitory effects of DPG on Hb–O_2 affinity (Mairbäurl and Weber, 2012).

In contrast to the hematological changes that are typically associated with the acclimatization response to hypoxia in lowland mammals, genetically based changes in Hb structure that increase intrinsic O_2 affinity or that suppress sensitivity to allosteric effectors are generally thought to make more important contributions to hypoxia tolerance in species that are high-altitude natives (Bunn, 1980; Monge and León-Velarde, 1991; Storz, 2007; Weber, 1995; Weber, 2007; Storz and Moriyama, 2008; Storz et al., 2010b; Mairbäurl and Weber, 2012). High-altitude deer mice seem to defy Monge and León-Velarde's proposed dichotomy between the hypoxia response strategies of highland and lowland natives (Monge and León-Velarde, 1991), as they possess a set of hematological traits that appear to fit both profiles. On the one hand, highland deer mice exhibit elevated Hb concentrations, Hcts and RBC DPG concentrations relative to lowland mice (Hock, 1964; Sawin, 1970; Snyder, 1982; Snyder et al., 1982), characteristics that seem to fit the profile of a hypoxia-acclimated lowland species. On the other hand, highland deer mice possess structurally distinct Hbs with slightly elevated O_2 affinity (Storz et al., 2009; Storz et al., 2010a), which fits the profile of a genetically adapted, hypoxia-tolerant highland species.

In the case of highly labile hematological traits like Hb concentration, Hct and RBC DPG concentration that are responsive to minute changes in arterial P_{O_2} and acid–base balance, it is probably often the case that altitude-related trait differences between species or between conspecific populations are purely attributable to physiological plasticity, reflecting hypoxia-induced regulatory changes in erythropoietic activity and RBC metabolism. The high degree of plasticity that we have documented for hematological traits in deer mice highlights the importance of using common-garden experiments to assess whether physiological differences between species or conspecific populations represent reversible, constitutively expressed traits or irreversible, fixed differences.

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Supplementary Table 1. Hematological data for population samples of *P. maniculatus* and other *Peromyscus* species.

Species Name	Altitude (m)	[Hb] g/dl (mM)	Hct (%)	RBC size (µm)	MCHC g/dl (mM)	DPG/Hb	Reference
Peromyscus maniculatus	4350	18.2 (2.8)	59.7	5.4	30.6 (4.7)	2.2	Present study
P. maniculatus	4340	21.3 (3.3)	64.2	-	33.2 (5.2)	1.7	Snyder 1982
P. maniculatus	3801	-	52.4	-	-	-	Hammond et al. 1999
P. maniculatus	3801	-	47.0	-	-	-	Hammond et al. 2001
P. maniculatus	3800	17.7 (2.8)	52.7	-	33.6 (5.2)	1.8	Snyder 1982
P. maniculatus	3800	12.8 (2.0)	42.8	-	30.0 (4.7)	-	Sawin 1970
P. maniculatus	3185	15.5 (2.4)	46.0	-	33.5 (5.2)	-	Thompson et al. 1966
P. maniculatus	3094	-	48.3	-	-	-	Hammond et al. 1999
P. maniculatus	3048†	15.5 (2.4)	46.4	-	33.5 (5.2)	-	Gough and Kilgore 1964
P. maniculatus	2905	17.9 (2.8)	52.0	-	34.4 (5.3)	1.8	Snyder 1982
P. maniculatus	2900	18.4 (2.9)	58.0	5.9	31.7 (4.9)	-	Wyckoff and Frase 1990
P. maniculatus	2896	15.9 (2.5)	49.1	6.4	32.5 (5.0)	-	Sealander 1964
P. maniculatus	2590	17.0 (2.6)	-	-	-	1.7	Snyder 1982
P. maniculatus	2286	14.9 (2.3)	47.0	-	31.6 (4.9)	-	Thompson et al. 1966
P. maniculatus	2210†	14.9 (2.3)	47.4	-	31.6 (4.9)	-	Gough and Kilgore 1964
P. maniculatus	2134	15.7 (2.4)	48.1	6.4	32.8 (5.1)	-	Sealander 1964
P. crinitus	2134	16.7 (2.6)	47.3	6.5	35.2 (5.5)	-	Sealander 1964
P. polionotus	1646	-	43.5	-	-	-	Dunaway and Lewis 1965
P. maniculatus	1234	-	42.1	-	-	-	Hammond et al. 1999
P. maniculatus	430	15.0 (2.3)	48.9	5.7	32.5 (5.1)	1.5	Present study
P. maniculatus	427	15.1 (2.3)	47.4	6.4	31.8 (4.9)	-	Sealander 1962
P. boylii	381	14.3 (2.2)	45.9	6.4	31.0 (4.8)	-	Sealander 1964
P. leucopus	381	15.3 (2.4)	47.2	6.3	32.5 (5.0)	-	Sealander 1964
P. maniculatus	381	16.3 (2.5)	50.3	6.3	32.4 (5.0)	-	Sealander 1964
P. maniculatus	370	-	38.0	-	-	-	Hammond et al. 2001
P. maniculatus	340	15.6 (2.4)	45.9	-	34.1 (5.3)	1.6	Snyder 1982
P. leucopus	259	-	46.0	-	-	-	Dunaway and Lewis 1965
P. nuttalli	259	-	46.0	-	-	-	Dunaway and Lewis 1965
P. leucopus	182	15.6 (2.4)	47.6	6.3	32.8 (5.2)	-	Wyckoff and Frase 1990
P. leucopus	152	15.1 (2.3)	46.1	6.4	32.8 (5.2)	-	Sealander 1964
P. polionotus	152	13.8 (2.1)	42.8	-	32.2 (5.0)	-	Sealander 1964
P. nuttalli	152	15.5 (2.4)	47.2	-	32.7 (5.1)	-	Sealander 1964
P. leucopus	122*	15.3 (2.4)	45.9	6.1	33.2 (5.2)	-	Foreman 1956
P. maniculatus	94	10.4 (1.6)	35.2	-	29.3 (4.6)	-	Sawin 1970
P. gossypinus	84*	13.7 (2.1)	43.5	-	31.6 (4.9)	-	Gough and Kilgore 1964
P. nuttalli	84*	14.0 (2.2)	43.3	-	32.4 (5.0)	-	Gough and Kilgore 1964

† Represents the elevational midpoint of collection localities reported by Gough and Kilgore (1964).
*Estimated altitude based on locality information provided in the text.

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