Contributions of phenotypic plasticity to differences in thermogenic performance between highland and lowland deer mice.

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Summary

Small mammals face especially severe thermoregulatory challenges at high-altitude because the reduced O_2 availability constrains the capacity for aerobic thermogenesis. Adaptive enhancement of thermogenic performance under hypoxic conditions may be achieved via physiological adjustments that occur within the lifetime of individuals (phenotypic plasticity) and/or genetically based changes that occur across generations, but their relative contributions to performance differences between highland and lowland natives is unclear. Here, we examined potentially evolved differences in thermogenic performance between populations of deer mice (*Peromyscus maniculatus*) that are native to different altitudes. The purpose of the study was to assess the contribution of phenotypic plasticity to population differences in thermogenic performance under hypoxia. We used a common-garden deacclimation experiment to demonstrate that highland deer mice have enhanced thermogenic capacities under hypoxia, and that performance differences between highland and lowland mice persist when individuals are born and reared under common-garden conditions, suggesting that differences in thermogenic capacity have a genetic basis. Conversely, population differences in thermogenic endurance appear to be entirely attributable to physiological plasticity during adulthood. These combined results reveal distinct sources of phenotypic plasticity for different aspects of thermogenic performance, and suggest that thermogenic capacity and endurance may have different mechanistic underpinnings.

INTRODUCTION

For small homeothermic endotherms, the capacity for metabolic heat production is a primary determinant of survival during periods of prolonged cold stress (Conley and Porter, 1986; Hayes and O'Connor, 1999). Like many complex phenotypic traits, changes in thermogenic performance can be achieved via physiological adjustments that occur within the lifetime of individuals (phenotypic plasticity) and/or genetically based changes that occur across generations (genetic adaptation) (Garland and Carter, 1994; Rezende *et al*, 2001; Hammond *et al*, 2002; Storz *et al*, 2010b; Swanson, 2010). Quantitative genetic studies have demonstrated that thermogenic capacity and similar measures of whole-organism metabolic performance have a heritable basis (Swallow *et al*, 1998; Dohm *et al*, 2001; Nespolo *et al*, 2003; Fontanillas *et al*, 2005; Nespolo *et al*, 2005; Sadowska *et al*, 2005; Wone *et al*, 2009), indicating that evolutionary changes in such traits could be brought about by directional selection. However, many changes in whole-organism metabolic performance could also occur through reversible physiological adjustments. For example, temperate-zone birds and mammals often exhibit marked changes in thermogenic capacity and cold hardiness with the onset of winter (Merritt, 1995; Liknes and Swanson, 1996; Liknes *et al*, 2002; Swanson, 2007; Swanson, 2010; Oelkrug *et al*, 2012). Changes in thermogenic performance can also be induced by environmental conditions experienced during prenatal development (Chappell *et al*, 2007; Russell *et al*, 2008), and in contrast to reversible acclimatization responses, the effects of developmental plasticity may persist throughout postnatal life (Dzialowski et al. 2001; Chappell et al. 2007; Russell et al 2008). Thus, a central goal of research in evolutionary physiology is to assess the relative contributions of genotypic specialization and phenotypic plasticity in enabling species to cope with changing environmental conditions (Garland and Adolph, 1991; Garland and

Carter, 1994; Kingslover and Huey, 1998; Feder *et al*, 2000; Storz *et al*, 2010b). Studies of altitude-related variation in aerobic thermogenic performance are particularly well-suited to this goal because ambient temperature and oxygen availability vary predictably as a function of altitude.

The deer mouse (*Peromyscus maniculatus*) has emerged as a particularly promising study organism for investigations of high-altitude adaptation. This is largely due to the fact that deer mice have the broadest altitudinal distribution of any North American mammal, occurring above 4300 m in mountain ranges of western North America to below sea-level in Death Valley, California (Hock, 1964). Deer mice also do not hibernate (Jones *et al* 1983), and as a result, they are highly dependent on aerobic thermogenesis to maintain a constant body temperature during periods of prolonged cold stress (Chappell and Hammond 2004). Because hypoxia can potentially constrain the metabolic scope for aerobic activity, deer mice face especially severe thermoregulatory challenges in cold, alpine and subalpine environments. Indeed, survivorship studies of free-ranging mice at high-altitude have documented strong directional selection on thermogenic capacity (Hayes and O'Connor, 1999), and thermogenic performance is strongly correlated with above-ground activity and foraging behavior in the cold (Sears *et al*, 2006; Sears *et al*, 2009). Thus, in high-altitude deer mice, thermogenic performance under hypoxia is clearly an ecologically-important trait that has a well-documented connection to Darwinian fitness (Hayes and O'Connor, 1999).

Most previous studies of environmental effects on aerobic performance and hypoxia tolerance in deer mice have focused on interindividual variation among mice collected from a single geographic locality (Chappell, 1985; Hammond *et al*, 2002; Chappell and Hammond, 2004; Chappell *et al*, 2007; Russell and Chappell, 2007; Russell *et al*, 2008; Van Sant and Hammond, 2008;

Rezende *et al*, 2009). Given that deer mice have such a broad altitudinal distribution, it is also of interest to assess the contribution of phenotypic plasticity to population differences in physiological traits that have distinct local optima in different elevational zones. Thus, the purpose of the present study was to assess the contribution of phenotypic plasticity to altitude-related population differences in thermogenic performance under hypoxia.

We used a common-garden deacclimation experiment to characterize the effects of environmental variation during pre- and post-natal life on two aspects of whole-organism thermogenic performance under hypoxia: thermogenic capacity and thermogenic endurance. We show that deer mice that are native to high altitude have elevated thermogenic capacities under hypoxia compared to those that are native to low altitude, and that these differences persist in mice that are born and reared under common-garden conditions, suggesting that they have a genetic basis. Conversely, differences in thermogenic endurance between highland and lowland deer mice appear to be entirely attributable to environmental effects that act during adulthood. These results reveal distinct sources of phenotypic plasticity for different aspects of thermogenic performance, and suggest that thermogenic capacity and endurance may have different mechanistic underpinnings.

MATERIALS AND METHODS

Experimental animals and deacclimation treatments

Adult deer mice were live trapped at one high-altitude locality in the Southern Rocky Mountains, the summit of Mt. Evans, Clear Creek Co., Colorado, USA (39° 35′ 18″ N, 105° 38′ 38″ W, 4350 m a.s.l., P_{O_2} ~ 95.6 mmHg) and one low-altitude locality in the Great Plains, 9-mile prairie; Lancaster Co, NE, USA (40° 52' 12" N, 96° 48' 20.3" W, 430 m a.s.l., P_{O_2} ~ 152.0 mmHg). This pair of high- and low-

altitude localities is separated by a linear distance of 770 km. Following capture, high- and low-altitude deer mice were either measured on-site within one to two days of capture [*in-situ* treatment; highland mice, n = 10; lowland mice, n = 10] or they were transferred from collection localities to a common-garden lab environment at the animal research facility at the University of Nebraska, Lincoln, NE (elevation 360 m, PO_2 ~ 153.3 mmHg). Mice that were transferred to the common-garden lab environment were assigned to one of two groups. Mice in the first group (highland mice, n = 10; lowland mice, n = 10) were housed for 6 wks with a constant ambient temperature (25°C) and light:dark cycle (12L:12D). We measured the thermogenic performance of all mice at the conclusion of this 6–wk deacclimation period. Mice assigned to the second group were used as parental stock to produce F_1 progeny that were born and reared in the common garden. Once these F_1 progeny reached adulthood (75 - 90 days), we measured thermogenic capacity under hypoxia in 10 full-sibling progeny from a pair of highland parents and 10 full-sibling progeny from a pair of lowland parents. This experimental design allowed us to control for two distinct sources of phenotypic plasticity in thermogenic performance, physiological plasticity during adulthood (through the comparison of wild-caught high- and low-altitude mice that underwent the 6-wk deacclimation under common garden conditions) and developmental plasticity (through comparison of the F_1 progeny of wild-caught high- and low-altitude mice that were born and reared under common garden conditions). Differences in thermogenic performance that persisted in the F_1 mice were assumed to represent genetically-based differences in thermogenic performance between highland and lowland mice.

Respirometry

We used open-flow respirometry to measure thermogenic capacity as the maximum rate of oxygen consumption ($VO_{2\,max}$) elicited by cold-exposure. The measurements performed in Lincoln were made in a hypoxic heliox atmosphere (12.6% O_2, 87.4% He), which simulates the atmospheric PO_2 on the summit of Mt. Evans. For the measurements made on the summit of Mt. Evans, we used a normoxic heliox atmosphere (21% O_2; 79% He). At both localities, heliox gas mixtures were equilibrated to local atmospheric pressure so that all of the experimental animals experienced an equivalent level of hypoxia during the thermogenic trials. The incurrent flow rate of heliox was approximately 450ml/min after correction (see below). All of the trials were conducted at ambient temperatures just below freezing (minimum -4°C). Rates of heat loss in heliox are several times greater than in ambient air, which makes it possible to elicit $VO_{2\,max}$ without risking cold injury to experimental animals (Rosenmann and Morrison, 1974). Similar protocols have been used to elicit $VO_{2\,max}$ in *P. maniculatus* in previous studies (Chappell and Hammond, 2004; Rezende *et al*, 2004; Cheviron *et al*, 2012). Heliox mixtures were obtained from a commercial supplier (Linweld, Lincoln, NE).

The respirometry setup for the thermogenic trials was identical to that described by Cheviron *et al*. (2012). Briefly, heliox gas mixtures were first equilibrated to atmospheric pressure, and were then pumped into copper coils inside a temperature control chamber using mass flow controllers (Sable Systems Inc, Las Vegas, NV). The cooled heliox was then pumped into the animal chamber and a baseline (empty) chamber at a rate of approximately 450 mL/min. The animal and baseline chambers were constructed of thin, airtight polypropylene with an internal volume of 180 mL, which minimized locomotion but still permitted postures normally adopted for shivering. We verified that the temperature inside the temperature control chamber was identical to that

experienced by a mouse inside the animal chamber by using a thermocouple to measure excurrent air at the junction of the excurrent tube and the animal chamber. Excurrent air from the animal and baseline chambers was sampled at a rate of approximately 130 mL/min; the air was dried with magnesium perchlorate, passed through a CO_2 analyzer (CO_2 data reported elsewhere, Cheviron et al. 2012), scrubbed of CO_2 with ascarite, redried with drierite, and passed through an oxygen analyzer (Sable Systems Inc. FoxBox). We monitored excurrent O_2 continuously, and each experimental animal was removed when values showed clear indications of dropping to baseline. We used a rectal thermometer to measure the body temperature of each mouse following the thermogenic trials, and we confirmed that all experimental mice were hypothermic when removed from the chamber. The O_2 analyzer was spanned daily with ambient air.

We calculated VO_2 using equation 10.1 in Lighton (2008). Gas conversion factors for two-gas mixtures (helium and oxygen) were calculated, and applied to the flow data prior to VO_2 calculations. We measured thermogenic capacity ($VO_{2\,max}$) as the maximum VO_2 averaged over a continuous 5 min period, and we measured thermogenic endurance as the length of time (min) that individuals maintained $\geq$ 90% of $VO_{2\,max}$ during the thermogenic trials. All experimental protocols were approved by the University of Nebraska Institutional Animal Care and Use Committee (IACUC #522).

Statistics

We tested for differences in body mass between populations and across the deacclimation treatments using a two-way ANOVA design. Body mass varied significantly among the six experimental groups, and was significantly correlated with $VO_{2\,max}$ (see Results). To control for the effects of body mass on thermogenic

capacity ($VO_{2\,max}$), we tested for mean differences in thermogenic capacity between samples of highland and lowland deer mice across deacclimation treatments using a two-way ANCOVA design with body mass as a covariate (Packard and Boardman 1988). In contrast to thermogenic capacity, thermogenic endurance was not correlated with body mass, and as result, we used a two-way ANOVA design to test for differences in thermogenic endurance. Upon detection of significant ANCOVA and ANOVA main effects, we performed *post hoc* Tukey HSD tests to identify significant pairwise differences between populations and treatments. Finally, we used linear regression to assess the relationship between thermogenic capacity and endurance in each of the experimental groups (*in situ*, 6-wk deacclimation, F_1 progeny of deacclimated mice). Consistent with previous studies of thermogenic performance in deer mice (Chappell *et al*, 2007), sex had no effect on either thermogenic capacity or endurance (data not shown). Thus, both sexes were combined in all analyses. All statistical analyses were performed in either R or JMP 501.

RESULTS

There was significant variation in body mass among experimental groups (Tables 1 and 2). The highland mice were significantly larger in the *in-situ* group, and this difference persisted in the six –week deacclimated group, although it was not statistically significant (Table 1). Interestingly, this relationship was reversed in the F_1 mice, with the lowland mice being significantly larger than highlanders, which resulted in a significant population x treatment interaction (Table 2). This reversal likely reflects sampling error; it is not attributable to biased sex ratios in the population samples, as we measured equal numbers of F_1 males (n=5/group) and females (n=5/group). Variation in body mass was also significantly correlated with thermogenic capacity (uncorrected $VO_{2\,max}$ values)

($r^2 = 0.081$, $p = 0.028$), but not thermogenic endurance ($r^2 = 0.006$, $p = 0.57$). Thus, we statistically controlled for body mass by using an ANCOVA design in the analysis of thermogenic capacity. We did not correct for body mass in the analysis of thermogenic endurance.

There were significant differences in thermogenic capacity between high- and low-altitude mice (ANCOVA $F_{1, 59} = 60.17$, $p = 2.74 \times 10^{-10}$; Table 3) and among treatment groups (ANCOVA $F_{2, 58} = 21.46$, $p = 1.49 \times 10^{-10}$; Table 3). Within each experimental group (*In-situ*, 6-wk deacclimation, and F_1), highland deer mice had significantly higher thermogenic capacities than their lowland counterparts (Fig. 1a), but the proportional difference in thermogenic capacity between highland and lowland mice increased across the deacclimation treatments (Fig 1b). Specifically, the proportional difference in thermogenic capacity was greatest in the high- vs. low-altitude comparison between the lab-reared F_1 progeny of wild-caught mice, and smallest in comparisons between highland and lowland mice that were tested at the site of capture.

However, within population samples, thermogenic capacity decreased across the three treatments, demonstrating that thermogenic capacity is highly plastic. The interaction between population of origin and treatment was not significant ($F_{2, 58} = 0.59$, $p = 0.56$) (Table 1), indicating that the decline in thermogenic capacity across the three treatments was qualitatively similar for highland and lowland mice (Fig. 1b).

We found a fundamentally different pattern for thermogenic endurance. Although both population of origin (ANOVA $F_{1, 59} = 22.03$, $p = 1.87 \times 10^{-5}$) and acclimation treatment (ANOVA $F_{1, 59} = 15.4$, $p = 5.01 \times 10^{-6}$) also had significant effects on endurance (Table 2), this result was largely driven by differences between the *in situ* groups. After the 6-wk deacclimation period, the difference in thermogenic endurance between the highland and lowland deer mice essentially

disappeared (Fig. 2). Thus, in contrast to thermogenic capacity, the difference in thermogenic endurance between the *in situ* highland and lowland mice appears to be entirely attributable to physiological plasticity during adulthood.

In contrast to the deacclimation changes in thermogenic capacity, the highland and lowland mice exhibited distinct patterns of change in thermogenic endurance across the three experimental treatments, as indicated by a significant population x treatment interaction ($F_{2, 58} = 14.36$; $p = 1 \times 10^{-5}$) (Table 2). In the highland mice, thermogenic endurance decreased steadily across treatments (Fig 2a). By contrast, endurance in the lowland 6-wk deacclimation group was slightly higher than both the lowland *in situ* and lowland F_1 groups, although this difference was not significant.

For the *in situ* mice, thermogenic capacity was strongly and positively correlated with thermogenic endurance ($r^2 = 0.44$, $p = 0.001$) (Fig. 3). Although this correlation approached significance in the 6-week deacclimation group ($r^2 = 0.17$, $p = 0.063$), there was no relationship between thermogenic capacity and endurance for the F_1 mice ($r^2 = 0.03$; $p = 0.467$). This pattern mainly reflects the strong influence of environmental effects on thermogenic endurance, and it demonstrates that there is no ineluctable functional linkage between thermogenic capacity and endurance.

DISCUSSION

Thermogenic capacity and endurance are constrained by the reduced PO_2 at high altitude (Hayes, 1989; Ward *et al*, 1995; Chappell and Hammond, 2004), and this hypoxic challenge can be offset through a combination of genetic adaptation, physiological plasticity and/or developmental plasticity. Our experiments were designed to assess the contribution of phenotypic plasticity to population differences in thermogenic performance under hypoxia, and to distinguish

283 between physiological adjustments that occur during adulthood and prenatal
284 development.
285 Results from our experiments on the *in situ* groups revealed that highland
286 deer mice have greater overall thermogenic capacities and endurances under
287 hypoxia relative to lowland deer mice (*in situ* groups; Figs. 1 and 2). The
288 common-garden deacclimation experiment revealed that although a fraction of
289 the *in situ* performance differences in both thermogenic capacity and endurance
290 could be attributed to phenotypic plasticity, the relative contributions and
291 sources of plasticity were different for each of the two traits. Thermogenic
292 capacity decreased across the three treatments, but trait values for the highland
293 mice were significantly higher than those of the lowland mice in each treatment
294 (Fig 1a). These results suggest that differences in thermogenic capacity have a
295 genetic basis, but the upper limits of capacity are affected by environmental
296 influences during both pre- and post-natal life.
297 In contrast to thermogenic capacity, the *in situ* differences in thermogenic
298 endurance were no longer evident after the six-week deacclimation period,
299 suggesting that the elevated thermogenic endurance of highland deer mice is
300 largely attributable to acclimatization to hypoxic cold stress during adulthood.
301 Although aerobic capacity and endurance tend to be positively correlated in
302 animals (Bennett, 1991), the mechanistic link between these two performance
303 measures is unclear. Like total aerobic capacity, thermogenic capacity appears to
304 be generally correlated with measures of thermogenic endurance in birds
305 (Swanson, 2001; Swanson and Liknes, 2006; Swanson and Garland, 2009). The
306 relationship between thermogenic capacity and endurance is less well-studied in
307 mammals, but there is some evidence for a positive correlation between maximal
308 aerobic capacity and exercise endurance in rodents (Bennett, 1991; Rezende *et al*,

2006). Our results suggest that under hypoxic conditions, this correlation is at least partially dependent on acclimation history (Fig. 3).

Given the observed differences in the plasticity of thermogenic capacity and thermogenic endurance under hypoxia, our results also suggest that these performance measures may have different mechanistic underpinnings. A number of factors are known to contribute to thermogenic capacity under hypoxia in deer mice. These include the relative sizes of thermogenic and respiratory organs (Rezende *et al*, 2009), differences in ventilatory traits (Rezende *et al*, 2004), blood-oxygen transport (Chappell and Snyder, 1984; Chappell *et al*, 1988; Hammond *et al*, 2001; Hammond *et al*, 2002), and the capacity for lipid oxidation (Cheviron *et al*, 2012). Although each of these traits displays a degree of phenotypic plasticity, blood-O_2 transport is associated with genetic differences in hemoglobin- O_2 affinity (Snyder *et al*, 1982; Chappell and Snyder, 1984; Snyder, 1985; Chappell *et al*, 1988; Storz *et al*, 2007; Storz *et al*, 2009; Storz *et al*, 2010a), and population differences in the capacity for metabolizing lipid fuels between highland and lowland deer mice persist for at least 6 wks of low-altitude deacclimation (Cheviron *et al*, 2012). Thus, genetically-based differences in hemoglobin function and metabolic capacities may contribute to the consistently elevated thermogenic capacities of high-altitude deer mice.

The mechanistic underpinnings of thermogenic endurance has received less empirical attention in deer mice, but studies from other rodents suggest that aerobic endurance is influenced by a number of factors including muscle fiber composition and respiratory capacity (Hoppeler *et al*, 1973; Hoppeler and Lindstedt, 1985; Kraemer *et al*, 1995), mitochondrial density and function (Holloszy and Coyle, 1984; Hoppeler and Lindstedt, 1985; Chow *et al*, 2007), decreased rates of glucose and glycogen utilization (Holloszy and Coyle, 1984) and increased rates of lipid oxidation (Holloszy and Coyle, 1984; Bjorntorp, 1991;

McClelland *et al*, 1994; Henriksson and Hickner, 1996; Bangsbo *et al*, 2006; Weber, 2011). All of these traits are highly plastic. Although these muscular phenotypes may enhance shivering endurance, rodents also rely heavily on non-shivering thermogenesis to regulate body temperature. Brown adipose tissue (BAT) is the primary site of non-shivering thermogenesis, and the mass of BAT depots in rodents is known to decrease dramatically with warm acclimation and seasonal acclimatization (Didow and Hayward, 1969; Himms-Hagen, 1985; Rafael *et al*, 1985; Klaus *et al*, 1988; Cannon and Nedergaad, 2004). Regression of BAT depots over the course of the 6-wk deacclimation period and the concomitant decrease in nonshivering thermogenic capacities could lead to an increased reliance on shivering thermogenesis in the warm-acclimated mice. An increased reliance on shivering thermogenesis would not only reduce total thermogenic capacity, but it could also compound the effects of muscular changes associated with inactivity that decrease thermogenic endurance, and could therefore help explain the rapid reduction in thermogenic endurance in high-altitude deer mice.

Finally, variation in body mass may also have an important consequences for thermoregulatory performance. In principle, larger mice should have a thermoregulatory advantage due to their decreased surface area to volume ratio and the concomitant reduction in rates of heat loss (Bartholomew, 1968; Conley and Porter, 1986). However, variation in body mass is not sufficient to explain all observed performance differences between high- and low-altitude deer mice. First, although body mass was significantly and positively associated with thermogenic capacity, there was no relationship between body mass and endurance. Second, although body mass did not vary systematically across the treatment groups (Table 1), the highland mice always had higher thermogenic capacities (Fig. 1). Finally, we statistically controlled for the correlation between body mass and thermogenic capacity in all comparisons between highland and

lowland mice. Taken together, these results suggest that population differences in body mass do not fully account for the observed performance differences between high- and low-altitude deer mice.

Interestingly, our estimates of hypoxic thermogenic capacity were considerably lower than those reported in highland deer mice from the White Mountains of eastern California (Chappell et al. 2007). For wild-caught mice sampled and measured at 3800 m (analogous to our in-situ treatment), Chappell et al. (2007) reported an uncorrected mean $VO_{2\,max}$ of 5.87 ml/min, which is nearly twice as high as our mean $VO_{2\,max}$ for the in situ highland mice measured at 4350 m (Table 1). These apparent differences do not seem to stem from methodological differences as our studies employed highly similar protocols. It is possible that the apparent differences in thermogenic capacity could stem from differences in the test altitude (3800 m vs 4350 m), and the differences could also reflect geographic variation in thermogenic performance among *P. maniculatus* populations. Highland deer mice from the White Mountains (subspecies *P. m. sonoriensis*) are genetically distinct from the highland Colorado mice (subspecies *P. m. rufinus*) and the lowland Nebraska mice (subspecies *P. m. nebracensis*) (Gering et al. 2009). Given that these subspecies/phylogroups vary with respect to body size and many other multifactorial traits (Osgood 1909), it would not be surprising if they also varied in measures of whole-animal physiological performance such as thermogenic capacity. Detailed studies of the mechanistic underpinnings of this geographic variation in thermogenic performance is likely to be a fruitful area of future research.

Recent years have witnessed a surge of interest in studies that explore the mechanistic underpinnings of adaptive trait variation in natural populations (Dalziel *et al*, 2009; Storz and Wheat, 2010). Much of this work has focused on the genetic basis of adaptive traits, while mechanisms of physiological and

developmental plasticity have received less attention (but see Whitehead *et al*, 2011). Our experimental results revealed the contributions of phenotypic plasticity to observed population differences in thermogenic capacity under hypoxia, a measure of whole-organism physiological performance that has a well-documented connection to Darwinian fitness (Hayes and O'Connor, 1999). After controlling for both physiological and developmental sources of plasticity, the highland and lowland deer mice exhibited persistent differences in thermogenic capacity, suggesting that genetically based physiological differentiation between populations may reflect local adaptation to different elevational zones.

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631

632

633 **Figure Legends**

634 **Fig 1.** A.) Mass-specific maximum rates of oxygen consumption ($VO_{2\,max}$) under
635 hypoxic cold stress for highland (black symbols) and lowland (gray symbols)
636 deer mice across three treatments. Letters denote population and treatment
637 combinations that are significantly different from one another. Data are
638 presented as means $\pm$ 1 SEM. B.) The proportional difference in $VO_{2\,max}$ between
639 highland and lowland deer mice across treatments.

640

641 **Fig 2.** A.) Thermogenic endurance [length of time (min) that individuals
642 maintained $\geq$ 90% of $VO_{2\,max}$] under hypoxic cold stress for highland (black
643 symbols) and lowland (gray symbols) deer mice across three treatments. Letters
644 denote population and treatment combinations that are significantly different
645 from one another. Data are presented as means $\pm$ 1 SEM. B.) The proportional
646 difference in thermogenic endurance between highland and lowland deermice
647 across treatments.

648

649 **Fig 3.** Correlations between thermogenic endurance and $VO_{2\,max}$ across three
650 treatments. Black and gray symbols represent highland and lowland deer mice
651 respectively.

652

Fig 1

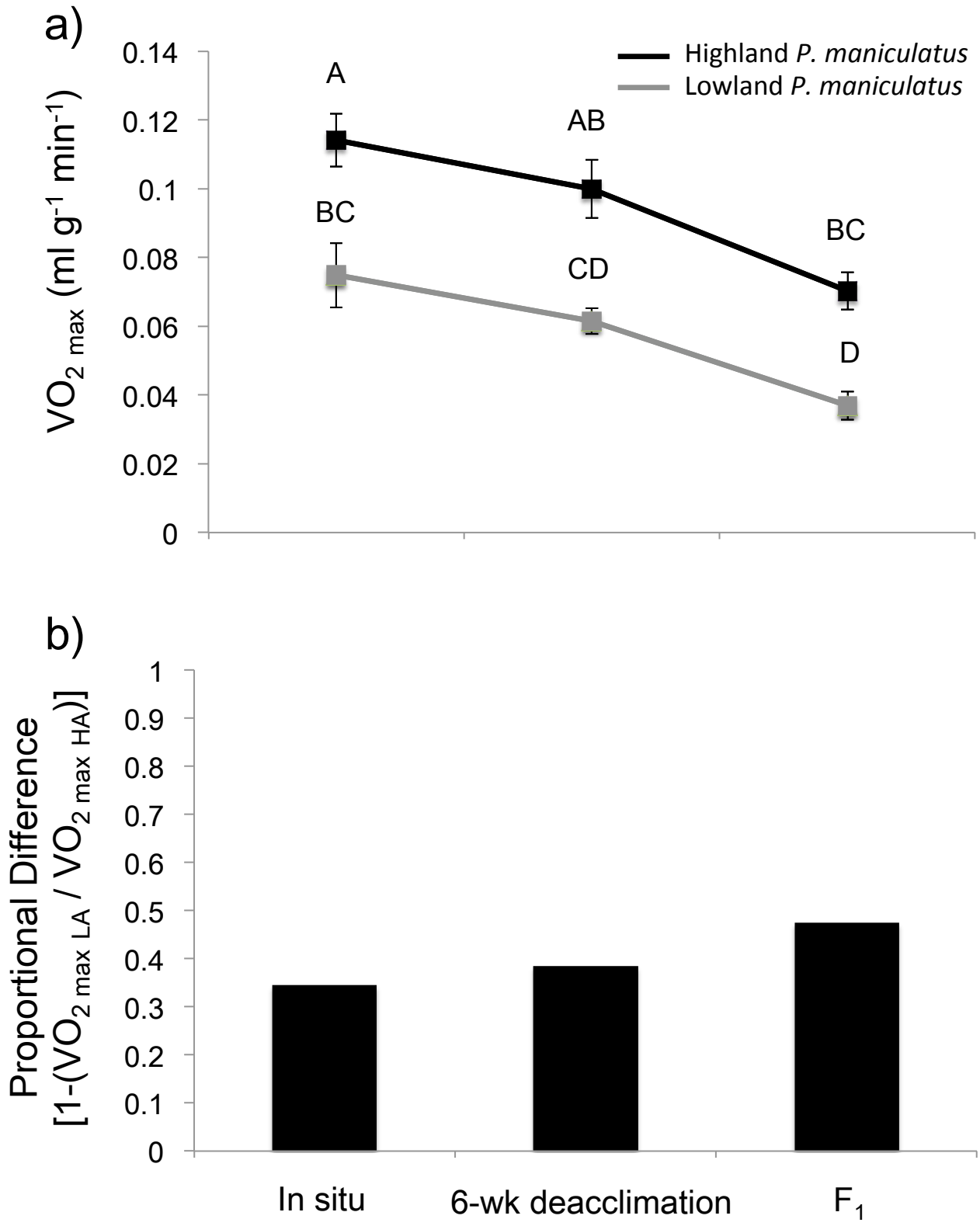
a)
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
$VO_{2\ max}$ (ml g^{-1} min^{-1})
Highland *P. maniculatus*
Lowland *P. maniculatus*
A
AB
BC
BC
CD
D
b)
Proportional Difference
[1-($VO_{2\ max\ LA}$ / $VO_{2\ max\ HA}$)]
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
In situ
6-wk deacclimation
F_1

Fig 2

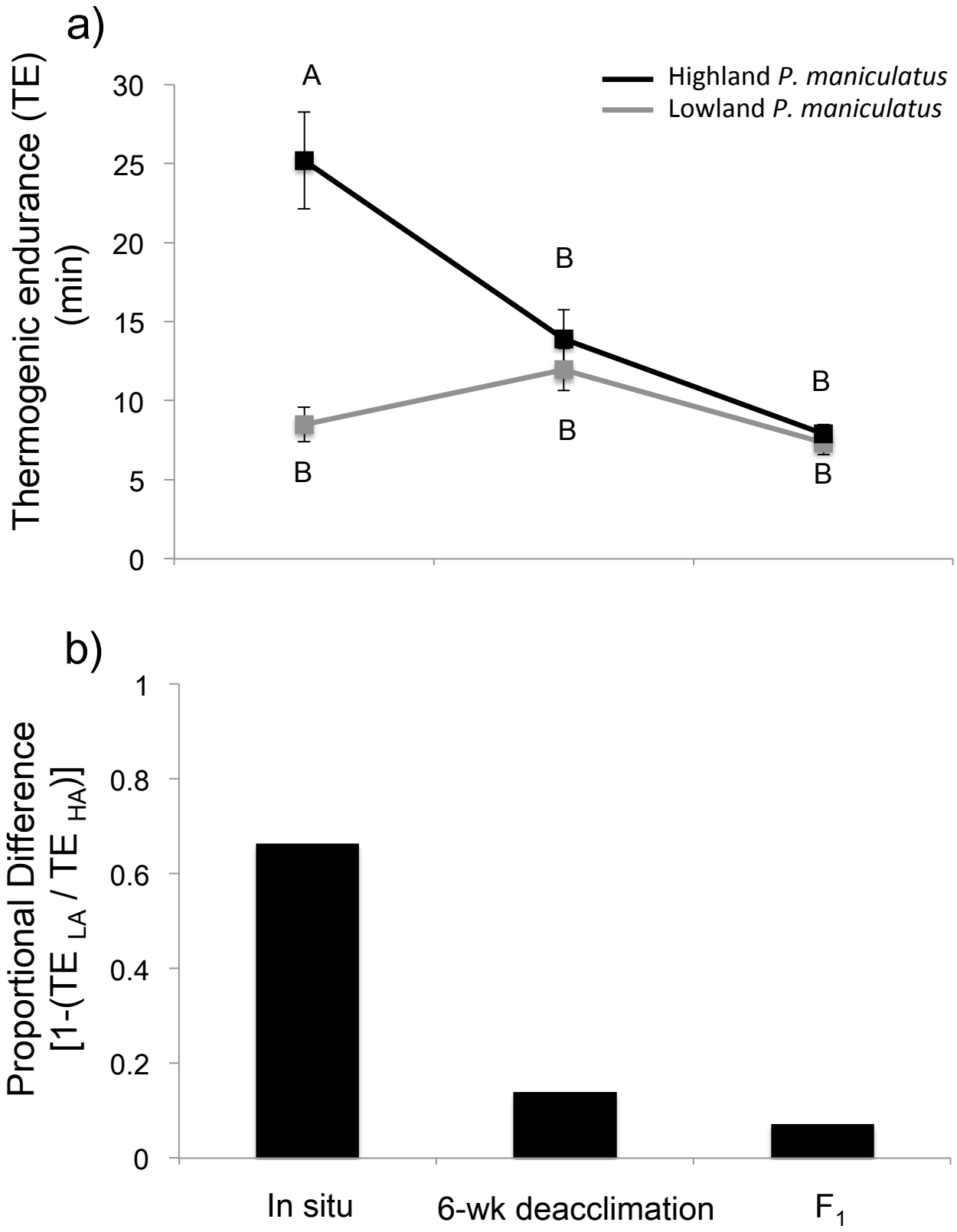
a)
Thermogenic endurance (TE) (min)
30
25
20
15
10
5
0
A
B
B
B
B
B
Highland P. maniculatus
Lowland P. maniculatus
b)
Proportional Difference [1-(TE LA / TE HA)]
1
0.8
0.6
0.4
0.2
0
In situ
6-wk deacclimation
F1

Fig 3

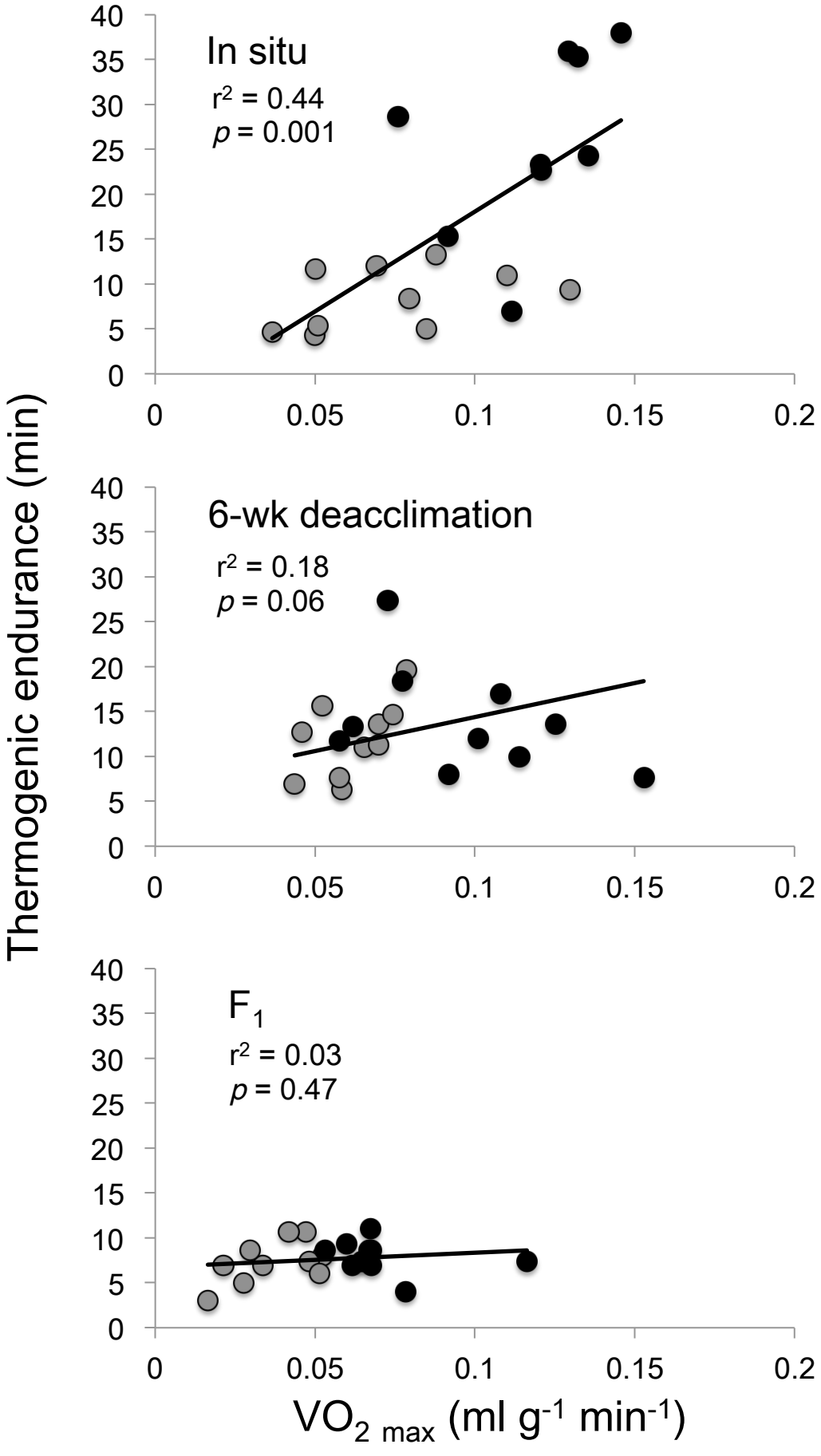
In situ
r2 = 0.44
p = 0.001
6-wk deacclimation
r2 = 0.18
p = 0.06
F1
r2 = 0.03
p = 0.47
Thermogenic endurance (min)
VO2 max (ml g-1 min-1)
0
5
10
15
20
25
30
35
40
0
0.05
0.1
0.15
0.2

Table 1. Altitudinal variation in body mass (g) across three treatments. HA = high-altitude; LA = low-altitude. Letters denote population and treatment combinations that are significantly different from one another (Two-way ANOVA followed by *post-hoc* Tukey HSD tests).

Population	Treatment	Mean	SE
HA	*In situ*	21.46 ^{A,B}	0.91
HA	6-wk deacclimation	21.01 ^{A,B,C}	1.04
HA	F_1	18.34 ^{B,C}	0.64
LA	*In situ*	17.38 ^{C}	0.80
LA	6-wk deacclimation	17.71 ^{B,C}	0.85
LA	F_1	22.91 ^{A}	1.16

Table 2. Effects of population of origin (high-altitude vs. low-altitude), and treatment (*in situ*, 6-wk deacclimation, and F_1 progeny of deacclimated mice) on body mass (g).

Factor	df	F	p
Population	1	1.57	0.2151
Treatment	2	1.21	0.3038
Population*Treatment	2	12.69	1.55x10-5

Table 3. Effects of body mass (g), population of origin (high-altitude vs. low-altitude), and treatment (*in situ*, 6-wk deacclimation, and F_1 progeny of deacclimated mice) on thermogenic capacity ($VO_{2\ max}$; ml O_2*min^{-1}). Tests of population of origin and treatment were performed using an ANCOVA model with body mass as a covariate

Factor	Df	F	*P*
Mass	1	13.77	0.0005
Population	1	60.17	2.74×10^{-10}
Treatment	2	21.46	1.49×10^{-10}
Population x Treatment	2	0.59	0.56

Table 4. Effects of population of origin (high-altitude vs. low-altitude) and treatment (in situ, 6-week acclimation, and F_1 progeny of deacclimated mice) on thermogenic endurance [length of time (min) that individuals maintained ≥ 90% of $VO_{2\,max}$].

Factor	Df	F	*P*
Population	1	22.03	1.87×10^{-5}
Treatment	2	15.40	5.10×10^{-6}
Population X Treatment	2	14.36	1.00×10^{-5}