Phenotypic Flexibility in a Novel Thermal Environment: Phylogenetic Inertia in Thermogenic Capacity and Evolutionary Adaptation in Organ Size

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ABSTRACT

The goal of our work was to understand the role of a novel thermal environment in shaping the phenotypic expression of thermogenic capacity and organ size. To examine this we compared two populations of the South American rodent *Phyllotis darwini* from different altitudes (Andean and valley populations), taking advantage of the fact that this genus originated at high altitude in the Andean plateau. DNA mitochondrial analysis showed that the two populations were separated and then experienced different thermal regimens for at least the last 450,000 yr. We expected the two populations of *P. darwini* to present more metabolic and organ size similarities if phylogenetic inertia had been an important factor. In this sense, phylogenetic inertia means that the valley population would retain evolutionary adaptations of high altitude: a greater phenotypic flexibility in both physiological and morphological traits. In general, our results indicate that the actual thermogenic capacities (magnitude and flexibility) of the valley population are a consequence of phylogenetic inertia. On the other hand, results for organ size (magnitude and flexibility) could suggest that this population would have adapted to the less seasonal central valley.

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Introduction

Phenotypic flexibility, or reversible phenotypic plasticity, allows organisms to match and increase their performance under changes in environmental conditions (Hammond et al. 2001). Acclimatization and acclimation are examples of phenotypic flexibility (Garland and Adolph 1991). In particular, seasonal changes in thermogenic capacity (maximum metabolic rate of thermoregulation, MMR) and thermal conductance allow small eutherian nonhibernating mammals to cope with variations in their thermal environments (Schmidt-Nielsen 1997; Hayes and Chappell 1986; Bozinovic et al. 1990; Heldmaier 1993). Theoretically, the thermogenic capacity is the sum of the basal metabolic rate (BMR), nonshivering thermogenesis (NST), and shivering thermogenesis (ST; Wunder and Gettinger 1996 and references therein). Hence, changes in MMR may be due to changes in any of these constituents (Jansky 1973; Heldmaier 1993; Piersma et al. 1996; Wunder and Gettinger 1996; Nespolo et al. 1999). In addition to these physiological changes, variations in the thermal environment have also been documented as causing morphological adjustments (Piersma and Lindström 1997). In fact, an increase in the mass of most metabolically active organs (i.e., the heart, lungs, small intestine, liver, and kidneys) has been observed in individuals acclimated or acclimatized to low temperatures (Chappell et al. 1999; Hammond et al. 1999, 2001). This increase in organ mass, which is a correlate of metabolic activity, represents the cost of the metabolic machinery needed to sustain metabolic rates above BMR during periods of elevated energy demands (Daan el al. 1990).

In this context, we are interested in understanding how a novel thermal environment experienced by an organism influences flexibility in thermogenic capacity and organ size, in comparison to individuals from the original environment. To achieve our goal, we studied two populations of the South American leaf-eared mouse, *Phyllotis darwini*. The tribe *Phyllotini* (Sigmodontinae) originated in the high-altitude plains of the Andes of northern Chile, Peru, and Bolivia during the Pliocene (Reig 1986; Engel et al. 1998). The Andean high-altitude population (1,500 m above sea level [asl]) experiences a seasonal thermal environment similar to that of the Andean plateau population (i.e., where *Phyllotis* originated), and there-

fore represents our *ancestral* population. On the other hand, the low-altitude, or valley, population that we studied (500 m asl) inhabits a much more stable Mediterranean-type environment, which is completely novel for this genus. We expect the two populations of *P. darwini* to present more metabolic and organ size similarities (after the confounding effects of body size are removed) if phylogenetic inertia is an important factor. In this sense, phylogenetic inertia means that the valley population would retain evolutionary adaptations of high altitude: greater absolute values of thermogenic capacity and organ mass and also greater phenotypic flexibility. This is because greater phenotypic flexibility seems to be more important than the maintenance of high, constant thermogenic capacity and organ size, since the latter would involve higher maintenance costs and comparatively lower energy savings. On the other hand, differences in thermogenic capacity and organ size between populations could suggest that the valley population has adapted to the reduced seasonality of the central valley (after the confounding effects of body size are removed). We are aware of the limitations associated with studies using only two populations (Garland and Adolph 1994). However, the main advantage of our study is that we know where this genus originated and what to expect regarding the flexibility of thermogenic capacity (Nespolo et al. 1999, 2001*b*) and organ size.

In addition, we estimated the divergence time between the populations to determine how long these populations have been experiencing different thermal environments.

Material and Methods

Animals and Acclimation

Twelve adult males and 13 females were captured with Sherman live traps in central Chile at Quebrada de la Plata (33°31′S, 70°50′W, 500 m asl) during austral autumn (April). This locality is characterized by a typical Mediterranean climate, with warm, dry summers and cold, wet winters (Gajardo 1994). In addition, 29 individuals (16 adult males and 13 females) were caught in the Andes, at Yerba Loca (33°10′S, 70°29′W, 1,500 m asl) during austral autumn (April). This locality presents a much more seasonal and cold thermal habitat than the Mediterranean one (Table 1). All individuals were transported to the laboratory (Santiago, 500 m asl) on the same day as their capture and maintained with rabbit-food pellets and water ad lib. at 22° ± 2°C and a 12L : 12D photoperiod for 1 mo.

After this period, individuals of each population were randomly divided into two groups. Twelve individuals (five females and seven males) from the valley and 15 individuals (seven females and eight males) from the Andean population were acclimated at 12° ± 2°C (i.e., cold conditions). Individuals were maintained under these conditions for 1 mo to ensure complete acclimation (Nespolo and Rosenmann 1997). The other treatment group consisted of 14 individuals (six females and eight males) from the Andes and 13 (eight females and five males) from the valley, which were acclimated to 30° ± 2°C (i.e., warm conditions) over 1 mo. All physiological measurements were made after the acclimation period. The 12L : 12D photoperiod was maintained for all temperature group treatments.

Table 1: Average temperatures for the two study locations (°C)

	Valley (Quebrada de la Plata)	Andean (Yerba Loca)
T_M	22.4 ± .6*	25.9 ± .4
T_m	7.6 ± .6*	1.6 ± .4
T_Y	14.8 ± .4*	13.8 ± .3
D_T	15.3 ± 2.4*	24.3 ± 2.2

Note. Records are from 1994 to 1999 for the Andean locality and from 1979 to 1997 for the valley locality. T_M = maximum temperature; T_m = minimum temperature; T_Y = annual temperature; D_T = maximum − minimum. Data are shown as means ± 1 SD.

* $P < 0.05$; Mann-Whitney *U*-test.

Basal Metabolic Rate and Nonshivering Thermogenesis

Upon completing each thermal acclimation, animals were fasted for 12 h (mean retention time of digesta is 10–18 h; Bozinovic and Nespolo 1997) before measurements of BMR and NST (Nespolo et al. 2003). Physiological measurements were made according to the protocol detailed below. Oxygen consumption ($\dot{V}O_2$) was measured in a computerized (Datacan V) open-flow respirometry system (Sable Systems, Henderson, Nev.) in steel metabolic chambers of 1 L, at an ambient temperature (T_a) of 30.0° ± 0.5°C, which is within the thermoneutral zone for this species (Bozinovic et al. 1988). The metabolic chamber received dried air at a rate of 505 mL min^{-1} from mass flow controllers (Sierra Instruments, Monterey, Calif.), enough to ensure adequate oxygen concentration in the chamber at all times (i.e., this flow rate is more than 10 times the peak oxygen demand) and to prevent partial oxygen pressure from falling to hypoxic conditions (Rosenmann and Morrison 1975). Mass flow meters are calibrated on a yearly basis by setting them in series configuration with the volumetric flowmeter and creating a calibration curve. So far there has been no need to introduce a correction factor for drifting. The air was passed through CO_2 (Baralyme) and H_2O (Drierite) absorbent granules, both before and after passing through the chamber, and was monitored every 5 s by an Applied Electrochemistry O_2 analyzer, model S-3A/I (Ametek, Pittsburgh, Pa.). This electrochemical sensor is cell-restored monthly. In addition, the baseline procedure carried out at every $\dot{V}O_2$ trial reveals the extent of drift that the sensor is accumulating, which is usually negligible. Oxygen consumption was calculated using equation (4a) of Withers (1977, p. 122). All metabolic trials were completed between 0800 and 1600 hours, since this species

is strictly nocturnal (Rezende and Bozinovic 2001). Before each metabolic measurement we recorded body mass (m_b) using an electronic balance (± 0.1 g), and after each measurement we recorded rectal body temperature (T_b) using a Cole-Parmer copper-constantan thermocouple connected to a Digi-Sense electronic thermometer.

For each individual we used the following experimental protocol: first, resting $\dot{V}O_2$ was recorded for 1 h; second, we made an intramuscular injection of norepinephrine (NE), and third, $\dot{V}O_2$ was recorded for an additional 30 min. We did not carry out a procedural control because previous work with this species indicated that $\dot{V}O_2$ is significantly lower following saline injection than after an NE injection (Nespolo et al. 1999, 2001*a*). To avoid possible death due to hyperthermia, we lowered the bath temperature by 5°C, following the NE injection, just after maximal $\dot{V}O_2$ was attained (i.e., from 30° to 25°C). NE doses were calculated according to Wunder and Gettinger's allometric equation (1996, p. 133). BMR was estimated as the continuous range of the lowest 5-min samples recorded during the first period (1 h) of $\dot{V}O_2$ recording. Measurements of BMR in this species indicate that $\dot{V}O_2$ reached a steady state after 15–20 min, without changes above 15% of $\dot{V}O_2$ during the following 4 h (Nespolo et al. 2003). We considered NST as the highest steady state 5-min $\dot{V}O_2$ measurement following NE injection minus BMR (Klaus et al. 1988; Wunder and Gettinger 1996). All animals showed hyperthermia at the end of the NST trial.

Maximum Metabolic Rate

MMR was measured in a mixed atmosphere of 80% He and 20% O_2 (Rosenmann and Morrison 1974), in a computerized (Datacan V) open-flow respirometry system (Sable Systems) at a T_a of 5.0° ± 0.5°C. The He-O_2 mixture passed through a volumetric flowmeter before entering the metabolic chamber at a rate of 1,002 mL min^{-1}. Before and after passing through the chamber the mixture was passed through CO_2- and H_2O-absorbent granules of Baralyme and Drierite. As in the case of BMR, $\dot{V}O_2$ was calculated using equation (4a) of Withers (1977, p. 122). After a recording period of 20 min, animals were removed and T_b was measured. All animals exhibited hypothermia, which indicated that MMR had been reached. Metabolic trials were conducted between 0800 and 1600 hours. MMR was estimated in two ways: (1) as the continuous range of the highest 3-min sample recorded during the period (MMR_{peak}) and (2) as the average oxygen consumption, including MMR_{peak}, during the period ($MMR_{stamina}$). We also calculated the aerobic scope (AS) as the difference between MMR_{peak} and BMR.

Shivering Thermogenesis

ST was calculated for each acclimation temperature using the following equation for small eutherian mammals:

$$MMR_{peak} = BMR + NST + ST$$

(Jansky 1973; Wunder and Gettinger 1996). That is, we considered thermogenic capacity to be additive, in spite of the controversy regarding whether it is additive or substitutive (e.g., Ricklefs et al. 1996; Weibel 2002).

Organ Morphometrics

Following physiological measurements, mice were killed and the gut (stomach, small intestine, cecum, and large intestine), heart, lungs, kidneys, and liver were removed and weighed (i.e., fresh masses) in an analytical electronic balance (Chyo JK-180, ±0.0001 g). Any residual digesta contained in the stomach, small intestine, cecum, and large intestine was removed before weighing, as well as any adherent fat. The organs and the remaining carcass (i.e., dry body mass) were dried to constant mass in an oven at 60°C for at least 7 d and then weighed.

DNA Extraction and Polymerase Chain Reaction Amplification

Total genomic DNA was extracted from liver samples preserved in ethanol from eight different individuals collected in the Andes (five individuals) and the valley (three individuals). The extraction procedure followed the phenol-chloroform standard protocol (Hillis et al. 1996).

To study the time of divergence between populations, the mitochondrial DNA control region was chosen to be amplified by PCR (polymerase chain reaction) using the following primers: 283 (forward, 5′-TACACTGGTCTTGTAAACC-3′) and 282 (reverse, 5′-AAGGCTAGGACCAAACCT-3′). For the valley population, after an initial denaturation step of 94°C for 3 min, 30 cycles alternating 94°C for 30 s, 60°C for 18 s, and 72°C for 90 s were followed, with a final extension step at 72°C for 5 min. All PCR reactions included negative controls. The same PCR conditions were held for the Andean population, but annealing was at 56°C for 50 s. Amplification products were analyzed through electrophoresis in an agarose gel at 1%. The sizes of the PCR products were determined against a standard. PCR products were cleaned using the QIAquick PCR purification kit (Qiagen, Valencia, Calif.) and sequenced using an ABI Prism 310 DNA sequencer (Applied Biosystems, Foster City, Calif.).

Divergence Time between Populations

Sequences were aligned with ClustalX (Thompson et al. 1999), and divergence time was estimated using transversions, which are less prone to saturation, in MEGA software (Kumar et al.

Table 2: Body mass, dry mass, and average body mass for each acclimation temperature and locality (g)

	Valley	Andean
Body mass:		
12°C	62.1 ± 15.0	59.6 ± 11.8
30°C	78.1 ± 10.8	58.9 ± 9.9
Dry body mass:		
12°C	17.9 ± 4.5	18.9 ± 6.2
30°C	26.2 ± 4.0	20.7 ± 7.3
Average body mass:		
12°C	59.1 ± 12.8	58.6 ± 11.2
30°C	73.9 ± 11.6	58.8 ± 10.4

Note. Body mass refers to the live animal mass when organ morphometrics were carried out. Dry body mass refers to the mass of the dry carcass. Average body mass is the average mass between the physiological measurements of BMR + NST and MMR. Data are shown as means ± 1 SD. See text for details.

2001). To calibrate the molecular clock we used estimates of the Sigmodontinae split of *Akodon*/*Necromys* at 3.7 million years ago and the Murinae split of *Mus*/*Rattus* at 12 million years ago. To test the constancy of the molecular clock, we conducted Tajima's relative rate test and evaluated the statistical significance using a χ^2 test (Tajima 1993).

Statistics

We performed all analyses both with and without sex as a factor. Since sex had no effect on the measured variables, we report results without including sex. Before statistical analyses, we examined data for assumptions of homogeneity of variances and normality, using Levene and Kolmogorov-Smirnov tests, respectively. When necessary, variables were $\log_{10}$-transformed. To analyze ANCOVA results, we checked for parallelism, using a test of interaction with the covariate. All data are presented as mean ± 1 SD. We designated $\alpha = 5\%$ as the probability level for falsely accepting a null hypothesis (Type I error). All statistical analyses were performed using STATISTICA 6.0 (StatSoft 2001) for Windows.

Results

Effects of Acclimation Temperature and Locality on Organ Dry Masses

Live animal body mass during organ mass measurements varied across locality, acclimation temperature, and sex (ANOVA, locality × temperature $F_{1,45} = 11.11$, $P = 0.0017$; sex $F_{1,45} = 18.89$, $P = 0.0001$; Table 2). Dry body mass also varied between localities and acclimation temperature (ANOVA, locality × temperature $F_{1,46} = 5.22$, $P = 0.03$; Table 2). Given that both fresh and dry body masses were highly correlated (Spearman $R = 0.92$, $t_{51} = 17.13$, $P < 0.0001$), we used only dry body mass (i.e., the carcass) as a covariate to test for differences in organ size between localities and acclimation temperatures (Christians 1999).

Organ water content differed significantly between localities in all cases (ANCOVA, $P < 0.05$); between acclimation temperature for small intestine, cecum, kidneys, and lungs (ANCOVA, $P < 0.05$); and between sexes for kidneys (ANCOVA, $P < 0.05$). Consequently, we decided not to evaluate possible differences in fresh organ masses, since these measurements would be confounded by differences in water content between factors (i.e., locality, sex, and acclimation temperature).

Descriptive data for dry organ masses are presented in Table 3, and ANCOVA results for each separate factor are provided in Table 4. Four organs presented higher masses under cold conditions: the kidneys, large intestine, cecum, and stomach. In addition, the masses of the kidneys and large intestine were higher in the valley population. A contrast analysis was performed to explore the interaction between acclimation temperature and population. Heart mass was higher in the valley population ($F_{1,48} = 9.26$, $P = 0.004$) under warm conditions, while the small intestine was heavier in the Andean population ($F_{1,48} = 13.36$, $P = 0.0001$) under cold conditions. Within-

Table 3: Descriptive data for organ dry masses (g)

	Valley (Quebrada de la Plata)		Andean (Yerba Loca)	
	30°C	12°C	30°C	12°C
Kidneys	.182 ± .064	.174 ± .037	.127 ± .022	.158 ± .026
Liver	.937 ± .302	.775 ± .152	.735 ± .180	.718 ± .134
Heart	.080 ± .016	.063 ± .013	.056 ± .016	.066 ± .016
Lungs	.123 ± .032	.098 ± .018	.118 ± .045	.099 ± .024
Small intestine	.264 ± .053	.264 ± .030	.253 ± .037	.325 ± .054
Large intestine	.099 ± .022	.125 ± .028	.077 ± .022	.097 ± .026
Cecum	.151 ± .039	.188 ± .039	.157 ± .025	.187 ± .043
Stomach	.179 ± .026	.171 ± .032	.160 ± .039	.188 ± .340

Note. Values are presented as means ± 1 SD.

Table 4: Results of an ANCOVA to test for the effects of temperature, locality, and their interaction on dry organ masses

	Mean Square Effect	Mean Square Error	$F_{1,48}$	P Level
Temperature:				
Kidneys	.0082	.0014	5.86	.02
Liver	.0005	.0316	.02	.89
Heart	.0001	.0002	.50	.48
Lungs	.0004	.0101	.04	.84
Small intestine	.0262	.0017	15.41	<.01
Large intestine	.0115	.0005	23.00	<.01
Cecum	.0223	.0013	17.15	<.01
Stomach	.0056	.0009	6.22	.02
Locality:				
Kidneys	.0102	.0014	7.29	.01
Liver	.0819	.0316	2.59	.11
Heart	.0007	.0002	3.50	.07
Lungs	.0003	.0101	.03	.86
Small intestine	.0155	.0017	9.12	<.01
Large intestine	.0055	.0005	11.00	<.01
Cecum	.0009	.0013	.69	.41
Stomach	.0004	.0009	.44	.51
Temperature-locality interaction:				
Kidneys	.0011	.0014	.79	.38
Liver	.0017	.0316	.05	.82
Heart	.0012	.0002	6.00	.02
Lungs	.0017	.0101	.17	.68
Small intestine	.0073	.0017	4.29	.04
Large intestine	.0008	.0005	1.60	.21
Cecum	.0017	.0013	1.31	.26
Stomach	.0011	.0009	1.22	.27

population differences in dry organ masses due to acclimation temperature are shown in Table 5.

Effects of Acclimation Temperature and Locality on Metabolic Variables

Body mass did not change significantly from BMR + NST to MMR (ANOVA, $F_{1,105} = 1.66$, $P = 0.200$). So we decided to use the average body mass (i.e., the average of body mass at MMR and body mass at BMR + NST) as a covariate in the subsequent analysis.

Descriptive data for metabolic variables are presented in Table 6 and ANCOVA results for each separate factor in Table 7. MMR_{peak} and $MMR_{stamina}$ were higher in the cold-acclimated groups. In addition, $MMR_{stamina}$ was higher in the Andean population. BMR, NST, and AS were the variables responsible for the statistical significance of the interaction. A contrast analysis showed differences between populations only under warm conditions. BMR was higher in the valley population ($F_{1,49} = 19.53$, $P < 0.001$), while NST ($F_{1,48} = 30.166$, $P < 0.0001$) and AS were higher in the Andean population ($F_{1,48} = 10.025$, $P < 0.01$). NST and AS were higher in cold-acclimated individuals ($F_{1,49} = 83.24$, $P < 0.01$ and $F_{1,48} = 66.74$, $P < 0.01$, respectively).

Within-population differences in metabolic variables due to acclimation temperature are shown in Table 8.

Phenotypic Flexibility in Metabolic and Morphometric Variables

Phenotypic flexibility was estimated in the following way. First, we obtained an average value for each trait under each acclimation temperature. For example, the average mass of the small intestine for the Andean population was 0.253 g at 30°C and 0.325 g at 12°C. Second, we computed the difference between these two values (i.e., $D_{12°C–30°C} = 0.325 - 0.253 = 0.072$). That

Table 5: Results of an ANCOVA to test for the effect of acclimation temperature on dry organ masses from the Andean and valley populations

	Mean Square Effect	Mean Square Error	$F_{1, 26}$	P Level
Andean population:				
Kidneys	.0086	.0004	21.50	<.01
Liver	.0001	.0186	.01	.92
Heart	.0010	.0002	5.00	.03
Lungs	.0013	.0107	.12	.73
Small intestine	.0376	.0016	23.50	<.01
Large intestine	.0039	.0005	7.80	.01
Cecum	.0075	.0012	6.25	.02
Stomach	.0067	.0012	5.58	.03
Valley population:				
Kidneys	.0047	.0024	1.96	.18
Liver	.0312	.0427	.73	.40
Heart	.00003	.0002	.15	.70
Lungs	.0058	.0090	.64	.43
Small intestine	.0008	.0019	.42	.52
Large intestine	.0081	.0005	16.20	<.01
Cecum	.0120	.0014	8.57	.01
Stomach	.0019	.0006	3.17	.09

is, 0.072 represents a 28.50% increase in the mass of the small intestine from 30°C to 12°C. To test for differences in phenotypic flexibility between the two populations we used a two-tailed *t*-test only for those traits that varied as a result of thermal acclimation (i.e., traits that changed from 30°C to 12°C within each population). Results are presented in Table 9.

Time of Divergence between Populations

The relative rate test (Tajima 1993) showed that in multiple comparisons, both groups behave according to the molecular clock. Divergence time was estimated to be between 4.4×10^5 and 5.5×10^5 yr. However, it is important to mention that the lower limit comes from the estimation of the sigmodontine split, the most reliable calibration point for this case. In addition, it should be mentioned that the accuracy of the molecular clock decreases with recent divergence.

Discussion

The goal of our work was to understand how a novel thermal environment experienced by an organism influences flexibility in thermogenic capacity and organ size, in comparison to individuals from the original environment. To examine this we compared two populations of the rodent *Phyllotis darwini* from different altitudes, taking advantage of the fact that this genus

Table 6: Descriptive data for metabolic traits (mL O_2 h^{-1})

	Valley (Quebrada de la Plata)		Andean (Yerba Loca)	
	30°C	12°C	30°C	12°C
BMR	92.6 ± 21.3	69.0 ± 9.5	64.1 ± 11.2	70.9 ± 9.6
NST	64.6 ± 23.4	180.0 ± 46.2	128.0 ± 53.2	190.7 ± 49.4
ST	135.4 ± 66.9	102.3 ± 54.5	72.0 ± 51.6	82.7 ± 70.5
MMR_{peak}	289.4 ± 68.6	351.3 ± 70.1	264.2 ± 64.9	344.3 ± 57.2
$MMR_{stamina}$	249.1 ± 57.1	320.3 ± 68.5	236.2 ± 43.4	327.8 ± 47.7
AS	197.4 ± 76.5	282.3 ± 62.8	200.1 ± 64.2	273.4 ± 54.0

Note. Values are presented as means ± 1 SD. BMR is basal metabolic rate, NST is nonshivering thermogenesis, ST is shivering thermogenesis, MMR_{peak} is peak maximum metabolic rate, $MMR_{stamina}$ is stamina maximum metabolic rate, and AS is aerobic scope.

Table 7: Results of an ANCOVA to test for the effects of temperature, locality, and their interaction on metabolic variables

	Mean Square Effect	Mean Square Error	$F_{1, 48}$	*P* Level
Temperature:				
BMR	479.63	187.37	2.56	.116
NST	131,667.8	1,520.2	86.61	<.001
ST	132.17	3,381.01	.039	.844
MMR_{peak}	124,225.7	1,943.0	63.93	<.001
$MMR_{stamina}$	140,854.1	1,161.1	121.31	<.001
AS	140,143.2	2,099.8	66.74	<.001
Locality:				
BMR	1,469.24	187.37	7.84	.007
NST	34,638.9	1,520.2	22.79	<.001
ST	8,051.46	3,381.01	2.38	.129
MMR_{peak}	3,370.3	1,943.0	1.73	.195
$MMR_{stamina}$	8,214.1	1,161.1	7.07	.011
AS	9,290.1	2,099.8	4.42	.040
Temperature-locality interaction:				
BMR	2,076.03	187.37	11.08	.002
NST	21,695.5	1,520.2	14.27	<.001
ST	694.4	3,381.01	.21	.649
MMR_{peak}	5,682.1	1,943.0	2.92	.093
$MMR_{stamina}$	3,503.2	1,161.1	3.017	.089
AS	14,627.2	2,099.8	6.97	.011

Note. BMR is basal metabolic rate, NST is nonshivering thermogenesis, ST is shivering thermogenesis, MMR_{peak} is peak maximum metabolic rate, $MMR_{stamina}$ is stamina maximum metabolic rate, and AS is aerobic scope.

originated at high altitude (Reig 1986). In our case, we did not consider hypoxia to be a confounding factor, since our high-altitude population (1,500 m asl) inhabits an environment with a Po_2 of approximately 130 Torr, a value far above what is considered to be the critical pressure (i.e., $Po_2 = 110$ Torr) for the maintenance of metabolic rate in this species (Rosenmann and Morrison 1975). Although we are aware that a fraction of the observed variability between populations may be due to another, unmeasured factor, it is possible to say that the main trends observed for thermogenic capacity and organ size arise mainly as a consequence of the different thermal environments these populations inhabit.

Intra- and Interpopulation Differences in Organ Dry Masses

Only the Andean population presented higher heart dry mass under cold conditions. Neither population showed differences in lung dry mass between acclimation temperatures. Only under warm conditions did organ masses differ between populations, in that heart mass was higher for the valley population. In addition, Hammond et al. (1999) found that cardiopulmonary organ masses were higher in individuals of *Peromyscus maniculatus bairdii* living at high altitudes, although in their study, the effects of temperature and hypoxic conditions were not analyzed separately. Hammond et al. (2001) explicitly tested the relative effect of ambient temperature and Po_2, finding that lung mass changes were mainly determined by hypoxic conditions, while heart mass changes were mainly driven by temperature.

Overall, considering both populations pooled together, mice exposed to colder temperatures had heavier kidneys, large intestines, ceca, and stomachs. On the other hand, populations differed only in the size of the kidneys and large intestine, both being heavier in individuals from the valley. In addition, in the Andean population, the kidneys, cecum, stomach, and small and large intestines were heavier under cold conditions. Nevertheless, in the valley population only the cecum and large intestine were heavier under cold temperatures. Similar changes in the gastrointestinal system as a result of lower ambient temperatures at higher altitudes have been reported by Hammond et al. (1999) for *Peromyscus*. In summary, our results indicate that both populations varied the masses of different organs as a result of thermal acclimation (i.e., Andean population: kidneys, small and large intestines, and cecum; valley population:

Table 8: Results of an ANCOVA to test for the effect of acclimation temperature on dry organ masses from the Andean and valley populations

	Mean Square Effect	Mean Square Error	$F_{1, 26}$	*P* Level
Andean population:				
BMR	343.98	98.38	3.50	.072
NST	28,834.99	1,976.63	14.59	<.001
ST	847.83	3,918.77	.22	.643
MMR_{peak}	47,294.38	2,246.31	21.05	<.001
$MMR_{stamina}$	61,478.11	1,177.66	52.20	<.001
AS	39,571.6	2,291.12	17.27	<.001
Valley population:				
BMR	1,916.01	302.87	6.33	.020
NST	85,996.35	971.31	88.54	<.001
ST	579.40	2,519.20	.23	.636
MMR_{peak}	74,829.5	1,569.62	47.67	<.001
$MMR_{stamina}$	82,169.18	979.52	83.89	<.001
AS	100,693.4	1,832.9	54.94	<.001

Note. See Table 7 for definition of abbreviations.

large intestine and cecum). But an important question is whether the pattern of change was similar in both populations. If phylogenetic inertia is important, we predicted similar flexibility in organ size between both populations. Contrary to our expectations, kidneys, heart, and small intestine presented a greater percentage of change in the Andean population (Table 7). These organs are known to contribute disproportionately to sustaining high nonbasal metabolic rates (Daan et al. 1990). On the other hand, we did not observe any change in phenotypic flexibility for the most expensive organs for individuals from the valley population.

Intra- and Interpopulation Differences in Thermogenic Capacity

Basal Metabolic Rate. In comparisons of the two populations, BMR was higher in individuals from the valley only under warm conditions. Interestingly, individuals from the valley also presented heavier kidneys (see above), which may explain this observed difference in BMR. Within localities, BMR was not affected by acclimation temperature in the Andean population; however, it was lower in cold-acclimated individuals from the valley. When thermal loads are high (i.e., in cold conditions), a reduction in BMR can be considered a strategy for minimizing energy expenditure. This reduction has also been reported following acclimation to other factors, such as low energy availability (Veloso and Bozinovic 1993). In addition, if we consider that the Andean population inhabits a colder environment than the valley population, then the observed difference in BMR under warmer conditions could reflect, comparatively, an important energy savings in a highly costly environment. On the other hand, Nespolo et al. (2001*b*) reported an increase in resting metabolic rate (RMR) following cold acclimation in individuals from the same valley population. These apparently contradictory results may be explained simply by whether or not animals were fasted (Nespolo et al. 2003).

In general, the effect of seasonal acclimatization, or thermal acclimation, on BMR is still a matter of great controversy (see McNab 2002 for a review). Other studies have reported a mixture of results, including a decrease (Corp et al. 1997; Deeremberg et al. 1998), an increase (Lynch 1973; Konarzewski and Diamond 1994, 1995; McDevitt and Speakman 1994; Nespolo and Rosenmann 1997), or even no changes in BMR following cold acclimation (Koteja 1996).

Maximum Metabolic Rate and Aerobic Scope. Before proceeding with the discussion, one point should be mentioned about using MMR_{peak} and $MMR_{stamina}$ as estimates of maximal thermogenic capacity. While MMR_{peak} has often been used as a measurement of ecological relevance (e.g., Bozinovic and Rosenmann 1989), the short duration of this measurement brings such relevance into question. For this reason we decided to incorporate $MMR_{stamina}$ as an additional, more indicative measure of performance over ecological timescales.

In our study, MMR_{peak}, $MMR_{stamina}$, and AS were higher in cold-acclimated animals for the two populations considered together, as well as separately. Such an increase following cold acclimation and acclimatization has been reported several times for other small mammals (Hayes and Chappell 1986; Bozinovic et al. 1990; Nespolo et al. 2001*a*; Rezende et al. 2001). In addition, $MMR_{stamina}$ presented higher values in the Andean pop-

Table 9: Differences in phenotypic plasticity between localities

	Valley (Quebrada de la Plata)	Andean (Yerba Loca)
Kidneys (g)	−.008 (−4.28)	.031 (+24.23)*,+
Liver (g)	−.162 (−17.30)	−.018 (−2.4)
Heart (g)	−.017 (−21.34)	.010 (+17.87)*
Lungs (g)	−.025 (−2.13)	−.018 (−15.55)
Small intestine (g)	−.001 (−.27)	.072 (+28.50)*
Large intestine (g)	.026 (+26.46)	.020 (+25.81)
Cecum (g)	.037 (+24.66)	.029 (+18.69)
Stomach (g)	−.008 (−4.45)	.028 (+17.60)*,+
BMR (mL O_2 h^{-1})	−23.55 (−24.55)	6.83 (+10.65)*
NST (mL O_2 h^{-1})	115.46 (+178.85)	62.64 (+48.93)*
ST (mL O_2 h^{-1})	−33.08 (−24.43)	10.67 (+14.81)
MMR_{peak} (mL O_2 h^{-1})	61.91 (+21.39)	80.14 (+30.33)
$MMR_{stamina}$ (mL O_2 h^{-1})	71.23 (+28.60)	91.61 (+38.79)
AS (mL O_2 h^{-1})	84.88 (+42.99)	73.31 (+36.64)

Note. Values are computed as average value $trait_{12°C}$ − average value $trait_{30°C}$. Values in parentheses denote the percent of change from 30°C to 12°C. See Table 7 for definition of abbreviations.

* $P < 0.05$; two-tailed *t*-test.

\+ Marginal significance.

ulation. Regarding plasticity, we expected to find similar flexibility between populations, if the thermogenic capacity of the valley population had been a result of the high Andean origin of the genus. According to our expectations, there were no population differences in flexibility in MMR_{peak}, $MMR_{stamina}$, and AS. This point is reinforced when considering AS. In fact, taking both acclimation temperatures together, the AS was 237.98 mL O_2 h^{-1} for the Andean population and 239.88 mL O_2 h^{-1} for the valley population. That is, the maintenance cost in the Andean population represents 22.1% of the thermogenic capacity, whereas in the valley population it represents 25.0%. This means that 77.9% and 75% of maximal aerobic capacity in the Andean and valley populations, respectively, are available for periods of high thermal requirements.

Nonshivering and Shivering Thermogenesis. Within populations, there were no differences in ST between acclimated and nonacclimated (i.e., 30°C) groups, and also ST did not differ between populations. Overall, this means that phenotypic flexibility in ST was statistically not different from zero. However, within both populations, NST was higher in the cold-acclimated groups, which has been reported in previous studies (Lynch 1973; Heldmaier 1993; Nespolo et al. 2001*a*, 2001*b*). In addition, NST was higher in cold-acclimated individuals, taking both populations together. Differences in NST between populations only appear under warm conditions, where the Andean population presented higher values. However, the valley population presented higher phenotypic flexibility than the Andean population. In general, these results are in accordance with patterns commonly reported in literature: NST represents the preferable strategy of heat production with respect to ST (Heldmaier 1993). Shivering, which is the main mechanism of thermogenesis in cold-acclimated or acclimatized birds (e.g., Saarela et al. 1995; Marjoniemi and Hohtola 2000), represents a rather inefficient mode of heat production in small mammals because of the increment in convective heat loss that it produces, and it is only used when maximal capacity for NST has been reached (Heldmaier et al. 1985; Heldmaier 1993; but see Böckler and Heldmaier 1983). Nespolo et al. (2001*b*) reported an increase of 130% in NST as a result of thermal acclimation in *Phyllotis darwini* from the same valley population as ours. These authors state that the high metabolic flexibility of NST was a consequence of phylogenetic inertia, since the percentage of change was similar to that exhibited by *P. xanthopygus* (113%), a congeneric species inhabiting the high Andean plateau where the genus originated. Nevertheless, Nespolo et al. (1999) reported that *P. xanthopygus* did show a small percentage of change in NST (49%) as a result of thermal acclimation. In addition, they observed that changes in ST (>200%) accounted for most of the thermogenic capacity in this species (Nespolo et al. 1999). In spite of the seemingly contradictory results between the two studies (Nespolo et al. 1999, 2001*b*), it is clear, in our case, that both populations of *P. darwini* rely on similar strategies to cope with the different thermal environments they inhabit. As in the cases of MMR and AS, these results would mean that the actual thermogenic capacities of the valley population are a consequence of the biogeographic origin of the genus (i.e., phylogenetic inertia).

Phenotypic Flexibility in Thermogenic Capacity and Organ Size

In temperate environments, where temperature varies not only between seasons but also on a daily basis, seasonal changes in thermogenic capacity and thermal conductance have been described as the most important mechanisms allowing small eutherian nonhibernating mammals to cope with such climatic variations. These physiological changes are generally accompanied by morphological adjustments in the masses of most metabolically active organs. In general, our results indicate that the actual thermogenic capacities (i.e., magnitude and flexibility) of the valley population are a consequence of phylogenetic inertia. That is, individuals from the valley population have retained the evolutionary adaptations of high altitude. On the other hand, results for organ size (i.e., magnitude and flexibility) could suggest that this population would have adapted to the less seasonal central valley. This is not contradictory, since it is well known that central organs are decoupled from thermogenic capacity (Weibel 2002). In general, our results mark the importance of considering thermal environment throughout evolutionary time, not only when comparing species (Rezende et al. 2001; Nespolo et al. 2001*b*), but also between populations within a species. Such a focus would allow for a better understanding of the role of phenotypic flexibility in seasonal environments.

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