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RESEARCH ARTICLE

Early life experience drives short-term acclimation of metabolic and osmoregulatory traits in the leaf-eared mouse

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ABSTRACT

We studied the putative effect of early life experience on the physiological flexibility of metabolic and osmoregulatory traits in the leaf-eared mouse, *Phyllotis darwini*, an altricial rodent inhabiting seasonal Mediterranean environments. Adult individuals were collected in central Chile and maintained in breeding pairs. Pups were isolated after weaning and acclimated to different temperatures (cold or warm) and water availability (unrestricted and restricted) until adulthood. Subsequently, individuals were re-acclimated to the opposite treatment. Rodents reared in the warm and subjected to water restriction had lower basal metabolic rate (BMR), total evaporative water loss (TEWL) and body mass (M_b) compared with those developing in the cold treatment; nevertheless, individuals subjected to warm temperatures had greater relative medullary thickness (RMT) and urine concentrating ability (UCA). Cold-reared rodents re-acclimated to warm conditions exhibited physiological flexibility of metabolic traits; however, their osmoregulatory attributes did not vary. Conversely, warm-reared rodents re-acclimated to cold had reduced RMT and UCA, but the metabolic traits of these individuals did not change. These results suggest a trade-off between metabolic performance and renal capabilities that might hinder physiological acclimation. Our results support the hypothesis of ontogenetic dependence of short-term acclimation in osmoregulatory and metabolic traits in *P. darwini*.

KEY WORDS: Metabolism, Osmoregulation, Phenotypic plasticity, *Phyllotis darwini*, Temperature, Water availability

INTRODUCTION

Phenotypic plasticity is an organism's ability to alter its phenotype in response to environmental factors (West-Eberhard, 2003). In an ecological context, phenotypic plasticity is fundamental to understanding physiological diversity (Miner et al., 2005; Pigliucci, 2001). Environmental cues can promote phenotypes that increase fitness in particular environments (Gilbert and Epel, 2009). Because organisms are highly sensitive to environmental changes during development, variability in developmental conditions can lead to irreversible phenotypic changes (Dufty et al., 2002; Hoverman and Relyea, 2007; West-Eberhard, 2005). When environmental conditions change rapidly over short time scales (e.g. seasonally), individuals can exhibit continuous and reversible phenotypic transformations (Piersma and Drent, 2003). And while phenotypic plasticity may be beneficial, it may be limited by the costs of maintenance and production of plastic structures (DeWitt et al., 1998; Pigliucci, 2001).

Temperature and water availability are likely the environmental variables that most impact animal performance (Nespolo and Rosenmann, 1997; Rezende et al., 2004a,b; Tirado et al., 2008). For endotherms, increased ambient temperatures along with decreased availability of drinking water favor physiological adjustments that promote water and energy storage (Al-Kahtani et al., 2004; Bozinovic and Gallardo, 2006; Bozinovic et al., 2003; Hinsley et al., 1993; Hudson and Kimzey, 1966; Krebs, 1950). Initial thermal conditions may also influence an organism's acclimation capability (Barceló et al., 2009). Some authors have found that thermal acclimation is dependent on pre-acclimation temperature in adult passerine birds. Studies in rodents suggest that during acclimation, the magnitude of phenotypic change is dependent on the temporal window in which environmental variability is acting; thus, changes in phenotypes are a result of phenotypic flexibility, developmental plasticity, natural selection and/or a combination of these factors (Oswald, 1998; Tracy and Walsberg, 2001b).

Despite an increase in studies of developmental plasticity in the last decade (Brzęk et al., 2009; Canals et al., 2009; Karmarkar and Dan, 2006; Russell et al., 2008; Terblanche and Chown, 2006), to the best of our knowledge there are no studies assessing the combined effect of multiple environmental variables (e.g. water and temperature) experienced during development on the physiological flexibility of different traits in adulthood. Here, we assessed the effects of both water availability and temperature on the physiological performance of *Phyllotis darwini*, an altricial rodent inhabiting seasonally variable semiarid and Mediterranean scrublands in central Chile (Iriarte, 2008). We measured two temporal windows of the ontogeny of this species: juvenile and adult. We predicted that high ambient temperature and limited water availability during development would decrease energy expenditure and increase adult renal functioning. Specifically, we hypothesized that the ability to modify physiological traits (in our case, the ability to acclimate osmoregulatory and metabolic traits) would be associated with the environmental conditions experienced during development. Thus, we predicted that the magnitude of short-term acclimation would be dependent on early life experience in our model system.

MATERIALS AND METHODS

Animals and experimental design

Adult male and female leaf-eared mice, *Phyllotis darwini* (Waterhouse 1837), were collected between November 2009 and March 2010 with Sherman traps in Quebrada de la Plata, central Chile (33°31′S. 70°50′W). The climate at this locality is Mediterranean with an annual mean precipitation of 380 mm concentrated in winter from June to August. Precipitation is minimal from December to March, accounting for only 3% of the annual

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List of symbols and abbreviations

BMR	basal metabolic rate
COX	cytochrome *c* oxidase
CS	citrate synthase
M_b	body mass
RMT	relative medullary thickness
TEWL	total evaporative water loss
UCA	urine concentrating ability

precipitation. Temperatures are highest from December to March (austral summer) and lowest from June to August during the austral winter. The combination of high temperatures and low precipitation from December to March characterizes the typical summer drought of this Mediterranean climate (Jaksic, 2001).

Two non-pregnant females were randomly assigned to a breeding male. The breeding aggregation (about 30 individuals) was allowed to interact for a period of 10 days, or until a clear state of pregnancy was observed. Because each female produces a litter of three to four pups, individuals from the same litter (and same mother) were separated in different treatments. Pregnant females were kept in enclosures maintained at 25°C, and individuals were given rat food (Champion S. A., Santiago, Chile) and water *ad libitum* during pregnancy and lactation (see Sabat and Bozinovic, 2000). Pups were isolated after weaning (22 days of age) and were acclimated to one of two different temperature treatments (cold: 15±1°C; warm: 30±1°C), and one of two water availability treatments (unrestricted: *ad libitum* water; restricted: 50% water restriction). We quantified the daily water consumption of rodents acclimated to *ad libitum* water conditions (at each temperature). Water consumption in those groups was used as a reference to establish the amount of water to be supplied to rodents from the restricted treatments (50% water restriction). The mean daily consumption for the two temperature treatments was 10±0.9 ml at 30°C and 8.9±0.7 ml at 15°C. In the restricted treatments, water was supplied once a day at approximately 12:00 h.

During the experimental period, animals were maintained on a 12 h:12 h light:dark photoperiod. Food was supplied *ad libitum*. Once pups reached adulthood (about 4 months of age), 10 individuals from each treatment were selected and killed by CO_2 exposure for further determination of functional and structural variables (see below). The association between short-term acclimation and early life experience was assessed by considering two scenarios of environmental variability: (1) changes in temperature and (2) changes in temperature and water availability. Briefly, after the initial acclimation treatment (until 4 months of age), rodents were re-assigned to the opposite treatment and then were re-acclimated to the new conditions for 4 weeks (Fig. 1). After the re-acclimation period, rodents were killed by exposure to CO_2 and were dissected. The large and small intestine, heart, lungs, pancreas, liver and kidneys were extracted. The mass of each organ was determined immediately (±0.05 g). The liver was frozen at −80°C for subsequent metabolic enzyme activity assays.

Rodents were captured under a permit issued by the Servicio Agricola Ganadero, Chile. The research was approved by the Ethics Committee of the Faculty of Science of the University of Chile, where the experiments were performed.

Basal metabolic rate (BMR) and total evaporative water loss (TEWL)

BMR and TEWL were determined while animals were inactive (08:00 h–17:00 h) and after they had fasted for 6 h, using a standard flow-through respirometry system and hygrometry methods (Nespolo et al., 2003). Animals were kept in 2000 ml steel metabolic chambers with a wire-mesh grid that allowed excreta to fall into a tray containing mineral oil, thus trapping water from this source. Oxygen consumption was measured using a computerized, open-flow respirometry system (Sable Systems, Henderson, NV, USA) calibrated with a known mix of oxygen (20%) and nitrogen (80%) that was certified by chromatography (INDURA, Santiago, Chile). Measurements were randomized and performed at an ambient temperature of 30.0±0.5°C, which is well within the thermoneutral zone for this species (Bozinovic and Rosenmann, 1988). Before passing through the metabolic chamber, air passed through CO_2-absorbent granules of Baralyme® and Drierite® at 750 ml min^{-1} from a mass flow controller (Sierra Instruments, Monterey, CA, USA). The mass flow meter was calibrated monthly with a volumetric (bubble) flow meter. After passing through a RH-200 relative humidity/dew point hygrometer (Sable Systems), the excurrent air passed through columns of CO_2-absorbent granules of Baralyme and Drierite before passing through an O_2-analyzer (model FC-10A, Sable Systems). The hygrometer was calibrated 1 week prior to the experiments following the manufacturer's instructions; 'zero' and 'span' values of water vapor pressure (kPa) were set using dry compressed nitrogen (0 kPa, zero value) and a saturated sample airstream at 20.0°C to calculate the water vapor pressure (2.339 kPa, span value). Output from the H_2O (kPa) and oxygen analyzers (%) was digitized using a Universal Interface II (Sable Systems) and recorded on a personal computer using EXPEDATA data acquisition software (Sable Systems). Our sampling interval was 5 s. We averaged the water vapor pressure and the O_2 concentration of the excurrent airstream over a 20 min period after a steady state was reached (Tieleman et al., 2002). Because CO_2 was scrubbed before entering the O_2 analyzer, oxygen consumption was calculated as (Withers, 1977, p. 122): $\dot{V}_{O_2}=[FR\times 60\times(F_{I_{O_2}}-F_{E_{O_2}})]/(1-F_{I_{O_2}})$, where FR is the flow rate in ml min^{-1} after standard temperature and pressure correction, and F_I and F_E are the fractional concentrations of O_2 entering and leaving the metabolic chamber, respectively. TEWL was calculated as $(\dot{V}_E\times\rho_{out})-(\dot{V}_I\times\rho_{in})$ in mg ml^{-1}, where ρ_{in} and ρ_{out} are the absolute humidity of the inlet and outlet air in kg m^{-3}, respectively, $\dot{V}_I$ is the flow rate of the air entering the chamber as given by the mass flow controller (750 ml min^{-1}), and $\dot{V}_E$ is the flow rate of exiting air. $\dot{V}_E$ was calculated as $\dot{V}_I-[\dot{V}_{O_2}(1-RQ)]+\dot{V}_{H_2O}$, where $\dot{V}_I$ and $\dot{V}_{O_2}$ (ml min^{-1}) are known, and we assumed a respiratory quotient (RQ) of 0.71. Absolute humidity was calculated as $\rho=P/(T\times R_w)$, where P is water vapor pressure of the air in Pa, T is the temperature of the dew-point hygrometer in K, and R_w is the gas constant for water vapor (461.5 J kg^{-1} K^{-1}; Lide, 2001). P_{in} was determined using the average vapor pressure of the air entering the empty chamber (i.e. the baseline period of 15 min) before and after each experiment. Using an electronic balance (±0.1 g), body mass (M_b) was measured before the metabolic measurements were taken. Rectal body temperature was recorded at the end of each measurement using a Cole–Palmer copper–constantan thermocouple attached to a Digi-Sense thermometer (model 92800-15, Cole Parmer Instrument Co., Vernon Hills, IL, USA).

Urine concentrating ability (UCA)

To assess renal function, we estimated the UCA after the acclimation period (after development and after re-acclimation). To do this, animals were placed in individual 25×25×30 cm metallic chambers and subjected to 24 h of water deprivation. After the restriction period, urine samples were taken each time the rodents

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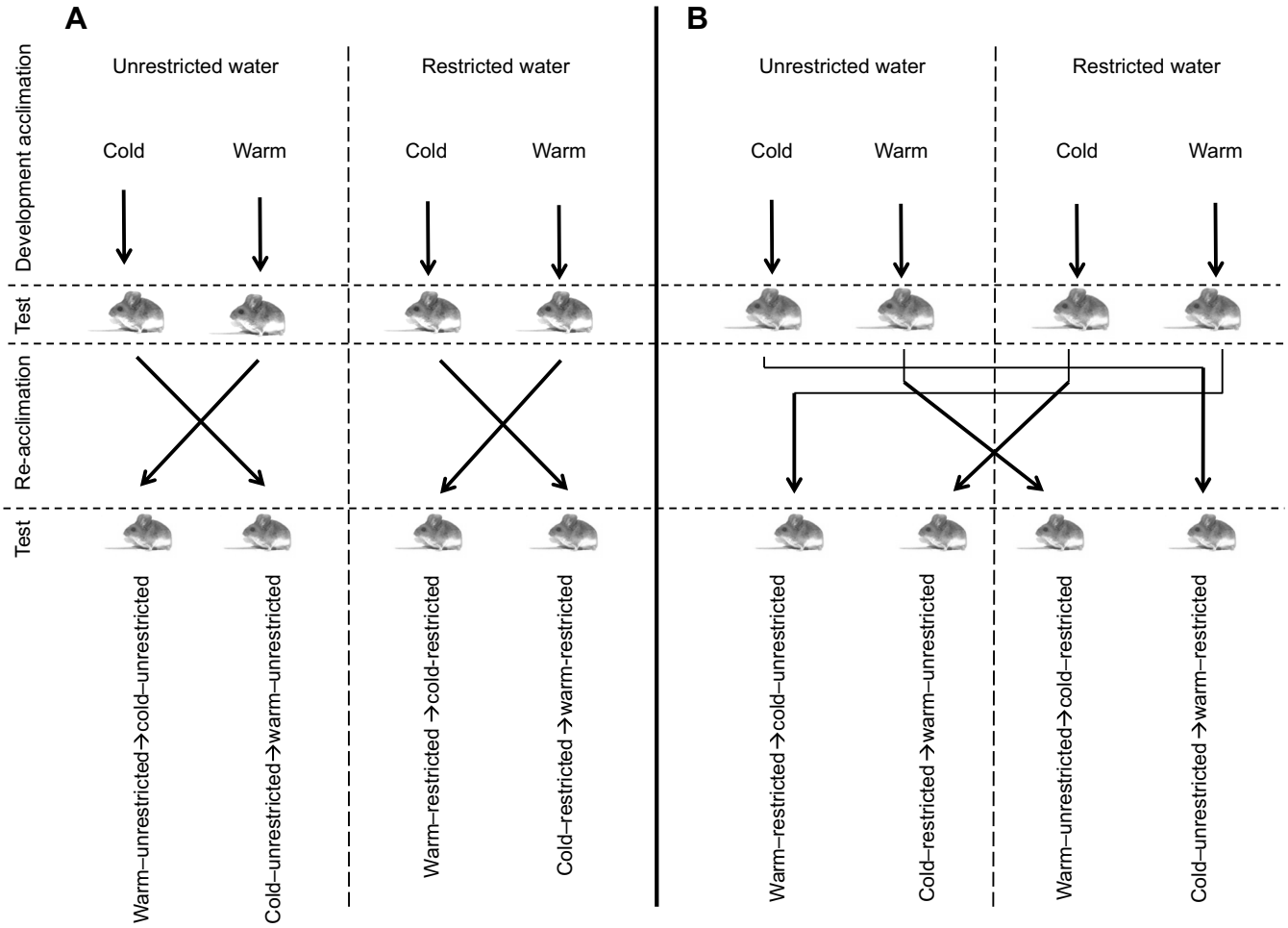

Fig. 1. Experimental design to determine the influence of temperature and water availability experienced during development on the short-term acclimation of *Phyllotis darwini*. (A) Mice underwent developmental acclimation at one temperature and were then re-acclimated to the opposite temperature under the same water availability conditions. (B) Mice were developmentally acclimated under one temperature and water availability regime then re-acclimated to the opposite temperature and water availability condition. During acclimation in A and B, water was unrestricted (available *ad libitum*) or 50% restricted; temperature was 15°C (cold) or 30°C (warm).

urinated. Mineral oil in the chambers allowed urine to be easily pipetted out of the cage along with the mineral oil. These samples were centrifuged at 9000 ***g*** for 5 min to separate the oil and urine. A urine sub-sample for each rodent was frozen at −40°C for posterior osmometric analysis using a vapor pressure osmometer (Wescor 5130B, Logan, UT, USA). Before each measurement, the osmometer was calibrated using Wescor NaCl standards at 100, 290 and 1000 mOsmol kg^{-1}.

Renal morphology

The kidneys from adult animals were examined. The length and breadth of sagittal half-sectioned kidneys were measured using a Vernier caliper (0.1 mm) (Cortes et al., 1990). Using a Wild M3 microscope, the total width and the medullary thickness of the kidneys were measured in sagittal slices taken from the cortex–medullary tips to the extreme end of the papilla (Heisinger and Breitenbach, 1969; Blake, 1977). Midsagittal cuts were made to maximize the medullary area visualized. Relative medullary thickness (RMT) was calculated following Sperber (1944):

$$\text{RMT} = 10(\text{medullary thickness})/(\text{length} \times \text{breadth} \times \text{width})^{1/3}. \quad (1)$$

Oxidative activity

We measured enzymatic activity to test the biochemical responses to temperature and water availability after acclimation. Frozen liver tissue isolated from individuals was weighed and homogenized in 10 volumes of 0.1 mol l^{-1} phosphate buffer with 0.002 mol l^{-1} (pH 7.3) EDTA using an Ultra Turrax (20,000 rpm) on ice to avoid enzymatic reactions. Using a VCX 130 Ultrasonic Processor, the sample was then sonicated on ice 14 times at 130 W for 20 s at 10 s intervals. Cellular debris was pelleted by centrifugation for 15 min at 15,000 rpm and 4°C. The supernatant was carefully transferred into a new tube, avoiding co-transferring the upper lipid layer present in the liver preparations. The concentration of proteins in the samples was determined following the method described by Bradford (1976), using bovine serum albumin as a standard. The activity of two mitochondrial enzymes was determined: (1) cytochrome *c* oxidase (COX; E.C. 1.9.3.1), an insoluble and membrane-bound enzyme, which is a terminal enzyme of the mitochondrial respiration chain and is indicative of mitochondrial energy capacity; and (2) citrate synthase (CS; E.C. 4.1.3.7), a soluble enzyme localized in the mitochondrial matrix that participates in the Krebs cycle. Increases in the activity of these enzymes would likely reflect modifications in both the properties and the number of mitochondria present (Guderley, 2007). COX

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activity was determined spectrophotometrically following Moyes et al. (1997) with slight modifications. In brief, enzyme activity was determined in 10 mmol l^{-1} Tris HCl pH 7, containing 120 mmol l^{-1} KCl, 250 mmol l^{-1} sucrose and with cytochrome *c* reduced with dithiothreitol, in a final volume of 0.2 ml. The absorption of COX at 550 nm changes with its oxidation state. The decrease in extinction at 550 nm was monitored in a Thermo Scientific Multiskan GO UV/VIS spectrophotometer monochromator at 25°C. Enzyme activity (U g^{-1} tissue) was calculated using an extinction coefficient of 21.84 l $mmol^{-1}$ cm^{-1} at 550 nm for cytochrome *c*. CS activity was measured according to Sidell et al. (1987) with minor modifications. The CS assay medium contained 10 mmol l^{-1} Tris HCl, pH 8.0, 10 mmol l^{-1} 5.5′dithio-bis-(2-nitrobenzoic acid) (DTNB), 30 mmol l^{-1} acetyl coenzyme A (acetyl CoA) and 10 mmol l^{-1} oxaloacetic acid (OAA; omitted for the control) in a final volume of 0.2 ml. CS catalyzes the reaction between acetyl CoA and OAA to form citric acid. The increase in extinction at 412 nm was measured in a Thermo Scientific Multiskan at 25°C. Enzyme activity (U g^{-1} tissue) was calculated using an extinction coefficient of 13.6 l $mmol^{-1}$ cm^{-1} at 412 nm. All enzyme activities are reported as specific activity per gram of protein (μmol min^{-1} g^{-1}).

Statistical analyses

Normality and homoscedasticity assumptions were fulfilled using log_{10} transformations. The non-independence of our data was accounted for in our models by controlling the response variable by individually fitting linear mixed models. Thus, to test the effect of treatment and sex on the response variable, we fitted mixed models including a variance structure (e.g. fixed variance structure) associated with each individual. Mixed models have a fixed part that explains the response variable as a function of the explanatory variables (as a linear regression). Additionally, they also have a random part, which contains components that allow for nested data (random effects) (Zuur et al., 2009). Specifically, to test for the effect of treatment and sex on energetic and osmoregulatory traits, we performed a mixed model with a random effect for individual (random intercept) to control by individual. To test the change in mass-specific BMR, mass-specific TEWL and M_b during adulthood, we performed repeated measures also using linear mixed models. Because the exponent of the allometric relationship between M_b and BMR and TEWL was not significantly different from 1 (all cases, $P>0.1$), it is appropriate to compare the mass-specific rates among weeks. To assess the effect of re-acclimation on each response variable, we contrasted the treatments during adulthood with the development treatment. We included treatment and sex as factors and M_b as a covariate (when the association between response variable and M_b was significant). Model selection was done using the Akaike information criterion for finite samples (AICc); both delta AIC (Δi) values, a measure of each model relative to the best model, and model weights (w_i), a measure of the evidence supporting a specific model, were used for selections and model comparison. When there were multiple models with $\Delta i<2$, selection was based on the difference in parameters present in each model, reduction in deviance, and log-likelihood values. The statistical analyses were carried out using R (http://www.R-project.org/).

RESULTS

M_b

Following the initial acclimation period post-weaning, the M_b of cold-reared rodents was significantly higher than that of warm-reared rodents (see Table 1). Additionally, the M_b of cold-reared rodents decreased 3 weeks after the rodents were re-acclimated to warm

Table 1. Coefficients of the linear model fitted to the energetic and osmoregulatory trait data of young *Phyllotis darwini* acclimated during development to different temperature and water availability treatments

Effect	Coefficient	s.e.	d.f.	*T*	*P*
Residuals BMR–M_b (ml h^{-1})					
Intercept (*N*=10)	−0.04	0.01	140	−4.41	**<0.001**
Warm–restricted water (*N*=10)	0.02	0.01	140	1.48	0.139
Cold–unrestricted water (*N*=10)	0.08	0.01	140	5.93	**<0.001**
Cold–restricted water (*N*=10)	0.11	0.01	140	6.39	**<0.001**
Residuals TEWL–M_b (mg h^{-1})					
Intercept (*N*=10)	−0.03	0.01	141	2.50	**0.01**
Warm–restricted water (*N*=10)	0.00	0.02	141	−0.07	0.943
Cold–unrestricted water (*N*=10)	0.07	0.01	141	3.78	**<0.001**
Cold–restricted water (*N*=10)	0.09	0.02	141	3.93	**<0.001**
M_b (g)					
Intercept	40.4	1.3	117	29.4	**<0.001**
Warm–restricted water	−1.75	2	135	−0.86	0.387
Cold–unrestricted water	6.20	1.7	130	3.57	**<0.001**
Cold–restricted water	0.25	2.1	137	0.12	0.904
Male	9.07	1.7	132	5.13	**<0.001**
Warm–restricted water × male	−1.30	2.6	130	−0.48	0.626
Cold–unrestricted water × male	−2.84	2.5	132	−1.13	0.259
Cold–restricted water × male	−4.10	3.1	131	−1.32	0.187
CS (μmol min^{-1} g^{-1})					
Intercept (*N*=9)	0.88	0.04	27	18.8	**<0.001**
Warm–restricted water (*N*=9)	0.17	0.06	27	2.85	**<0.001**
Cold–unrestricted water (*N*=7)	0.00	0.06	27	0.01	0.99
Cold–restricted water (*N*=7)	0.36	0.06	27	5.68	**<0.001**
Male	0.14	0.04	27	3.14	**<0.001**
COX (μmol min^{-1} g^{-1})					
Intercept (*N*=9)	0.53	0.05	27	9.03	**<0.001**
Warm–restricted water (*N*=8)	0.53	0.08	27	6.36	**<0.001**
Cold–unrestricted water (*N*=8)	−0.24	0.08	27	−2.92	**<0.001**
Cold–restricted water (*N*=7)	0.32	0.08	27	3.75	**<0.001**
RMT (g^{-1})					
Intercept (*N*=10)	1.97	0.13	28	14.94	**<0.001**
Warm–restricted water (*N*=10)	−0.21	0.05	29	−3.91	**<0.001**
Cold–unrestricted water (*N*=8)	−0.23	0.04	23	−4.90	**<0.001**
Cold–restricted water (*N*=6)	−0.30	0.05	28	−5.12	**<0.001**
M_b (g)	−0.01	0	28	−7.30	**<0.001**
UCA (mOsmol kg^{-1})					
Intercept (*N*=12)	3623	209	31	17.33	**<0.001**
Warm–restricted water (*N*=11)	43	283	31	0.15	0.88
Cold–unrestricted water (*N*=11)	−890	296	31	−3.01	**0.005**
Cold–restricted water (*N*=11)	−857	341	31	−2.51	**0.017**
Male (*N*=20)	−130	274	31	−0.48	0.637
Warm–restricted water × male	−544	407	31	−1.34	0.191
Cold–unrestricted water × male	92	403	31	0.23	0.82
Cold–restricted water × male	190	450	31	0.42	0.675
Small intestine					
Intercept (*N*=10)	1.14	0.04	31	25.51	**<0.001**
Warm–restricted water (*N*=10)	−0.3	0.06	31	−4.72	**<0.001**
Cold–unrestricted water (*N*=8)	0.16	0.07	31	2.35	**0.025**
Cold–restricted water (*N*=8)	0.20	0.07	31	2.90	**0.007**
Kidney					
Intercept	0.15	0.05	31	2.81	**0.009**
M_b (g)	0.01	0.00	30	5.50	**<0.001**
Heart					
Intercept	72.1	35.77	33	2.02	0.052
M_b (g)	2.91	0.79	33	3.67	**0.001**
Lung					
Intercept	110	20.06	26	5.50	**<0.001**
M_b (g)	2.58	0.44	24	5.82	**<0.001**

BMR, basal metabolic rate; TEWL, total evaporative water loss; M_b, body mass; CS, citrate synthase activity; COX, cytochrome *c* oxidase activity; RMT, relative medullary thickness; UCA, urine concentrating ability. For each analysis, only the best model is shown (for details, see Table 2). Significant *P*-values are in bold. *N*, sample size.

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conditions, while their M_b decreased 1 week after being re-acclimated to the warm and water-restricted treatment. In addition, M_b increased when animals were re-acclimated to unrestricted water (Table S1).

BMR and TEWL

As expected, BMR (ml O_2 h^{-1}) and TEWL (ml h^{-1}) were allometrically correlated with M_b (g). The equations for the fixed effects were:

$$\mathrm{BMR} = 7.25 M_b^{0.59\pm0.1}, \quad (2)$$

T=5.63, d.f.=138, P<0.0001:

$$\mathrm{TEWL} = 10.36 M_b^{0.54\pm0.1}, \quad (3)$$

T=4.28, d.f.=128, P<0.0001.

There was a significant and positive association between the residuals of TEWL and BMR against M_b (residuals BMR=1.01 residuals $\mathrm{TEWL}^{0.3\pm0.06}$, T=5.23, d.f.=141, P<0.0001). Furthermore, developmental acclimation had a significant effect on the residuals of the relationship between BMR and TEWL with M_b. After development, cold-reared rodents had higher BMR and TEWL values than warm-reared animals (Table 1). BMR and TEWL were also affected by re-acclimation treatment. BMR of cold-reared rodents decreased during the first and second week of re-acclimation to the warm treatment (restricted and unrestricted water, respectively; Fig. 2A,C; Table S2). Meanwhile, TEWL decreased significantly in rodents re-acclimated to the warm treatment (cold unrestricted→warm unrestricted and cold unrestricted→warm restricted) while TEWL

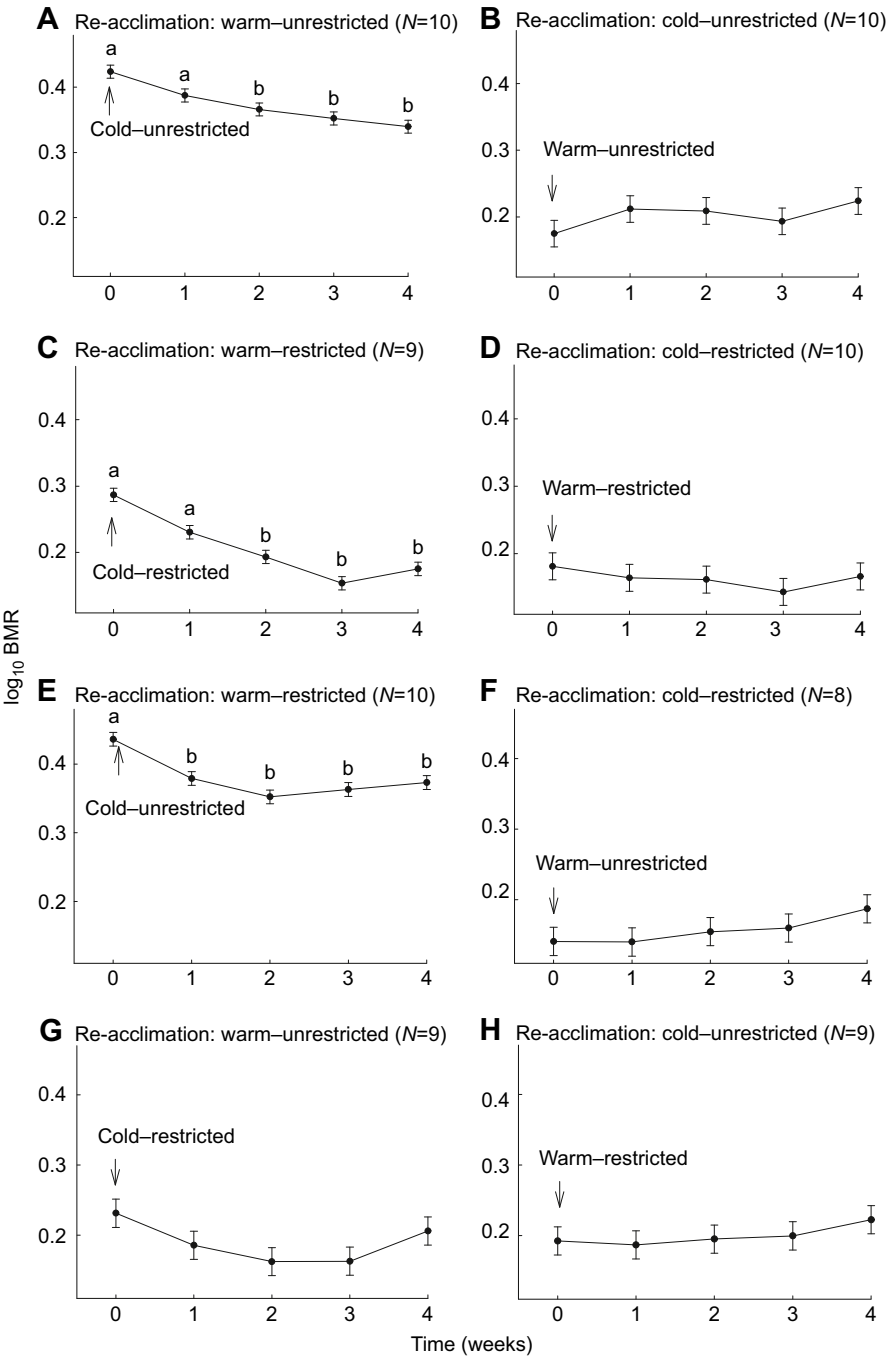

Fig. 2. Basal metabolic rate (BMR) of *P. darwini* subjected to different acclimation treatments over the course of 4 weeks. (A–D) Re-acclimation to the opposite temperature treatment. Mice were developmentally acclimated (week 0) to cold (A,C) or warm (B,D) temperature with unrestricted (A,B) or restricted (C,D) water then re-acclimated (weeks 1–4) to the opposite temperature under the same water availability conditions. (E–H) Re-acclimation to the opposite temperature and water availability treatment. Mice were developmentally (week 0) acclimated to cold (E,G) or warm (F,H) temperature with unrestricted (E,F) or restricted (G,H) water then re-acclimated (weeks 1–4) to the opposite temperature and water availability conditions. BMR was measured as ml O_2 h^{-1} g^{-1}. Different letters indicate a significant difference between values. Numbers in parentheses are sample size.

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increased in cold re-acclimated rodents (warm→cold unrestricted and warm→cold restricted; Table S3).

Oxidative activity of CS and COX

Post-development, the enzymatic activity of CS and COX was not associated with M_b (CS, T=0.58, d.f.=30, P=0.56; COX, T=−1.71, d.f.=29, P=0.1). The activity of CS and COX was significantly affected by development treatment (Table 1). CS activity was significantly higher in the water restriction treatments. COX activity increased in both the water restriction and warm treatments (Table 1). In addition, the activity of both enzymes was significantly affected by re-acclimation treatment: COX activity increased in both cold-reared rodents re-acclimated to warm conditions and warm-reared rodents re-acclimated to cold conditions and water restriction. Furthermore, COX activity decreased in adults re-acclimated to cold. Similarly, CS activity increased significantly in rodents re-acclimated to cold and subjected to water restriction. (Table 2; Table S5).

Organ mass

After developmental acclimation, the mass of rodent heart, liver, kidney, stomach, pancreas, lung and small and large intestine was positively associated with M_b (lung, T=5.8, d.f.=24, P≤0.001; small intestine, T=2.8, d.f.=33, P=0.008; large intestine, T=3.22, d.f.=33, P=0.004; kidney, T=5.5, d.f.=30, P<0.001; stomach, T=3.4, d.f.=33, P=0.001; pancreas, T=4.08, d.f.=25, P<0.001; heart, T=3.67, d.f.=33, P<0.001; and liver, T=0.4.2, d.f.=32, P<0.001). The multiple regression analyses between BMR and organ mass residuals showed that 32% of the variation in BMR can be explained by small intestine mass (pseudo r^2=0.32). Also, the small intestines of warm-reared and water-restricted rodents were of significantly lower mass than those of cold-reared rodents (Tables 1 and 3).

After re-acclimation to warm conditions and restricted water, there were significant decreases in the mass of the small intestines of adults that were initially subjected to the cold and unrestricted water treatments. Furthermore, the mass of the kidneys and the small intestines was significantly greater in rodents re-acclimated to cold (Tables S4 and S5).

Table 2. Coefficients of the linear model fitted to the enzymatic activity data of *P. darwini* re-acclimated during adulthood

Effect	Coefficient	s.e.	d.f.	T	P
COX (μmol min^{-1} g^{-1})					
Warm–unrestricted water					
Intercept (*N*=8)	0.54	0.04	11	12.40	**<0.001**
Cold–unrestricted water (*N*=5)	−0.22	0.07	18	−3.41	**0.003**
Cold–restricted water (*N*=8)	0.50	0.06	12	7.99	**<0.001**
Cold–unrestricted water					
Intercept (*N*=8)	2.01	0.78	21	2.58	**0.018**
Warm–unrestricted water (*N*=9)	4.01	1.06	24	3.79	**<0.001**
Warm–restricted water (*N*=11)	5.28	1.01	25	5.22	**<0.001**
CS (μmol min^{-1} g^{-1})					
Warm–unrestricted water					
Intercept (*N*=9)	0.95	0.06	12	16.35	**<0.001**
Cold–unrestricted water (*N*=7)	0.05	0.08	15	0.56	0.583
Cold–restricted water (*N*=8)	0.45	0.08	17	5.61	**<0.001**
Cold–restricted water					
Intercept (*N*=7)	1.32	0.07	17	17.62	**<0.001**
Warm–unrestricted water (*N*=7)	−0.51	0.11	18	−4.82	**<0.001**
Warm–restricted water (*N*=7)	−0.19	0.10	17	−1.84	0.083

COX, cytochrome *c* oxidase; CS, citrate synthase. For each analysis, only the best model is shown. *N*, sample size.

Table 3. Model selection for data from young *P. darwini* acclimated during development to different temperature and water availability treatments

Variable	Model	d.f.	log-likelihood	AICc	Δi	w_i
BMR (ml O_2 h^{-1} g^{-1})	1	7	168	321	0.00	0.97
TEWL (mg h^{-1} g^{-1})	1	7	129	243	0.00	0.71
M_b (g)	1, 2, 1:2	11	454	931	0.00	0.96
CS (μmol min^{-1} g^{-1})	1, 2	8	12.1	1.9	0.00	0.51
CS (μmol min^{-1} g^{-1})	1	7	9.9	1.1	0.79	0.34
COX (μmol min^{-1} g^{-1})	1	7	5.7	7.6	0.00	0.58
RMT (g^{-1})	1, 3	8	15.4	9	0.00	0.65
UCA (mOsmol kg^{-1})	1, 2, 1:2	11	241	513	0.00	1
Lung (g)	3	5	73.3	−135	0.00	0.92
Small intestine (g)	1	7	12.4	−6.6	0.00	0.59
Small intestine (g)	1, 3	8	13.5	−5.5	1.07	0.34
Large intestine (g)	Null	4	32.9	−56.5	0.00	0.91
Kidney (g)	3	5	40.6	−69.2	0.00	0.85
Stomach (g)	Null	4	19.7	−30	0.00	0.74
Pancreas (g)	Null	4	86.9	−165	0.00	0.85
Heart (g)	Null	4	56.5	−104	0.00	0.76
Liver (g)	3	5	−16.2	44.5	0.00	0.50
Liver (g)	2	5	−16.8	45.7	1.27	0.27

Variables included in each model: d.f., degrees of freedom; AICc, Akaike's information criterion for finite samples; Δi, delta AIC; w_i, model weights. Variables tested within each model: BMR, basal metabolic rate; TEWL, total evaporative water loss; M_b, body mass; CS, citrate synthase activity; COX, cytochrome *c* oxidase activity; RMT, relative medullary thickness; UCA, urine concentrating ability; organ mass (liver, lung, kidney, heart, pancreas, stomach, small intestine and large intestine). Shaded rows represent the models with substantial support (Δi). The predictor variables for each model are: treatment (1), sex (2) and M_b (3).

Renal structure and function (RMT and UCA)

After development, there was a significant negative relationship between RMT and M_b (RMT, T=−4.81, d.f.=31, P<0.01). RMT was significantly lower in rodents reared under cold and unrestricted water conditions (Table 1). UCA was not associated with M_b (T=0.28, d.f.=38, P=0.77). Cold-reared rodents (restricted and unrestricted water) had significantly lower UCA values (Table 1). There were significant decreases in RMT and UCA in rodents re-acclimated to cold (Fig. 3). There were no significant changes in RMT and UCA for rodents re-acclimated to warm treatment (Fig. 3; Tables S4 and S5).

DISCUSSION

Here, we assessed how two environmental variables experienced during post-weaning development affected short-term acclimation in adult *P. darwini* individuals. We observed that both the magnitude and the direction of phenotypic plasticity were dependent on the trait analyzed, temporal scale of environmental variability and developmental thermal experience (Sultan and Stearns, 2005; McKechnie et al., 2006; Barceló et al., 2009). Several studies have posited that the mechanisms determining acclimation differ among organization levels, and these mechanisms can include structural (Diaz et al., 2006; McDevitt and Speakman, 1994), physiological, biochemical (Golozoubova et al., 2001; Nedergaard et al., 2001) and genetic modifications, among others (Osovitz and Hofmann, 2005; Podrabsky and Somero, 2004). Accordingly, in the study presented here of *P. darwini*, biochemical (enzymatic activity), morphological (organ mass, M_b and RMT) and functional (through UCA, BMR and TEWL) modifications were detected.

Our results reveal that thermal acclimation and water availability significantly affect individuals' M_b. Specifically, rodents reared in warm conditions and/or with restricted water exhibited lower M_b than

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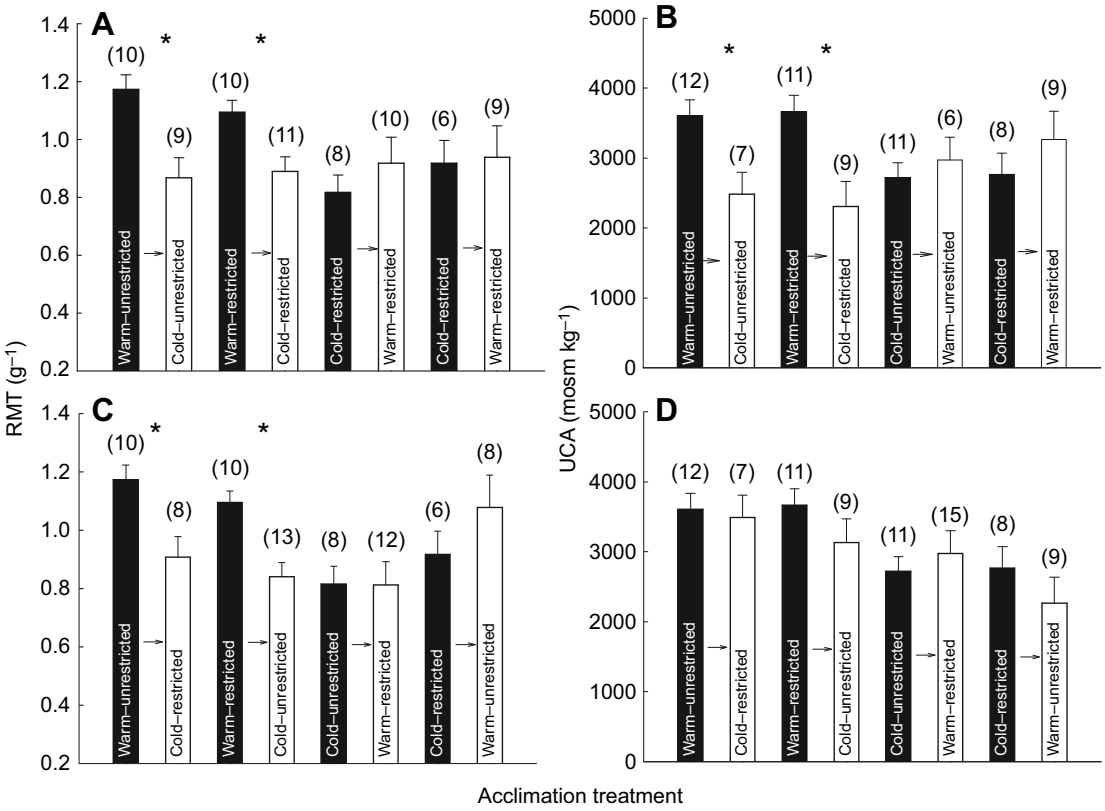

Fig. 3. Relative medullar thickness (RMT) and the urine osmolality of *P. darwini* following 24 h of water deprivation. (A,B) Mice were acclimated during development to cold or warm temperature with restricted or unrestricted water and then re-acclimated to the opposite temperature treatment under the same water availability conditions. RMT (A) and urine concentrating ability (UCA; B) are shown. (C,D) Mice were acclimated during development to cold or warm temperature with restricted or unrestricted water and then re-acclimated to the opposite treatment during adulthood. RMT (C) and UCA (D) are shown. Asterisks denote significant differences between treatments. Numbers in parentheses are sample size.

rodents reared in cold conditions. Several comparative studies have demonstrated that M_b is a highly plastic trait that can be modified as a result of adjustments in energy acquisition and energetic demands (Goldstein and Ellis, 1991; Nespolo et al., 2003; Swanson, 2001; Tirado et al., 2008; Wang et al., 2001). For instance, small animals have a relatively large body surface area that serves as an efficient heat dissipater in warm environments (Hinsley et al., 1993). In this sense, Yom-Tov (2001) reported that, as a result of increases in environmental temperature, the M_b of *Pycnonotus xanthopygos* has decreased significantly. Also, several studies have shown that water availability affects body mass (Oswald, 1998; Tracy and Walsberg, 2000, 2001a). Al-Kahtani (2003) reported a decrease of up to 50% of M_b in rodents with reduced water availability. Here, we have shown that high temperatures and low water availability during development hinder *P. darwini* M_b gains during adulthood (see Tracy and Walsberg, 2001b).

BMR represents the minimal maintenance costs of endothermic organisms and is strongly affected by M_b (Schmidt-Nielsen, 1997; Nespolo et al., 2003; White and Seymour, 2005). Our results show that the BMR and TEWL of rodents is low when individuals are reared in the warm and with restricted water (Tables S2 and S3). In this context, reductions in BMR and TEWL in desert mammals have been shown to be related to the need to maintain heat and water balance (Van Sant et al., 2012; Baldo et al., 2015). Similarly, Ostrowski et al. (2006) found a reduction in the mass-specific resting metabolic rate (16%) and the TEWL (25.7%) of desert antelope (*Oryx leucoryx*) acclimated to water and food shortage. Although we assessed the short-term response to variation in temperature and water availability, our results are consistent with those suggested by Dawson (1984), who proposed that high temperatures and water scarcity can favor phenotypes with lower metabolic rates in order to diminish evaporative water loss and endogenous heat production and thereby reduce the water requirements of evaporative cooling.

Differences in BMR among acclimation treatments can be attributed to the size of internal organs (Konarzewski and Książek, 2013). Indeed, we found that decreases in the specific energy expenditure of warm-reared rodents were coupled with a reduction in the size of metabolically active organs such as intestines. Others have noted that the size and activity of energetically costly organs are probably associated with the demands and nutrient processing of the entire animal (Gross et al., 1985; Hammond and Janes, 1998). The multiple regression analyses we conducted revealed that 32% of the variance in BMR could be explained by variations in intestine mass. Indeed, the mass-specific metabolic rates of cold-reared rodents re-acclimated to warm temperatures decreased, accompanied by decreases in small intestine mass (see Table 1; Table S5). Furthermore, we found that the BMR of warm-reared rodents re-acclimated to cold increased, as did the size of the kidneys and small intestines, all organs having relatively high tissue-specific metabolic rates (Heroux and Gridgeman, 1958; Krebs, 1950).

Contrary to previous studies that have evaluated the adaptive role of liver enzymes in thermogenesis during cold acclimation (Wrutniak-Cabello et al., 2001; Zaninovich et al., 2003), our results show that the temperature experienced during development only affects COX activity. Interestingly though, the effect on COX

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activity was opposite to what was expected; COX activity per gram of protein was 55% lower in individuals in the cold than in the warm development treatment. In addition, COX and CS activities were greater in individuals in the water-restricted treatments compared with those in individuals with unrestricted water; CS of individuals with restricted water was 30% and 40% greater in the warm and cold treatments, respectively, and COX activity of the same individuals was 2 and 3 times higher in the warm and cold treatments, respectively (Table 1). These results suggest that greater enzyme efficiency is related to water balance. Indeed, animals subjected to water stress would have to rely primarily on metabolic water to maintain proper water balance. In this regard, the reduction of one oxygen molecule produces two water molecules as a product of COX activity (Lehninger et al., 2005). This was directly seen in our results; COX activity increased 3-fold when the animals were re-acclimated to warm treatment and 3.6-fold in animals re-acclimated to warmth and water restriction (see Table 2 for cold-reared, water-restricted rodents). Our results also show that, as a developmental consequence, adult phenotypic flexibility may not be fully compensated or reversible (see Russell et al., 2008). Adults re-acclimated to cold and subjected to water restriction maintained high levels of COX activity (Table 2), while those re-acclimated to cold and with unrestricted water availability had significantly reduced enzymatic activity.

As expected, *P. darwini* renal functioning was affected by water availability and temperature. Rodents reared in warm conditions had higher RMT and UCA values, indicating that renal structure and function are coupled (Al-Kahtani et al., 2004). When *P. darwini* individuals experienced an increase in water availability or a decrease in ambient temperature, they exhibited short-term acclimation in terms of their osmoregulatory traits. For instance, rodents re-acclimated to cold had decreased RMT and UCA, but the osmoregulatory capabilities of adult individuals did not increase after re-acclimation to warm conditions (Fig. 3; Table S4). In addition, rodents reared in warm conditions and re-acclimated to cold (restricted and unrestricted water) showed an increase in kidney mass and a decreased in RMT, likely as a result of improved water intake (Selman et al., 2001).

Supporting the idea that phenotypic plasticity is a property of a trait, not of an individual, we found differences in the acclimation response of *P. darwini* at biochemical to organismal levels. In fact, some of the *P. darwini* traits were plastic only during development (irreversible plasticity) while others were phenotypically flexible. It has been described that plastic phenotypes could be restricted by costs (and limits), including the maintenance costs of sustaining the sensory and response pathways that induce plastic responses (Relyea, 2002; Chevin et al., 2013). Our results show that rodents re-acclimated to warm treatment had decreased BMRs, but counter to expectations, they did not increase their renal capabilities. Because maintaining osmoregulatory machinery is energetically expensive (Peña-Villalobos et al., 2013), a possible explanation for our results is the existence of a functional trade-off between the need to reduce energy expenditure and the need to concentrate urine in warm environments.

Our results support the hypothesis that in small endotherms, short-term acclimation is dependent on early life experience. These results highlight the importance of incorporating ontogeny in physiological studies to avoid underestimating the potential plastic response of organisms. In this vein, several authors have proposed that the environment not only selects for but also generates phenotypic variation (Schlichting and Pigliucci, 1998; West-Eberhard, 2003; Gilbert and Epel, 2009). Accordingly, we confirm that the impact of the environment on development may contribute to the origin of so-called 'phenotypic novelties' (see Sultan, 2015; Yom-Tov, 1993), such as an increase in thermal and osmoregulatory capabilities to adjust to novel conditions (Sultan, 2015).

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: G.C., F.B., P.S.; Methodology: G.C., M.N.-V., P.S.; Formal analysis: G.C.; Writing - original draft: G.C.; Writing - review & editing: G.C., M.N.-V., F.B., P.S.; Supervision: P.S.; Funding acquisition: G.C., F.B.

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Data availability
Data are available at the Dryad Digital Repository (Cavieres et al., 2017): http://dx.doi.org/10.5061/dryad.cn8mk

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