

Randall L. Tracy · Glenn E. Walsberg

Developmental and acclimatory contributions to water loss in a desert rodent: investigating the time course of adaptive change

Accepted: 19 June 2001 / Published online: 8 August 2001
© Springer-Verlag 2001

Abstract Understanding the evolution of physiological traits requires considering three nonexclusive mechanisms that underlie phenotypes and cause their change over different time scales: acclimation, developmental plasticity, and natural selection for genetically fixed traits. Physiological adjustments to changes in the desiccating potential of the environment were investigated with one subspecies of common desert rodent, *Dipodomys merriami merriami* (Merriam's kangaroo rat). We raised young whose parents originated from environments that differ in both temperature and humidity. These young were raised under either desiccating or water-abundant conditions, and their water loss was measured at a series of temperatures to determine the effect developmental conditions have on resistance to desiccation. We then determined the contribution of acclimation to desiccation resistance by keeping the differentially raised young in conditions opposite to those during their development and again measuring water loss. We found that developmental plasticity and acclimation can completely account for the existing intraspecific variability in desiccation resistance under certain conditions. In fact, developmental and acclimatory changes can equal genetically based differences of the populations. This phenotypic plasticity can operate relatively quickly and therefore may attenuate the actions of natural selection. Understanding the extent and nature of such flexibility is critical to our understanding

intraspecific variability and the consequences of changing climate.

Keywords Acclimation · Developmental plasticity · Evaporation · Kangaroo rats · Water loss

Abbreviations *DRY*→*WET* kangaroo rats raised under dry conditions and acclimated as adults under wet conditions · *EWL* evaporative water loss · *MWP* metabolic water production · *RER* respiratory exchange ratio · *WET*→*DRY* kangaroo rats raised under wet conditions and acclimated as adults under dry conditions

Introduction

Exploring variation in physiology and how it affects the distribution of animals, and the patterns and processes by which it evolves, are fundamental components of physiological ecology and environmental physiology (Feder and Block 1991). Investigating the extent to which variation in physiology exists within and among species is not a new trend (see Kleiber 1975). Comparisons showing that a particular physiological capacity is greater among individuals exposed to more extreme conditions than others are commonplace (Withers 1992). Often though, these differences are thought to be strictly genetic in origin, and geographic variation is held as evidence that natural selection has acted directly on individuals with certain innate physiological capacities (e.g., Wang et al. 1973; Pyörnilä et al. 1992). More cautious interpretations simply state that these differences in physiology are consistent with the physical conditions of the habitat (MacMillen and Hinds 1998). However, phenotypes may be shaped by two modes of flexibility. One is acclimatory responses that allow adults to change in response to changes in their environments. The second mode of response is ontogenetic lability. Developmental plasticity can define individual physiological capacities early during growth and development, which may have consequences for later survival.

Communicated by G. Heldmaier

R.L. Tracy (✉) · G.E. Walsberg
Department of Biology, Arizona State University,
Tempe, AZ 85287-1501, USA
E-mail: randytracy@email.com
Tel.: +1-860-4861882
Fax: +1-860-4866364

Present address: R.L. Tracy
The University of Connecticut,
Department of Ecology and Evolutionary Biology,
75 North Eagleville Road, Storrs, CT 06269-3043, USA

Contradictions exist in the literature with respect to the origins of intraspecific variation in physiology. Some suggest much of this variation results from short-term physiological acclimation (see Burggren and Bemis 1990), while other studies indicate that physiological capacities often show substantial direct heritabilities (Garland and Carter 1994). Developmental plasticity, however, has been virtually ignored as a source of variability. Nevertheless, some knowledge of the relative contributions of these mechanisms is fundamental to understanding species distributions and the evolutionary origins of physiological traits.

Small rodents are the dominant mammals in many deserts and, because physiological adaptations are prominent at environmental extremes, these animals have been excellent models for studying physiological traits. In particular, kangaroo rats are the best-studied models for desert mammals. These rodents are nocturnal, reside in burrows, do not have access to free-standing water, and are active throughout the year (see French 1993).

An important physiological trait affecting a kangaroo rat's survival in desert environments is resistance to desiccation. To resist desiccation, water output should be minimized and water input maximized in those organisms (such as kangaroo rats) that cannot resist desiccation by tolerating steep declines in body water (see Schmidt-Nielsen et al. 1948). The capabilities of Merriam's kangaroo rat (*Dipodomys merriami*) to conserve water are well documented. It produces highly concentrated urine (Schmidt-Nielsen et al. 1948; Carpenter 1966; Kenagy 1973a) and extremely dry feces (Schmidt-Nielsen and Schmidt-Nielsen 1951). A common theme in the biology of desert-dwelling rodents is reduction of evaporative water loss (MacMillen 1972), which is reduced in this species compared to mesic forms (Schmidt-Nielsen and Schmidt-Nielsen 1952; Hinds and MacMillen 1985).

D. merriami, because of its extensive range (south-central Mexico to northern Nevada), is exposed to widely varying environments (Schmidly et al. 1993) and, therefore, is also an ideal species with which to analyze geographic variability in physiological parameters. For example, one subspecies, *D. m. merriami*, ranges from areas of extreme aridity and temperature in the Sonoran Desert to milder areas in central and northwestern Arizona (Hoffmeister 1986; Turner and Brown 1994; Tracy and Walsberg 2000, 2001). Because evaporation is the major route of water loss from this species (accounting for 76% of its total water loss; Schmidt-Nielsen and Schmidt-Nielsen 1952), we previously investigated this trait and found that animals of this subspecies from an extremely xeric location possess significantly lower evaporative water loss than those from a more mesic location (Tracy and Walsberg 2000, 2001).

We chose therefore to address the time course of adaptive changes in desiccation tolerance in this subspecies by determining the relative contributions of short-term acclimation, developmental plasticity, and

genetic differences to intraspecific variation. We attempt to provide a clearer understanding of intraspecific variability in dealing with desiccating conditions by examining the mechanisms behind such phenotypic variation. This knowledge is necessary not only for understanding the evolutionary origin of adaptations, but also for evaluating the magnitude of variation, either individually through plasticity or intraspecifically through genetic variation, available to a species that is subjected to climate change.

Materials and methods

Field sites

Two field sites that exhibit the broad range of conditions faced by *D. merriami* throughout its range were used. The xeric site is located in the heart of the Sonoran Desert in Yuma County, southwestern Arizona, at 150 m elevation. It is typified by Aeolian sand dunes, with sparse mesquite and creosote bushes, and is one of the most arid locations inhabited by *D. merriami* (Hoffmeister 1986; Tracy and Walsberg 2001). Mean annual maximum and minimum temperatures are 31.9°C and 14.7°C, respectively (Green and Sellers 1964). Mean annual precipitation averages only 10.6 cm (Green and Sellers 1964). The mesic site is located in north-central Arizona, within Gila County, at 1200 m elevation, and contains creosote bushes, and is bordered by pinyon-juniper woodland. Maximum and minimum temperatures annually average 23.5°C and 6.2°C, respectively, and yearly precipitation averages 43.6 cm (Green and Sellers 1964; Sellers et al. 1985).

Animal care and handling/acclimation regime

Kangaroo rats do not readily breed in captivity (Eisenburg 1993); there is only one study reporting successful captive breeding (Daly et al. 1984). However, this species breeds opportunistically after rainfall (Beatley 1969; Kenagy 1973a), especially in the spring, when succulent vegetation is available (Kenagy 1973b; Reichman and Van De Graaf 1975; Soholt 1977). Therefore, pregnant females belonging to one subspecies of Merriam's kangaroo rat (*D. m. merriami*) were trapped using Sherman live-traps at each site from March until June 1999. Females were then transported to Arizona State University, weighed, and maintained on a 12 h:12 h light-dark photoperiod (lights on at 0700 hours and lights off at 1900 hours) in an environmental chamber at 30°C and a vapor density of approximately 6 g m⁻³. Each animal was individually caged with a dirt floor and provided with a section of plastic piping for shelter. Kangaroo rats were given dry seed diet (Hartz Cockatiel Seed, Hartz Mountain Corporation, N.J., USA) that was comprised of millet seed, oat groats, red millet seed, sunflower seed, and canary seed ad libitum. Initially, many females neglected or killed their young. Therefore, all females were provided with cotton bedding material and a 5 cm×5 cm square of lettuce for the first 10 days postpartum to reduce pup rejection. Even with these conditions, 4 of 26 litters were rejected. After day 10, mothers were provided with dry seed only. Day 22 initiates post-weaning in this species (Butterworth 1961). At day 25 postpartum, young were separated from their mothers, weighed, and forced to develop individually in one of two regimes. A total of 22 litters were tested: 13 from the xeric site and 9 from the mesic site. Of the viable litters that were used in this study, all had two young per litter except 5 litters from mesic-site mothers, which had 3 young per litter. A total of 24 xeric-lineage and 22 mesic-lineage animals were used in this study.

Young born to xeric- or mesic-site animals are referred to as xeric- or mesic-lineage animals, respectively. Those young raised under relaxed hydric conditions were maintained at 30°C and 12 g m⁻³. They were also fed dry seed ad libitum and allowed

access to 10 ml of distilled water daily, which they readily consumed with the seed. They are referred to as “wet-raised” animals. The conditions for the wet-raised animals were chosen because they are known to elicit increases in mass in adults (Tracy and Walsberg 2000). Those young raised under restrictive hydric conditions were given only dry seeds ad libitum, without supplemental water, and were continuously subjected to air (flow = 2.01 min^{-1}) that was dried to 3 g m^{-3} by an industrial chiller-drier (Model WRCM10-1 Series Refrigerated Dryer, Wilkerson, Colo., USA) as measured with a digital thermohygrometer (Model 35519-050 RH, Omega Engineering, Conn., USA). They are referred to as “dry-raised” animals. 30°C was chosen as the housing temperature because it lies within the zone of thermal neutrality for this species (Tracy and Walsberg 2000).

Animals were tested when they reached sexual maturity (60 days; Butterworth 1961). After initial testing, these adults were placed into the opposite environment, acclimated for 45 days under these conditions, and retested (Fig. 1). Animals raised under “wet” conditions and then acclimated in “dry” conditions are referred to as “WET→DRY” animals. Those animals raised under “dry” conditions and then acclimated under “wet” conditions are referred to as “DRY→WET” animals. $n=11$ and $n=12$ for DRY→WET xeric- and mesic-lineage animals, respectively, and $n=13$ and $n=10$ for WET→DRY xeric- and mesic-lineage animals, respectively.

Measurements of gas exchange and evaporative water loss

Measurements of O_2 consumption and CO_2 production were coupled to simultaneous measurements of evaporative water loss (EWL) to determine metabolic rates and, therefore, inferred met-

abolic water production (MWP). Measurements were made in four open-flow metabolic chambers (each 1.13 l). Each chamber was fitted with a wire mesh floor, allowing excreta to fall into a layer of mineral oil. All animals became calm within the chamber within 2 min of beginning experimentation, and no differences in activity between the subpopulations were observed.

All measurements were made between 0800 hours and 1800 hours, during the inactive phase of each animal's daily cycle, within 1 h of access to food being denied to that animal. Instrument signals were recorded by a solid-state data logger (CR 23x, Campbell Scientific, Utah, USA) and averaged at 1-min intervals. Animals remained quiescent within the chambers, as viewed with a video camera mounted inside the temperature-controlled cabinet. A fluorescent light illuminated the cabinet. Values reported are from those periods when each animal was completely inactive for 5 min prior to data collection.

Temperatures within the chamber were measured with 26-gauge, type-T thermocouples and controlled at $10 \pm 1^\circ\text{C}$, $15 \pm 1^\circ\text{C}$, $20 \pm 1^\circ\text{C}$, $25 \pm 1^\circ\text{C}$, $30 \pm 1^\circ\text{C}$, $35 \pm 1^\circ\text{C}$, and $40 \pm 1^\circ\text{C}$ by placing the chambers within a temperature-controlled cabinet. Air was passed separately through the chamber at $200\text{--}300 \text{ ml min}^{-1}$ after being dried and scrubbed of CO_2 by an absorber system (CDA112 air dryer/ CO_2 , Puregas Equipment Company, Colo., USA). Air flow was measured with rotameters (N112-02G, Omega Engineering, Conn., USA) that were calibrated to $\pm 1\%$ with a 100-ml soap-bubble flow meter. These flow rates allowed the entire respiratory apparatus to equilibrate in less than 20 min, following the calculations of Lasiewski et al. (1966). A subsample of gas was dried with anhydrous calcium sulfate and passed to a CO_2 analyzer (LI 6252, Li-Cor, Neb., USA) that had been factory-recalibrated 3 months earlier. The CO_2 analyzer resolved CO_2 concentration to 0.1 ppm, or less than 0.1% of measured values, and was calibrated daily using both CO_2 -free air and a calibration gas known to contain 2780 ppm CO_2 . Noise level of this analyzer is typically 0.2 ppm, with a maximum of 0.4 ppm. Characteristic readings exceeded 1200 ppm, giving a signal to noise ratio of approximately 4000:1. CO_2 production was calculated using Eq. 3 of Walsberg and Wolf (1995) and corrected to STP (0°C , 101 kPa).

The O_2 concentration of air entering and leaving the chamber was determined with an O_2 analyzer (S3a, Applied Electrochemistry, Calif., USA) that was calibrated using atmospheric air drawn in from outside of the building and positioned upstream of the CO_2 analyzer in a serial arrangement. The O_2 analyzer has a sensitivity of 0.001% O_2 and an accuracy of $\pm 0.1\%$. Air drawn into the O_2 analyzer was dried with anhydrous calcium sulfate (Drierite). Although calcium sulfate acts as a capacitor for CO_2 to some degree in measurements of rapid transients, we opted for this desiccator because of its negligible impact in our steady-state measurements (G.E. Walsberg, personal observation). The subsample routed to the CO_2 and O_2 analyzers did not exceed that of the flow rate into the chamber. O_2 consumption was calculated using Eq. 2 of Hill (1972) after taking into consideration the CO_2 content as measured by the CO_2 analyzer. Respiratory exchange ratios (ratios of CO_2 production to O_2 consumption, RER) then were determined with these O_2 consumption and CO_2 production values for each animal to identify potential differences in substrate catabolism and MWP between animals from the two sites or treatment groups. Though O_2 and CO_2 measurements were used to determine metabolism-specific rates of EWL, measurements of O_2 and CO_2 levels also were used to determine the presence of leaks in the chamber during pre-experiment baseline measurements. Depressed O_2 levels or elevated CO_2 levels were monitored to identify leaks. Typical baseline CO_2 levels did not exceed 5 ppm and thereby verified chamber integrity.

EWL was measured using a hygrometer (PC-2101C, Thunder Scientific, N.M., USA) that measures water vapor concentration and was calibrated by artificially creating known vapor densities (see Walsberg et al. 1997). These values were matched with corresponding airflow rates into the chamber and the mass of the kangaroo rat to arrive at mass-specific rates of whole-body EWL. Flow rates to the chamber were maintained high enough to prevent the vapor density from building up within the respiratory chambers in excess of 7 g m^{-3} and low enough to depress O_2 by 0.65–1.0%.

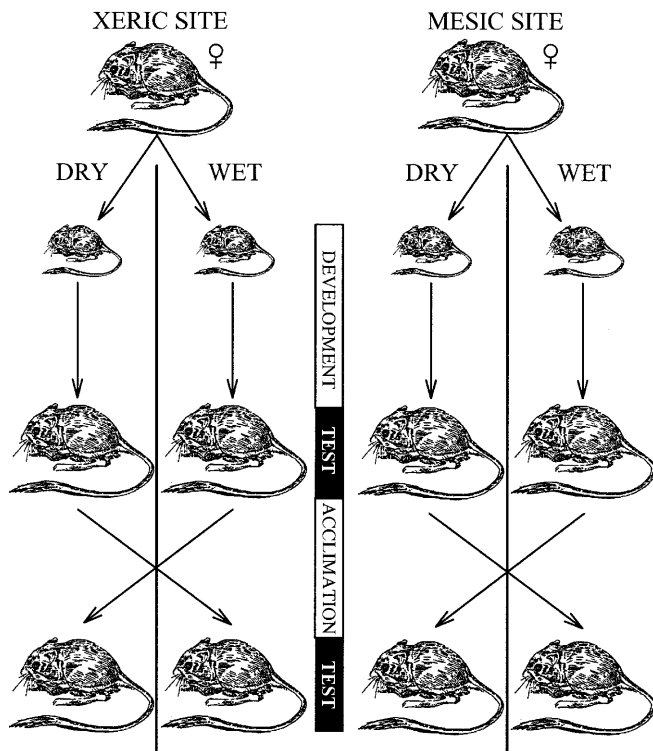


Fig. 1 Diagrammatic representation of the protocol used for these experiments. Pregnant female *D. merriami* from each site gave birth in the laboratory to young that were placed under one of two conditions (WET or DRY) post-weaning until adulthood. These offspring were tested to determine developmental and lineage-specific differences, and then switched to opposite conditions, acclimated, and then retested to determine the relative contributions of acclimation to measured differences

Body size determination

In addition to body mass, tarsus length was used as a robust, noninvasive index of animal size to investigate differences between lineages or treatment groups. Tarsus length was measured to the nearest 0.1 mm from the tip of the middle toe, excluding the nail, to the medial end of the tarsus of all kangaroo rats after development and then again after acclimation under opposite conditions with a digital caliper (500–351, Mitutoyo, Ill., USA). Also, after acclimation, all experimental animals were sacrificed, and cranial length was measured in the frontal plane (from tip of the nasal bone to the most posterior portions of the tympanic bullae).

Statistics

General linear model repeated measures ANOVA were used when investigating differences in physiological responses at different temperatures and for data collected post-development versus post-acclimation using SPSS 7.0 (1996). The model included four between-subject factors: developmental/acclimatory conditions, lineage, size of the litter into which the individual was born (as the only measurable index of maternal effects for this model), and gender. Ambient temperature was considered a within-subject factor for these physiological parameters initially, but (because there were no significant effects of ambient temperature on these variables) was removed from analyses. Therefore, data were combined for each variable from all these ambient temperatures, and repeated measures ANOVA were performed for these values between post-development and post-acclimation (see individual results). In this way, time (either post-development or post-acclimation) was the within-subject factor for these averaged values for physiological parameters for individuals. Statistics for rates of water loss were performed on all values from ambient temperatures up to, but not including 40°C, because of the highly variable and significantly elevated levels of evaporation at this temperature. Data for post-weaning body mass and skull length at the completion of this study (because only one measure was taken for these parameters) and differences in mass, tarsus length, EWL, and mass-specific EWL between post-development and post-acclimation studies were analyzed by one-way ANOVA using SPSS 7.0 (1996) with the same between-subjects factors mentioned previously. Significance was accepted at the $P < 0.05$ level. Mean values are reported with standard errors.

Results

Body mass

Post-weaning body mass was slightly greater among mesic- than xeric-lineage animals ($F = 5.264$; $df = 1$; $P = 0.027$), but did not differ by litter size ($F = 3.083$; $df = 1$; $P = 0.087$) nor gender ($F = 1.518$; $df = 1$; $P = 0.225$). Post-weaning body mass of mesic-lineage animals averaged 18.45 ± 0.545 g, while that of xeric-lineage animals averaged 17.16 ± 0.690 g. There was no effect of ambient temperature on body mass throughout this study ($F = 1.708$; $df = 5$; $P = 0.132$). Therefore, values for each animal were averaged from those measured at each testing temperature after development and then after acclimation. Body mass varied significantly with development and acclimation conditions ($F = 4.472$; $df = 1$; $P = 0.043$), but not by lineage ($F = 0.051$; $df = 1$; $P = 0.824$), litter size ($F = 0.039$; $df = 1$; $P = 0.844$) nor gender ($F = 2.293$; $df = 1$; $P = 0.140$). Therefore, masses for xeric- and mesic-lineage animals were combined for

presentation at each development and acclimation regime (Fig. 2A, B). After development, wet-raised animals were significantly heavier than dry-raised animals and, after acclimation in opposite conditions from that of development, DRY→WET animals were significantly heavier than WET→DRY animals.

There was a significant effect of development/acclimation conditions ($F = 126.916$; $df = 1$; $P < 0.001$) and litter size ($F = 4.498$; $df = 1$; $P = 0.042$), but not lineage ($F = 0.400$, $df = 1$; $P = 0.532$) nor gender ($F = 0.052$; $df = 1$; $P = 0.821$) on the change in mass from post-development to post-acclimation in opposite conditions. DRY→WET animals gained mass after acclimation (gain = 16.5 ± 1.32 g), while WET→DRY animals lost mass after acclimation (loss = 2.0 ± 0.92 g; Fig. 2A, B).

Body size

There was no effect of development/acclimation conditions ($F = 2.706$; $df = 1$; $P = 0.110$), litter size ($F = 2.826$; $df = 1$; $P = 0.103$), nor gender ($F = 3.221$; $df = 1$; $P = 0.083$) on tarsus length for all animals. However, tarsus length was significantly greater in mesic- than xeric-lineage animals ($F = 22.167$; $df = 1$; $P < 0.001$) and increased significantly between post-development and post-acclimation ($F = 19.002$; $df = 1$; $P < 0.001$). Therefore, tarsus length data for DRY→WET and WET→DRY animals were pooled for the distinct xeric- and mesic-lineage animals before and after acclimation (Fig. 3A). There was no significant interaction between testing time (post-development or post-acclimation) and the conditions of development/acclimation ($F = 2.501$; $df = 1$; $P = 0.124$), lineage ($F = 0.145$; $df = 1$; $P = 0.706$), litter size ($F = 0.174$; $df = 1$; $P = 0.680$), nor gender ($F = 0.137$; $df = 1$; $P = 0.714$) on tarsus length. Although tarsus length increased between post-development and post-acclimation, this increase did not vary by development/acclimation conditions ($F = 2.501$, $df = 1$; $P = 0.124$), lineage ($F = 0.145$; $df = 1$; $P = 0.706$), litter size ($F = 0.174$; $df = 1$; $P = 0.680$), nor gender ($F = 0.137$; $df = 1$; $P = 0.714$), and averaged 0.4 ± 0.08 mm for all animals.

There was no effect of development/acclimation conditions ($F = 0.252$; $df = 1$; $P = 0.620$) nor litter size ($F = 0.300$; $df = 1$; $P = 0.588$) on final skull length as well. There was, however, a significant effect of lineage ($F = 8.506$; $df = 1$; $P = 0.007$) and gender ($F = 4.531$; $df = 1$; $P = 0.042$). Despite identical development regimes, mesic-lineage animals still maintained larger skulls than xeric-lineage animals. Male animals maintained larger skulls than female animals from both sites. Female and male skulls of xeric-lineage animals averaged 34.44 ± 0.217 mm ($n = 11$) and 35.26 ± 0.366 mm ($n = 11$), respectively, and female and male skulls of mesic-lineage animals averaged 35.76 ± 0.430 mm ($n = 6$) and 36.59 ± 0.213 mm ($n = 12$), respectively. Data for skull length for DRY→WET and WET→DRY animals were pooled for both xeric- and mesic-lineage animals (Fig. 3B).

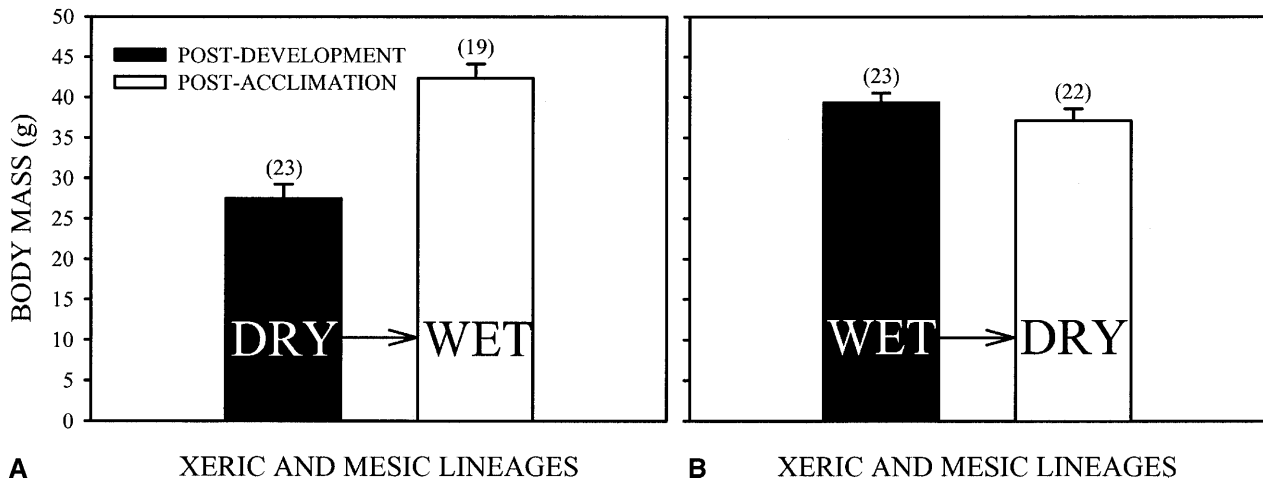


Fig. 2 **A** Body masses of xeric- and mesic-lineage animals raised under dry conditions and then acclimated under wet conditions. **B** Body masses of xeric- and mesic-lineage animals raised under wet conditions and then acclimated under dry conditions. Means \pm SE are represented. Significant differences exist between all groups. Sample sizes are shown above each histogram

acclimation, while that from mesic-lineage animals decreased $0.302 \pm 0.094 \text{ mg g}^{-1} \text{ h}^{-1}$. That is to say, for instance, an additive slight comparative increase in mass-specific EWL among xeric-lineage, DRY \rightarrow WET animals and a slight comparative decrease among mesic-lineage, WET \rightarrow DRY animals after acclimation, could

Mass-specific evaporative water loss

There was no within-subjects effect of temperature on mass-specific EWL ($F=0.937$; $df=5$; $P=0.457$) and no significant interactions of temperature and developmental conditions ($F=1.285$; $df=5$; $P=0.270$), lineage ($F=1.053$; $df=5$; $P=0.386$), litter size ($F=0.557$; $df=5$; $P=0.733$), nor gender ($F=1.508$; $df=5$; $P=0.187$). Therefore, values for all six temperatures (10–35°C) were pooled for each individual post-development and post-acclimation for repeated measures ANOVA. There were no significant effects of development/acclimation conditions ($F=1.496$; $df=1$; $P=0.231$), litter size ($F=0.002$; $df=1$; $P=0.961$), nor gender ($F=1.136$; $df=1$; $P=0.295$) on mass-specific EWL. There was, however, a significant effect of lineage ($F=6.569$; $df=1$; $P=0.016$) and a significant interaction between lineage and development/acclimation conditions ($F=5.272$; $df=1$; $P=0.029$) on mass-specific EWL. Therefore, DRY \rightarrow WET and WET \rightarrow DRY values for mass-specific evaporation were combined for the respective xeric- and mesic-lineage groups both post-development and post-acclimation ($\text{mg g}^{-1} \text{ h}^{-1}$; Fig. 4A, B). Mesic-lineage animals possessed greater mass-specific evaporation than xeric-lineage animals after development and, to a lesser extent, after acclimation (Fig. 4A, B).

There was a significant effect of lineage ($F=4.815$, $df=1$; $P=0.036$), but not development ($F=1.421$; $df=1$; $P=0.243$), litter size ($F=1.470$; $df=1$; $P=0.235$), nor gender ($F=1.242$; $df=1$; $P=0.274$) on the change in mass-specific EWL from post-development to post-acclimation in opposite conditions. Mass-specific EWL of xeric-lineage animals (as a grand, pooled average of DRY \rightarrow WET and WET \rightarrow DRY animals from this lineage) decreased only $0.037 \pm 0.066 \text{ mg g}^{-1} \text{ h}^{-1}$ post-

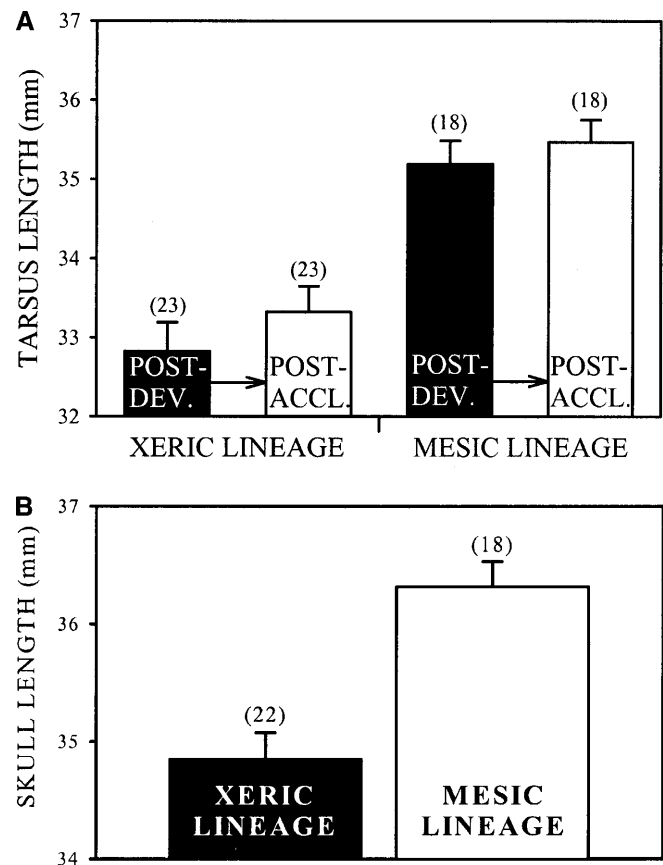


Fig. 3 **A** Tarsi lengths of xeric- and mesic-lineage animals post-development (POST-DEV.) and post-acclimation (POST-ACCL.). **B** Skull lengths of xeric- and mesic-lineage animals. Because no differences between treatment groups (WET/DRY) were detected, they were combined for each lineage. Means \pm SE are represented. Sample sizes are shown above each histogram

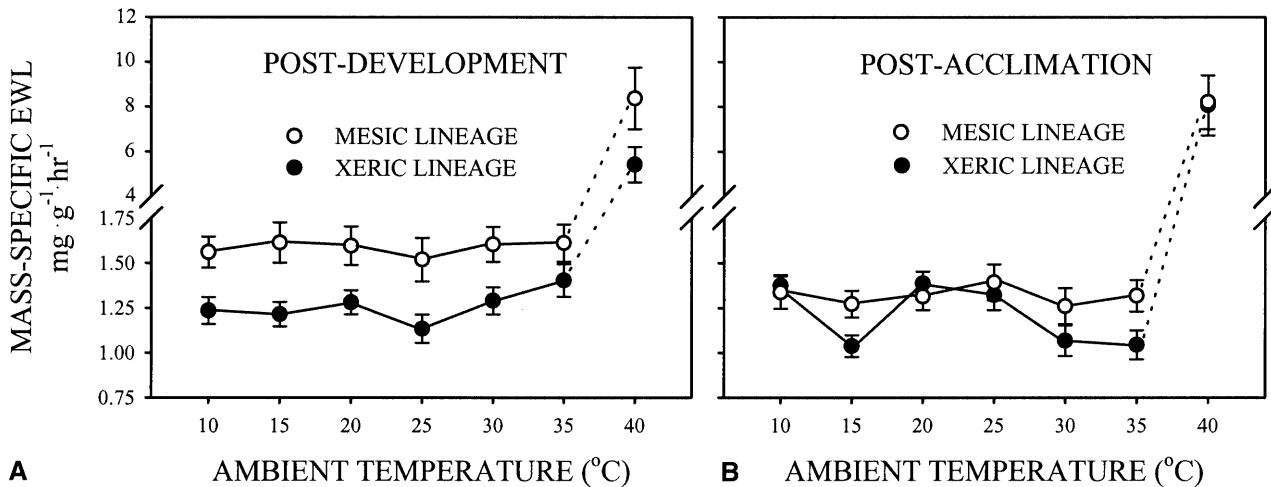


Fig. 4 **A** Mass-specific evaporative water loss (EWL) of adults of the two lineages over a series of temperatures. Because no significant effects were detected between treatment groups (WET/DRY), they were combined for each lineage. $n=22$ for mesic-lineage animals and $n=24$ for xeric-lineage animals. **B** Mass-specific EWL post-acclimation for the two acclimation regimes. Because no significant effects were detected between treatment groups (WET/DRY), they were combined for each lineage. $n=18$ for mesic-lineage animals and $n=23$ for xeric-lineage animals. Means \pm SE are represented for both figures

have contributed to the observed effect of lineage and lack of an effect of development/acclimation conditions (Fig. 4B).

Total evaporative water loss

There was no within-subjects significant effect of ambient temperature on total EWL ($F=1.888$; $df=5$; $P=0.096$), and no significant interactions between ambient temperature and developmental/acclimation conditions ($F=1.631$; $df=5$; $P=0.151$), lineage ($F=1.451$; $df=5$; $P=0.206$), litter size ($F=0.350$; $df=5$; $P=0.882$), nor gender ($F=0.886$; $df=5$; $P=0.490$) on total EWL. Therefore, just as with mass-specific EWL, general linear model repeated measures were used with the average total EWL for these temperatures for each individual as the dependent variable, developmental/acclimatory conditions, lineage, litter size, and gender as between-subjects factors, and post-development/post-acclimation as within-subjects factors. There was a significant effect of development/acclimation conditions ($F=4.027$; $df=1$; $P=0.050$) and lineage ($F=7.916$; $df=1$; $P=0.009$), but not litter size ($F=0.415$; $df=1$; $P=0.524$) nor gender of the animal ($F=0.591$; $df=1$; $P=0.448$), on total EWL (mg min^{-1} ; Fig. 5A, B). More-massive, mesic-lineage, and wet-raised or wet-acclimated animals exhibited greater total EWL than less massive, xeric-lineage, and dry-raised or dry-acclimated animals, respectively.

There was a significant effect of development/acclimation conditions ($F=69.390$; $df=1$; $P<0.001$) and

litter size ($F=4.864$; $df=1$; $P=0.035$), but not lineage ($P=0.051$) nor gender ($F=2.762$; $df=1$; $P=0.107$), on the change in total EWL after acclimation into opposite conditions. Therefore, xeric- and mesic-lineage values for % change in total EWL after acclimation were combined for the respective DRY \rightarrow WET and WET \rightarrow DRY groups. Those animals that were dry-raised and then wet-acclimated (DRY \rightarrow WET) increased their total EWL, while wet-raised and dry-acclimated (WET \rightarrow DRY) animals decreased their total EWL after acclimation. These increases in total EWL exhibited by DRY \rightarrow WET animals were significantly greater than the decreases in total EWL exhibited by WET \rightarrow DRY animals after acclimation.

Discussion

Body mass and body size

Body mass of mesic-site animals is greater than that of xeric-site animals in the field (Tracy and Walsberg 2000, 2001). Similarly, post-weaning body mass was greater in mesic- than xeric- lineage animals. However, there was no effect of lineage on the mass of young raised under either of the two extreme treatments, either before or after acclimation. Nonetheless, while the dry developmental regime hindered mass gain, the wet developmental regime facilitated mass gain in both xeric- and mesic-lineage animals (Fig. 2A, B). When placed under water-rich conditions during acclimation, the dry-raised animals rapidly gained mass and surpassed the adult masses of wet-raised animals. The opposite effect occurred with WET \rightarrow DRY animals after acclimation, as they lost several grams body mass. Nonetheless, the decrease in mass exhibited by WET \rightarrow DRY animals after acclimation was not as dramatic as the increase in mass in DRY \rightarrow WET animals.

Other body size differences did not mirror body mass differences. We observed effects on tarsus length only by lineage (Fig. 3A). Though tarsus length increased during acclimation of both xeric and mesic lineages, it did not

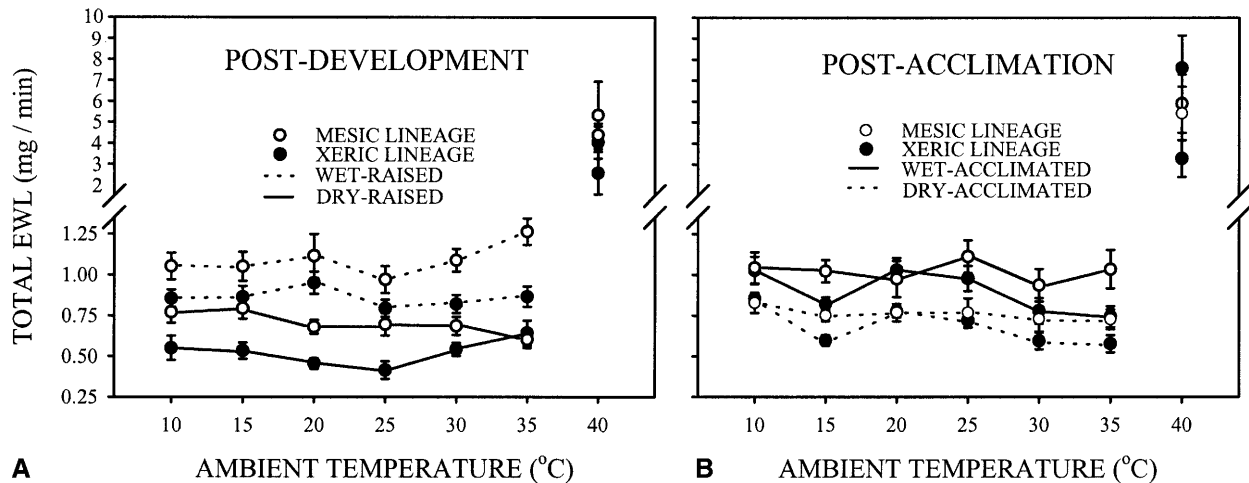


Fig. 5 **A** Whole animal rates of evaporative water loss (EWL) post-development for the two lineages and their two treatment groups over a series of temperatures. Significant effects of lineage and development regime were detected. $n=10$ for mesic-lineage, wet-raised animals and $n=12$ for xeric-lineage, wet-raised animals. $n=8$ for mesic-lineage, dry-raised animals and $n=11$ for xeric-lineage, dry-raised animals. **B** Whole animal rates of EWL post-acclimation for the two lineages and their two treatment groups over a series of temperatures. Significant effects of lineage and development regime were detected. $n=8$ for mesic-lineage, wet-acclimated animals and $n=11$ for xeric-lineage, wet-acclimated animals. $n=10$ for mesic-lineage, dry-acclimated animals and $n=12$ for xeric-lineage, dry-acclimated animals. Means \pm SE are represented for both figures

increase differentially between the two acclimatory regimes and most likely represents the small amount of skeletal growth that occurred after 60 days of age. Skull length at termination of this study paralleled tarsus length: mesic-lineage animals were always skeletally larger than xeric-lineage animals, regardless of developmental regime/acclimatory regime or total body mass associated with the different conditions (Fig. 3B). We detected neither developmental nor acclimatory contributions to this trait, but only the contributions of those traits that were strictly inherited (but see Summary and perspectives). Skeletal size differences were conserved for each lineage, despite that animals varied in mass under desiccating conditions of development or acclimation. Therefore, the intraspecific variation in skull length observed in wild-caught individuals of this subspecies (Tracy and Walsberg 2000) arises from lineage differences alone.

Mass-specific evaporative water loss

It is interesting that the magnitude of genetic differences in mass-specific EWL between the two lineages is maintained in spite of different conditions of development and acclimation (Fig. 4A, B). It is also paradoxical that there are no effects of development on this parameter.

This scenario suggests that development prior to adulthood is critical for xeric-site animals, and that there

has been direct selection for a fixed, lower mass-specific rate of EWL early during the ontogeny of these individuals. That conditions of development do not affect this capacity suggests that thermal and hydric environments during this period commonly are challenging for xeric-site animals and, therefore, there is an adaptive advantage of a fixed, reduced mass-specific EWL. Likewise, occasional episodes of extremely dry and hot weather may select against animals that are developmentally labile and that may have relaxed their capacities to resist desiccation during short, comparatively mild periods.

Conversely, environmental conditions at the mesic site during development may be consistently mild or these animals are not subjected to such acute bottleneck events. Therefore, there may not be any selective forces acting on mesic-site animals that would require developmental plasticity or a fixed inherited capacity for reduced mass-specific EWL.

These rodents are displaced by their parents from their burrows as juveniles and forced to construct new burrows, often during the hottest and driest parts of summer (French 1993). This period may be critical and would select for maximal resistance to desiccation. This would explain why, even as young adults, the two lineages exhibit different rates of evaporation.

Total evaporative water loss

We present EWL in both mass-specific and total rates, because the selective use of one or the other can lead to contradictory analyses of physiological states (McNab 1999; see Fig. 5A, B). It has been argued that mass-specific units are useful with certain assumptions, but that total units are the ecologically and evolutionary-relevant units (see McNab 1999). Focusing on differences in total EWL reveals that lineage effects seen in mass-specific rates of EWL are reflected by differences in total rates of EWL. However, developmental conditions contribute nearly equally to whole animal evaporative loss between xeric- and mesic-lineage

animals in this parameter. That is, differences in wild-caught animals could be mistakenly ascribed strictly to genetic effects, when, in fact, there is an equal component attributable to development. For example, wet-raised, xeric-lineage animals have nearly the same total EWL as dry-raised, mesic-lineage animals after development. After acclimation, differences between xeric- and mesic-lineage animals are not as great as differences between dry- and wet- acclimated animals. Differences credited to opposite acclimation regimes, though similar to those differences found post-development, are less pronounced after acclimation for this parameter.

It is possible that the genetic differences in total evaporation between xeric- and mesic-lineage animals originates from genetic differences in skeletal size and its associated parameters. For instance, cutaneous surface area and respiratory surface area may scale with skeletal size within this subspecies. This relationship remains untested. Assuming equal rates of surface area-specific evaporation between the two lineages, the mesic-lineage animals may possess higher rates of mass-specific loss than xeric-lineage animals because of this possible greater surface area. Importantly though, acclimation mutes earlier developmental and genetic differences in total EWL.

Evaporative water loss and metabolic water production

In heteromyid rodents, MWP is the principal source of water gain and EWL is the predominant source of water loss (Schmidt-Nielsen and Schmidt-Nielsen 1952). Although respiratory exchange ratios (RER) ranged from 0.7 CO_2/O_2 to 0.8 CO_2/O_2 at different temperatures (with the higher RER found at the higher ambient temperatures), the indistinguishable RER displayed by individuals from both lineages and both developmental conditions suggest similar substrate utilization. We calculated MWP assuming a strict catabolism of lipids, although RER values greater than 0.7 represent catabolism of unknown fractions of carbohydrates or proteins.

The environmental temperature at which MWP balances evaporation (Fig. 6A, B) is a useful index of the thermal conditions required for maintenance of hydration (MacMillen and Hinds 1983). Below the temperature at which production equals loss, the animal is in positive water balance. Above this temperature, the animal dehydrates. This average transition temperature differs between animals from each lineage and between animals raised under different conditions. Mesic lineage animals always had lower transition temperatures than xeric-lineage animals, and wet-raised animals always had lower temperatures of water balance than dry-raised animals (Fig. 6A). The average transition temperature for mesic-lineage animals that were wet-raised was only 22°C, while that for xeric-lineage animals raised under the same conditions was 25.3°C. While mesic-lineage

animals that were raised under dry conditions can maintain hydric balance up to 28.6°C, xeric-lineage animals raised under the same conditions can do so up to 30°C. Because inferred MWP rates differed only slightly between kangaroo rats from the two extreme sites, it is differences in EWL that contribute to different temperatures of net water balance. The 8°C difference in transition temperatures between the extreme groups (xeric animals raised in dry conditions and mesic animals raised with ample water) appears mostly due to development, as the differences between wet-raised and

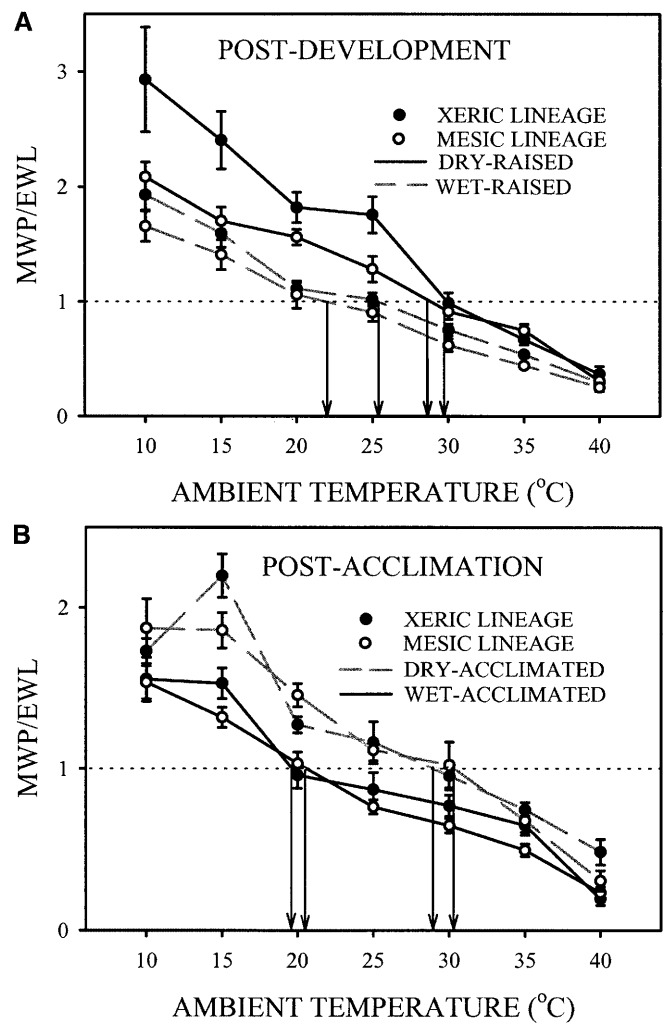


Fig. 6 A Ratios of metabolic water production (MWP) to EWL over a series of temperatures for the two lineages and two treatment groups after development. $n=10$ for mesic-lineage, wet-raised animals and $n=12$ for xeric-lineage, wet-raised animals. $n=8$ for mesic-lineage, dry-raised animals and $n=11$ for xeric-lineage, dry-raised animals. B Ratios of MWP to EWL over a series of temperatures for the two lineages and two treatment groups after acclimation. Arrows point towards the highest ambient temperature at which water balance can be met. $n=11$ for xeric-lineage, wet-acclimated animals and $n=8$ for mesic-lineage, wet-acclimated animals. $n=12$ for xeric-lineage, dry-acclimated animals and $n=10$ for mesic-lineage, dry-acclimated animals. MWP does not significantly vary between any groups or lineages. Means \pm SE are represented for both figures

dry-raised animals are greater than the differences between xeric-lineage and the mesic-lineage animals independent of developmental conditions.

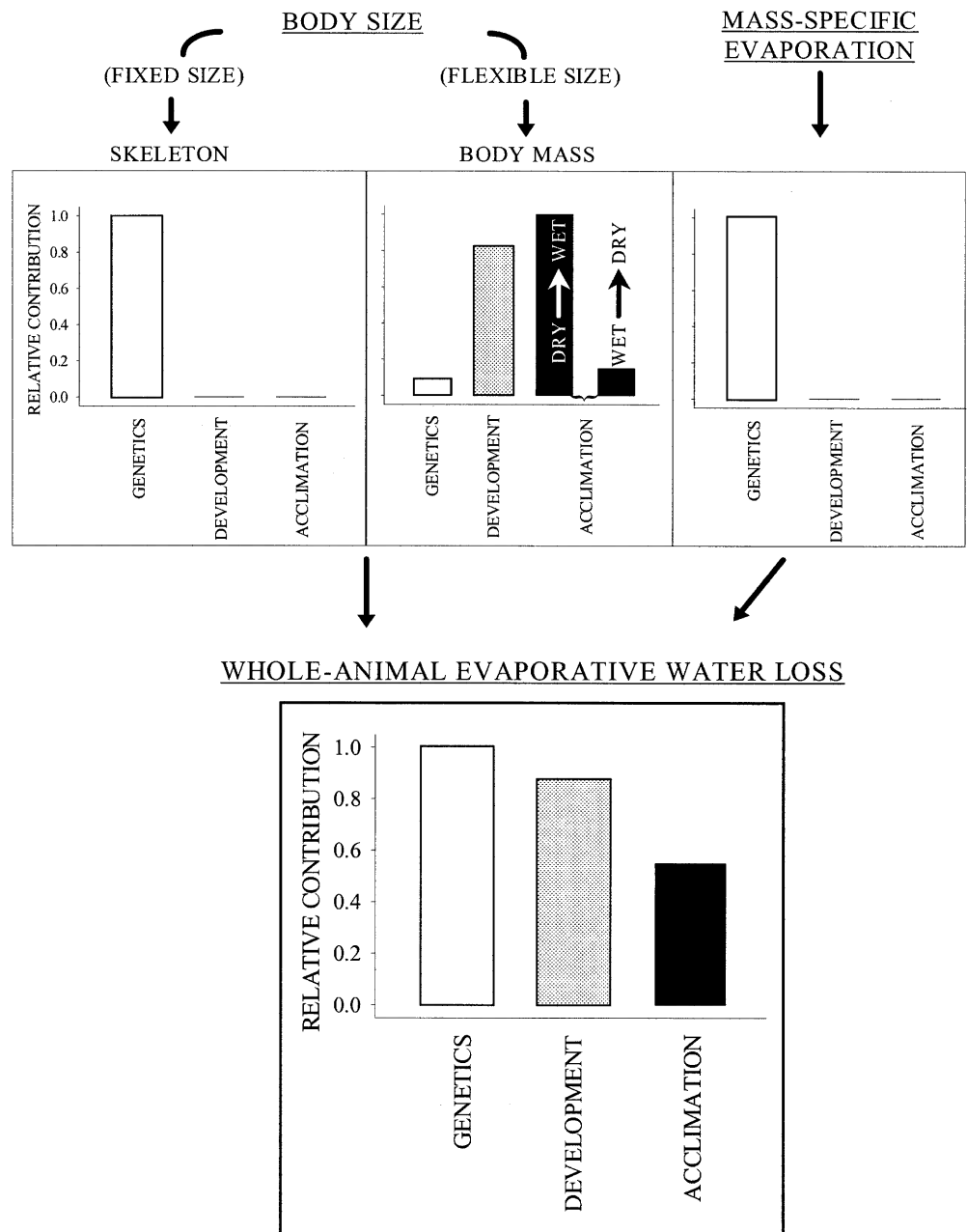
After acclimation, however, lineage effects on total EWL (although statistically significant) appear to be of questionable biological relevance (Fig. 6B), and acclimation conditions alone nearly completely account for differences in the temperatures of unity and desiccation resistance (Fig. 6B). Wet-acclimated animals (regardless of lineage) had an average temperature of unity of approximately 20.2°C, while dry-acclimated animals averaged about 29.4°C. Water balance therefore is achieved at notably higher temperatures for xeric-lineage animals during development and for dry-acclimated animals at

all times during their ontogeny in this subspecies (Fig. 6A, B).

Summary and perspectives

Figure 7 describes the relative contributions that strict genetic inheritance, developmental plasticity, and acclimation make to intraspecific variation in EWL. Although we included litter size in an attempt to incorporate maternal effects into our statistical model, this factor is inextricably linked to lineage because all of the xeric-lineage animals were raised in litters of two and over half of the mesic-lineage animals were raised

Fig. 7 Relative contributions of inflexible inheritance (and/or maternal effects), developmental plasticity, and acclimation to intraspecific variability in body size and evaporative water loss in *D. m. merriami*



in litters of three until they were post-weaned. Maternal differences certainly might have contributed to differences we have credited to strictly genetic differences between the two lineages. For instance, wild-caught pregnant females from the mesic site might have been in better overall hydric states than those from the xeric site, and these differences may have caused differential physiological capacities or size by inertia from unequal development in utero or pre-weaning conditions. Logistical and behavioral barriers aside, cross-fostering of young between the two lineages would have added more control for possible post-natal maternal effects as well. Nevertheless, for our purposes, we assumed maternal effects beyond litter size to be insignificant to illustrate the relative contributions of genetics, development, and acclimation to the measured physiological parameters.

While intraspecific differences in skeletal size are not developmentally or temporally plastic, differences in body mass are. Indeed, acclimation can overwhelm the effects of development on body mass. Intraspecific differences in mass-specific evaporation are mainly due to genetic differences. Development and acclimation have no measurable effects on this parameter.

In contrast, intraspecific variation in whole-animal evaporation can have contributions from all three modes. Genetic differences between the two lineages contribute the most to these differences, and there are smaller contributions from developmental plasticity and acclimation. The developmental component of whole-animal EWL differences originates from body mass differences during development under different conditions. It is also clear that the genetic component of whole-animal EWL arises from genetic differences in mass-specific EWL that may be associated with skeletal differences between the two lineages and possibly some effect from body mass early during their ontogenies. Acclimatory differences in whole-animal EWL originate from body mass differences during acclimation.

The developmental plasticity in body mass, whole-animal EWL, and (indirectly determined) temperature of MWP/EWL balance is similar to that found in other physiological parameters. For example, post-weaning water restriction causes kidney hypertrophy in domestic (Blount and Blount 1968) and desert-adapted rodent species (Hewitt 1981; Buffenstein and Jarvis 1985) and results in an elevated capacity to concentrate urine.

Data for other *Dipodomys* species demonstrate that individuals also have significant capacities to acclimate. In *D. venustus*, pulmocutaneous water loss decreased after 1 month when animals subsisted on air-dried seeds (Church 1969). Mass and mass-specific O₂ consumption (Scelza and Knoll 1980), hematological indices (Scelza and Knoll 1982a), and rate of EWL (Scelza and Knoll 1982b) vary seasonally in Panamint kangaroo rats (*D. panamintinus*). Acclimation also is suspected in populations of *D. microps* that do not have the typical

access to succulent leaves of saltbush that most populations do, but only seeds, and therefore survive with little preformed water (Csuti 1979).

Differences in water economy between xeric- and mesic-lineage animals, and between animals raised or acclimated under different conditions, may reside in epidermal differences or adjustments. For example, when adult Zebra Finches are exposed to either hydrating or desiccating conditions during acclimation, intercellular deposition of multigranular bodies decreases or increases, respectively, and results in either increased or decreased transepidermal water loss (Menon et al. 1989). Because the major route of water loss from *D. merriami* is evaporative (76%; Schmidt-Nielsen and Schmidt-Nielsen 1952) and cutaneous evaporation can account for between 56% and 71% of the total evaporation from this species (Tracy and Walsberg 2000), similar epidermal adjustments could occur during development and acclimation in these rodents. This possibility, and the possibility that xeric- and mesic-site animals have inherently different epidermal properties, remain untested.

Had we included replicates of both mesic and xeric lineages in this study, we could more convincingly assert that the genetic differences in morphology and physiology were a result of selection to different habitat water availabilities, and not drift caused by geographic distance. However, we have demonstrated in an earlier study clinal differences in physiology and morphology in *D. m. merriami* among three sites (xeric, intermediate, and mesic) that differ dramatically in their aridity (Tracy and Walsberg 2001). Individuals from the more arid localities lost proportionately less mass under dehydrating conditions than those from less arid localities. We also showed that increased aridity is correlated with a decrease in size, mass-specific EWL, and total EWL among kangaroo rats from the three locations (Tracy and Walsberg 2001). Therefore, it appears unlikely that the differences in these parameters result merely from drift caused by geographic separation.

Importantly, though, flexible components of an animal's phenotype must be considered in addition to the default assumption of differential inherited capacities when considering the selection and evolution of traits in physiological analyses. For instance, geographic differences may not represent capacities that are specific to individuals, but rather variation in the innate capacity to acclimate to different environments. By extension, populations of other species could exhibit different degrees of plasticity.

Acknowledgements We thank P. Dockens for assistance with animal husbandry. We also thank J.F. Harrison, J.R. Hazel, T.C. M. Hoffman, F.H. Pough, A.T. Smith, K.M. Wooden, and H.A. Woods for their comments relating to this study. We trapped all animals under Arizona Game and Fish Scientific Collecting Permit No. SP785298, and conducted all studies in compliance with ASU's Institutional Animal Care and Use Committee Protocols No. 96-367R and No. 98-417R. All experiments complied with the legal requirements of the state of AZ, USA, and UK. Funding was assisted by NSF grant IBN 9725211 to GW.

References

- Beatley JC (1969) Dependence of desert rodents on winter annuals and precipitation. *Ecology* 50:721–724
- Blount RF, Blount IH (1968) Adaptive changes in size of renal papilla with altered function. *Tex Rep Biol Med* 26:473–484
- Buffenstein R, Jarvis JUM (1985) Thermoregulation and metabolism in the smallest African gerbil, *Gerbillus pusillus*. *J Zool* 205:107–121
- Burggren WW, Bemis WE (1990) Studying physiological evolution: paradigms and pitfalls. In: Nitecki MH (ed) *Evolutionary innovations*. University of Chicago Press, Chicago
- Butterworth BB (1961) A comparative study of growth and development of the kangaroo rats, *Dipodomys deserti* Stephens and *Dipodomys merriami* Mearns. *Growth* 25:127–139
- Carpenter RE (1966) A comparison of thermoregulation and water metabolism in the kangaroo rats *Dipodomys agilis* and *Dipodomys merriami*. *Univ Calif Berkeley Publ Zool* 78:1–36
- Church RL (1969) Evaporative water loss and gross effects of water privation in the kangaroo rat, *Dipodomys venustus*. *J Mammal* 50:514–523
- Csuti BA (1979) Patterns of adaptation and variation in the Great Basin kangaroo rat (*Dipodomys microps*). *Univ Calif Publ Zool* 111:1–69
- Daly M, Wilson MI, Behrends P (1984) Breeding of captive kangaroo rats, *Dipodomys merriami* and *D. microps*. *J Mammal* 65:338–341
- Eisenberg JF (1993) Ontogeny. In: Genoways HH, Brown JH (eds) *Biology of the Heteromyidae*. *Spec Publ Am Soc Mammal* 10:479–490
- Feder ME, Block BA (1991) On the future of animal physiological ecology. *Funct Ecol* 5:136–144
- French AR (1993) Physiological ecology of the Heteromyidae: economics of energy and water utilization. In: Genoways HH, Brown JH (eds) *Biology of the Heteromyidae*. *Spec Publ Am Soc Mammal* 10:509–538
- Garland T Jr, Carter PA (1994) Evolutionary physiology. *Annu Rev Physiol* 56:579–621
- Green CR, Sellers WD (eds) (1964) *Arizona climate*. University of Arizona Press, Tucson, Arizona
- Hewitt S (1981) Plasticity of renal function in the Australian desert rodent *Notomys alexis*. *Comp Biochem Physiol* 69A:297–304
- Hill RW (1972) Determination of oxygen consumption by use of the paramagnetic oxygen analyzer. *J Appl Physiol* 33:261–263
- Hinds DS, MacMillen RE (1985) Scaling of energy metabolism and evaporative water loss in heteromyid rodents. *Physiol Zool* 58:282–298
- Hoffmeister DF (1986) *Mammals of Arizona*. University of Arizona Press and the Arizona Game and Fish Department, Tucson, Arizona
- Kenagy GJ (1973a) Adaptations for leaf eating in the Great Basin kangaroo rat, *Dipodomys microps*. *Oecologia* 12:383–412
- Kenagy GJ (1973b) Daily and seasonal patterns of activity and energetics in a heteromyid rodent community. *Ecology* 54:1201–1219
- Kleiber M (1975) *The fire of life: an introduction to animal energetics*. Wiley, New York
- Lasiewski RC, Acosta AL, Bernstein ML (1966) Evaporative water loss in birds. I. Characterization of the open flow method of determination, and their relation to estimates of thermoregulatory ability. *Comp Biochem Physiol* 19:445–457
- MacMillen RE (1972) Water economy of nocturnal desert rodents. *Symp Zool Soc Lond* 31:147–174
- MacMillen RE, Hinds DS (1983) Water regulatory efficiency in heteromyid rodents: a model and its application. *Ecology* 64:152–164
- MacMillen RE, Hinds DS (1998) Water economy of granivorous birds: California house finches. *Condor* 100:493–503
- McNab BK (1999) On the comparative ecological and evolutionary significance of total and mass-specific rates of metabolism. *Physiol Biochem Zool* 72:642–644
- Menon GK, Baptista LF, Brown BE, Elias PM (1989) Avian epidermal differentiation. II. Adaptive response of permeability barrier to water-deprivation and replenishment. *Tissue Cell* 21:83–92
- Pyörnilä A, Putaala A, Hissa R, Sulkava S (1992) Adaptations to environment in the mountain hare (*Lepus timidus*): thermal physiology and histochemical properties of locomotory muscles. *Can J Zool* 70:1325–1330
- Reichman OJ, Van De Graaf KM (1975) Association between ingestion of green vegetation and desert rodent reproduction. *J Mammal* 56:503–506
- Scelza J, Knoll J (1980) The effects of acclimatization on body weight and oxygen consumption in *Dipodomys panamintinus*. *Comp Biochem Physiol* 65A:77–84
- Scelza J, Knoll J (1982a) Seasonal variation in various blood indices of the kangaroo rat, *Dipodomys panamintinus*. *Comp Biochem Physiol* 71A:237–241
- Scelza J, Knoll J (1982b) Seasonal variation in the rate of evaporative water loss in the kangaroo rat, *Dipodomys panamintinus*. *Comp Biochem Physiol* 71A:579–584
- Schmidly DJ, Wilkens KT, Derr JN (1993) Biogeography. In: Genoways HH, Brown JH (eds) *Biology of the Heteromyidae*. *Spec Publ Am Soc Mammal* 10:319–356
- Schmidt-Nielsen B, Schmidt-Nielsen K (1951) A complete account of the water metabolism in kangaroo rats and an experimental verification. *J Cell Comp Physiol* 38:165–181
- Schmidt-Nielsen KB, Schmidt-Nielsen B (1952) Water metabolism of desert mammals. *Physiol Rev* 32:135–166
- Schmidt-Nielsen K, Schmidt-Nielsen B, Brokaw A (1948) Urea excretion in desert rodents exposed to high protein diets. *J Cell Comp Physiol* 32:361–380
- Sellers WD, Hill RH, Sanderson-Rae M (eds) (1985) *Arizona climate: the first hundred years*. University of Arizona Press, Tucson, Arizona
- Soholt LF (1977) Consumption of herbaceous vegetation and water during reproduction and development of Merriam's kangaroo rat, *Dipodomys merriami*. *Am Midl Nat* 98:445–457
- SPSS (1997) *SPSS Base 7.5 for Windows user's guide*. SPSS, Chicago
- Tracy RL, Walsberg GE (2000) Prevalence of cutaneous evaporation in Merriam's kangaroo rat, and its adaptive variation at the subspecific level. *J Exp Biol* 203:773–781
- Tracy RL, Walsberg GE (2001) Intraspecific variation in water loss in a desert rodent, *Dipodomys merriami*. *Ecology* 82:1130–1137
- Turner RM, Brown DE (1994) Desertlands: tropical-subtropical desertlands. In: Brown DE (ed) *Biotic communities: southwestern United States and northwestern Mexico*. University of Utah Press, Salt Lake City, Utah
- Walsberg GE, Wolf BO (1995) Variation in the respiratory quotient of birds and implication for indirect calorimetry using measurements of carbon dioxide production. *J Exp Biol* 198:213–219
- Walsberg GE, Weaver T, Wolf BO (1997) Seasonal adjustments of solar heat gain independent of coat coloration in a desert mammal. *Physiol Zool* 70:150–157
- Wang LCH, Jones DL, MacArthur RA, Fuller WA (1973) Adaptation to cold: energy metabolism in an atypical lagomorph, the arctic hare (*Lepus arcticus*). *Can J Zool* 51:841–846
- Withers PC (1992) *Comparative animal physiology*. Saunders College Publishing, San Diego, California