

PREVALENCE OF CUTANEOUS EVAPORATION IN MERRIAM'S KANGAROO RAT AND ITS ADAPTIVE VARIATION AT THE SUBSPECIFIC LEVEL

RANDALL L. TRACY^{1,*} AND GLENN E. WALSBERG²

¹Arizona State University, Department of Biology, LSC448, Tempe, AZ 85287-1501, USA and ²Arizona State University, Department of Biology, LSC444, Tempe, AZ 85287-1501, USA

*e-mail: randyt@imap2.asu.edu

Accepted 22 November 1999; published on WWW 26 January 2000

Summary

Previous estimates suggested that ventilatory evaporation constitutes the major source of water loss in kangaroo rats (*Dipodomys* spp.). We quantified rates of water loss in Merriam's kangaroo rat (*Dipodomys merriami*) and demonstrate the degree to which acclimation to a particular thermal and hydric environment plays a role in the intraspecific variation in water loss evident in this species. We draw the following conclusions: (1) that water loss varies intraspecifically in Merriam's kangaroo rat, in association with habitats of contrasting aridity and temperature; (2) that animals from more xeric locations have lower water loss rates than those from more mesic

sites; (3) that most water loss is cutaneous, with ventilatory evaporative water loss contributing, at most, only 44 % to total evaporative water loss; and (4) that intraspecific differences in rates of water loss are not acclimatory, but fixed. After acclimating under the same conditions, xeric-site animals still show a 33 % lower rate of evaporative water loss than mesic-site animals.

Key words: acclimation, cutaneous water loss, desert adaptation, *Dipodomys merriami*, evaporative water loss, intraspecific variation, kangaroo rat, urine concentration, ventilatory water loss.

Introduction

How physiology affects the distribution of animals and the patterns and processes by which physiological variation evolves are fundamental components of ecological physiology (see Feder and Block, 1991). Historically, water balance has been a major focus of investigations dealing with desert animals. Quantifying geographic variability in physiological variables related to desiccation is essential to understand the time frame through which adjustments act in dealing with a harsh environment and to determine the origins of potential variation in these physiological variables. Adjustments to changing conditions may take place through three non-exclusive modes. The first is acclimational responses that allow individuals to change their physiological characteristics in response to environmental changes. A second mode of response is ontogenetic lability. Developmental plasticity can define individual physiological capacities early during development and can have consequences for the survival of individuals later in their ontogenies. 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. Finally, it is possible that physiological changes relating to desiccation occur at the level of natural selection, whereby populations exhibit adaptation to local conditions.

Within the North American deserts, free-standing water is

not readily available to rodents such as Merriam's kangaroo rat (*Dipodomys merriami*), and water input is restricted to metabolic production from the catabolism of foodstuffs and preformed input from the diet. Consequently, water losses must be minimized. Routes of water loss include urine production, fecal water loss, lactation and evaporation (either ventilatory or insensible cutaneous evaporative water loss, as these species lack sweat glands on all but their feet). One subspecies of Merriam's kangaroo rat, *Dipodomys merriami merriami*, ranges from areas of extreme aridity and temperature in southwestern Arizona to milder areas in central and northwestern Arizona (Hoffmeister, 1986; Schmidly et al., 1993; Turner and Brown, 1994). In a recent study of this subspecies, R. L. Tracy and G. E. Walsberg (in preparation) found that, while fecal and urinary loss did not vary within this subspecies, evaporative water loss varied among individuals from locations of differing aridity. Evaporative water loss was significantly lower among individuals from more xeric areas than among those from the more mesic areas. These differences are important because evaporative loss typically accounts for 75 % of the total water loss from this species (Schmidt-Nielsen and Schmidt-Nielsen, 1952).

Two significant questions remain unaddressed regarding this dominant mode of water loss. The first is the source of the intraspecific variation observed, i.e. whether it reflects acclimation, developmental plasticity or genetically fixed

differences. Establishing the relative roles of such modes is important because this may yield insights into the pattern and time course of populations adapting to environments that are altered as a result of either range expansion or climate change. We therefore tested the hypothesis that acclimation is the primary mechanism behind intraspecific variation in the capacity of this species to withstand desiccation.

The second major question deals with the relative importance of the skin and respiratory surfaces as sites for evaporation. Although data are not extensive, previous studies have concluded that ventilatory evaporation accounts for 70–84% of total evaporative loss in kangaroo rats (Schmidt-Nielsen, 1964; Chew and Dammann, 1961). These conclusions, however, were derived from measurements of the temperature of exhaled air combined with estimates of tidal volume. Direct measurements of the relative contributions of ventilatory and cutaneous water loss in small mammals are conspicuously lacking. We therefore partitioned evaporative water loss into its ventilatory and cutaneous components and tested the hypothesis that ventilatory water loss is the dominant route of evaporative loss in this species.

Materials and methods

Field sites

Two field sites that exhibit the broad range of conditions faced by *D. merriami* (Mearns) throughout its species range were used. The xeric site is located in the heart of the Sonoran Desert in Yuma County, southwestern Arizona, USA, 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*. Mean annual maximum and minimum daily temperatures are 31.9 and 14.7 °C, respectively (Green and Sellers, 1964). Mean annual precipitation averages only 10.6 cm, with the greatest accumulation during the winter months and summer monsoon season (Green and Sellers, 1964). The mesic site is located in north-central Arizona, within Gila County, at 1200 m elevation, and contains creosote bushes, bordered by pinyon-juniper woodland. Daily maximum and minimum temperatures annually average 23.5 and 6.2 °C, respectively, and yearly precipitation averages 43.6 cm (Green and Sellers, 1964; Sellers et al., 1985).

Individuals belonging to one subspecies of Merriam's kangaroo rat (*D. m. merriami*) were trapped using Sherman live-traps at each site from May until June 1998 for all metabolic chamber and urinary water loss measurements. Juveniles were excluded from all experiments. Data were analyzed by applying either one-way analysis of variance (ANOVA) using SPSS 7.0 (1996) when examining potential differences among the animals from the two locations or paired *t*-tests when investigating individual responses at different temperatures or after acclimation. Significance was accepted at the level $P < 0.05$. Mean values are reported with standard errors of the mean.

Animal care and handling/acclimation regime

Animals captured from the xeric and mesic sites ($N=13$ and

$N=11$, respectively) were transported to Arizona State University, weighed and maintained on a 12h:12h light:dark photoperiod in an environmental chamber at 30 °C and approximately 30% relative humidity (RH) (minimum 5.2 mg l⁻¹, maximum 12.9 mg l⁻¹, mean 9.0 ± 0.4 mg l⁻¹; measured with an Omega, model 35519-050, RH digital thermohygrometer and by calculating vapor density; Campbell, 1977). These conditions provided an adequate test for the effects of acclimation because 30 °C lies within thermal neutrality for this species and 30% represents a moderate relative humidity. Kangaroo rats were allowed access to moistened seeds for 12 h after capture, and were then maintained on a dry seed diet (Hartz cockatiel seed consisting of millet seed, oat groats, red millet seed, sunflower seed and canary seed) *ad libitum* for the duration of the studies. Each animal was individually caged with a dirt floor and provided with a section of plastic piping for shelter. Kangaroo rats were tested 1 week after capture, housed under identical conditions for 6 weeks of acclimation, and then retested for metabolic chamber and urinary experiments. $N=12$ for pre-acclimation xeric-site animals and $N=10$ for pre-acclimation mesic-site animals, and $N=13$ and $N=11$ for post-acclimation kangaroo rats, respectively. Two animals that were initially fouled with mineral oil were precluded from pre-acclimation experiments. Animals were weighed daily throughout the acclimation period and for 10 days following the post-acclimation evaporative water loss measurements.

Urinary water loss

We investigated the role acclimation may play in urine concentration, and used these measurements as indices of urinary water loss within this subspecies. Two days prior to metabolic chamber measurements before and after the acclimation period, kangaroo rats were placed inside urine collection chambers, weighed, maintained at 30 °C and fasted for the duration of the urine collection procedure. Each collection chamber consisted of a 3.81 aluminum can with a wire mesh floor suspended over mineral oil. The vapor density of incurrent air did not exceed 0.1 mg l⁻¹, and flow rate was maintained at 800 ml min⁻¹. After 12 h, individuals were weighed again, and urine that was not contaminated by feces was collected using glass capillary tubes. These tubes were sealed using hematocrit sealer and frozen until later analysis. For analysis, samples were thawed, centrifuged for 1 min at 11 700 revs min⁻¹ in an Adams Microhematocrit centrifuge, and emptied into and diluted with distilled water in Eppendorf tubes. Osmolality was measured with a Wescor model 5500 ER vapor pressure osmometer calibrated with standard sodium chloride solutions. At least three samples from each animal were measured, and the mean of these values was used as a single measurement for that individual.

Ventilatory-cutaneous chamber measurements (gas exchange/evaporation)

All measurements were made between 08:00 h and 18:00 h, 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 using a Campbell 23x datalogger and averaged at 1 min intervals. Animals remained quiescent within the chambers, as viewed with a Magnavox observation 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.

Measurements of rates of O₂ consumption and CO₂ production were coupled to simultaneous measurements of evaporative water loss to determine metabolism-specific rates of ventilatory evaporative water loss. Measurements were made at 10, 20 and 30 °C in an open-flow metabolic chamber (1.13 l) partitioned to measure both respiratory and cutaneous water loss. The chamber itself was oriented horizontally and consisted of a body section (0.48 l) and a separate head section (0.65 l). The body section possessed a wire mesh floor to allow excreta to fall into the mineral oil beneath, while the head section contained a metal frame and latex barrier (HCM-Hygienic Co. dental dam) that was tightened onto the body section of the chamber with wing nuts after the animal had been positioned inside. Prior to this, the animal's head was placed through a small hole created in the latex barrier that was sandwiched between two flat metal frames. The hole perimeter was snug around the animal's neck, but not overly constrictive. A steel bar on one metal frame acted as a perch for the animal's hind limbs, while two metal plates with semicircular notches acted as a yoke by being slid through slotted grooves on metal bars positioned on either side of the animal's head. This yoke prevented the animal from pulling its head through the flexible latex barrier. Finally, two clasps locked the head portion of the chamber onto the body portion, compressing a rubber gasket. All animals became calm within the chamber within 2 min of experimentation, and no differences in activity between the subpopulations were observed.

Temperatures within the chamber were measured using 26 gauge, type-T thermocouples and controlled at 10±1 °C, 20±1 °C and 30±1 °C by placing the chambers within a temperature-controlled cabinet (animals were tested at these three temperatures in separate experiments to determine possible temperature-dependent differences in evaporative water loss between individuals from the two sites). Air was passed separately through each side of the chamber at 200–300 ml min⁻¹ after being dried and scrubbed of CO₂ by a Puregas model CDA112 air dryer/CO₂ absorber system. Air flow was measured using Omega N112-02G rotameters, calibrated to ±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 using anhydrous calcium sulfate and passed to a Li-Cor (model LI 6252) carbon dioxide analyzer that had been factory-recalibrated 3 months previously. The carbon dioxide analyzer resolved CO₂ concentration to 0.1 p.p.m., or less than 0.1 % of measured values, and was calibrated daily using both CO₂-free air and a calibration gas known to contain 2780 p.p.m. CO₂. The noise level of this analyzer is typically 0.2 p.p.m., with a maximum of 0.4 p.p.m. Characteristic readings exceeded 1200 p.p.m., giving a signal-to-noise ratio of

approximately 4000:1. The rate of carbon dioxide production was calculated using equation 3 of Walsberg and Wolf (1995) and corrected to STP (0 °C, 101 kPa).

The O₂ concentration of air entering and leaving the chamber was determined using an Applied Electrochemistry S3a oxygen analyzer calibrated using atmospheric air drawn from outside the building and positioned upstream of the CO₂ analyzer in a serial arrangement. The oxygen analyzer has a sensitivity of 0.001 % O₂ and an accuracy of ±0.1 % of the O₂ reading. Air drawn into the oxygen analyzer was dried using anhydrous calcium sulfate (Drierite). The flow rate of the subsample routed to the CO₂ and O₂ analyzers did not exceed that into the chamber. The rate of O₂ consumption was calculated using equation 2 of Hill (1972) after taking into consideration the CO₂ content measured by the CO₂ analyzer. Respiratory exchange ratios (ratios of carbon dioxide production to oxygen consumption, RER) were then determined with these rates of oxygen consumption and CO₂ production for each animal to identify potential differences in metabolic water production between animals from the two sites, assuming that only carbohydrates and lipids were metabolized during measurements. Although O₂ and CO₂ measurements were used to determine metabolism-specific rates of ventilatory evaporative water loss, measurements of O₂ and CO₂ levels from the cutaneous portion of this chamber were also used to determine the presence of leaks between the two partitions, both while an animal was present within the chamber and when the chamber was sealed without an animal during pre-experiment baseline measurements. Depressed O₂ levels or elevated CO₂ levels in the cutaneous section were monitored to identify contamination from the respiratory partition. Typical CO₂ levels did not exceed 30 p.p.m. in the cutaneous section, thereby verifying chamber integrity.

Evaporative water loss was measured using a Thunder Scientific (model PC-2101C) hygrometer that measures water vapor concentration in mg l⁻¹ 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 mean mass of the kangaroo rat to arrive at mass-specific rates of whole-body evaporative water loss. Flow rates to the chamber were maintained at a level high enough to prevent the vapor density from building up within the respiratory chambers in excess of 7 mg l⁻¹ and low enough to depress oxygen content by 0.65–1.0 %. Flow rates between the respiratory and cutaneous partitions were matched to prevent pressure differences from stretching the latex barrier and compromising the seal between the partitions.

Body temperature was recorded by a Digi-sense thermocouple thermometer and 40 gauge type T thermocouple probe inserted 2.5 cm rectally immediately before experimentation and within 10 s of experimental completion at each temperature.

Surface area and body size determination

Because cutaneous water loss is a surface-area-specific phenomenon, it was imperative to determine the total body surface area of these animals, especially to correct for the cranial surface area exposed in the ventilatory portion of the

chamber during evaporative water loss measurements. All experimental animals were killed and measured for surface area determination ($N=13$ for the xeric sites and $N=11$ for the mesic sites). The skins from each animal's torso and separated head were delicately removed to minimize stretching and immediately flattened and traced onto white paper. These two areas were excised, placed upon a black background including a reference scale and digitally scanned using a Microteck Scanmaker (model V310). After calibrating with the associated scale, the surface area for each image was determined using NIH Image 1.6/ppc analysis software.

We assumed that surface-area-specific rates of evaporation were the same for cranial skin as for the rest of the body. Water loss from the eyes was assumed to equal that of an equivalent area of skin. Values for total cutaneous evaporative water loss were corrected for whole-body surface area by determining surface-area-specific rates of evaporation from the body partition of the chamber and multiplying this value by the total surface area of that animal, and values for ventilatory evaporation were calculated by subtracting the product of the surface area of the head and the surface-area-specific cutaneous water loss of the rest of the body from that evaporation recorded from the head partition.

To determine intraspecific differences in body size, skulls from dead xeric- and mesic-site animals were measured. The overall cranial length in the frontal plane (from the tip of the nasal bone to the most posterior portions of the tympanic bullae) was measured using a Mitutoyo Digimatic (model 500-351) digital caliper and recorded to the nearest 0.01 mm.

Results

Body temperature

Presumably as a pathological result of restricted movement and lack of postural adjustments, animals became moribund during experiments conducted at 10 and 20 °C. Measurements indicate that body temperature decreased considerably at 10 °C for both xeric- and mesic-site animals. Body temperature also decreased at 20 °C for animals from both sites. Consequently, the data from these temperatures were excluded from analyses and are not presented here. Although body temperature at 30 °C increased somewhat during experimentation for animals from

each site, it did not differ between sites for kangaroo rats either before or after experimentation (Table 1). As this temperature is within the thermoneutral zone for this species (Carpenter, 1966), all subsequent data and discussion refer to measurements made at 30 °C.

Mass and body size

Mass varied significantly with respect to location (Figs 1, 2). Xeric-site animals were significantly smaller than mesic-site animals at the start of this study, an intraspecific difference well established for *D. merriami* throughout the year from these two sites (R. L. Tracy and G. E. Walsberg, in preparation). Animals initially lost mass, then regained mass during a brief 5-day period in which vapor density within the housing chamber increased as a result of seasonal humidity changes in the building. While xeric-site animals maintained mass throughout the remainder of this study, mesic-site animals remained only as massive as xeric-site animals until experimental completion ($P>0.05$). The mean value of 2.38 ± 1.01 g mass lost from each mesic-site animal during the 6 weeks amounts to 5.75% of the mean initial mass, a mean loss of 57 mg day^{-1} .

Cranial length measured at the end of experimentation varied between subpopulations ($P<0.001$). Mean cranial length for xeric animals (35.13 ± 0.14 mm) was significantly smaller than for mesic animals (37.00 ± 0.23 mm).

Surface area

Head, torso and total surface areas did not vary by location upon study completion. Total surface area averaged $83.06\pm 2.55\text{ cm}^2$ and $88.56\pm 2.82\text{ cm}^2$ for the xeric- and mesic-site animals, respectively, and did not differ ($P=0.162$). Tail, head and torso surface areas averaged 10.09 ± 0.32 , 20.23 ± 0.63 and $52.74\pm 2.23\text{ cm}^2$, respectively, for the xeric-site animals and 9.23 ± 0.76 , 21.51 ± 0.91 and $57.81\pm 2.67\text{ cm}^2$, respectively, for the mesic-site animals. These three respective areas averaged 13, 24 and 64% of total surface area for the xeric-site animals and 11, 24 and 65% for mesic-site animals, and none of them differed between the two sites ($P=0.281$, $P=0.247$ and $P=0.155$, respectively). Fractional contributions of these three areas were calculated for individuals and then averaged for each site. The sum of these averages exceeds 100% for xeric-site animals because of errors in determining means of

Table 1. *Body temperatures of Dipodomys merriami merriami from contrasting locations before and after experimentation at an ambient temperature of 30 °C*

	Xeric site		Mesic site		P (Xeric versus Mesic)
	Mean	N	Mean	N	
Pre-experiment body temperature (°C)	37.0±0.15	13	37.1±0.18	10	0.70
Post-experiment body temperature (°C)	37.6±0.22	13	37.9±0.33	11	0.38
P (Pre versus Post)	0.035		0.002		

Values are means ± S.E.M.

P values are from ANOVAs testing for significant differences between animals from the two locations both before and after experimentation or from paired *t*-tests testing for significant changes in individuals from each site following experimentation.

Significant P values are given in bold type.

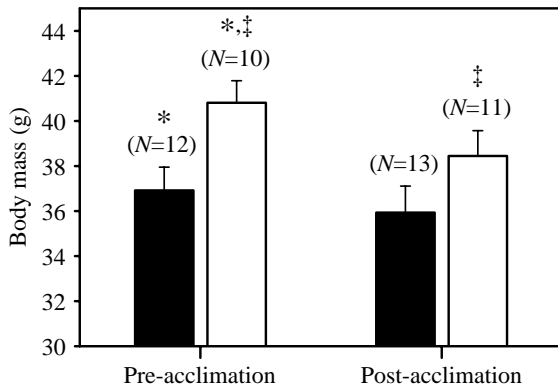


Fig. 1. Body mass of kangaroo rats from each location before and after acclimation. Values are means + S.E.M. Significant differences between individuals from the two sites are identified by *, while differences due to acclimation for mesic-site animals are shown with †. Filled columns, xeric site; open columns, mesic site.

the fractions. Surface areas before acclimation could not be measured directly and, therefore, may have differed from values after acclimation.

Gas exchange and metabolic water production

Mesic site animals possessed a 21 % greater rate of total volumetric oxygen consumption (\dot{V}_{O_2}) and a 23 % greater rate of volumetric carbon dioxide production (\dot{V}_{CO_2}) than xeric-site animals before acclimation, yet animals from the two sites manifested similar rates of gas exchange after acclimation (Table 2). However, mass-specific \dot{V}_{O_2} and \dot{V}_{CO_2} did not vary

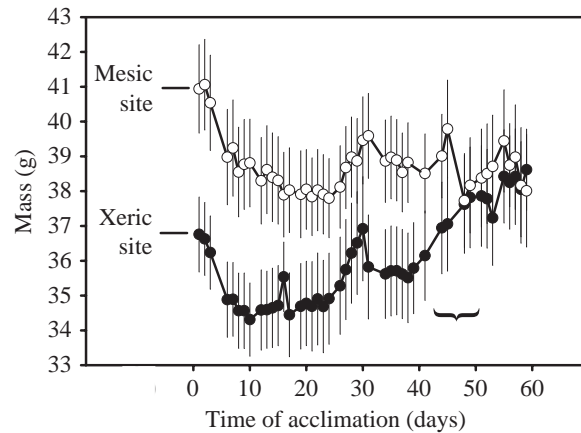


Fig. 2. Body mass versus day of acclimation for xeric- and mesic-site animals. Values are means \pm S.E.M., $N=10$ for each site. Post-acclimation measurement days are indicated by a bracket.

by location either before or after acclimation. \dot{V}_{CO_2} increased by 15 % after acclimation for xeric-site animals (Table 2). Respiratory exchange ratios, and therefore probably substrate catabolism and relative metabolic water production, did not vary by location or state of acclimation and averaged 0.79 ± 0.01 .

Evaporative water loss

Cutaneous evaporative water loss

Total cutaneous evaporative water loss (CEWL) for mesic-site animals was 77 % greater than that for xeric-site animals

Table 2. Physiological variables relating to water loss in *Dipodomys merriami merriami* from contrasting locations

	Pre-acclimation			Post-acclimation			Pre-acclimation versus post-acclimation	
	Xeric site	Mesic site	<i>P</i>	Xeric site	Mesic site	<i>P</i>	Xeric site	Mesic site
\dot{V}_{CO_2} (ml min ⁻¹)	0.75 \pm 0.05	0.92 \pm 0.05	0.027	0.86 \pm 0.05	0.99 \pm 0.09	0.218	0.039	0.361
Mass-specific \dot{V}_{CO_2} (ml CO ₂ g ⁻¹ h ⁻¹)	1.22 \pm 0.08	1.38 \pm 0.09	0.204	1.44 \pm 0.06	1.55 \pm 0.16	0.483	0.064	0.178
\dot{V}_{O_2} (ml min ⁻¹)	0.97 \pm 0.06	1.17 \pm 0.06	0.027	1.12 \pm 0.07	1.24 \pm 0.13	0.416	0.126	0.506
Mass-specific \dot{V}_{O_2} (ml O ₂ g ⁻¹ h ⁻¹)	1.59 \pm 0.09	1.75 \pm 0.10	0.248	1.86 \pm 0.11	1.94 \pm 0.22	0.72	0.169	0.262
Respiratory exchange ratio	0.77 \pm 0.01	0.79 \pm 0.02	0.301	0.79 \pm 0.03	0.82 \pm 0.03	0.521	0.377	0.382
Total cutaneous EWL (mg min ⁻¹)	0.74 \pm 0.05	1.31 \pm 0.14	<0.001	1.18 \pm 0.12	1.49 \pm 0.12	0.077	0.013	0.351
Total ventilatory EWL (mg min ⁻¹)	0.578 \pm 0.048	0.799 \pm 0.125	0.093	0.475 \pm 0.070	0.720 \pm 0.120	0.081	0.226	0.827
Total EWL (mg min ⁻¹)	1.32 \pm 0.08	2.11 \pm 0.22	0.002	1.66 \pm 0.14	2.21 \pm 0.18	0.023	0.066	0.629
Urine concentration (mosmol kg ⁻¹)	4516 \pm 194	4323 \pm 231	0.527	3380 \pm 243	3780 \pm 410	0.383	0.009	0.362
Mass (g)	36.92 \pm 1.03	40.81 \pm 0.98	0.014	35.93 \pm 1.18	38.45 \pm 1.12	0.140	0.401	0.043

Values and means \pm S.E.M.

P-values are from ANOVAs testing for significant differences between animals from the two locations both before and after acclimation or from paired *t*-tests testing for significant changes in individuals from each site after acclimation.

EWL, evaporative water loss.

Significant *P* values are given in bold type.

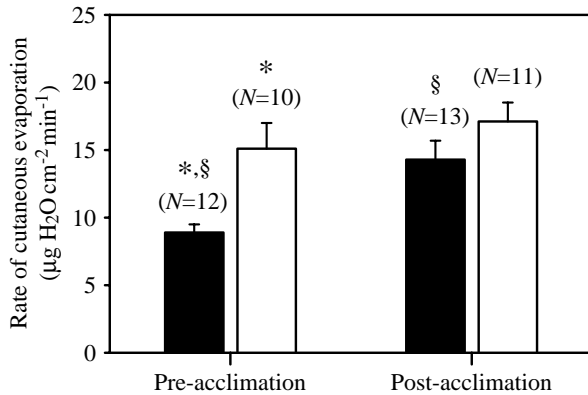


Fig. 3. Surface area-specific rates of cutaneous evaporative water loss (CEWL) for xeric-site (filled columns) and mesic-site (open columns) animals. Values are means + S.E.M. Significant differences between individuals from the two sites are identified by *, while differences due to acclimation for xeric-site animals are shown with §.

before acclimation, and surface-area-specific CEWL for mesic-site animals was 70% greater than that for xeric-site animals. Total CEWL and surface-area-specific CEWL did not differ significantly between sites after acclimation (Table 2; Fig. 3). Correspondingly, total CEWL increased by 59% and surface-area-specific CEWL increased by 61% for xeric-site animals after acclimation, yet remained unchanged after acclimation for mesic animals (Table 2). The fractional contribution of total CEWL to total EWL increased by 15% after acclimation for xeric-site animals (see Fig. 5).

Ventilatory evaporative water loss

Ventilatory evaporative water loss (VEWL) did not differ between animals from the two sites at either acclimation state (Table 2; Fig. 4). While total VEWL did not change after acclimation for xeric-site animals, metabolism-specific VEWL decreased by 32% after acclimation (Fig. 4). Both total VEWL and metabolism-specific VEWL for mesic-site animals remained unchanged after acclimation. The fractional contribution of total VEWL to total EWL decreased by 15% after acclimation in xeric-site animals as a result of increased fractional CEWL (Fig. 5), but remained unchanged for mesic-site animals.

Total evaporative water loss

Total evaporative water loss (EWL) and mass-specific total EWL were significantly greater for mesic-site animals than for xeric-site animals before and after acclimation (Table 2; Figs 5, 6). Mesic-site animals manifested a 60% greater EWL and a 45% greater mass-specific EWL than xeric-site animals before acclimation. Despite acclimation and the increases in evaporative water loss from xeric-site animals, mesic-site animals still possessed a 33% greater total EWL and a 25% greater mass-specific EWL than xeric-site animals. Mass-specific EWL increased by 27% for xeric site animals after acclimation (Table 2).

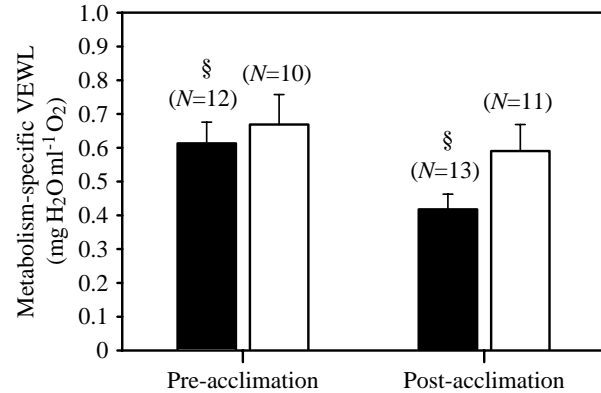


Fig. 4. Metabolism-specific rates of ventilatory evaporative water loss (VEWL) for xeric-site (filled columns) and mesic-site (open columns) animals. Values are means + S.E.M. Significant differences due to acclimation for xeric-site animals are shown with §.

Urine concentration

Urine osmolality did not differ between animals from the xeric and mesic sites before or after acclimation (Table 2). Urine osmolality also did not change for mesic-site animals after acclimation. However, urine concentration of xeric-site animals decreased after acclimation by 25% (Table 2).

Discussion

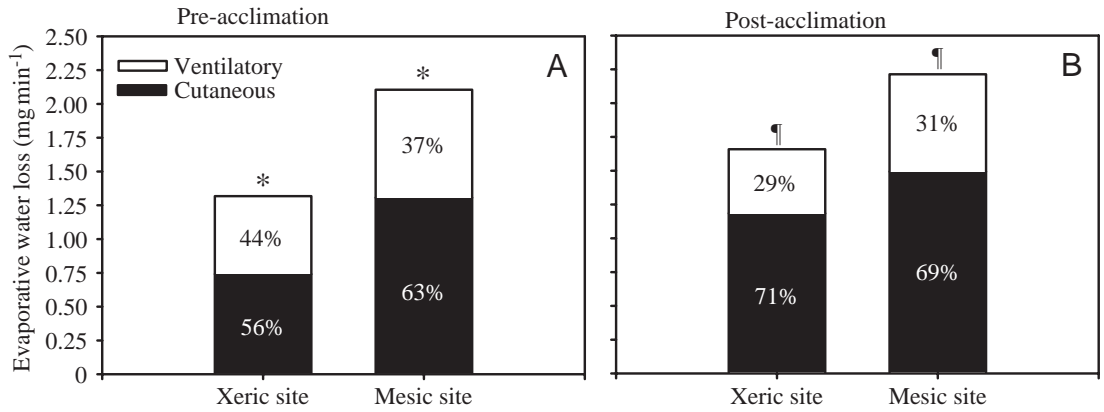
In contrast to previous suggestions that cutaneous evaporation is an insignificant source of water loss in kangaroo rats (Schmidt-Nielsen and Schmidt-Nielsen, 1950), our results clearly demonstrate that cutaneous water loss is the major avenue of evaporative water loss in this species. Given the overall importance of evaporation to water balance in kangaroo rats, therefore, cutaneous evaporation presents the single largest source of loss to these animals.

Acclimation (Withers, 1992) represents a special type of phenotypic plasticity describing the ability of an organism to make variable responses after 'short-term' exposure to different conditions in the laboratory, while acclimatization refers to the same ability in response to altered environments in nature. Our results also do not support the hypothesis that acclimation alone accounts for the differences in evaporative water loss apparent within this subspecies. Although there was a limited effect of acclimation on the level of evaporative losses on individuals from these two sites, these individuals still maintained distinct evaporative losses.

Surface area

The lack of geographic differences in skin surface areas in this subspecies is paradoxical. Wild mesic-site animals are both heavier and skeletally larger than xeric-site animals (this study; R. L. Tracy and G. E. Walsberg, in preparation). Even when excluding tail surface areas and reducing analyses only to central areas of CEWL, no differences were found. Although care was taken when removing the skins from recently killed animals, it is still possible that error introduced

Fig. 5. Mean relative contributions of cutaneous and ventilatory evaporative water loss (EWL) to total EWL for xeric- and mesic-site animals. Significant differences between animals from the two locations are represented by * before acclimation and by ¶ after acclimation. S.E.M. values for pre-acclimation xeric- and mesic-site animals are 2 and 3 %, respectively. S.E.M. values for post-acclimation xeric- and mesic-site animals are 3 and 4 %, respectively.



while removing the skins masked any differences that exist between these two groups of kangaroo rats. To address this issue, we used an empirically derived equation representing the relationship between mass (M in g) and surface area (A in cm^2) from xeric-site animals ($A=7.63M^{2/3}$) and applied it to mesic-site animals. The constant for this equation ($7.63\text{cm}^2\text{g}^{-2/3}$) was determined by averaging values calculated for individual xeric-site animals using each one's mass and measured surface area ($N=13$). Our measured surface area values for the mesic-site animals deviated, on average, by only 5.6% from those estimated using this empirically derived equation. Using these values to calculate surface-area-specific rates of CEWL did not yield significant differences between sites. The empirically derived coefficient for a similar equation for the mesic-site animals ($7.75\text{cm}^2\text{g}^{-2/3}$; $N=11$) differed by less than 2% from the coefficient for the equation for the xeric-site animals. Therefore, it appears that skins from one group of animals were not unduly stretched more than those from the other group and that any errors associated with measurement of surface area rested in the same direction for each group.

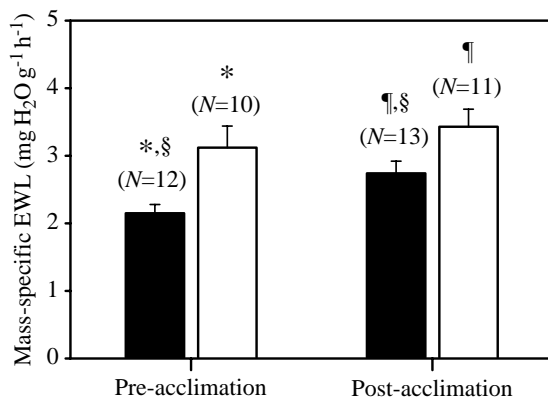


Fig. 6. Mass-specific rates of total evaporative water loss (EWL) for *Dipodomys merriami*. Values are means + S.E.M. Significant differences between animals from the two sites are represented by * before acclimation and by ¶ after acclimation, and differences in xeric-site animals as a result of acclimation are represented by §. Filled columns, xeric site; open columns, mesic site.

Evaporative water loss

The increase in cutaneous evaporation among xeric-site animals after acclimation, combined with their ability to maintain body mass, suggests a more positive water balance among these animals. Maintenance of body mass may reflect comparatively mild housing conditions. Similarly, the increased CEWL may represent relaxed cutaneous resistance during acclimation. In contrast, the mass loss of mesic-site animals during acclimation, combined with their invariant CEWL, suggests an inability to decrease CEWL to prevent overall water and mass loss through this route.

Different cutaneous patterns of circulation could at least partially account for the differences observed in cutaneous evaporation. Another possible mechanism is through changes in lipid distribution in the skin, as has been found in zebra finches *Poephila guttata* (Menon et al., 1989). Sebaceous glands, which may reduce water loss (Brylski, 1993), are larger and more active in desert heteromyids than in tropical heteromyids (Quay, 1965), and it is possible that similar differences may exist between xeric- and mesic-site animals. It is unlikely that the differences were due to different short-term stress responses among the two subpopulations because the testing condition (30°C) lies within each group's thermoneutral zone (R. L. Tracy and G. E. Walsberg, unpublished data).

Ventilatory evaporation contributed the least to water loss for animals from both sites and did not differ between the two sites before or after acclimation. Additive effects of slightly increased \dot{V}_{O_2} and slightly decreased total VEWL values for xeric-site animals (although not significant) appeared to contribute to the significant decrease in metabolism-specific VEWL after acclimation. Greater oxygen-extraction efficiencies at the lungs could also have accounted for the lower ventilatory water loss through reduced ventilation while concurrently maintaining similar metabolic rates. However, the decrease is of questionable significance because of the confounding effects of increased \dot{V}_{O_2} and the slightly decreased (although not significantly) total VEWL for xeric-site animals after acclimation.

Even when combining CEWL and VEWL and exploring differences in mass-specific rates of water loss, xeric-site animals exhibited lower rates than mesic-site animals. Despite acclimation and increases in CEWL, xeric-site animals still

possessed significantly lower total and mass-specific rates of evaporative water loss, illustrating that acclimation cannot fully account for the intraspecific differences in water loss between animals from the two sites. These findings parallel those of the study of MacMillen and Hinds (1998), which showed significant differences in total EWL between coastal and desert populations of California house finches (*Carpodacus mexicanus*), both on an *ad libitum* and a water-restricted regimen.

Urine production

The results from the urine-collection experiments strengthen the argument that there are fundamental differences between xeric- and mesic-site animals in their abilities to prevent desiccation. If urine concentration is an accurate index of urinary water loss, then it is clear that xeric-site animals are labile in their physiological responses to water loss. A more positive water balance in xeric-site animals after acclimation appears to be reflected in their lower urine osmolality. However, the urine concentration of mesic-site animals remained unchanged. If the mass losses evident in mesic-site animals after acclimation were at least partly due to water loss, then these mesic-site animals appear to be less able to reduce urine production by acclimation.

Metabolic water production

Metabolism is intimately tied to water balance because of its effects on water production and water loss. In addition to low metabolic rates being common among desert heteromyid rodents (Dawson, 1955; McNab, 1966), the aridity or the maximum temperature of a species' habitat is correlated with the reduction in that species' resting metabolic rate among species within this family (McNab, 1979). It is possible that this correlation also applies within a species and, thus, one would expect those individuals living in the most arid habitats to manifest the lowest resting metabolic rates. This does not appear to be the case within *D. m. merriami*. Although xeric-site animals possessed significantly lower \dot{V}_{O_2} and \dot{V}_{CO_2} values than mesic-site animals before acclimation, mass-specific \dot{V}_{O_2} and \dot{V}_{CO_2} did not differ between animals from the two sites. Differences in initial mass between xeric- and mesic-site animals probably account for differences in total \dot{V}_{O_2} and \dot{V}_{CO_2} . Animals trapped in the autumn from these two sites in a previous study also possessed indistinguishable mass-specific metabolic rates (R. L. Tracy and G. E. Walsberg, in preparation). Also, after acclimation, there were no differences in total \dot{V}_{O_2} or in total \dot{V}_{CO_2} between xeric- and mesic-site animals in the present study. There was, however, a significant increase in total \dot{V}_{CO_2} (but not in mass or in mass-specific \dot{V}_{O_2}) among xeric-site animals. Increases in metabolic rate should translate into differences in ventilatory water loss. However, total ventilatory and metabolism-specific ventilatory evaporative water loss did not differ for animals from either of the sites. Acclimation may play a role in metabolic adjustments in this species, yet it does not appear that large metabolic shifts for increasing metabolically produced water occurred in these experiments.

Preformed water input

In general, there is an inverse correlation between the reliance of heteromyid rodents on dietary water and the aridity of that species' habitat (French, 1993). Tropical heteromyids and *Dipodomys microps* survive only a few days without free water (Flemming, 1977), but the degree of seasonal dependence on free water for desert forms is unknown. Levels of preformed water in their diet may be variable, either seasonally or geographically. Therefore, one ultimate hypothesis to explain differences in water loss in *D. m. merriami* from variable environments is that geographic variation in water input accompanies and drives these physiological differences.

At the onset of this study, it was unclear how these animals would respond to the thermal and hydric conditions of housing. It was soon evident that, while the xeric-site animals fared well under these conditions, the mesic-site animals lost mass. This was presumably due to both direct and indirect results of constant and relatively high rates of evaporative water loss. Mesic-site animals may be exposed to milder conditions in the field. However, it is also possible that they may have a significant preformed water input in the form of green vegetation and insects that balances their apparently fixed and comparatively high evaporative water loss, that they maintain mass in the field and that selection does not act as strongly for decreases in evaporative water loss as in the xeric site. Intraspecific variation in preformed water input has yet to be determined for animals in the wild.

Concluding comments

Our results indicate that cutaneous loss is the major contributor to evaporative water loss. Because evaporation is the major route of water loss for *D. merriami*, cutaneous evaporation can therefore have a significant effect on their water balance as a whole. It is also clear that water loss varies within Merriam's kangaroo rat associated with contrasting habitats and that xeric-site animals show lower rates of water loss throughout the year. Even within a kangaroo rat subspecies, there is physiological variability consistent with environmental constraints. Finally, our results indicate that this variability cannot be attributed entirely to acclimation.

During the acclimation period, xeric- and mesic-site animals converged in mass and apparently maintained positive water balance. Although animals from both sites showed increases in evaporative water loss, they also showed some increases in \dot{V}_{O_2} and, therefore, metabolic water production. It appears that the xeric-site animals may be better able to offset evaporative water loss with metabolic water production, as reflected by their more dilute urine after acclimation. However, despite the subpopulations undergoing physiological acclimation of a similar magnitude and direction during the period of study, they maintained their subpopulational differences.

Investigating whether acclimation contributes to the survival of these animals in such a harsh environment yields information critical in determining the time course for basic evolutionary processes by identifying the degree to which this

species is physiologically labile with respect to water loss. If individuals from these different locations had converged on indistinguishable capacities to resist desiccation after acclimation, then this acclimational response could have explained the differences observed between locations immediately after capture. Because acclimation does not account for the differences in the major route of water loss in this subspecies, there must also exist fixed differences in their ability to resist desiccation. These could be the result of developmental plasticity, fixed genetic differences or a combination of the two. In addition to evaporative water loss, other avenues of water loss have been shown to be developmentally determined in some rodent species (Blount and Blount, 1968; Hewitt, 1981; Buffenstein and Jarvis, 1985). Future experiments will determine the extent to which conditions of development are formative in the ability of individuals of this species to resist desiccation. This should allow better determinations of the time course of adaptive change to climatic shifts and the evolution of such responses.

We thank A. Borek, T. C. M. Hoffman and K. M. Wooden for their assistance with animal trapping and husbandry. We would also like to thank J. F. Harrison, J. R. Hazel, F. H. Pough and A. T. Smith for their comments relating to this study. Trapping was enabled by Arizona Game and Fish Scientific Collecting Permit SP785298, and all studies were conducted in compliance with ASU's Institutional Animal Care and Use Committee Protocols 96-367R and 98-417R. All experiments complied with the legal requirements of the state of Arizona, the USA and the UK. Funding was assisted by the Research Support Program at Associated Students of Arizona State University, the Graduate College and Vice President for Research (grant 97-410) to R.L.T. and NSF grant IBN 9725211 to G.E.W.

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