

## Partitioning of evaporative water loss in white-winged doves: plasticity in response to short-term thermal acclimation

Andrew E. McKechnie\* and Blair O. Wolf

UNM Biology Department, MSC03-2020, 1 University of New Mexico, Albuquerque, NM 87131-0001, USA

\*Author for correspondence (e-mail: aemckech@unm.edu)

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### Summary

We investigated changes in the relative contributions of respiratory evaporative water loss (REWL) and cutaneous evaporative water loss (CEWL) to total evaporative water loss (TEWL) in response to short-term thermal acclimation in western white-winged doves *Zenaida asiatica mearnsii*. We measured REWL, CEWL, oxygen consumption and carbon dioxide production in a partitioned chamber using flow-through respirometry. In doves housed for 2–4 weeks in a room heated to *ca.* 43°C during the day, TEWL increased from  $5.5 \pm 1.3 \text{ mg g}^{-1} \text{ h}^{-1}$  at an air temperature ( $T_a$ ) of 35°C to  $19.3 \pm 2.5 \text{ mg g}^{-1} \text{ h}^{-1}$  at  $T_a=45^\circ\text{C}$ . In doves housed at room temperature for the same period, TEWL increased from  $4.6 \pm 1.1 \text{ mg g}^{-1} \text{ h}^{-1}$  at  $T_a=35^\circ\text{C}$  to  $16.1 \pm 4.6 \text{ mg g}^{-1} \text{ h}^{-1}$  at  $T_a=45^\circ\text{C}$ . The CEWL of heat-acclimated doves increased from  $3.6 \pm 1.2 \text{ mg g}^{-1} \text{ h}^{-1}$  (64% of TEWL) at 35°C to  $15.0 \pm 2.1 \text{ mg g}^{-1} \text{ h}^{-1}$  (78% of TEWL) at  $T_a=45^\circ\text{C}$ . Cool-acclimated doves exhibited more modest increases in CEWL, from  $2.7 \pm 0.7 \text{ mg g}^{-1} \text{ h}^{-1}$  at  $T_a=35^\circ\text{C}$  to  $7.8 \pm 3.4 \text{ mg g}^{-1} \text{ h}^{-1}$  at  $T_a=45^\circ\text{C}$ , with the contribution of CEWL to TEWL averaging 53% over

this  $T_a$  range. Cool-acclimated doves became mildly hyperthermic (body temperature  $T_b=42.9 \pm 0.4^\circ\text{C}$ ) and expended 35% more energy relative to heat-acclimated doves ( $T_b=41.9 \pm 0.6^\circ\text{C}$ ) at  $T_a=45^\circ\text{C}$ , even though TEWL in the two groups was similar. In each of the two groups, metabolic rate did not vary with  $T_a$ , and averaged  $7.1 \pm 0.5 \text{ mW g}^{-1}$  in cool-acclimated doves and  $6.3 \pm 0.8 \text{ mW g}^{-1}$  in heat-acclimated doves. The differences in TEWL partitioning we observed between the two experimental groups resulted from a consistently lower skin water vapour diffusion resistance ( $r_v$ ) in the heat-acclimated doves. At  $T_a=45^\circ\text{C}$ ,  $r_v$  in the cool-acclimated doves was  $120 \pm 81 \text{ s cm}^{-1}$ , whereas  $r_v$  in the heat-acclimated doves was  $38 \pm 8 \text{ s cm}^{-1}$ . Our data reveal that in *Z. a. mearnsii*, TEWL partitioning varies in response to short-term thermal acclimation.

Key words: acclimation, cutaneous evaporative water loss, respiratory evaporative water loss, water vapour diffusion resistance, thermoregulation, *Zenaida asiatica mearnsii*.

### Introduction

The rates at which terrestrial animals lose water through evaporation across the skin and respiratory surfaces have important consequences for their water balance, thermoregulation and survival. Because of the desiccating nature of terrestrial environments, adaptation to a terrestrial existence has required the minimization of evaporative water losses. In response to these demands, animals have evolved a number of structures and mechanisms, such as nasal turbinates and epidermal water-proofing, to moderate rates of evaporation (Gordon and Olson, 1995). For birds and other endotherms living in hot environments, however, evaporative water loss becomes an essential means of heat transfer when environmental temperatures exceed body temperature (Dawson and Whittow, 2000). Under these conditions, a conflict thus arises between the need to conserve water and maintain an adequate state of hydration, and the requirement to maintain body temperature below critical limits through increased evaporative water losses. In this study we focus on the relative importance of respiratory (REWL) and cutaneous

(CEWL) evaporative water losses to avian thermoregulation and how they are modified with changing environmental conditions through time.

Current data provide limited insight into the variation in REWL and CEWL associated with phylogenetic position, environmental temperature and acclimation period. In most avian species investigated to date, CEWL has been found to represent at least 50% of total evaporative water loss (TEWL) at moderate air temperatures ( $T_a$ ) (Bernstein, 1971; Lasiewski et al., 1971; Dawson, 1982; Webster and Bernstein, 1987; Webster and King, 1987; Wolf and Walsberg, 1996; Tieleman and Williams, 2002). Considerably less attention has been focused on the relative importance of respiratory and cutaneous evaporative heat loss in response to high air temperatures ( $T_a \geq 35^\circ\text{C}$ ). The limited data available on the relative contributions of CEWL and REWL to TEWL at high  $T_a$  values reveal considerable variation among taxa (Wolf and Walsberg, 1996). For instance, in the few members of the Columbiformes (pigeons and doves) examined, CEWL represented more than

40% of TEWL at  $35^{\circ}\text{C} \leq T_a \leq 45^{\circ}\text{C}$  (Webster and King, 1987; Withers and Williams, 1990; Hoffman and Walsberg, 1999). In contrast, in passerine birds the relative contribution of cutaneous evaporation decreases with increasing  $T_a$  (Dawson, 1982; Wolf and Walsberg, 1996; Tieleman and Williams, 2002). In verdins (*Auriparus flaviceps*: Passeriformes: Remizidae), for example, the contribution of CEWL decreased from ca. 60% of TEWL at  $T_a=35^{\circ}\text{C}$  to ca. 15% at  $T_a=50^{\circ}\text{C}$  (Wolf and Walsberg, 1996). Tieleman and Williams (2002) observed similar decreases in the relative contribution of CEWL with increasing  $T_a$  in four species of larks (Passeriformes: Alaudidae).

An understanding of factors other than taxonomic affiliation that influence patterns of TEWL partitioning is critical for understanding avian thermoregulation and water balance at high environmental temperatures. Of particular interest are potentially adaptive changes in CEWL in response to acclimatization, acclimation and/or short-term changes in evaporative cooling requirements. Tieleman and Williams (2002) found little evidence for changes in CEWL/TEWL of larks following thermal acclimation. However, other researchers have found large changes in the role of cutaneous evaporation with acclimation. Adult rock doves acclimated to high  $T_a$  values from hatching exhibited elevated rates of CEWL compared to non-acclimated doves, and dissipated virtually their entire heat load cutaneously at  $T_a=60^{\circ}\text{C}$  (Marder and Arieli, 1988; Ophir et al., 2002). Evidence that CEWL can be adjusted over much shorter time scales is provided by Hoffman and Walsberg (1999), who found that mourning doves (*Zenaida macroura*) responded within minutes to experimental inhibition of REWL by increasing CEWL by 72% at  $T_a=35^{\circ}\text{C}$ . Nevertheless, the periods over which changes in CEWL associated with thermal acclimation occur remain relatively unknown.

We investigated the effect of short-term (days to weeks) thermal acclimation on the partitioning of TEWL in western white-winged doves (*Zenaida asiatica mearnsii*). We also examined the interactions between modes of evaporative water loss, body temperature, skin temperature and metabolic rate, in an attempt to provide an integrative view of how heat-acclimated and non-heat-acclimated doves respond to high levels of heat stress. We chose white-winged doves for this study because they winter in southern Mexico under moderate environmental conditions, but breed during the summer in the Sonoran Desert of the southwestern United States and northwestern Mexico, where they frequently experience environmental temperatures approaching or exceeding  $50^{\circ}\text{C}$  (B. O. Wolf and A. E. McKechnie, personal observation).

## Materials and methods

### Study animals

Fourteen western white-winged doves *Zenaida asiatica mearnsii* L. were trapped using mist nets during July 2002 in the Sand Tanks Mountains on the US Air Force Barry M. Goldwater Bombing Range ( $32^{\circ}49'\text{N}$ ;  $112^{\circ}26'\text{W}$ ) in southern

Arizona, USA. After capture, the doves were transported by road to the Department of Biology, University of New Mexico, Albuquerque, USA. They were housed in an outdoor aviary with *ad libitum* access to wild bird seed, grit and drinking water from mid-July 2002 until late March 2003. Thereafter, they were transferred indoors, with seven doves housed in each of two identical rooms (1.8 m long  $\times$  1.1 m wide  $\times$  2.4 m high). Each bird occupied a separate cage (0.39 m long  $\times$  0.23 m wide  $\times$  0.28 m high) and was provided with food and water *ad libitum*. The photoperiod in the rooms (13 h:11 h L:D) approximately matched the outdoor photoperiod. Air temperature in each room was measured using a Stowaway XTI Temperature Logger (Onset Computer Corporation, Bourne, MA, USA). Beginning 4 days after the doves were transferred indoors, the day-time air temperature in one of the rooms ('hot room') was elevated to  $42.6 \pm 0.1^{\circ}\text{C}$  (mean  $\pm$  95% confidence interval) using three commercially available fan heaters. The air temperature in the other room ('cool room') and in the hot room at night, averaged  $21.7 \pm 0.1^{\circ}\text{C}$ . Hereafter, we refer to the doves housed in the cool and hot rooms as 'cool-acclimated' and 'heat-acclimated', respectively. Measurements of evaporative water loss were conducted during an 11-day period in April 2003. The duration of thermal acclimation to the conditions in the rooms varied from 17–28 days at the time of experiments. The mean body mass of the doves at the time of the experiments was  $142.2 \pm 7.4$  g and did not differ among treatment groups.

### Measurements of evaporative water loss, $O_2$ consumption and $CO_2$ production

We measured the respiratory and cutaneous components of evaporative water loss in a 14.6 l metabolic chamber, constructed from 5 mm-thick glass. During measurements, the bird perched on a stainless steel wire mesh screen placed at a height that allowed for normal perching postures. Any faeces produced fell through the mesh into a 2 cm layer of mineral oil, preventing evaporation. REWL and CEWL were partitioned using a chamber that was divided into upper (4.3 l) and lower (10.3 l) compartments by a 1 mm-thick aluminum partition. A 80 mm  $\times$  120 mm rectangle was cut from the partition, and a piece of 0.25 mm-thick stretchable latex membrane (Semantodontics Dental Dam, Phoenix, AZ, USA) secured over the opening using an aluminum frame bolted to the partition. The bird's head and neck protruded through a hole punched through the membrane. The diameter of the hole was slightly less than the diameter of the bird's neck, and provided a snug fit. An illustration of a similar chamber may be found in Wolf and Walsberg (1996). Tieleman and Williams (2002) suggested that the flow-through mask system they used to measure REWL and CEWL in four species of lark was an improvement over the partitioned chamber system used by previous investigators and in the present study, on the grounds that cutaneous losses from the head and neck are included in the respiratory component measured in the upper compartment. However, this source of error is small and can be minimized by making corrections to REWL and CEWL measurements

based on the surface area of the head and neck that protrude into the upper chamber (Wolf and Walsberg, 1996; see below).

The experimental air temperature ( $T_a$ ) was controlled by placing the metabolism chamber in an insulated 200 l environmental chamber. The  $T_a$  within the environmental chamber was regulated using a temperature-controlled circulator (Model 1187, VWR Scientific Products, West Chester, PA, USA), which pumped fluid through copper tubing. Air within the environmental chamber was mixed using a small electric fan.  $T_a$  within the metabolism chamber was measured using a 21-gauge Cu–Cn thermocouple and a TC-1000 thermocouple meter (Sable Systems, Las Vegas, NV, USA).

Dry, CO<sub>2</sub>-free air (dewpoint =  $-73^\circ\text{C}$ ) produced using a FT-IR Purge Gas Generator (Whatman, Newton, MA, USA) flowed through the chamber at flow rates of 6–10 l min<sup>-1</sup>, chosen to maintain dewpoints below 1°C in the chamber during all measurements. Flow rates into the lower compartment of the chamber were regulated using a FMA-series mass flow controller (Omega, Bridgeport, NJ, USA), and flow rates into the upper chamber by a FMA-series mass flow controller and model 246B mass flow controller (MKS Instruments, Andover, MA, USA) plumbed in parallel. The mass flow controllers were calibrated using a 1 l soap bubble flowmeter (Baker and Pouchot, 1983). Because the partition between the upper and lower compartments was not completely airtight, we controlled flow rates to the upper and lower compartments so as to ensure that no pressure gradient existed between them. During all measurements, we maintained CO<sub>2</sub> concentrations of <20 p.p.m. in the lower compartment, indicating that leakage between the compartments was negligible.

Dry, CO<sub>2</sub>-free control air and excurrent air from the upper and lower compartments of the metabolism chamber were sequentially sampled using a TR-RM8 Respirometer Multiplexer (Sable Systems). The partial pressure of water vapour and carbon dioxide concentration in sampled air was measured using a LI-7000 CO<sub>2</sub>/H<sub>2</sub>O analyzer (Li-Cor, Lincoln, NE, USA), calibrated daily using dry, CO<sub>2</sub>-free air and a span gas for water vapour generated using a LI-610 portable dew point generator (Li-Cor) and a certified span gas containing 1020.0 p.p.m. CO<sub>2</sub> (Matheson Tri-Gas, Houston, TX, USA). Dewpoint temperatures of the air samples were well below those of the ambient environment, ensuring that no condensation occurred within the sampling system. After the sample left the LI-7000 CO<sub>2</sub>/H<sub>2</sub>O analyzer, water vapour and CO<sub>2</sub> were scrubbed using a Drierite® and Ascarite® column. The fractional O<sub>2</sub> concentration was then determined using a FC-1B oxygen analyzer (Sable Systems). Output from the CO<sub>2</sub>/H<sub>2</sub>O and oxygen analyzers, and the thermocouple meter, was digitized using a Universal Interface II (Sable Systems) and recorded on a personal computer using Datacan V data acquisition software (Sable Systems), with a sampling interval of 1 s.

#### Measurements of skin and body temperature

Body temperature ( $T_b$ ) was measured by inserting a 36-

gauge Cu–Cn Teflon-coated thermocouple (Physitemp, Clifton, NJ, USA) approximately 1.5 cm into the cloaca, at which depth a slight withdrawal did not result in a decrease in the  $T_b$  reading. The thermocouple wire was secured to the feathers immediately behind the cloaca by a small piece of adhesive tape. Skin temperature ( $T_{\text{skin}}$ ) was measured dorsally, by attaching a similar thermocouple to the skin between the scapulars using cyanoacrylic adhesive.

#### Experimental protocol

REWL, CEWL, oxygen consumption ( $\dot{V}_{\text{O}_2}$ ) and carbon dioxide production ( $\dot{V}_{\text{CO}_2}$ ) were measured at  $T_a=34.8\pm 0.5$ ,  $40.0\pm 0.5$  and  $45.9\pm 0.4^\circ\text{C}$  (means  $\pm$  95% confidence intervals). For convenience, we refer to these experimental  $T_a$  values as 35, 40 and 45°C, respectively. Measurements at each  $T_a$  were carried out during separate 2–3 day periods. At each experimental  $T_a$ , measurements were made in five cool-acclimated and five heat-acclimated doves, in random order. All measurements were made in darkness on fed doves, during the active-phase of their circadian cycle. Typically, each set of measurements lasted 2 h, and data recorded during the first 30 min of each run were not included in the analyses. In a few cases, cool-acclimated doves exhibited rapid, apparently uncontrolled increases in  $T_b$  at  $T_a>45^\circ\text{C}$ , and in such cases we removed the bird prior to the end of the 2 h period. For each bird, the interval between each set of measurements was at least 3 days.

Because REWL and CEWL were measured sequentially, there was a time lag of 5 min between the respective measurements. However, 99% equilibrium times for the two compartments, calculated using the relevant equation in Lasiewski et al. (1966), were 7–14 min. Since the equilibrium times (and hence the periods over which gas measurements were integrated) were greater than the time lag between the REWL and CEWL measurements, the data are essentially equivalent to simultaneous measurements. Moreover, we only used EWL data that were stable during the entire measurement period.

#### Data analysis

We calculated REWL and CEWL from the water vapour partial pressure in excurrent air from the upper and lower compartments respectively. We corrected these measurements for the surface area of the head and neck in the upper compartment by subtracting a surface-area specific estimate of CEWL from the head and neck from the measured REWL, following Wolf and Walsberg (1996). The surface area of the head and neck in the upper compartment averaged 2.5% of the total skin surface area, estimated from the equation provided by Walsberg and King (1978). We calculated whole-body water vapour diffusion resistance ( $r_v$ ) as  $r_v=(\rho'_{v(T_{\text{skin}})}-\rho_{va})/\text{CEWL}$ , where  $\rho'_{v(T_{\text{skin}})}$  is the saturation water vapour density at skin temperature ( $\text{g cm}^{-3}$ ),  $\rho_{va}$  is the water vapour density of the air in the metabolism chamber ( $\text{g cm}^{-3}$ ), and CEWL is surface area-specific cutaneous evaporative water loss ( $\text{g cm}^{-2} \text{s}^{-1}$ ) (Webster et al., 1985). Although the effective

evaporative surface area could conceivably have varied slightly through postural adjustments, we assumed that it remained constant during all measurements. We also assumed that the dorsal  $T_{\text{skin}}$  measurements were representative of the entire skin surface. During the experiments, the water vapour partial pressure in the chamber varied from 0.097 kPa to 0.616 kPa, corresponding to dewpoints of  $-22.9^{\circ}\text{C}$  and  $0.1^{\circ}\text{C}$ . It is unlikely that these low levels of ambient humidity affected any properties of the skin that potentially determine  $r_v$ , such as the hydration state of surface skin layers.

$\dot{V}_{\text{O}_2}$  was calculated using the relevant equation in Withers (1977) and  $\dot{V}_{\text{CO}_2}$  using equation 3 in Walsberg and Wolf (1995). Respiratory exchange ratios were calculated as  $\dot{V}_{\text{CO}_2}/\dot{V}_{\text{O}_2}$  and gas exchange measurements were converted to metabolic rates using thermal equivalence data from table 4-2 in Withers (1992). This approach assumes that only carbohydrates and lipids are metabolized, and a maximum error of 6% is associated with protein metabolism (Walsberg and Wolf, 1995).

We subjectively examined all data, and discarded any traces of gas exchange and/or EWL that were not stable. For each bird at each  $T_a$ , we used the lowest value recorded over 1 min, representing a mean of 60 measurements. All values are presented as means  $\pm$  95% confidence intervals. We compared data using t-tests and repeated-measures analyses of variance (RM-ANOVA; Zar, 1999). Unless otherwise stated, the sample size for each experimental group was five doves.

## Results

### Evaporative water loss

The TEWL of cool-acclimated doves increased from  $4.6 \pm 1.1 \text{ mg g}^{-1} \text{ h}^{-1}$  at  $T_a=35^{\circ}\text{C}$  to  $16.1 \pm 4.6 \text{ mg g}^{-1} \text{ h}^{-1}$  at  $T_a=45^{\circ}\text{C}$ , and the TEWL of heat-acclimated doves increased from  $5.5 \pm 1.3 \text{ mg g}^{-1} \text{ h}^{-1}$  at  $T_a=35^{\circ}\text{C}$  to  $19.3 \pm 2.5 \text{ mg g}^{-1} \text{ h}^{-1}$  at  $T_a=45^{\circ}\text{C}$ . The TEWL of heat-acclimated doves was significantly higher than that of cool-acclimated doves at  $T_a=40^{\circ}\text{C}$  ( $t=4.592$ ;  $P<0.001$ ), but not at  $T_a=35^{\circ}\text{C}$  or  $45^{\circ}\text{C}$  (Fig. 1A).

The REWL of heat-acclimated doves increased from  $1.9 \pm 0.2 \text{ mg g}^{-1} \text{ h}^{-1}$  at  $T_a=35^{\circ}\text{C}$  to  $4.3 \pm 0.6 \text{ mg g}^{-1} \text{ h}^{-1}$  at  $T_a=45^{\circ}\text{C}$ , whereas REWL in cool-acclimated doves increased from  $1.9 \pm 0.5 \text{ mg g}^{-1} \text{ h}^{-1}$  at  $T_a=35^{\circ}\text{C}$  to  $8.3 \pm 1.9 \text{ mg g}^{-1} \text{ h}^{-1}$  at  $T_a=45^{\circ}\text{C}$  (Fig. 1B). REWL did not differ between the two groups at  $T_a=35^{\circ}\text{C}$  or  $40^{\circ}\text{C}$ , but was significantly higher in cool-acclimated doves at  $T_a=45^{\circ}\text{C}$  ( $t=3.951$ ;  $P=0.002$ ).

The CEWL of heat-acclimated doves increased from  $3.6 \pm 1.2 \text{ mg g}^{-1} \text{ h}^{-1}$  (64% of TEWL) at  $T_a=35^{\circ}\text{C}$  to  $15.0 \pm 2.1 \text{ mg g}^{-1} \text{ h}^{-1}$  (78% of TEWL) at  $T_a=45^{\circ}\text{C}$ , and the CEWL of cool-acclimated doves increased from  $2.7 \pm 0.7 \text{ mg g}^{-1} \text{ h}^{-1}$  at  $T_a=35^{\circ}\text{C}$  to  $7.8 \pm 3.4 \text{ mg g}^{-1} \text{ h}^{-1}$  at  $T_a=45^{\circ}\text{C}$ . CEWL in heat-acclimated doves was significantly higher than that of cool-acclimated doves at  $T_a=40^{\circ}\text{C}$  ( $t=6.337$ ;  $P<0.001$ ) and at  $T_a=45^{\circ}\text{C}$  ( $t=3.517$ ;  $P=0.003$ ), but not at  $T_a=35^{\circ}\text{C}$  (Fig. 1C). At  $T_a=35^{\circ}\text{C}$ ,  $40^{\circ}\text{C}$  and  $45^{\circ}\text{C}$ , the mean CEWL in heat-acclimated doves was equivalent to 136%,

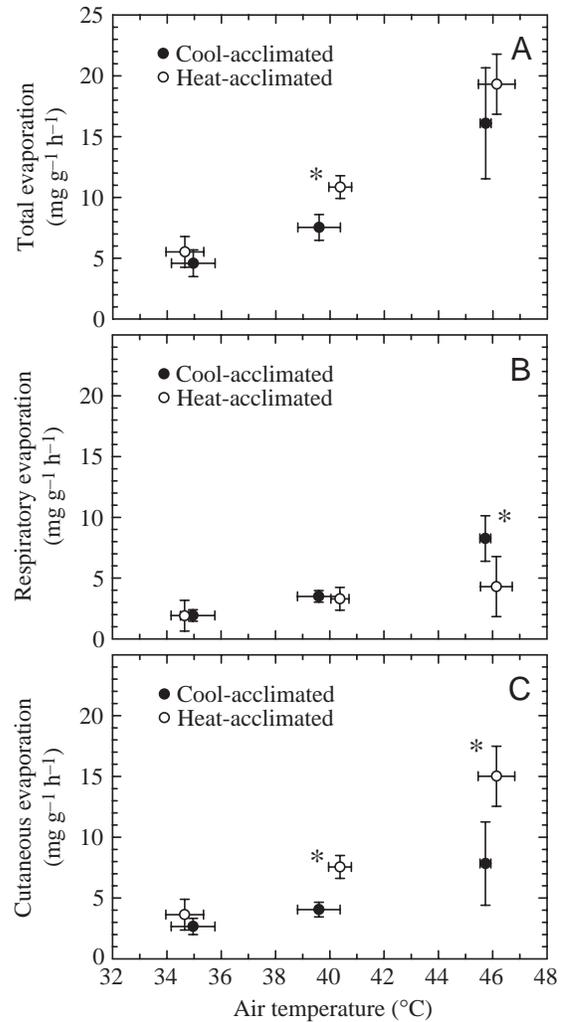


Fig. 1. Mean total evaporative water loss (A), respiratory evaporative water loss (B) and cutaneous evaporative water loss (C) ( $\text{mg H}_2\text{O g}^{-1} \text{ body mass h}^{-1}$ ) as a function of air temperature in cool- and heat-acclimated western white-winged doves *Zenaida asiatica mearnsii*. The error bars represent 95% confidence intervals. \*Significant difference between values ( $P<0.05$ ).

187% and 192%, respectively, of the corresponding rates in cool-acclimated doves (Fig. 1C). Surface area-specific CEWL increased from  $1.4 \pm 0.4 \text{ mg cm}^{-2} \text{ h}^{-1}$  at  $T_a=35^{\circ}\text{C}$  to  $4.1 \pm 1.7 \text{ mg cm}^{-2} \text{ h}^{-1}$  at  $T_a=45^{\circ}\text{C}$  in the cool-acclimated doves, and from  $1.9 \pm 0.6 \text{ mg cm}^{-2} \text{ h}^{-1}$  at  $T_a=35^{\circ}\text{C}$  to  $7.7 \pm 1.0 \text{ mg cm}^{-2} \text{ h}^{-1}$  at  $T_a=45^{\circ}\text{C}$  in the heat-acclimated doves.

The contribution of CEWL to TEWL did not vary significantly between the three experimental  $T_a$  values in cool-acclimated doves (RM-ANOVA,  $F_{2,4}=3.119$ ;  $P=0.100$ ), although CEWL/TEWL decreased slightly with increasing  $T_a$  (Fig. 2). In contrast, the contribution of CEWL increased significantly with increasing  $T_a$  in the heat-acclimated doves (RM-ANOVA,  $F_{2,4}=13.281$ ;  $P=0.003$ ) from  $64 \pm 7\%$  of TEWL at  $T_a=35^{\circ}\text{C}$  to  $78 \pm 2\%$  of TEWL at  $T_a=45^{\circ}\text{C}$  (Fig. 2). The contribution of CEWL to TEWL did not differ between the cool- and heat-acclimated groups at  $T_a=35^{\circ}\text{C}$  ( $t=1.614$ ;

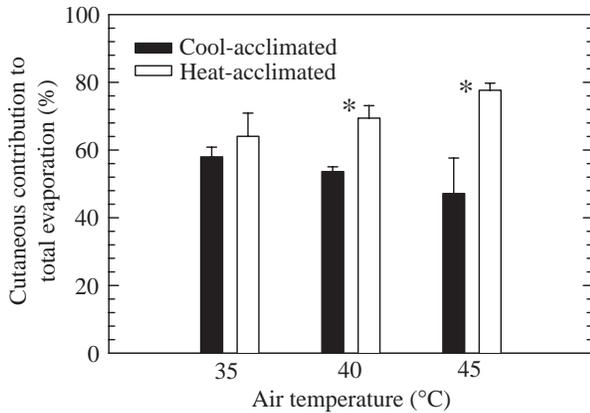


Fig. 2. The contribution of cutaneous evaporation to total evaporative water loss at three experimental air temperatures in cool- and heat-acclimated western white-winged doves *Zenaida asiatica mearnsii*. The error bars represent 95% confidence intervals. \*Significant difference between values ( $P < 0.001$ ).

$P = 0.09$ ), but was significantly greater in heat-acclimated doves at  $T_a = 40^\circ\text{C}$  ( $t = 7.833$ ;  $P < 0.001$ ) and  $T_a = 45^\circ\text{C}$  ( $t = 5.593$ ;  $P < 0.001$ ; Fig. 2).

#### Metabolic rate

Metabolic rates did not show significant variation between the three experimental  $T_a$  values in either the cool- (RM-ANOVA,  $F_{2,4} = 0.480$ ;  $P = 0.636$ ) or heat-acclimated groups (RM-ANOVA,  $F_{2,4} = 1.139$ ;  $P = 0.367$ ; Fig. 3). The mean metabolic rate for cool-acclimated doves was  $7.1 \pm 0.5 \text{ mW g}^{-1}$ , and the mean metabolic rate for heat-acclimated doves was  $6.3 \pm 0.8 \text{ mW g}^{-1}$ . At  $T_a = 45^\circ\text{C}$ , the mean metabolic rate of the cool-acclimated doves was significantly higher ( $t = 2.726$ ;  $P = 0.013$ ) than that of the heat-acclimated doves (Fig. 3).

#### Body temperature and skin temperature

Our sample sizes for  $T_b$  were in some cases smaller than

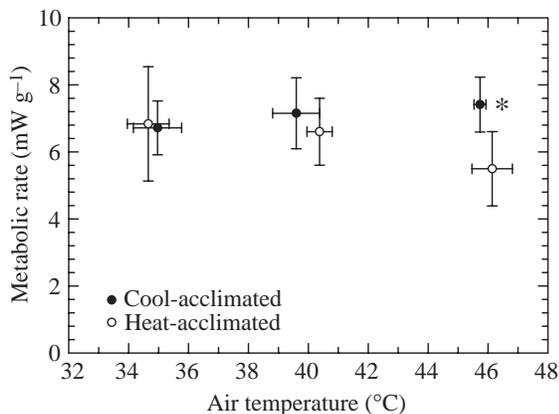


Fig. 3. Mean metabolic rate as a function of air temperature in cool- and heat-acclimated western white-winged doves *Zenaida asiatica mearnsii*. The error bars represent 95% confidence intervals. \*Significant difference between values ( $P < 0.05$ ).

Table 1. Cloacal body temperature and dorsal skin temperature at three air temperatures ( $T_a$ ) in cool-acclimated (Cool) and heat-acclimated (Heat) western white-winged doves *Zenaida asiatica mearnsii*

$T_a$ (°C)	Body temperature (°C)		Skin temperature (°C)	
	Cool	Heat	Cool	Heat
35	41.9±0.4 (5)		40.4±0.6 (4)	
40	43.0±0.1 (3)	42.3±0.3 (4)	40.8±0.8 (8)	
45	42.9±0.4 (4)	41.9±0.6 (5)	42.6±1.0 (8)	

Where no significant differences occurred between cool- and heat-acclimated doves, pooled values are given.

Values are means  $\pm$  95% confidence intervals;  $N$  is given in parentheses.

those for EWL and metabolic rate because doves sometimes initially struggled and dislodged the thermocouples shortly after being placed in the metabolism chamber. The mean  $T_b$  of cool-acclimated doves was significantly higher than that of the heat-acclimated doves (Table 1) at both  $T_a = 40^\circ\text{C}$  ( $t = 4.314$ ;  $P = 0.004$ ) and  $T_a = 45^\circ\text{C}$  ( $t = 2.537$ ;  $P = 0.019$ ). This observation is consistent with the doves' behavior upon removal from the metabolic chamber at the termination of the measurements at  $T_a = 45^\circ\text{C}$ . Whereas heat-acclimated doves showed no indication of panting or gular flutter, all five cool-acclimated doves exhibited pronounced panting behavior.

#### Whole-body water vapour diffusion resistance

In both the heat- and cool-acclimated doves,  $r_v$  decreased significantly with increasing  $T_a$  (Fig. 4). In the heat-acclimated doves, mean  $r_v$  decreased by 78% from  $172 \pm 78 \text{ s cm}^{-1}$  ( $N = 3$ ) at  $T_a = 35^\circ\text{C}$  to  $38 \pm 8 \text{ s cm}^{-1}$  ( $N = 5$ ) at  $T_a = 45^\circ\text{C}$ .

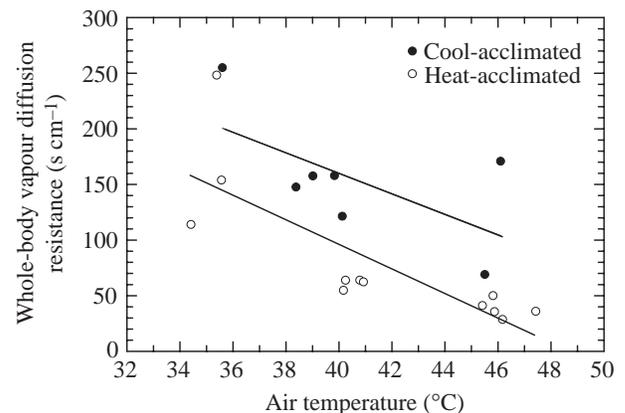


Fig. 4. Whole-body water vapour diffusion resistance  $r_v$  as a function of air temperature in cool- and heat-acclimated western white-winged doves *Zenaida asiatica mearnsii*. The solid lines are linear regressions fitted to the data. The regression for the heat-acclimated doves (lower line) is  $r_v = 536.59 - 11.01T_a$  ( $r^2 = 0.634$ ) and that for the cool-acclimated doves (upper line) is  $r_v = 529.06 - 9.22T_a$  ( $r^2 = 0.395$ ). The slopes of these regressions did not differ significantly.

### Discussion

#### *Plasticity in the partitioning of evaporative water losses*

Our results describe significant variation in cutaneous evaporation relative to total evaporation in response to short-term thermal acclimation in *Z. a. mearnsii*. Doves acclimated to high  $T_a$  for 2–4 weeks exhibited elevated rates of CEWL, and hence higher CEWL/TEWL ratios, compared to doves held in a cooler environment. Levels of CEWL also increased with increasing heat stress and varied significantly between cool- and heat-acclimated doves. CEWL in cool-acclimated doves increased approximately threefold between 35°C and 45°C, whereas CEWL in heat-acclimated doves increased fourfold over the same range of air temperatures. In cool-acclimated doves, the modest increases in cutaneous evaporation were accompanied by a fourfold increase in REWL. Heat-acclimated doves, in contrast, showed only a twofold increase in REWL. Although CEWL and REWL were partitioned very differently among treatments, rates of total evaporation among groups were essentially the same. These acclimation-associated differences in the partitioning of evaporative losses between the skin and respiratory tract were accompanied by differences in body temperature. At  $T_a=45^\circ\text{C}$ ,  $T_b$  in cool-acclimated doves was higher than in heat-acclimated doves, even though TEWL rates were nearly equal. In spite of these increases in  $T_b$ , cool-acclimated doves had difficulty compensating for their lower CEWL through increases in REWL. Our data suggest that increases in CEWL in response to short-term thermal acclimation have important thermoregulatory implications. Based on the observations above, we suggest that rapid acclimation to changing thermal environments is a critical factor in maintaining thermoregulatory competence at high environmental temperatures and is also important for water conservation. In *Z. a. mearnsii*, it appears that both the quantity of water lost through evaporation and the route by which evaporative water is lost importantly affects thermoregulation at high  $T_a$  values.

Another way that partitioning of evaporative losses affects thermoregulation and water balance is through the increased heat loads associated with panting and gular flutter. At  $T_a=45^\circ\text{C}$ , cool-acclimated doves had REWL rates that were approximately double those of heat-acclimated doves, and metabolic rates 35% greater than those of heat-acclimated doves, suggesting that a reliance on respiratory heat loss is more energetically costly than cutaneous heat loss. However, an analysis of the relative energetic costs and benefits of different patterns of evaporative water loss partitioning requires a complete understanding of the mechanisms responsible for adaptive decreases in skin water vapour diffusion resistance. Changes in the physical properties of the skin and/or the circulatory system may entail longer-term energetic costs associated with structural changes in the epidermis that are currently unquantified.

The  $T_b$  values we observed in heat-acclimated white-winged doves were similar to those reported by Marder and Arieli

(1988) for heat-acclimated rock doves (*Columba livia*) at 30°C >  $T_a$  > 60°C. At  $T_a=45^\circ\text{C}$ , the mean  $T_b$  of cool-acclimated white-winged doves (42.9±0.4°C) was slightly lower than that observed in non-acclimated *C. livia* (43.4±0.7°C; Marder and Arieli, 1988).

The increases that we observed in the CEWL of heat-acclimated doves primarily resulted from decreases in whole-body water vapour diffusion resistance (Fig. 4). Whole-body water vapour diffusion resistance  $r_v$  is the sum of skin ( $r_{vs}$ ), plumage ( $r_{vc}$ ) and boundary layer ( $r_{va}$ ) resistances. Of these resistances, however, the plumage and boundary layer comprise a small fraction of the total resistance, and that of the skin ( $r_{vs}$ ) comprises 75–94% of  $r_v$  (Webster et al., 1985). Hence, the lower  $r_v$  values that we observed in heat-acclimated doves primarily reflect changes in the resistance of the skin. Two broad categories of mechanisms can potentially lead to changes in skin resistance to water vapour diffusion (Webster et al., 1985). The first category includes structural changes affecting the permeability of the skin, and the second includes mechanisms affecting peripheral blood supply. The available evidence suggests that mechanisms from both these categories may be involved in increasing avian CEWL in response to thermal acclimation. Lipid bodies in the epidermis are the major determinant of skin permeability to water in vertebrates (Hadley, 1989) and several studies have demonstrated that acclimation-induced changes in avian CEWL correspond with changes in epidermal lipid structure and abundance (Menon et al., 1988, 1989, 1996). The epidermis of heat-acclimated rock doves included modified areas of greater thickness and different intracellular structure compared to non-acclimated and cold-acclimated birds (Peltonen et al., 1998). Recently, Haugen et al. (2003) attributed acclimation-induced variation in the CEWL of hoopoe larks (*Alaemon alaudipes*) to differences in the lipid composition of the epidermis. These authors found that CEWL was negatively correlated with the proportion of ceramides in the stratum corneum. Hoopoe larks acclimated to  $T_a=35^\circ\text{C}$  exhibited higher proportions of ceramides, lower proportions of free fatty acids and sterols, and reduced CEWL compared to larks acclimated to  $T_a=15^\circ\text{C}$  (Haugen et al., 2003).

Ultrastructural changes permit more rapid water vapour diffusion across the avian epidermis, but a second critical component of acclimation-induced increases in CEWL appears to be an increased supply of water to the epidermis. In rock doves, increased skin capillary hydrostatic pressure, rather than increased blood flow *per se*, appears to be an important driving force behind acclimation-induced elevated CEWL (Ophir et al., 2002). The inhibition of  $\beta$ -adrenergic receptors or the stimulation of  $\alpha_2$ -adrenergic receptors (by propranolol and clonidine administration, respectively) led to increased arterial blood flow and decreased venous blood flow in heat-acclimated doves, measured using laser Doppler flowmetry (Ophir et al., 2002). These authors argued that in heat-acclimated rock doves, increased hydrostatic pressure in the skin microvasculature is achieved by adrenergic control of arterial and venous blood flow, and results in elevated water outflow

from capillaries leading to elevated CEWL. These adjustments in epidermal water supply presumably occur over shorter time scales than structural changes in skin permeability, and may well be responsible for rapid adjustments in CEWL such as those reported by Hoffman and Walsberg (1999) in mourning doves. In the present study, we did not investigate the mechanism(s) responsible for increased CEWL in heat-acclimated white-winged doves. At best, we can speculate that similar mechanisms to those discussed above led to higher CEWL/TEWL. Our data do reveal, however, that the differences in skin resistance between cool- and heat-acclimated white-winged doves were approximately constant at all three experimental  $T_a$  values (Fig. 4).

#### *Ecological and evolutionary implications*

As in other Columbiforme species (Webster and King, 1987; Withers and Williams, 1990; Hoffman and Walsberg, 1999), a large proportion of TEWL in *Z. a. mearnsii* occurred cutaneously. The CEWL/TEWL ratios we observed in cool-acclimated *Z. a. mearnsii* were similar to the ratios exhibited by mourning doves *Z. macroura* housed indoors at  $T_a=30^\circ\text{C}$  (Hoffman and Walsberg, 1999). The CEWL/TEWL ratios in heat-acclimated *Z. a. mearnsii* are among the highest recorded (Wolf and Walsberg, 1996), and are comparable to the ratios observed in Australian Spinifex pigeons (*Geophaps plumifera*; Withers and Williams, 1990). In the latter study, CEWL represented 70–80% of TEWL at all  $T_a$  values between  $0^\circ\text{C}$  and  $40^\circ\text{C}$  (Withers and Williams, 1990). Our data, together with recent data on TEWL partitioning in mourning doves (Hoffman and Walsberg, 1999) and four species of larks (Tieleman and Williams, 2002), support the view that avian patterns of TEWL partitioning exhibit considerable taxonomic variation (Wolf and Walsberg, 1996). Differences in the relative importance of CEWL appear to be particularly pronounced between Columbiformes and Passeriformes (Wolf and Walsberg, 1996).

Our data suggest that in addition to taxonomic differences in patterns of TEWL partitioning, there may be variation in the phenotypic plasticity of these patterns. Tieleman and Williams (2002) investigated changes in the partitioning of TEWL in response to thermal acclimation in four species of larks, namely hoopoe larks *Alaemon alaudipes*, Dunn's larks *Eremalauda dunni*, skylarks *Alauda arvensis* and woodlarks *Lullula arborea*. CEWL in *A. alaudipes* was 22% lower in individuals acclimated to  $T_a=35^\circ\text{C}$  for 3 weeks than in individuals acclimated to  $T_a=15^\circ\text{C}$ , but no differences were evident in any of the other species (Tieleman and Williams, 2002). On the basis of these results, Tieleman and Williams (2002) concluded that acclimatization plays a minor role in determining CEWL.

Extrapolating physiological data collected in the laboratory to field conditions is potentially problematic and often speculative. For instance, the variable ambient water vapour pressures experienced by wild birds may affect TEWL partitioning, and patterns of partitioning in active birds are probably different from those of resting birds. Nevertheless,

our data suggest that in free-ranging *Z. a. mearnsii*, CEWL varies in response to thermal acclimatization, and that the relative importance of REWL and CEWL at any particular time of year reflects the preceding thermal conditions. Moreover, we argue that the ability of *Z. a. mearnsii* to tolerate high  $T_a$  values, together with the high radiative heat loads associated with foraging in the open during mid-summer in the Sonoran Desert, is at least partly dependent on thermal acclimatization earlier in the season.

Currently data on the partitioning of TEWL at  $T_a \geq 35^\circ\text{C}$  are available for 12 species from four avian orders (Galliformes, Anseriformes, Columbiformes and Passeriformes; reviewed in Wolf and Walsberg, 1996). Hence, there are currently too few data to generate hypotheses concerning the phylogenetic distribution of patterns of TEWL partitioning, or the evolution of these patterns. The observation that in *Z. a. mearnsii* the relative contributions of REWL and CEWL vary in response to short-term thermal acclimation has important implications for future work. Firstly, measurements of REWL and CEWL need to account for the possible influence of thermal acclimation prior to the measurements. Secondly, data used for comparative analyses need to be carefully selected to ensure that taxonomic variation in TEWL partitioning is not confounded by the effects of acclimation/acclimatization. A related issue concerns comparing data from species in which TEWL partitioning is sensitive to thermal acclimation to data from species in which it is not.

In summary, our data provide evidence of plasticity in the partitioning of avian evaporative water losses in response to short-term thermal acclimation. The capacity of white-winged doves, and possibly other species, to alter TEWL partitioning over relatively short time scales has important implications for thermoregulation and water balance at high environmental temperatures. Our data also reveal that the degree of plasticity in the partitioning of TEWL varies greatly between avian taxa.

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