The Journal of Experimental Biology 210, 741-749
Published by The Company of Biologists 2007
doi:10.1242/jeb.02705

Cloacal evaporation: an important and previously undescribed mechanism for avian thermoregulation

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Accepted 2 January 2007

Summary

We present the first experimental evidence that a bird is capable of evaporating enough water from the cloaca to be important for thermoregulation. We measured rates of evaporation occurring from the mouth, the skin, and the cloaca of Inca doves *Columbina inca* Lesson and Eurasian quail *Coturnix coturnix* Linnaeus. Inca doves showed no significant increase in cutaneous evaporation in response to curtailment of buccopharyngeal evaporation. Cloacal evaporation in doves was negligible at ambient temperatures of 30°, 35° and 40°C. However, at 42°C, the apportionment of total evaporation in doves was 53.4% cutaneous, 25.4% buccopharyngeal and 21.2% cloacal, with cloacal evaporation shedding, on average, 150 mW of heat. In contrast, the evaporative apportionment in quail at 32°C (the highest ambient temperature tolerated by this species) was 58.2% cutaneous, 35.4% buccopharyngeal and 6.4% cloacal. These results suggest that, for some birds, cloacal evaporation can be controlled and could serve as an important emergency tactic for thermoregulation at high ambient temperatures.

Key words: cloaca, cutaneous, evaporative, water loss, metabolism, bird, Inca dove, *Columbina inca*, Eurasian quail, *Coturnix coturnix*.

Introduction

Organisms are able to exchange heat with the environment *via* four modes: conduction, convection, radiation and evaporation (Porter and Gates, 1969). Of these modes, evaporation holds a place of peculiar ecological interest. First, evaporation from an organism always results in a decrease in the temperature of the surface from which evaporation takes place. Evaporation is therefore a one-way transfer, always representing a loss of heat from the organism. In contrast, heat can be lost or gained either conductively, convectively or radiatively, depending on the direction of the gradient for each respective mode of transfer. Second, biological evaporation always involves the loss of water, a vital resource on which nearly all biochemical processes depend. Evaporation, then, is loss of heat *via* loss of mass. Among the four modes of heat transfer, evaporation is unique in its coupling of heat loss with resource loss. These fundamental differences underlie an important biological conflict of interests: the animals with the least access to water for hydrostasis (such as desert forms) are the animals with the greatest need to lose water for thermostasis. The competing needs for water retention and water evaporation lead one to expect many desert animals to adjust the rate of evaporation as a trade-off between avoiding overheating and avoiding dehydration.

Adjustment of evaporation can be made either by changing the evaporative conductance of (and therefore the rate of evaporation from) any specific epithelium or by changing the surface area of exposed epithelia. Experimental partitioning of total evaporation into components, or evaporative routes, has been done for many species using various methods in studies that have used a variety of terms to describe those evaporative routes (e.g. Bernstein, 1971a; Richards, 1976; Maloney and Dawson, 1998; Webster and Bernstein, 1987; Taylor et al., 1971; Arieli et al., 1999; Menon et al., 1986; Lee and Schmidt-Nielsen, 1971; McKechnie and Wolf, 2004; Tieleman and Williams, 2002). Birds possess three anatomically distinct epithelia from which evaporation can occur: the mouth and pharynx, the dry skin, and the cloaca. We therefore categorize avian evaporative routes as either buccopharyngeal, cutaneous, or cloacal. The present study is the first to measure avian rates of cloacal evaporation. Buccopharyngeal evaporation includes gular fluttering and evaporation due to breathing, whether by panting or not. For simplicity, we include ocular evaporation within cutaneous evaporation. Because previous studies did not discriminate between evaporation from the dry skin and from the cloaca, we describe the sum of cutaneous and cloacal evaporation as non-buccopharyngeal evaporation.

Despite the avian lack of sweat glands, several bird species have been shown to exhibit rates of non-buccopharyngeal evaporation that rival or exceed buccopharyngeal rates (e.g. Hoffman and Walsberg, 1999; McKechnie and Wolf, 2004; Marder et al., 1989; Webster and King, 1987; Arad et al., 1987; Wolf and Walsberg, 1996; Withers and Williams, 1990; Marder and Gavrieli-Levin, 1987; Smith, 1969). Historically,

workers (Bernstein, 1969; Smith and Suthers, 1969) have assumed that all but a negligible portion of this non-buccopharyngeal evaporation occurs from the skin or from the conjunctivae. Terms such as 'cutaneous' (Lasiewski et al., 1971; Bernstein, 1969; Smith and Suthers, 1969), 'peripheral' (Dawson, 1982) and 'transepidermal' (Hattingh, 1972; Menon et al., 1989; Muñoz-Garcia and Williams, 2005) were thus used to describe the remainder of a bird's evaporative output, after evaporation due to ventilation and gular fluttering were subtracted. Though some workers (Cade and Dybas, Jr, 1962) have conducted hygrometric measurements in which the avian cloaca was occluded, the rationale for such experimental treatment was to prevent urination and defecation, either of which would render a hygrometric measurement unusable in analyses of evaporation from the skin. A recent study of a desert reptile, the Gila monster *Heloderma suspectum* Cope (DeNardo et al., 2004), demonstrated for the first time in any animal that cloacal rates of evaporation can rid the body of enough heat to be important for thermoregulation. Those results raised the possibility that birds (which, like reptiles, possess cloacae) are similarly able to exploit this previously undescribed evaporative route.

Columbiform species, which can tolerate high ambient temperatures without panting (Arieli et al., 1988; Marder and Arieli, 1988; Ophir et al., 2002), show some of the highest non-buccopharyngeal rates of evaporation for any bird (Hoffman and Walsberg, 1999; Marder and Ben-Asher, 1983; McKechnie and Wolf, 2004). We have demonstrated previously (Hoffman and Walsberg, 1999) that mourning doves *Zenaida macroura* Linnaeus are able to make rapid adjustments to the rate of non-buccopharyngeal evaporation in response to an experimental suppression of evaporation from the mouth. Here, to add insight regarding the generality of the results observed in mourning doves, we investigate the response to suppression of buccopharyngeal evaporation in a different columbiform, the Inca dove *Columbina inca* Lesson. In addition, we refine the experimental technique to quantify the apportionment of non-buccopharyngeal evaporation into its cutaneous and cloacal components. For comparison, we present values for all three evaporative rates in a gallinaceous bird, the Eurasian quail *Coturnix coturnix* Linnaeus. Both of the test species are easily obtained and are widely distributed, occurring in arid and semiarid habitats, but they represent distinct taxonomic orders.

Materials and methods

Animals

Adult Inca doves *Columbina inca* Lesson of undetermined sex were captured using drop traps in Phoenix, Arizona, USA in June 2004. Adult male Eurasian quail *Coturnix coturnix* Linnaeus were purchased (Pratt's Feed and Supply, Phoenix, AZ, USA) in January 2005. The birds were housed in wire cages (1–5 doves or 1–2 quail per cage) in a temperature-controlled room on the campus of Arizona State University in Tempe, Arizona, and the room provided a 12 h:12 h L:D artificial photoperiod. Ambient temperature (T_a) was maintained at 25°C. All birds had continuous access to water and food (seed for doves and game bird feed for quail), except during trials. A few downy feathers occurring near the cloaca were trimmed to allow for safe and consistent access for cloacal manipulation and to prevent retention of wet feces during trials. Feather trimming did not differ between types of trials, and the removal of such a small fraction of plumage is unlikely to have made any appreciable change to evaporative conductance (Webster et al., 1985).

Respirohygrometry

Inca doves

We used the flow-through method to measure evaporative rates, which allowed us also to measure rates of change in oxygen and carbon dioxide. To minimize hygroscopicity, we constructed the test chamber of plate glass with aluminum corner supports. The chamber included two compartments – one for the head and one for the torso – separated by an aluminum partition that supported a thin sheet of latex (4 cm×4 cm) into which a hole was cut to allow for passage of the head and neck. With the bird in place the latex was stretched slightly, forming a barrier between the two compartments while not interfering with the bird's breathing. The head compartment (426 ml) was contained by a borosilicate bell jar fitted with borosilicate ports that accepted minimally hygroscopic tubing (3 mm i.d., Bev-a-Line IV, Thermoplastic Processes, Inc., Stirling, NJ, USA) for both influent and effluent. Identical ports were attached to the plate glass of the torso compartment (17.72 l) using epoxy, and the influent port was equipped with a copper–constantan (type T) thermocouple for measurement of ambient temperature. A steel rod hanging from the aluminum partition was equipped to support a removable polypropylene shackle that was placed on the bird's legs prior to placement into the chamber. An aluminum neck stock positioned immediately below the latex sheet prevented the bird from pulling its head through the neck hole. An illustration of a similar chamber appears elsewhere (Wolf and Walsberg, 1996).

Air entering the two compartments was first passed through an industrial air purifier (PCDA11129022, Puregas, Denver, CO, USA) that removed carbon dioxide and water vapor. Flux through each of the two influent lines was controlled and measured by separate mass flow controllers (FMA-A2406 and FMA-A2409, Omega Engineering, Stamford, CT, USA) positioned upstream of the compartments. Flux into the head compartment and torso compartment was maintained at ca. 1300 ml min^{-1} and ca. 6700 ml min^{-1}, respectively. A borosilicate U-tube containing mineral oil was interposed between tubes connecting the compartments. The U-tube served as a manometer to allow for minimization of any intercompartmental pressure gradient due to unequal flow rates, thus minimizing the possibility of a gas leak from one compartment to the other. We occasionally verified that leaking was not occurring by sending air subsampled from the torso compartment to the CO_2 analyzer and ensuring that the air was virtually free of carbon dioxide. To avoid any appreciable

increase of chamber air pressure beyond barometric pressure, both effluent lines were kept short and allowed to empty into spill tubes from which separate subsampling pumps drew air and delivered it to the downstream instruments.

Sample air from the two compartments was pumped to separate dewpoint hygrometers (RH100, Sable Systems International, Las Vegas, NV, USA). Effluent from the torso-compartment hygrometer was vented to the temperature-controlled room in which the test chamber sat. Effluent from the head-compartment hygrometer was sent through anhydrous calcium sulfate to rid it of water vapor, and the dried air then passed through a carbon dioxide analyzer (LI-6252, Li-Cor Biosciences, Lincoln, NE, USA) and an oxygen analyzer (FC-1B, Sable Systems International, Las Vegas, NV, USA). Prevailing barometric pressure was continuously measured using an electronic manometer.

For half of the trials, the acapnic air supplying the head compartment was diverted to a series of three copper water columns through which it was bubbled to saturate the air with water vapor. Condensate, visible in the tubing that exited the water columns, assured us of saturation. The water-saturated air was then sent to the test chamber, just as for dry air in all other trials. To avoid condensation in the mass flow controller, and because we calibrated the controller for dry air, it was placed upstream of the water columns. We used the value for saturation vapor density at the temperature of the water to calculate the volumetric rate at which water vapor was added to the air stream, and we added that rate to the flux through the mass flow controller to determine head-compartment influx for those trials.

Measurements from all sensors were sampled every second by a datalogger (CR23X, Campbell Scientific, Logan, UT, USA) and then averaged for output every minute. The effective volumes (Lasiewski et al., 1966) of the compartments were calculated as 1960 ml (head) and 81.5 l (torso), yielding 99% equilibration periods of 1.5 min and 12.2 min, respectively.

Eurasian quail

Because of the mass and body geometry of Eurasian quail, we were not able to conduct trials in the compartmentalized chamber. Instead, quail were placed in a cylindrical chamber made of borosilicate glass (5.1 l) with an aluminum lid and a glass floor. A cylindrical, polycarbonate mask (open on one end) was placed over the bird's head and secured at the neck by nylon twine. The distal (closed) end of the mask was attached to a flexible tube connected to a miniature air swivel that allowed the bird to move about the cage without tangling the air line. The effluent line from the swivel was attached to a pump that drew air from the chamber, through the mask, and into a dewpoint hygrometer (Sable Systems RH100), from which it was sent through anhydrous calcium sulfate and then through a carbon dioxide analyzer (Li-Cor 6252) and an oxygen analyzer (Sable Systems FC-1B).

The cylindrical chamber was fitted with three borosilicate ports, each of which connected to minimally hygroscopic tubing (Bev-a-Line IV). Thus, there were separate air lines for chamber influent, chamber effluent and mask effluent. The influent line was equipped with a copper–constantan thermocouple for measurement of ambient temperature. Negative-pressure flux through the mask was maintained by a mass flow controller (Omega Engineering FMA-A2406) at ca. 630 ml min^{-1}, sufficient to capture the expired air and prevent it from escaping at the junction between the mask and the neck. Positive-pressure flux into the chamber was maintained at ca. 6730 ml min^{-1} by a separate mass flow controller (Omega Engineering FMA-A2409), resulting in a 3.5 min period for gaseous equilibration (Lasiewski et al., 1966). Collecting all of the expired air at the mask served to effectively partition the chamber into torso and head compartments. The baseline gas for the torso compartment was dry, acapnic air as described above for the Inca dove experiment. The chamber effluent provided for measurement of non-buccopharyngeal evaporation. In addition, this effluent served as the baseline gas for the mask, because air drawn through the mask included water vapor added to the chamber from the bird's torso. As for the Inca dove experiment, the chamber effluent line was allowed to empty into a spill tube from which air was subsampled and sent to a dewpoint hygrometer (Sable Systems RH100). This chamber effluent could also be routed to the carbon dioxide and oxygen analyzers. By ensuring that there was a negligible change in the dried fractions of respiratory gases sampled from the body compartment, we were assured that leaking from the mask did not occur.

The specifics of data acquisition for Eurasian quail were the same as for Inca doves.

Experimental protocol

Inca doves

The experiment included three treatment variables: ambient temperature, ventilatory humidity and cloacal patency (N=8 to 13; see Table 1). Trials were conducted at four ambient temperatures (30°C, 35°C, 40°C and 42°C), two levels of ventilatory humidity ('dry trials' and 'humid trials'), and two levels of cloacal patency ('unsealed trials' and 'sealed trials'). For humid trials, the torso compartment was supplied with dry air, and the head compartment was supplied with air saturated at the respective ambient temperature with water vapor. Immediately prior to placement of the bird into the chamber for sealed trials, the cloaca was occluded with cyanoacrylic glue. The resulting cloacal cap remained in place throughout the trial and was removed using acetone immediately after the trial. Any feces released during unsealed trials fell into a layer of mineral oil on the floor of the chamber, thereby eliminating fecal water from hygrometric measurements.

During unsealed trials, the hygrometers directly measured buccopharyngeal and non-buccopharyngeal evaporation; during sealed trials, they directly measured buccopharyngeal and cutaneous evaporation. These direct measurements allowed us to calculate cloacal evaporation as the difference between non-buccopharyngeal and cutaneous evaporation. During humid trials, buccopharyngeal evaporation was eliminated (or at least severely reduced), because the influent was already

saturated with water vapor. This required the bird to either store that extra heat or dissipate it by increasing evaporative flux elsewhere. The bird remained in the test chamber for 2 h. For the first 60 min, dry air was delivered to both compartments. A remote switch then triggered a re-routing of the influent without disturbing the bird, thereby delivering water-saturated air to the head chamber for an additional 60 min, before the bird was removed from the chamber. Data used in analyses were averages of measurements taken over the last 10 min of each portion (dry or wet) of the overall time spent in the chamber. All trials were conducted in darkness during daylight hours. Darkness reduced the unnatural level of stress experienced by the birds. Conducting trials in darkness during daylight hours results in a modest, circadian increase in total evaporation (MacMillen and Trost, 1967). We feel this is more representative of field conditions under which thermoregulatory evaporation is employed by Inca doves.

Eurasian quail

The experiment included two treatment variables: ambient temperature and cloacal patency (N=8). Trials were conducted at two ambient temperatures (30°C and 32°C) and two levels of cloacal patency ('unsealed trials' and 'sealed trials'). We did not conduct trials at T_a>32°C, because quail apparently became distressed at higher temperatures, as evidenced by observation of persistent struggling. Because quail were allowed to stand on the floor of the chamber, no mineral oil was used; if defecation occurred during any trial, the resulting data were discarded. For sealed trials, the cloaca was sealed with cyanoacrylic glue for the duration of the trial, after which the glue was removed using acetone. Except for differences in the method of partitioning evaporative routes and in the ambient temperatures of trials, the protocol for the Eurasian quail experiment was the same as for the dry trials using Inca doves. All trials were conducted in darkness during daylight hours.

Calculations

Evaporation represents an input of gas into the chamber, so that the efflux and influx differ. Similarly, rate of oxygen consumption, $\dot{V}_{O_2}$, and carbon dioxide production, $\dot{V}_{CO_2}$, alter the flux. To incorporate these changes into our data, we derived the following equations for calculating evaporative rates. All symbols are defined in the List of symbols.

$$\dot{V}_A = \dot{V}'_A + \dot{V}_{H_2O} + \dot{V}_{CO_2} - \dot{V}_{O_2}\,, \tag{1}$$

$$\dot{V}_{H_2O} = \frac{\dot{V}'_A(F_{H_2O} - F'_{H_2O}) + F_{H_2O}(\dot{V}_{O_2} - \dot{V}_{CO_2})}{1 - F_{H_2O}}\,, \tag{2}$$

$$\begin{aligned}\dot{V}_A &= \dot{V}'_A + \frac{\dot{V}'_A(F_{H_2O} - F'_{H_2O}) + F_{H_2O}(\dot{V}_{O_2} - \dot{V}_{CO_2})}{1 - F_{H_2O}} + \dot{V}_{CO_2} - \dot{V}_{O_2} \\ &= \dot{V}'_A\left[1 + \frac{(F_{H_2O} - F'_{H_2O})}{1 - F_{H_2O}}\right] + (\dot{V}_{O_2} - \dot{V}_{CO_2})\left(\frac{F_{H_2O}}{1 - F_{H_2O}} - 1\right)\,,\end{aligned} \tag{3}$$

$$\begin{aligned}\dot{M}_{H_2O} &= \dot{V}_A\rho_V - \dot{V}'_A\rho'_V \\ &= \left\{\dot{V}'_A\left[1 + \frac{(F_{H_2O} - F'_{H_2O})}{1 - F_{H_2O}}\right] + (\dot{V}_{O_2} - \dot{V}_{CO_2})\left(\frac{F_{H_2O}}{1 - F_{H_2O}} - 1\right)\right\}\rho_V - \dot{V}'_A\rho'_V \\ &= \dot{V}'_A\left\{\left[1 + \frac{(F_{H_2O} - F'_{H_2O})}{1 - F_{H_2O}}\right]\rho_V - \rho'_V\right\} + (\dot{V}_{O_2} - \dot{V}_{CO_2})\left(\frac{F_{H_2O}}{1 - F_{H_2O}} - 1\right)\rho_V\,,\end{aligned} \tag{4}$$

$$F_{H_2O} = \frac{P_V}{P_B}\,, \tag{5}$$

$$F'_{H_2O} = \frac{P'_V}{P_B}\,, \tag{6}$$

$$\begin{aligned}\dot{M}_{H_2O} &= \left\{\dot{V}'_A\left[1 + \frac{\left(\frac{P_V}{P_B} - \frac{P'_V}{P_B}\right)}{\left(\frac{P_B}{P_B} - \frac{P_V}{P_B}\right)}\right] + (\dot{V}_{O_2} - \dot{V}_{CO_2})\left[\frac{\left(\frac{P_V}{P_B}\right)}{\left(\frac{P_B - P_V}{P_B}\right)} - 1\right]\right\}\rho_V - \dot{V}'_A\rho'_V \\ &= \dot{V}'_A\left[\left(1 + \frac{P_V - P'_V}{P_B - P_V}\right)\rho_V - \rho'_V\right] + (\dot{V}_{O_2} - \dot{V}_{CO_2})\left(\frac{P_V}{P_B - P_V} - 1\right)\rho_V\,.\end{aligned} \tag{7}$$

For non-buccopharyngeal evaporation, we assumed $\dot{V}_{O_2}$=0 and $\dot{V}_{CO_2}$=0, thereby simplifying Eqn 7 as:

$$\dot{M}_{H_2O} = \dot{V}'_A\left[\left(1 + \frac{P_V - P'_V}{P_B - P_V}\right)\rho_V - \rho'_V\right]\,. \tag{8}$$

For respirometric measurements, we used the following:

$$\dot{V}_{O_2} = \dot{V}'_A\left[F'_{O_2} - F_{O_2}\frac{(1 - F'_{O_2} - F'_{CO_2} - F'_{H_2O})}{(1 - F_{O_2} - F_{CO_2} - F_{H_2O})}\right]\,, \tag{9}$$

$$\dot{V}_{CO_2} = \dot{V}'_A\left[F_{CO_2}\frac{(1 - F'_{O_2} - F'_{CO_2} - F'_{H_2O})}{(1 - F_{O_2} - F_{CO_2} - F_{H_2O})} - F'_{CO_2}\right]\,. \tag{10}$$

The derivation of Eqn 9 and Eqn 10 can be found elsewhere (Walsberg and Hoffman, 2006).

We calculated sampled water vapor pressure from the measured dewpoint, using the eighth-order polynomial of Flatau

et al. (Flatau et al., 1992), and we calculated vapor density from vapor pressure using the Ideal Gas Law (Campbell and Norman, 1998).

Analysis of data

We used SAS (Version 9.1, SAS Institute, Cary, NC, USA) to perform all statistical tests. For Inca doves, the MIXED procedure was used to perform repeated-measures analyses of variance (RM-ANOVA) and Tukey–Kramer *post-hoc* comparisons, with non-buccopharyngeal evaporation (*NBE*), buccopharyngeal evaporation (*BE*), oxygen consumption ($\dot{V}_{O_2}$), and carbon dioxide production ($\dot{V}_{CO_2}$) separately defined as dependent variables. For each of these tests, the within-subjects factors were ambient temperature, humidity of the head-chamber influent and cloacal patency. The same tests were performed for Eurasian quail, but humidity was not included as a within-subjects factor, because humidity was not adjusted in trials using quail. In all tests for both species, we specified the Compound Symmetry covariance structure, because it yielded the lowest values for both Akaike's Information Criterion and Schwartz' Bayesian Criterion.

Results

Inca doves

Table 1 provides means and standard errors for hygrometric and respirometric measurements, along with numbers of individuals on which measurements were made. Values for non-buccopharyngeal evaporation (*NBE*) and buccopharyngeal evaporation (*BE*) are plotted in Fig. 1. There was a significant effect of T_a on *BE* (F=15.30, P<0.0001) and on *NBE* (F=88.88, P<0.0001), but the effect of temperature on the two measures differed dramatically. Over the range of experimental ambient temperatures, *NBE* changed by 219.5 μg g^{-1} min^{-1} during dry, unsealed trials, 254.9 μg g^{-1} min^{-1} during wet, unsealed trials, 125.5 μg g^{-1} min^{-1} during dry, sealed trials, and 146.8 μg g^{-1} min^{-1} during wet, sealed trials. These changes in *NBE* represent increases by 235.3%, 279.6%, 83.3% and 127.6%, respectively. The large differences in these percentages between unsealed trials and sealed trials reflect the magnitude of cloacal evaporation, which is a component of *NBE*. In contrast, the corresponding changes in *BE* were much smaller (dry, unsealed trials: 27.5 μg g^{-1} min^{-1} change, 43.5% increase; dry, sealed trials: 58.3 μg g^{-1} min^{-1} change, 91.3% increase).

The overall fixed effect of cloacal patency was not significant for either *BE* (F=3.16, P=0.0988) or *NBE* (F=3.09, P=0.1020). However, there was a significant interaction between cloacal patency and ambient temperature (F=6.09, P=0.0052), and *post-hoc* analysis revealed that cloacal patency

Table 1. *Hygrometric and respirometric measurements*

Species	T_a (°C)	Humidity	Cloacal patency	*BE* (μg g^{-1} min^{-1})	*NBE* (μg g^{-1} min^{-1})	*CutE* (μg g^{-1} min^{-1})	*CloE** (μg g^{-1} min^{-1})	$\dot{V}_{O_2}$ (μl g^{-1} min^{-1})	$\dot{V}_{CO_2}$ (μl g^{-1} min^{-1})	*BE*:$\dot{V}_{O_2}$
Columbina inca Lesson	30	Dry	Unsealed	63.2 ±6.2 (10)	93.3±10.6 (10)	120.3±19.3 (10)	–27.1±17.7 (10)	65.3±3.9 (10)	61.8±4.0 (10)	1.72±0.07 (10)
			Sealed	63.9±3.4 (10)	120.3±19.3 (10)			71.0±4.1 (9)	66.4±3.2 (9)	1.64±0.11 (9)
		Wet	Unsealed	n/a	91.1±8.6 (10)	113.0±18.4 (10)	–21.84±15.0	62.8±3.2 (10)	55.8±2.7 (10)	n/a
			Sealed	n/a	113.0±18.4 (10)		(10)	63.6±3.2 (9)	55.4±2.9 (9)	n/a
	35	Dry	Unsealed	69.9±7.0 (13)	117.3±18.8 (13)	90.8±38.1 (8)	7.3±35.6 (8)	43.1±3.1 (12)	45.1±3.4 (12)	3.05±0.20 (12)
			Sealed	73.1±6.6 (9)	95.1±33.9 (9)			44.3±3.5 (9)	41.7±3.0 (9)	3.03±0.24 (9)
		Wet	Unsealed	n/a	113.2±15.8 (13)	108.8±42.7 (8)	–8.2±33.8 (8)	42.1±3.0 (12)	42.0±2.9 (12)	n/a
			Sealed	n/a	110.3±37.6 (9)			43.8±3.6 (9)	40.9±3.4 (9)	n/a
	40	Dry	Unsealed	83.6±5.4 (10)	201.2±25.3 (10)	194.3±21.5 (9)	7.9±27.7 (9)	41.9±4.5 (10)	38.8±3.5 (10)	4.48±0.42 (10)
			Sealed	83.4±5.8 (11)	189.3±18.3 (11)			37.5±2.0 (11)	35.7±1.6 (11)	4.53±0.23 (11)
		Wet	Unsealed	n/a	236.6±26.9 (10)	242.2±25.9 (9)	–6.9±30.0 (9)	47.1±4.1 (10)	40.9±3.6 (10)	n/a
			Sealed	n/a	256.7±24.0 (11)			45.4±3.6 (11)	41.0±3.3 (11)	n/a
	42	Dry	Unsealed	90.7±5.1 (8)	312.8±28.5 (8)	222.4±33.4 (7)	91.3±28.4 (7)	38.9±3.1 (8)	36.5±2.5 (8)	5.44±0.41 (8)
			Sealed	122.1±13.0 (9)	220.6±26.1 (9)			38.1±3.7 (9)	35.9±3.0 (9)	6.25±0.71 (9)
		Wet	Unsealed	n/a	346.0±38.3 (8)	256.6±23.9 (7)	85.0±34.6 (7)	48.0±3.4 (8)	45.0±3.7 (8)	n/a
			Sealed	n/a	257.1±19.7 (9)			45.2±5.3 (9)	42.8±6.7 (9)	n/a
Coturnix coturnix Linnaeus	30	Dry	Unsealed	18.7±1.4 (8)	56.9±7.4 (8)	48.7±4.7 (8)	8.1±5.8 (8)	28.2±1.7 (8)	21.7±1.1 (8)	1.52±0.16 (8)
		Dry	Sealed	33.2±8.3 (8)	48.7±4.7 (8)			32.4±2.9 (8)	23.6±2.1 (8)	1.67±0.28 (8)
	32	Dry	Unsealed	36.8±12.0 (8)	55.9±5.6 (8)	48.9±4.5 (8)	7.0±5.5 (8)	30.1±3.5 (8)	23.5±2.1 (8)	2.06±0.32 (8)
		Dry	Sealed	31.3±4.9 (8)	48.9±4.5 (8)			30.9±2.4 (8)	22.0±1.7 (8)	1.95±0.28 (8)

Values shown are means ± s.e.m.; numbers in parentheses indicate numbers of individuals used in analyses; symbols are explained in the List of symbols.
*Calculation of *CloE* can yield negative values when *CloE* is negligible, because of extensive overlap of variances in *NBE* means for unsealed and sealed trials.

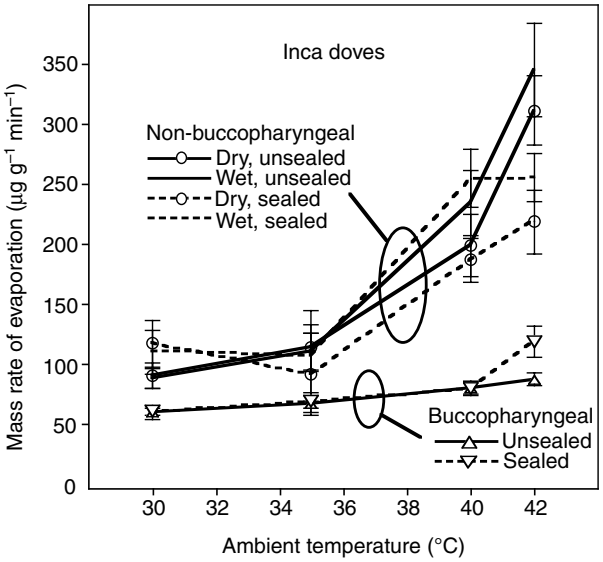

Fig. 1. Rates of evaporation measured in Inca doves at four ambient temperatures. During 'Sealed' trials cloacae were occluded with cyanoacrylic glue; during 'Unsealed' trials cloacae were not occluded. Relative humidity of the head-compartment influent was near 0% during 'Dry' trials and near 100% during 'Wet' trials. The differences between non-buccopharyngeal traces for 'Unsealed' and 'Sealed' trials indicate rates of cloacal evaporation. Those differences (and therefore the rates of cloacal evaporation) were negligible at $T_a \leqslant 40$°C and significant at T_a=42°C. The differences between traces for 'Dry' and 'Wet' trials indicate compensatory adjustment of cutaneous evaporation; the differences were non-significant at all four ambient temperatures. Values shown are means ± s.e.m. (N=7–13).

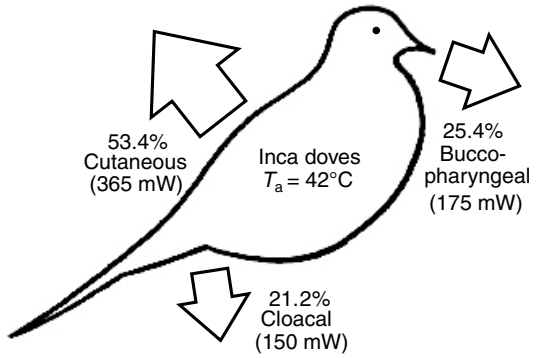

Fig. 2. Average apportionment of total evaporation in Inca doves at 42°C. Buccopharyngeal and non-buccopharyngeal evaporation were directly and separately measured. Cutaneous evaporation was defined as the whole of non-buccopharyngeal evaporation during 'Sealed' trials, in which cloacae were occluded. Cloacal evaporation was calculated as non-buccopharyngeal evaporation during 'Unsealed' trials minus non-buccopharyngeal evaporation during 'Sealed' trials. Values in parentheses indicate average rates of evaporative heat loss.

significantly affected *NBE* at T_a=42°C (t=–4.29, adjusted P=0.0091). This is clearly indicated in Fig. 1, in which the values for sealed trials diverge from those for unsealed trials at T_a=42°C. All other interactions (temperature × patency for *BE* and *NBE*; humidity × patency, humidity × temperature, and humidity × temperature × patency for *NBE*) were non-significant. The overall effect of humidity on *NBE* was significant (F=5.61, P=0.0308). However, *post-hoc* tests could identify no significant effect at any fixed level of temperature or patency. This is illustrated in Fig. 1, in which values for wet trials appear only marginally greater than values for dry trials.

Cloacal evaporation (*CloE*) was negligible at $T_a \leqslant 40$°C. However, at T_a=42°C, mean values for *CloE* were 91.3 μg g^{-1} min^{-1} during dry trials and 85.0 μg g^{-1} min^{-1} during wet trials. These values are similar to mean *BE* at T_a=42°C during dry trials (90.7 μg g^{-1} min^{-1}) and slightly less than half of mean cutaneous evaporation (*CutE*, 222.4 μg g^{-1} min^{-1} during dry trials, 256.6 μg g^{-1} min^{-1} during wet trials). That is, for trials at 42°C, total evaporation was apportioned as 25.4% buccopharyngeal, 21.2% cloacal and 53.4% cutaneous (Fig. 2). This indicates that cloacal evaporation was thermoregulatorily important at the highest experimental temperature, on a par with buccopharyngeal evaporation. The heat liberated by cloacal evaporation at T_a=42°C averaged 3.7 mW g^{-1}, or 27.6% of mean metabolic heat (13.4 mW g^{-1}) at that temperature.

We separately calculated the volumetric rate (μl g^{-1} min^{-1}) of *BE*, so we could relate buccopharyngeal evaporation to oxygen consumption as the dimensionless evaporespiratory ratio, $BE{:}\dot{V}_{O_2}$. A temperature-dependent change in this ratio indicates an uncoupling of the rate of buccopharyngeal evaporation from the rate of ventilation. This, in turn, can be partially caused by an attempt to increase evaporation from the rate that would occur just as a result of breathing. The evaporespiratory ratio increased by more than threefold with ambient temperature from 30°C to 42°C (F=62.9, P<0.0001), and the ratio at each temperature differed significantly from that at all other temperatures ($P \leqslant 0.0011$ at all temperatures). This reflects the significant decrease in $\dot{V}_{O_2}$ as T_a increased from 30°C to 35°C (t=8.69, adjusted P<0.0001) and the temperature-dependent increase in *BE*, along with the birds' use of panting or gular fluttering that we observed at the higher temperatures.

Eurasian quail

Table 1 provides means and standard errors for hygrometric and respirometric measurements, along with numbers of individuals on which measurements were made. There were no significant effects of treatment variables on *NBE* (T_a: F=0.01, P=0.9215; patency: F=3.57, P=0.1009; T_a × patency: F=0.02, P=0.8908). Similarly, *BE* did not change with treatment (T_a: F=1.16, P=0.3163; patency: F=0.36, P=0.5689; T_a × patency: F=1.79, P=0.2226), nor did the evaporespiratory ratio (T_a: F=3.77, P=0.0932; patency: F=0.15, P=0.7075; T_a × patency: F=1.00, P=0.3513). Despite frequent observations of panting, cloacal evaporation remained comparatively low, accounting for only 8.3% (T_a=30°C) and 6.4% (T_a=32°C) of total evaporation, and *CloE* did not change with T_a (F=0.01, P=0.9151). Evaporation from the cloaca was about one-fifth to one-third of *BE*, the latter of which accounted for 26.2% (T_a=30°C) and 35.4% (T_a=32°C) of total evaporation. Thus, the majority of evaporation from Eurasian quail was cutaneous

(65.4% and 58.2% at 30°C and 32°C, respectively). The relatively constant rate of cloacal evaporation liberated 330 μW g^{-1} of heat at T_a=30°C and 283 μW g^{-1} at T_a=32°C, corresponding to presumably negligible portions (2.8% and 2.5%) of metabolic heat at the respective ambient temperatures.

Discussion

Our results indicate for the first time that the rate of evaporation from the avian cloaca can be high enough to be important for thermoregulation, accounting for the loss of more than one quarter of metabolic heat. Moreover, we have demonstrated that at least Inca doves are able to control the rate of cloacal evaporation, greatly increasing evaporative heat loss at high ambient temperatures, while virtually preventing cloacal evaporation at lower temperatures. The results of the Inca dove study show that, at 42°C, as much water can be evaporated from the cloacal epithelium as from the buccal epithelium. Yet, buccopharyngeal evaporation has always been recognized as being important for thermoregulation, while cloacal evaporation has always been assumed to be negligible. We view these results as the foundation of a major revision of our knowledge of hydric and thermal relations in birds.

The earliest accepted standard view of avian evaporation was driven by the anatomical discovery that birds do not possess sweat glands; workers therefore assumed that a lack of sweat glands indicated a corresponding lack of evaporation from the avian integument, and that effectively all of the water lost evaporatively from a bird's body was lost from its mouth (Bartholomew and Cade, 1963; Bartholomew and Dawson, 1953; Bartholomew et al., 1962; Cowles and Dawson, 1951; Schmidt-Nielsen et al., 1969; Calder, Jr and Schmidt-Nielsen, 1966; Lasiewski and Dawson, 1964). This assumption was challenged by subsequent studies in which separate hygrometric measurements were made from the head and from the rest of the body (Bernstein, 1971a; Bernstein, 1971b; Smith and Suthers, 1969; Lasiewski et al., 1971; Lee and Schmidt-Nielsen, 1971; Marder and Ben-Asher, 1983; Taylor et al., 1971). These newer results threw into question the original assumption of negligible evaporation from the skin of birds, and they prompted microanatomical investigations (Arieli et al., 1999; Menon et al., 1989; Menon et al., 1986; Menon et al., 1996) that revealed major differences between mammalian and avian epidermis, helping to explain the observed rates of cutaneous evaporation in the absence of sweating. Yet with cutaneous evaporation having been clearly established as occurring in birds, researchers continued to assume that evaporation from the cloaca was negligible (Marder and Ben-Asher, 1983; Marder, 1983; Crawford, Jr and Lasiewski, 1968). That is, any measurement of avian evaporation that was not occurring from the mouth was assumed to be a measurement of cutaneous evaporation. Our results demonstrate that non-buccopharyngeal evaporation in birds can be subdivided into cutaneous and cloacal components, and that avian evaporation should now be considered on a tripartite basis.

Rates of cloacal evaporation in Eurasian quail and Inca doves differed markedly. Unfortunately, quail became thermally stressed in the test chamber at ambient temperatures much lower than we anticipated, and we were forced to restrict our measurements to two, relatively low and closely spaced temperatures. This did not afford us the experimental resolution necessary for determining whether these birds make any thermally driven adjustment to the rate of cloacal evaporation. Nevertheless, two interesting findings emerge. First, evaporation is dominated just as strongly by the cutaneous route in Eurasian quail as it is in Inca doves, despite the fact that the Eurasian quail is a non-columbiform bird. However, despite the predominance of cutaneous evaporation, Eurasian quail exhibited mass-specific rates of cutaneous evaporation at 30°C that were only about 42% of those measured in Inca doves at the same air temperature. This is in agreement with the observation by others (Degen et al., 1982; Roberts and Baudinette, 1986) that rates of evaporation and water-turnover in quail occur near the minimum of the predicted range for bird species. Second, cloacal evaporation accounts for about 7% of total evaporation in Eurasian quail. This fraction is lower than the cloacal fraction observed in Inca doves, despite the large anatomical difference between the cloacae of these species. The Eurasian quail has a cloaca appearing as a semilunar slit, the orifice of which is much larger in relation to the body than that of the small, circular sphincter occurring in the Inca dove.

For Inca doves at all four experimental temperatures, the majority of total evaporation was non-buccopharyngeal, ranging from 58.9% of total evaporation at 30°C to 76.8% at 42°C. Below 42°C, virtually all of the non-buccopharyngeal evaporation was cutaneous. However, at 42°C, cloacal evaporation accounted for over one-quarter of non-buccopharyngeal evaporation and over

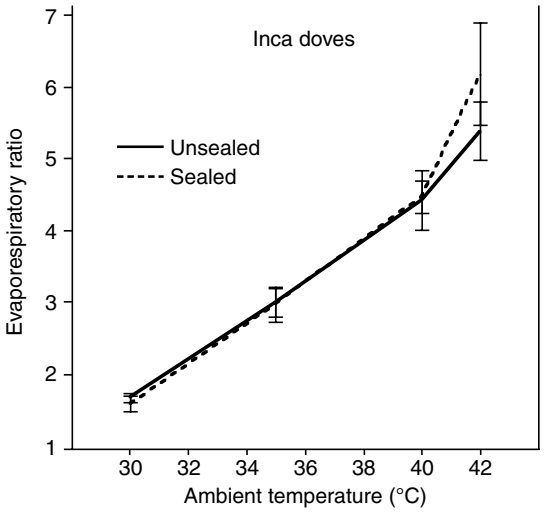

Fig. 3. The ratio of volumetric rate of buccopharyngeal evaporation to volumetric rate of oxygen consumption in Inca doves at four ambient temperatures. This evaporespiratory ratio was nearly quadrupled as ambient temperature increased from 30° to 42°C, indicating that birds were elevating buccopharyngeal evaporation above rates that would occur just as a result of breathing. There is no statistical difference between traces for 'Unsealed' and 'Sealed' trials. Values shown are means ± s.e.m. (*N*=8–12).

one-fifth of total evaporation. MacMillen and Trost (MacMillen and Trost, 1967) measured total evaporation in Inca doves at seven air temperatures ranging from 5° to 44°C. In that study, rates of evaporation underwent large increases at air temperatures above 35°C, and the investigators attributed those increases to gular fluttering. MacMillen and Trost's calculations revealed that thermal conductance underwent an approximately threefold increase over the wide range of air temperatures from 5°C to 40°C, but the increase in thermal conductance resulting from the comparatively small increase in air temperature from 40°C to 44°C was greater than tenfold. While the use of gular fluttering at 44°C in that study doubtless contributed to the observed increases, it is interesting to note the parallels between our observations and those of MacMillen and Trost. Our data do not allow statistical evaluation of evaporative partitioning with respect to changes in body temperature or breathing rates, but we observed gular fluttering at both 40°C and 42°C. Perhaps the steep rise in evaporation that MacMillen and Trost measured above 40°C was largely or primarily due to the facultative use of cloacal evaporation.

Our results suggest that Inca doves could employ a three-stage approach toward evaporative thermoregulation. At lower temperatures, at which breathing might rid the body of sufficient heat for thermostasis, cutaneous evaporation is minimized and cloacal evaporation is virtually eliminated by constricting the cloacal sphincter. As temperature increases beyond a point at which buccopharyngeal evaporation is inadequate, cutaneous evaporation is increased to make up for the thermoregulatory deficit. At still higher temperatures, when evaporation by panting and from the skin might be maximized, the cloacal epithelium is exposed to provide for increased latitude with respect to the range of survivable microenvironments.

Hoffman and Walsberg previously showed that another columbiform, the mourning dove, is able to make temperature-dependent adjustments to rates of non-buccopharyngeal evaporation, and that those adjustments are larger than any that could be explained passively, or simply on the basis of a change in skin-surface temperature (Hoffman and Walsberg, 1999). Because that experiment did not discriminate between cloacal and cutaneous evaporation, it is uncertain how much of the observed change in non-buccopharyngeal evaporation resulted from a change in cutaneous evaporation. The present study of Inca doves is intriguing in light of those earlier results for mourning doves, because suppression of buccopharyngeal evaporation in Inca doves did not significantly increase cutaneous evaporation at any individual temperature, though cutaneous evaporation increased greatly with increasing temperature. Whether mourning doves possess a greater capacity than Inca doves for adjusting rates of cutaneous evaporation or whether the adjustment of evaporation in mourning doves was largely due to adjustment of cloacal evaporation remains to be tested.

It is interesting to note that the response of cloacal evaporation to increase in ambient temperature is similar in Inca doves and Gila monsters (DeNardo et al., 2004), the two species for which cloacal evaporation has been demonstrated at magnitudes sufficient for thermoregulation. Both of these species are able to tolerate very high temperatures, and in both of these species cloacal evaporation remains negligibly low until a critically high ambient temperature prompts a steep rise in cloacal evaporation. This is in keeping with the notion that cloacal evaporation might be used by some animals as a last resort, when the only alternatives are an immediate change of microenvironment or a potentially life-threatening increase in body temperature.

These novel observations of avian cloacal evaporation raise several interesting questions. Perhaps most obvious is the question of how cloacal evaporation is controlled. Apart from simply relaxing the cloacal sphincter, is the bird everting the cloaca? If so, then how much of the cloacal surface is exposed? Whether or not the cloaca is everted, the rate of evaporation therefrom could be altered by changes in such properties as the surface temperature and degree of perfusion of the cloacal epithelium. Independent of all of these factors, a rhythmic ventilation of the cloaca could increase the rate of evaporation, as could postural adjustments that take advantage of the convective air currents to which the bird is exposed.

A second set of important questions raised by these findings involves possible trade-offs that might occur. Traditionally, the cloaca has been viewed as a fairly simple repository for excretory, digestive and reproductive products. Given its additional function of serving as an evaporative organ, perhaps the cloaca will prove to possess unforeseen complexities. Since avian urine can undergo postrenal processing, how might the resorption of water into the hindgut interfere with cloacal evaporation, and how quickly can changes be made to these seemingly competing processes? Similarly, how might the demands for cloacal evaporation affect (and be affected by) the digestive and reproductive functions of the cloaca?

Indeed, since such high rates of cloacal evaporation have now been observed in Inca doves and Gila monsters, most of these questions apply to both birds and reptiles. Further refinement of measurement techniques and testing of other taxa will provide much needed insight.

List of symbols

BE	buccopharyngeal evaporation
$CloE$	cloacal evaporation
$CutE$	cutaneous evaporation
F'_{X}	fractional content of Gas X in influent
F_{X}	fractional content of Gas X in effluent
$\dot{M}_{H_2O}$	mass rate of water evaporation
NBE	non-buccopharyngeal evaporation
P_{B}	barometric pressure
P'_{V}	water-vapor pressure of influent
P_{V}	water-vapor pressure of effluent
T_{a}	ambient temperature
$\dot{V}'_{A}$	volumetric flux of influent air
$\dot{V}_{A}$	volumetric flux of effluent air
$\dot{V}_{O_2}$	volumetric rate of oxygen consumption
$\dot{V}_{CO_2}$	volumetric rate of carbon dioxide production
ρ'_{V}	water-vapor density of influent
ρ_{V}	water-vapor density of effluent

We thank J. F. Harrison and K. J. McGraw for their help in the preparation of the manuscript. C. A. Roeger and M. D. Wheeler provided invaluable assistance in chamber construction. All work was approved by the ASU Institutional Animal Care and Use Committee. Support for this research was provided by NSF Grant No. 0210804 to G.E.W.

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