Cutaneous water loss and lipids of the stratum corneum in house sparrows *Passer domesticus* from arid and mesic environments

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Summary

Birds that live in hot, dry environments must balance water intake with losses in order to maintain water homeostasis. The outer layer of the integument, called the stratum corneum (SC), consists of corneocytes embedded in a matrix of lipids. The SC serves as a barrier to water vapor diffusion through the skin. We measured cutaneous water loss (CWL) in two populations of house sparrow *Passer domesticus* L., one living in a desert environment in Saudi Arabia, and another living in a mesic environment in Ohio, USA. We found that CWL rates at 30°C were lower in desert individuals (11.9±2.2 mg H_2O cm^{-2} day^{-1}; N=11) than in mesic birds (16.0±2.6 mg H_2O cm^{-2} day^{-1}; N=14). We hypothesized that changes in the lipid composition of the SC could affect CWL. We analyzed four classes of lipids in the SC: ceramides, cerebrosides, cholesterol and free fatty acids, by thin layer chromatography. Compared to mesic sparrows, desert birds had a higher amount of ceramides (49.2±10.3 mg g^{-1} SC dry mass in Saudi Arabia; 38.2±18.0 mg g^{-1} SC dry mass in Ohio) and cerebrosides (101.2±48.9 mg g^{-1} SC dry mass in Saudi Arabia; 56.5±34.0 mg g^{-1} SC dry mass in Ohio), and a lower percentage of cholesterol (4.1±3.6% in Saudi Arabia; 5.4±2.5% in Ohio) in their SC.

Although CWL was lower in sparrows from Arabia, and lipid composition of their SC differed, we could not detect differences between rates of water loss through non-living skin attached to glass vials (46.0±15.7 mg H_2O cm^{-2} day^{-1} for sparrows in Saudi Arabia; 45.8±27.2 mg H_2O cm^{-2} day^{-1} for sparrows in Ohio). These results suggest that biological control mechanisms interact with layers of lipids in the stratum corneum to adjust CWL to the environment.

Key words: cutaneous water loss, lipid, house sparrow, *Passer domesticus*, stratum corneum, desert.

Introduction

Given that water is the *sine qua non* of life, it is intriguing that animals can live in deserts, environments with little drinking water, high ambient temperatures (T_a), intense solar radiation, low humidity and desiccating winds. Because desert birds are typically diurnal, at times they experience high ambient temperatures, requiring them to evaporate water from their respiratory passages and skin to keep body temperature below lethal limits (Williams and Tieleman, 2005). Birds also have high mass-specific rates of metabolism, which drive respiratory water loss, further exacerbating problems of water balance in birds living in deserts (Dawson and Bartholomew, 1968; Noy-Meir, 1973; Williams and Tieleman, 2002). One might envisage that natural selection has, and continues to, favor those phenotypes that can minimize rates of evaporative water loss under normal circumstances. But when T_a values exceed body temperatures, phenotypes will be favored if evaporation from skin and respiratory passages successfully regulates body temperature. Our understanding of the mechanisms that might be acted on by natural selection to prevent excessive water loss, or to ensure sufficient evaporative losses to maintain body temperatures during heat stress, remains rudimentary.

Total evaporative water loss, the sum of cutaneous water loss (CWL) and respiratory water loss, is reduced in birds living in deserts compared to those from mesic environments (Williams, 1996; Williams and Tieleman, 2000). Evidence thus far indicates that this reduction in total evaporative water loss cannot be explained by a decrease in respiratory water loss (Tieleman et al., 1999; Tieleman and Williams, 1999). Exploring the idea that natural selection has influenced CWL in desert birds, Tieleman and Williams (2002) measured CWL of four species of larks, two from mesic and two from arid environments, and found that CWL was reduced in larks from arid environments.

CWL is a function of the water vapor gradient between skin and air, and the total resistance to vapor diffusion through skin, feathers and boundary layer (Webster and King, 1987; Wolf and Walsberg, 1996; Williams and Tieleman, 2001).

Resistance across the skin contributes 70–95% of the total resistance (Webster et al., 1985). Tieleman and Williams (2002) suggested that reduced CWL observed in desert larks could be achieved by increasing resistance through the skin, and they proposed that changes in the lipid content of the stratum corneum (SC) would serve this purpose.

The epidermis of the skin of birds consists of four layers, all derived mitotically from the cells in the basal layer. Cells of the stratum basale, the innermost layer, have a large Golgi apparatus that apparently synthesizes lipids (Menon et al., 1986). Cells of the stratum intermedium, the layer above basal cells, form multigranular bodies (MGB), homologous to the lamellar bodies of mammals (Landmann, 1980; Menon et al., 1986). MGB are membrane-bounded organelles about 0.5 μm in diameter that contain lipids, mainly glycosphingolipids, cholesterol and phospholipids, which are thought to be stacked in layers called lamellae (Elias and Menon, 1991). In the stratum transitivum, it is thought that lamellae inside the MGB deteriorate and MGB coalesce to form membrane-free neutral lipid droplets, at least under normal circumstances (Landmann, 1986; Elias and Menon, 1991). At the stratum transitivum–stratum corneum interface, lipid droplets are apparently extruded into the intercellular spaces of the SC, creating the barrier to water vapor diffusion (Scheuplein and Blank, 1971; Elias et al., 1981; Blank et al., 1984; Grubauer et al., 1989; Elias and Menon, 1991; Menon and Menon, 2000).

The SC consists of flattened, dead corneocytes embedded in a matrix of lipids (Elias and Friend, 1975; Menon, 2002; Wertz, 2000). The main lipid classes found in the SC of birds are cholesterol, free fatty acids, ceramides (a molecule of sphingosine linked to a fatty acid) and cerebrosides (a ceramide bound to a sugar; Menon et al., 1986; Wertz et al., 1986). If the SC is the barrier to water vapor diffusion, and if the layer of dead corneocytes and lipids forms this barrier, it would seem that the barrier properties would be the same even when skin is removed from the bird.

Few studies have explored the association between the lipid composition of the SC and CWL in birds. Working with eight species of larks along an environmental gradient from mesic to arid, Haugen et al. (2003a) found that larks living in arid environments have a reduced CWL, a higher proportion of ceramides, and a smaller proportion of free fatty acids in their SC. They concluded that reduced CWL observed in desert larks was associated with changes in ratios of lipid classes in the SC.

Acclimation experiments on single species suggest that CWL is associated with the type and arrangement of lipids in the SC. Menon et al. (1989) found that zebra finches (*Taeniopygia guttata*) that were water-deprived for 45 days reduced their transepidermal water loss by 50% compared to control birds. This decrease was associated with changes in the lipid composition of the SC. When birds were rehydrated, changes were apparently reversed (Menon et al., 1989). Haugen et al. (2003b) found that Hoopoe larks *Alaemon alaudipes* L. acclimated to 35°C increased the proportion of polar ceramides and decreased the proportion of free fatty acids

in the SC with respect to individuals acclimated to 15°C, and that these changes in lipid composition were associated with a reduction in CWL.

Transepidermal water loss is thought to be a passive diffusion process influenced by the type and arrangement of lipids in the SC (Pinnagoda, 1994; Wilson and Maibach, 1994; Hoffman and Walsberg, 1999). If evaporation through the skin is a passive process, then rates of water loss through the skin of a living bird, and rates of water loss through the same skin removed from the bird, ought to be nearly the same. Although the physical properties of the SC do influence CWL, biological mechanisms may also operate in the live animal, enhancing the water barrier (Elias, 2004). Most of these processes involve the creation of gradients of ions across the SC that influence water permeation through lipid layers. For example, changes in the pH and calcium gradient across the SC alter the permeability of the skin, and affect barrier recovery after disruption (Menon et al., 1994; Bernard et al., 2003; Fluhr et al., 2004a,b). Despite the variety of biological mechanisms known to modify the performance of the permeability barrier in the skin, no studies have attempted to separate the contribution of physical and biological factors in the formation of the epidermal water barrier.

In this study we compared CWL of populations of house sparrows, one living in deserts (Saudi Arabia) and one living in a mesic environment (Ohio), and related CWL to lipid composition of the SC. We hypothesized that desert sparrows would have a lower CWL and that this reduction would be associated with changes in the lipids of the SC. We attempted to separate the physical properties of the barrier to water vapor diffusion from biological properties active in a living bird. We found that desert sparrows had a reduced CWL compared with sparrows from Ohio. Further, desert sparrows had larger concentrations of ceramides and cerebrosides in their SC and the proportion of cholesterol was lower in their SC. Despite this variation in lipid composition of the SC, when we measured water permeation through the dead skin in both groups of sparrows, we detected no differences between desert and mesic individuals. We concluded that water loss through the skin is not simply a passive process but rather an interaction between living cells of the epidermis and the non-living layers of the stratum corneum.

Materials and methods

We mist-netted house sparrows *Passer domesticus* L. near Taif, Saudi Arabia, at the National Wildlife Research Center (22°15′N, 41°50′E) and in Columbus (Ohio, USA, 40°00′N, 83°10′W), during October–November 2003. Prior to measurements, sparrows were held in captivity for 1–3 weeks; they were provided with a mixture of seeds, mealworms and egg yolk, and had unrestricted access to water.

House sparrows in North America, members of the subspecies *P. d. domesticus*, were introduced at the end of the 19th century from England (Summers-Smith, 1988). House sparrows in Saudi Arabia, *P. d. indicus*, are apparently native

to the area, although they depend on humans for access to water (Cramp and Perrins, 1994). The population of house sparrows that we studied in west-central Saudi Arabia had daily access to water, and probably would not be able to survive without it. These two subspecies have been isolated from one another for about 5000 years (Summers-Smith, 1988).

Measurement of metabolic rate and evaporative water losses

We measured oxygen consumption, respiratory water loss and CWL using an open-flow mask system, during November and December of 2003 (Gessaman, 1987; Tieleman and Williams, 2002). We removed food from the cages of birds 2–3 h prior to measurements to ensure postabsorptive conditions. Dissection of specimens at the end of measurements confirmed that the digestive tract was empty. In Ohio, measurements were made at night; in Saudi Arabia they were made during either the day or night. Because we did not find significant differences between measurements made during the day or night in Saudi Arabia, we pooled results (t=1.04, d.f.=11, P>0.33).

Birds were placed in a water-jacketed steel metabolic chamber (29.5 cm×21.5 cm×28 cm) that had a Plexiglas lid rendered airtight by a rubber gasket. We controlled the temperature of the air in the chamber using a Neslab circulating water bath (model RTE-140) set at 30.0°C, a temperature within the thermoneutral zone of sparrows (Hudson and Kimzey, 1966). Sparrows stood on a wire mesh platform over a layer of mineral oil that trapped feces, eliminating them as a source of water in our measurements. We quantified CWL and respiratory water loss separately using a plastic mask system (Tieleman and Williams, 2002). The mask covered the bill and nares, but did not cover the eyes or head of the bird, so evaporation from these areas contributed to CWL.

In our system, air coursed through Drierite®, soda lime and Drierite® to remove water and CO_2, then into the chamber. Air was drawn through the mask, then routed to a dewpoint hygrometer (EdgeTech, model 2001-C1-S3, Milford, MA, USA, in Ohio; General Eastern, model M4, Woburn, MA, USA, in Saudi Arabia), columns of Drierite® and ascarite, a mass-flow controller (Tylan, model FC 260, Billerica, MA, USA, in Ohio; Brooks, model 5850, Hatfield, PA, USA, in Saudi Arabia), set at 600 ml min^{-1}, both calibrated with a bubble meter (Levy, 1964), and into an oxygen analyzer (Applied Electrochemistry S3A-II, Naperville, IL, USA). Another air stream exited the chamber itself, and was directed through a second circuit identical to the first at a flow rate of 400 ml min^{-1}, except that air was vented to the room after passing through the dewpoint hygrometer and the vacuum pump. We verified that all respiratory gases were captured by the mask by directing air from the chamber to the oxygen analyzer: the fraction of O_2 in chamber air was always identical to inlet air.

Oxygen consumption, calculated using equation 4a of Withers (1977), was converted to kJ day^{-1} using 20.08 J ml^{-1} O_2 (Schmidt-Nielsen, 1997). To estimate respiratory water loss, we used the equation RWL=(ρ_{mask}–$\rho_{chamber}$) (V'_{e1}),

where ρ_{mask} is absolute humidity (g m^{-3}) of air leaving the mask corrected to standard temperature and pressure (STP), $\rho_{chamber}$ is the absolute humidity of air in the chamber (g m^{-3}, STP), and V'_{e1} is the flow rate of air leaving the mask (Tieleman and Williams, 2002). To calculate V'_{e1}, we assumed a respiratory quotient of 0.71 (King and Farner, 1961). CWL was determined as CWL=($\rho_{chamber}$–ρ_{in})(V'_{e1}+V'_{e2}), where ρ_{in} is the absolute humidity of the air entering the chamber (STP), and V'_{e2} is the flow rate of the air leaving the chamber (Tieleman and Williams, 2002). After 2–3 h, when traces of O_2 consumption were stable, we recorded oxygen consumption, dewpoint temperatures, and temperature of the air in the dewpoint hygrometers. We averaged data for oxygen consumption and dewpoint temperatures from traces that remained stable for at least 10 min.

Dewpoint hygrometers were factory calibrated against a primary standard traceable to the National Institute of Standards and Technology, less than 1 year prior to measurements. However, to confirm the accuracy of dewpoint hygrometers at the time of measurements, compressed air was routed through a column of Drierite® to remove water, then through a Brooks mass-flow controller (model 5850E), calibrated with a bubble meter, at a rate of 1000 ml min^{-1}. To saturate this air stream with water, we bubbled air through a 25 cm high column of distilled water at 20°C. Next we bubbled air through a water jacketed chamber filled with distilled water controlled at 12°C by a Neslab circulating water bath. Wet saturated air exited the chamber was directed to a dewpoint hygrometer. Air temperature into the chamber, measured with a thermocouple, was 12.0°C, whereas dewpoint temperatures were 11.7°C (General Eastern) and 11.3°C (EdgeTech), a deviation of 1–3% for General Eastern and 3–6% for our EdgeTech dewpoint hygrometer. These measurements were made in August 2005.

We also validated our ability to predict water loss from a bird using dewpoint hygrometry. Dry air was pushed through a mass-flow controller set at 1000 ml min^{-1}. Air was then directed into a 125 ml sealed flask partially filled with about 75 ml of distilled water. Air exited the flask to a dewpoint hygrometer. We wanted to estimate the error using our system to calculate evaporative water loss. To do so, we calculated water loss gravimetrically, weighing the water in the flask when the system reached equilibrium and 2–3 h after, and compared the mass difference with the total evaporative water loss obtained from the dewpoint temperature using the equations given above. The average error was 0.86±1.96% (N=5 trials).

Passive water loss through skin removed from sparrows

To estimate the passive permeability barrier of the skin attributable to the non-living SC, apart from active processes maintained by the living bird, we affixed skin of the ventral apterium to a glass vial (surface area of skin=2.13 cm^2) filled with a solution of phosphate-buffered saline (PBS; $Na_2HPO_4 \cdot 7H_2O$, $NaH_2PO_4 \cdot H_2O$ monobasic, and NaCl in distilled water, pH 7.4, 370 mOsm). Skin was glued to the edges of the vial with cyanoacrylate glue. We ensured a

complete seal of our preparation by turning the vial upside down and examining for leaks. We then placed the vial in a sealed container over a layer of Drierite® to ensure a low and constant water vapor pressure. The container and contents were then set in an incubator at 36°C, a temperature selected because it is near skin temperature of a desert bird. An uncovered vial filled with PBS was also placed in the same container and used as a control. After 8 h, a period previously determined for rates of water loss from the vial to stabilize, we recorded the weight of the vials using a balance (Metler, model AB204; 0.1 mg). We re-weighed the vials 2–3 h after the initial weighing.

Separation and identification of skin lipids

To isolate and quantify lipids of the SC of the sparrows, we weighed birds, killed them, plucked their feathers and removed their skin (Wertz et al., 1986; Haugen et al., 2003a,b). We pinned the skin to a thin sheet of Teflon®, and immersed it into a distilled water bath at 65°C for 3 min. Then, we gently peeled the epidermis from the dermis. The epidermis was incubated at 4°C overnight in a solution of 0.5% trypsin in PBS, allowing us to separate the SC from the rest of the epidermis. The following day, we rinsed the SC with distilled water, and re-immersed it in fresh 0.5% trypsin solution for 3 h at 38°C. We then rinsed the SC with distilled water over a fine mesh of silk cloth to remove any remaining feathers, and then froze the SC at –20°C in an atmosphere of argon or nitrogen. Thereafter, we freeze-dried the SC for 12 h, and stored it in a test tube at –20°C, again in an atmosphere of argon or nitrogen.

After determining dry mass of the SC (±0.01 mg), we extracted lipids with a mixture of chloroform:methanol 2:1, 1:1 and 1:2 v/v for 2 h, each step containing 50 mg l^{-1} of the antioxidant butylated hydroxytoluene (BHT; Law et al., 1995). We then combined extracts and dried the solution using a stream of nitrogen with an evaporimeter (N-EVAP, model 11155-O, Organomation Associates, Inc., Berlin, MA, USA).

Lipid classes were separated using analytical thin layer chromatography (TLC) on 20 cm×20 cm glass plates coated with silicic acid (0.25 mm thick; Adsorbosil-Plus 1, Altech, Deerfield, IL, USA). We removed contaminants from the plates by developing them with a mixture of chloroform:methanol (2:1, v/v) to the top, and thereafter activated plates in an oven at 110°C for 30 min. Then, we divided each plate into 29 6 mm-wide lanes. We prepared a series of five standards of known concentration, each containing nonhydroxy fatty acid ceramides (a sphingosine base with a mixture of octodecanoic and cis-15-tetracosenoic acids as the N-acyl fatty acid groups), galactocerebrosides, cholesterol, and a mixture of free fatty acids. We dissolved standards in chloroform:methanol (2:1, v/v) in concentrations ranging from 0.30 mg ml^{-1} to 10 mg ml^{-1}. Previous work indicated that this range covered concentrations of lipids found in our TLC plates. A duplicate series of standards was run on each plate. To prepare our samples for TLC, we re-dissolved the extracted lipids in 200 μl of chloroform:methanol (2:1, v/v) containing BHT. We pipeted 5 μl of each lipid extract in triplicate in the pre-adsorbent area of the plates using a Teflon-tipped Hamilton syringe. Two solvent systems were used, one for polar lipids, such as ceramides and cerebrosides, and another for non-polar lipids, free fatty acids and cholesterol. To separate ceramides and cerebrosides, we developed plates with a mixture of chloroform:methanol:acetic acid (190:9:1, v/v) to the top, followed by development with hexane:ethyl ether:acetic acid (70:30:1, v/v) run to 12 cm from the bottom. For sparrows, this procedure yielded four bands of ceramides and three bands of cerebrosides. Cholesterol and free fatty acids were separated by development with hexane to the top of the plate, followed by toluene to the top, and finally a development with hexane:ethyl ether:acetic acid run to 12 cm from the bottom. We visualized bands of lipids by spraying the plates with a solution of 3% cupric acetate in 8% phosphoric acid, and then placing the plates on a 20 cm×20 cm aluminum hotplate slowly raised to 220°C over the course of 2 h.

To quantify the concentration of lipid classes, we scanned the plates with a Hewlett Packard scanner, and measured the amount of each class by photodensitometry using the computer software TN-Image (T. J. Nelson, 2003: Shareware software available at http://entropy.brni-jhu.org/tnimage.html). Because Haugen et al. (2003a,b) found that proportions of the main lipid classes in the SC changed along an aridity gradient, we also calculated percentages of lipids as the amount (mg) divided by the sum of the total amount of the four lipid classes analyzed.

To validate our ability to measure the quantity of lipids in solution, we followed the same protocol but used known concentrations of cholesterol as our unknown. The average error, calculated as [(observed–actual/actual)×100], was –0.88±4.47% (N=8).

Statistics

All statistical tests were performed using SPSS 12.0. We rejected the null hypothesis at $P>0.05$. Values are means ± 1 s.D. We tested for differences between means using a two-tailed t-test for independent samples. Concentrations of cholesterol and free fatty acids were not normally distributed (Kolmogorov–Smirnov test, KS=0.18, $P<0.04$, and KS=0.20, $P<0.01$, respectively). We log-transformed these variables to normalize the data (KS=0.10, $P>0.15$, and KS=0.09, $P>0.15$). Percentages were logit transformed [$\ln(Y/1–Y)$; Zar, 1996] prior to analyses to normalize data. We performed regressions using a general linear model. To test for differences between regressions for lipids from desert and mesic sparrows, we first tested for the significance of the interaction term. If the interaction was not significant, we removed it from the model, and tested for differences in intercepts assuming a common slope.

Results

Body mass and surface area

For six males and five females in Saudi Arabia, and eight males and five females in Ohio, average mass did not differ between sexes ($P>0.7$ Saudi Arabia, $P>0.09$ Ohio). Combining data for sexes, mean mass of sparrows in Saudi Arabia was

20.1±1.6 g (*N*=11) and in Ohio was 23.2±2.8 g (*N*=15), a difference that was significant (*t*=–3.25, d.f.=24, *P*<0.01). Using Meeh's equation (Walsberg and King, 1978), the surface area of sparrows from Saudi Arabia was calculated as 74.0±4.0 cm^2, whereas from Ohio it was 81.3±6.6 cm^2.

Oxygen consumption

Desert sparrows consumed oxygen at a lower rate than did Ohio sparrows: 56.9±5.9 ml h^{-1} for sparrows in Saudi Arabia, 64.8±6.0 ml h^{-1} for birds in Ohio (*t*=–2.7, d.f.=17, *P*<0.02). Heat production was 27.5±3.0 kJ day^{-1} for sparrows from Saudi Arabia, and 31.3±3.3 kJ day^{-1} for birds from Ohio. Mass-specific metabolic rates were not significantly different between groups: 1.37±0.14 kJ g^{-1} day^{-1} (Saudi Arabia) and 1.32±0.18 kJ g^{-1} day^{-1} (Ohio; *t*=0.65, d.f.=17, *P*>0.5).

Respiratory water loss

Respiratory water loss was 0.97±0.23 and 1.25±0.32 g H$_2$O day^{-1} for sparrows from Saudi Arabia and from Ohio, respectively, values that differed significantly (*t*=–2.5, d.f.=20, *P*<0.02). When these values were expressed per unit body mass, however, we did not detect significant differences: 48±12 mg H$_2$O g^{-1} day^{-1} (Saudi Arabia), 54±16 mg H$_2$O g^{-1} day^{-1} (Ohio); *t*=–0.997, d.f.=20, *P*>0.3 (Fig. 1A).

Cutaneous water loss of live sparrows

With a CWL of 0.88±0.18 g H$_2$O day^{-1}, desert sparrows lost significantly less water through their skin than did sparrows from Ohio (1.32±0.14 g H$_2$O day^{-1}; *t*=–5.2, d.f.=23, *P*<0.001).
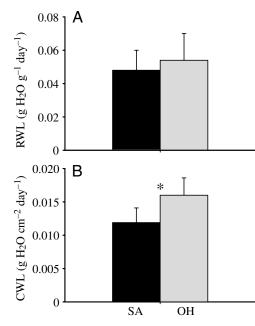

Fig. 1. (A) Mass-specific respiratory water loss (RWL) and (B) surface-specific cutaneous water loss (CWL) in sparrows from Saudi Arabia (SA) and Ohio (OH). Asterisks represent significant differences between groups (*P*<0.05).

When CWL was adjusted for differences in surface area, CWL remained significantly different between desert and mesic sparrows: 11.9±2.2 mg H$_2$O cm^{-2} day^{-1} (Saudi Arabia), 16.0±2.6 mg H$_2$O cm^{-2} day^{-1} (Ohio; *t*=–2.8, d.f.=23, *P*<0.01; Fig. 1B).

Passive water loss through non-living skin

In vials covered with skin, water loss was 46.0±15.7 mg H$_2$O cm^{-2} day^{-1} for sparrows from Saudi Arabia (*N*=12), and 45.8±27.2 mg H$_2$O cm^{-2} day^{-1} for sparrows from Ohio (*N*=11), values that were not significantly different (d.f.=21, *P*>0.9). The rate of water loss in the uncovered vial was 456.4±37.1 mg cm^{-2} day^{-1} in Saudi Arabia, and 440.5±59.0 mg cm^{-2} day^{-1} in Ohio (*t*=0.77, d.f.=16; *P*>0.45). When compared with an open vial, a free water surface, skin-covered vials lost 10.1% and 10.4% as much water in Saudi Arabia and Ohio, respectively.

Lipids in the stratum corneum

For both groups of sparrows, our procedure using TLC revealed distinct bands of lipids corresponding to standards of cholesterol, free fatty acids, ceramides and cerebrosides. The ceramides that separated into bands were named in order of increasing polarity (ceramides 1–4), as were the cerebrosides 1–3 (Fig. 2).

Using photodensitometry to quantify lipids (mg lipid g^{-1} dry mass SC), we did not find significant differences in the quantity of total lipids, cholesterol or free-fatty acids between desert and mesic sparrows, but desert house sparrows had a larger concentration of total ceramides and total cerebrosides per g dry mass SC than did sparrows from Ohio (Table 1). For specific sphingolipid classes, desert sparrows had significantly more ceramide 3 and cerebroside 1 in their SC. In general, cerebrosides were more abundant in the SC of sparrows than has been previously reported for either birds or mammals.

Because it is thought that subtle changes in the proportions of lipid classes in the SC can influence the fluidity of the lipid layer and therefore water permeation (Haugen et al., 2003a,b; Bouwstra et al., 2003), we also expressed lipid classes as a percentage of the total lipids extracted. Desert sparrows had a significantly lower proportion of cholesterol and ceramide 2 than mesic birds (Table 2).

CWL and lipids

CWL did not vary with the quantity of total lipids in the SC in either group of sparrows (*r*^{2}<0.06, *P*>0.27, for all cases). However, among sparrows from Ohio, CWL was positively correlated with the percentage of free fatty acids (*r*^{2}=0.50, *P*<0.01), and it varied negatively with the percentage of total ceramides, ceramide 3 and total cerebrosides (*r*^{2}=0.46, *P*<0.02; *r*^{2}=0.36, *P*<0.04; *r*^{2}=0.39, *P*<0.04, respectively; Fig. 3). There were no significant correlations between CWL and percentages of the different lipid classes in sparrows from Saudi Arabia, but this may not be surprising since variation tended to be lower in desert sparrows.
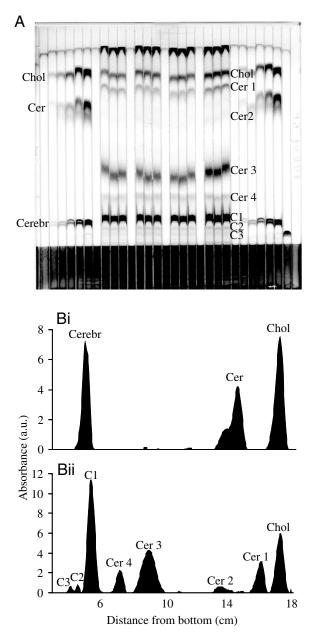
Fig. 2. (A) Thin layer chromatograph of the polar lipids in the SC of house sparrows. (B) Profile of lipid standards (Bi) and cerebrosides, ceramides and cholesterol from the extracted lipids in the SC of house sparrows obtained using densitometry (Bii). Chol, cholesterol; Cer, ceramide; Cerebr, cerebroside; C1–C3, cerebrosides 1–3. a.u., arbitrary units.

Correlations among lipids in the SC

Given that adjustments in the relative proportions of lipids in the SC may have important consequences for barrier function, we explored covariation between the percentages of the various lipids in the SC; proportions of some classes of lipids were significantly correlated in both groups (Table 3). Interestingly, variation in lipids among individuals was low for desert sparrows. In general, free fatty acids are negatively correlated with the rest of the lipid classes in the SC.

Discussion

A number of features of this study are noteworthy. For a single species of free-living house sparrow, our study provides evidence that total evaporative water loss is lower in populations living in desert environments, a diminution correlated with a reduction in CWL. Reduction in CWL was coincident with sparrows in Saudi Arabia having larger concentrations of ceramides and cerebrosides in their SC. Variation in CWL among individuals was related to changes in the lipids within the SC for sparrows from Ohio, but not those from Saudi Arabia, which tended to have little variation in CWL. For birds in Ohio, free fatty acids were positively associated with CWL, whereas ceramides and cerebrosides were negatively correlated with CWL. In both populations of sparrows, we have found high concentrations of cerebrosides in their SC, a lipid class that seems to be unimportant in the SC of mammals, but may serve a prominent role in integument barrier formation in birds. Although we have detected changes in the lipids of the SC in desert sparrows compared to mesic individuals, these alterations do not seem to significantly affect passive diffusion of water through skin removed from the bird. Thus, we think that biological processes in concert with the physical lipid barrier operate to reduce CWL.

In order to compare house sparrows with other birds, we compiled data for CWL from the literature (Table 4). As one might expect, amongst 21 species of birds, CWL (mg H_2O cm^{-2} day^{-1}) varied positively with body mass (M_b, in g) as described by CWL=$0.011M_b$+11.946 (r^2=0.98, P<0.01). However, to our surprise, we found that surface-specific CWL (mg H_2O cm^{-2} day^{-1}) also varied with body mass: CWL=11.44+7.93logM_b (r^2=0.45, P<0.001; Fig. 4). Larger birds tend to lose more water per unit surface area than do small birds. Therefore, expressing CWL on a surface-specific basis does not standardize comparisons for interspecific data

Table 1. *Quantities of each lipid class in the stratum corneum of house sparrows from Saudi Arabia and Ohio*

				Lipid concentration (mg lipid g^{-1} dry mass SC)								
Site	N	Cholesterol	FFA	Total ceramides	Cer 1	Cer 2	Cer 3	Cer 4	Total cerebrosides	Cerebr 1	Cerebr 2	Cerebr 3
Saudi Arabia	11	11.9±2.1	246.4±167.8	49.2±10.3*	9.7±1.1	5.6±1.5	26.7±8.6*	9.9±8.4	101.2±48.9*	103.0±40.1*	4.0±1.5	3.8±1.5
Ohio	15	13.9±7.3	206.5±204.7	38.2±18.0*	8.0±4.6	5.5±3.0	13.0±9.4*	11.8±11.1	56.5±34.0*	45.5±31.3*	3.8±2.1	3.8±2.6

FFA, free fatty acids; Cer 1–4, ceramide of class 1–4; Cerebr 1–3, cerebroside of class 1–3; SC, stratum corneum.

Values are means ± S.D. Asterisks indicate significant differences between populations (P<0.05).

Table 2. *Mean proportions of each lipid class in the stratum corneum of house sparrows in Saudi Arabia and Ohio*

Site	N	Cholesterol	FFA	Total ceramides	Cer 1	Cer 2	Cer 3	Cer 4	Total cerebrosides	Cerebr 1	Cerebr 2	Cerebr 3
Saudi Arabia	10	4.1±3.6*	55.3±12.3	15.2±7.3	2.5±0.7	1.4±0.4*	7.1±3.7	2.4±1.6	25.4±6.6	24.3±6.7	0.9±0.3	0.9±0.3
Ohio	12	5.4±2.5*	58.5±18.7	14.5±5.9	3.2±1.1	2.0±0.7*	5.1±4.0	4.5±4.8	21.6±12.7	18.0±12.7	1.3±0.7	1.3±0.6

FFA, Free fatty acids; Cer 1–4, ceramide of class 1–4; Cerebr 1–3, cerebroside of class 1–3.
Proportions were calculated as the amount of a given lipid class divided by the sum of the amounts of all four lipid classes. Values are means ± S.D. Asterisks indicate significant differences between populations ($P<0.05$).

sets. Using analysis of covariance (ANCOVA) with M_b as a covariate, we could not find any significant difference between desert and non-desert birds ($P>0.45$). These data suggest that large birds, having a smaller surface to volume ratio, need higher rates of water loss for thermoregulation than birds of smaller size, a hypothesis in need of testing.

Desert house sparrows had significantly lower rates of CWL than did sparrows from Ohio (about 33% less). At this point we do not know if this reduction can be attributed to
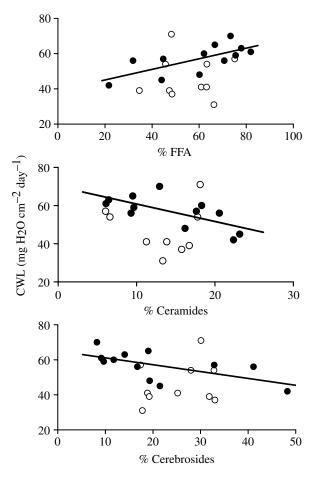

Fig. 3. Cutaneous water loss (CWL) in desert (open circles) and mesic (filled circles, solid lines) sparrows as a function of the percentage of free fatty acids (FFA), ceramides and cerebrosides. Only the correlations for sparrows from Ohio were significant; for regression equations, see text.

acclimation, genetic differences, or both. Using our allometric equation for surface-specific CWL, sparrows in Saudi Arabia and Ohio should lose 21.8 and 22.3 mg H_2O cm^{-2} day^{-1}, respectively. Sparrows from Saudi Arabia lost 11.9 mg H_2O cm^{-2} day^{-1}, whereas in Ohio house sparrows evaporated 16.0 mg H_2O cm^{-2} day^{-1} through their skin. The reduction in CWL among sparrows in Arabia can be attributed in part to a smaller body size, but also to changes in the rates of CWL through skin. Our calculations from allometric curves indicate that reduction in body size only accounts for a difference of 0.17 g H_2O day^{-1}, with the remainder being an alteration in CWL. Changes in the lipid composition of the skin associated with factors operating in the live animal may explain these different water loss rates in sparrows from different environments.

The lipid composition of the SC of house sparrows differed from that found in other species of birds. In pigeons and chickens, free fatty acids comprised about 20–30% of the dry weight of the SC (Menon et al., 1986; Wertz et al., 1986). The percentage of free fatty acids in our samples exceeded 55% in both desert and mesic house sparrows. Sphingolipids accounted for about 25% of the dry mass of the SC in pigeons, with ceramides and cerebrosides accounting for equal proportions (Menon et al., 1986). The percentage of sphingolipids in the SC in both populations of sparrows was around 40%. Moreover, in our samples almost two thirds of the sphingolipids were cerebrosides. A high proportion of glucosylceramides apparently precludes the formation of lamellae in the intercellular spaces of the SC in mammals (Holleran et al., 1993; Proksch et al., 1993). This effect is not caused by a decrease in ceramides, so it does not imply a negative association between these two lipid classes. In fact, we found a positive relationship between ceramides and cerebrosides in house sparrows (Table 3). Cerebrosides can be cleaved to form ceramides by glycosidases (Wertz and Downing, 1989). Therefore, accumulation of cerebrosides could lead to a mobilization of a higher amount of ceramides whenever it is necessary by increasing the activity of this enzyme.

The high concentration of glycosphingolipids within the SC of desert sparrows seems counterintuitive because in mammals an increase in glycosphingolipids decreases barrier function of skin (Holleran et al., 1993). Birds in general are thought to have a less competent barrier than mammals, and they typically

Table 3. *Correlations between the proportions of the different lipid classes in the SC of house sparrows from Saudi Arabia and Ohio*

	Saudi Arabia			Ohio		
	b	a	r^2	b	a	r^2
Free fatty acids *vs*						
Cholesterol	–2.5[a]	65.4	0.50	–4.61[a]	83.4	0.39
Total ceramides[b]	–1.49[a]	78.0	0.78	–2.87[a]	100.2	0.80
Ceramide 3	–1.65	66.6	0.24	–4.02[a]	78.9	0.75
Total cerebrosides	–0.96	79.6	0.26	–1.38[a]	88.4	0.88
Cerebroside 1	–0.48	66.3	0.09	–1.30[a]	82.0	0.79
Total ceramides *vs*						
Cholesterol[b]	1.87[a]	7.6	0.82	1.92[a]	4.2	0.71
Ceramide 3 *vs*						
Cholesterol	–0.07	7.1	0.01	0.30	3.4	0.04
Total cerebrosides *vs*						
Cholesterol	–0.40	27.1	0.05	1.69	12.4	0.12
Total ceramides	0.06	24.6	0.01	1.50[a]	–0.2	0.47
Ceramide 3	1.10	17.9	0.37	3.04[a]	6.2	0.93
Cerebroside 1 *vs*						
Cholesterol	–0.95	26.9	0.20	1.57	9.6	0.10
Total ceramides	–0.18	25.7	0.03	1.33[a]	–1.2	0.36
Ceramide 3	1.23	14.6	0.35	2.88[a]	3.5	0.83

Equations are of the form $y=bx+a$.
[a]Significant parameter ($P<0.05$).
[b]Significant differences between desert and mesic house sparrows ($P<0.05$).

have higher concentrations of glycosphingolipids in their SC (Elias and Menon, 1991). So how can we reconcile desert sparrows having lower water loss, yet at the same time higher concentrations of cerebrosides in their SC? We propose a model to explain our results, based on requirements for thermoregulation and for water conservation (Fig. 5).

The SC serves multiple purposes in vertebrates and is not used solely for reduction in water permeation through the skin. Another important aspect of CWL is that it may be a significant component of the thermoregulatory apparatus of a living bird. House sparrows in deserts of Saudi Arabia have access to water because they are associated with Bedu camps where water is provided to livestock. At the same time, these sparrows are subjected to high ambient temperature (T_a), especially during summer when T_a can reach 45°C each day. Thus selection for their ability to maintain body temperature (T_b) may be important. In our model we hypothesize that the SC is divided into two regions, the upper SC with a high concentration of ceramides, and a lower region that has a higher concentration of cerebrosides (Fig. 5). The polar heads of cerebrosides would interact with water molecules, creating a pathway of diffusion of water through the skin; every cerebroside molecule can interact with 5–10 water molecules (Bach and Miller, 1998). It is also likely that the upper SC exists in a gel phase and the lower SC in a crystalline phase; infrared spectroscopy and X-ray diffraction showed that different lipid phases coexist in the SC in mammals (Bouwstra et al., 2003). The structure of the

SC in desert and mesic sparrows would be essentially the same, but there would be a higher quantity of ceramides in the upper SC and a higher amount of cerebrosides in the lower SC in desert sparrows than in mesic birds. The higher quantity of ceramides would explain the reduction in CWL observed in desert sparrows. During periods of heat stress, sparrows would be compelled to increase their CWL. Under these demands, the activity of glycosidases would decrease, leading to an accumulation of cerebrosides in the SC, therefore increasing CWL. On the other hand, when the birds are water-stressed, the activity of glycosidases and other hydrolytic enzymes would increase, and cerebrosides would be transformed into ceramides. It is likely that a given quantity of ceramides in the SC would promote the arrangement of the intercellular lipids in lamellae in the SC. The net result would be a decrease in CWL, so the bird would be able to conserve water. Enzymatic responses to environmental cues operate in less than 6 h (Schaefer and Redelmeier, 1996).

Experimental work on birds partially supports predictions of the model. Menon et al. (1989) found that cerebrosides in water-stressed zebra finches represented 5.9% of the epidermal lipid dry mass, compared with 8.7% in the controls, and 9.9% in rehydrated birds. In addition, water-deprived zebra finches showed fewer lipid droplets in the corneocytes, more intercellular lamellae in their SC, and a higher number of MGB when compared to control birds. These changes led to an enhancement of the permeability barrier through the skin;

Table 4. *Body mass and cutaneous water loss for 21 species of birds*

Species	Body mass (g)	CWL (mg H_2O day^{-1} cm^{-2})	% of TEWL	Deviation from prediction	Source
Struthioniformes					
Dromaius novaehollandiae[a]	52 500[e]	40.6		−16.9	Webster (1991)
Galliformes					
Excalfactoria chinensis	43.2	17.3	44.7	−29.1	Bernstein (1971)
Gallus domesticus	2040	43.8		16.3	Webster (1991)
Anseriformes					
Anas platyrynchos	2500	55.6		44.9	Webster (1991)
Ciconiformes					
Cathartes aura	1470[e]	29.9		−18.17	Webster (1991)
Phalacrocorax auritus	1460	38.4		5.2	Webster (1991)
Psittaciformes					
Melopsittacus undulates	31.6	40.2	58.9	72.4	Bernstein (1971)
Columbiformes					
Columba livia	473	33.7		3.3	Webster (1991)
Streptopelia risoria	146	9.7		−66.1	Webster (1991)
Zenaida macroura[c]	109	28.0		1.5	Webster (1991)
Cuculiformes					
Geococcyx californicus	274	25.6		−16.8	Webster (1991)
Strigiformes					
Phanaeloptilus nutalli[d]	43	28.3		16.1	Webster (1991)
Passeriformes					
Corvus cryptoleucus[b]	534[e]	38.3		15.9	Webster (1991)
Taenopygia castanotis	12.5	31.2	62.9	55.0	Bernstein (1971)
Ploceus cucullatus	42.6	28.5	50.8	17.0	Bernstein (1971)
Auriparus flaviceps	7.0	15.0	61.1	−17.3	Wolf and Walsberg (1996)
Alaemon alaudipes[c]	36.5	18.5	67.1	−22.3	Tieleman and Williams (2002)
Eremalauda dunni[c]	20.5	20.2	56.7	−7.5	Tieleman and Williams (2002)
Lullula arborea[c]	25.5	23.9	67.4	5.8	Tieleman and Williams (2002)
Alauda arvensis[c]	31.5	25.7	66.7	10.3	Tieleman and Williams (2002)
Passer domesticus (desert)	20.1	11.9	49.5	−28.3	This study
Passer domesticus (mesic)	23.5	16.0	51.4	−42.6	This study

CWL, cutaneous water loss; TEWL, total evaporative water loss.

Predicted values for CWL were calculated using the allometric equation reported in Fig. 6. Deviation from the prediction was computed as [(observed CWL–predicted CWL)×100]/predicted CWL. All the species were measured at 30°C, except where noted: [a]27°C; [b]32°C; [c]25°C; [d]35°C.

[e]Estimated mass.

water-deprived zebra finches exhibited a 50% reduction of their trans-epidermal water loss. Peltonen et al. (1998, 2000) found that cold-acclimated pigeons (*Columba livia* L.) substituted the amorphous lipoid material of the intercellular spaces in the SC by lipid lamellae, a response similar to that found in zebra finches under xeric stress. Unfortunately, they did not report any values for CWL. Heat acclimated pigeons did not show lamellar material in the SC (Peltonen et al., 1998, 2000), a result consistent with the idea that effective theromoregulation by CWL requires more cerebrosides. Desert birds without access to water should be selected to maintain high concentrations of ceramides in their SC and rely on respiratory water loss to control body temperature (Tieleman and Williams, 2002). In eight species of larks, the proportion of ceramides in the SC increases with the aridity of the environment (Haugen et al., 2003a).

Although we found that the lipid composition of the SC differs in desert house sparrows, this alteration by itself does not seem to make the physical permeability barrier more effective when compared to their mesic counterparts. If only passive diffusion through the skin were acting on water loss rates in house sparrows, we would predict differences in water loss rates through the dead skin of the sparrows from desert and mesic populations. However, water evaporation rate through the non-living epidermis attached to vials was not significantly different between the two populations. If passive diffusion were the only factor accounting for evaporation through the skin of the sparrows, birds in Saudi Arabia would lose 3.24 g day^{-1}, whereas sparrows in Ohio would evaporate 3.73 g day^{-1}. Live animals lose 28.7% and 35.4% of these values, respectively. Therefore, biological factors operating in the live animal influence CWL significantly. According to our
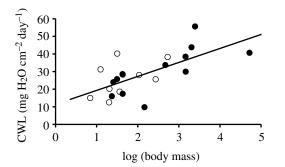
Fig. 4. Relationship between surface-specific cutaneous water loss (CWL), calculated by Meeh's equation (Walsberg and King 1978), and log(body mass) for 21 species of birds. Desert species are represented by open circles, whereas mesic species are indicated by filled circles.
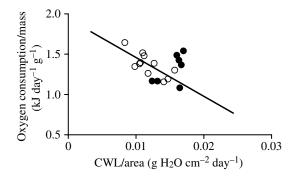

Fig. 6. Relationship between surface-specific CWL and mass-specific oxygen consumption in the sparrows from Saudi Arabia (open circles) and Ohio (solid circles). Regression was only significant in the sparrows from Saudi Arabia; mass-specific oxygen consumption (kJ g^{-1} day^{-1})=–48.1×Surface-specific CWL (g H_2O cm^{-2} day^{-1})+1.94 (solid line; r^2=0.56, $P<0.01$).

model, activation of the metabolic machinery would produce a reduction of water loss through the skin (Fig. 5). For example, pH is lower in the upper layers of the SC than in the lower SC (Elias, 2004). Most of the enzymes that convert lipids in the multigranular bodies to those lipid classes that form the permeability barrier have a maximum activity within a pH of 4–6. It is likely that active processes are responsible for creating and maintaining this pH gradient, allowing regulation of the biochemical properties of the SC. Consistent with the idea that there is a metabolic cost in maintaining the barrier to vapor diffusion, we found a negative correlation between surface-specific CWL and oxygen consumption in desert house sparrows (Fig. 6).

In conclusion, CWL in house sparrows living in a desert environment was reduced compared to mesic house sparrows, and this decrease in CWL was responsible for the reduction of total evaporative water loss in desert house sparrows when compared to the mesic population. We found an alteration of the lipid composition in the SC, yet we did not find any significant difference in the properties of the physical barrier in both populations. Thus, biological control mechanisms must play a crucial role enhancing the permeability barrier. The balance between requirements for thermoregulation by evaporative means and water conservation might have played an important role in the evolution of the composition of the skin in desert species.
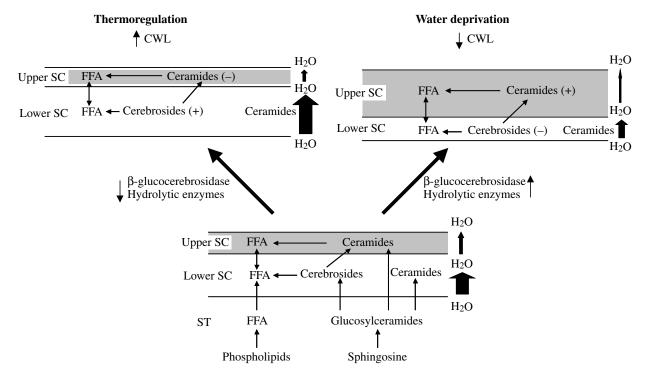

Fig. 5. Functional model for the structure of the SC in desert house sparrows. See text for explanation.

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