Phenotypic Flexibility in Cutaneous Water Loss and Lipids of the Stratum Corneum in House Sparrows (*Passer domesticus*) following Acclimation to High and Low Humidity

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ABSTRACT

Resistance to water-vapor diffusion through the skin is thought to be conferred by lipids in the stratum corneum (SC), the outer layer of the epidermis. We tested the effect of ambient humidity on cutaneous water loss (CWL) and lipid composition of the SC by acclimating house sparrows (*Passer domesticus*) to either a dry (6.5 g/m^3 absolute humidity) or a humid (31 g/m^3) environment for 3 wk at a thermoneutral temperature (30°C). Sparrows in the dry-acclimated group reduced CWL by 36% compared with those in the humid environment. Relative to initial values, both groups of sparrows decreased CWL, 45% in the dry-acclimated group and 23% in the humid group, suggesting that temperature is also an important stimulus for CWL apart from humidity. Both groups of acclimated sparrows decreased quantities of cholesterol, free fatty acids, and cerebrosides and increased the proportion of ceramides in their SC. Lipid amounts or proportions in the SC did not differ between dry- and humid-acclimated sparrows, but the free fatty acid : ceramide ratio was significantly lower in dry-acclimated birds. Also, lipid composition was only correlated with CWL in dry-acclimated sparrows, suggesting that structural changes to SC lipids are more tightly linked to CWL regulation in response to low humidity. Our results demonstrate phenotypic flexibility in CWL and lipid composition of the SC and provide support for a functional relationship between these traits.

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Introduction

Balancing water intake with losses can determine survival and reproduction, and thus fitness, for many animals. Although it was once thought that cutaneous water loss (CWL) represented a small fraction of total water loss in mammals and birds (Bartholomew and Cade 1963; Schmidt-Nielsen 1970; Mount 1979), it is now appreciated that CWL represents a substantial contribution to total water efflux. As such, regulation of CWL is an essential aspect of water balance (Bernstein 1971*a*, 1971*b*; Tracy and Walsberg 2000).

For mammals, birds, and reptiles, the outer layer of the epidermis, the stratum corneum (SC), forms a barrier to water vapor diffusion from the animal to its environment, thereby regulating CWL and preventing desiccation (Scheuplein and Blank 1971; Elias et al. 1981; Blank et al. 1984; Lillywhite 2006). The SC of mammals is composed of corneocytes, flattened dead cells embedded in a matrix of lipids (primarily ceramides, cholesterol, and free fatty acids) in near equimolar amounts (Bouwstra et al. 2003). Lipids in the mammalian SC are organized in bilayers called lamellae that retard water permeation through the skin (Menon and Ghadially 1997; Wertz 2000; Bouwstra et al. 2003). Although how each lipid class is involved in the formation of lamellae is controversial, there is general agreement that ceramides, molecules of sphingosine amide linked to a long-chain free fatty acid, are the structural backbone of the bilayers and are thus essential for the formation of a tight barrier to water vapor diffusion through the skin (Forslind et al. 1997; Norlén 2001; Bouwstra et al. 2003; Hill and Wertz 2003).

One important difference between the SC of birds and mammals is that the former contains substantial quantities of cerebrosides, ceramides bound to a sugar, with a concomitant reduction in ceramides. A lack of cerebrosides is associated with the absence of intercellular lipid lamellae in birds that are normally hydrated, a feature thought to result in a less competent permeability barrier (Menon and Menon 2000). Cerebrosides may increase water permeability of the SC because hydroxyl groups of the sugar tend to attract water molecules rather than repel them, potentially creating a water channel, an analogous process to what appears to happen in cell membranes (Carruthers and Melchior 1983).

Unlike mammals, the skin in birds is an important component of the thermoregulatory apparatus in some species (Bernstein 1971*b*; Peltonen et al. 2000; Webster et al. 1985; Webster and King 1987). At thermoneutral temperatures, av-

erage surface-specific CWL for 21 species of birds was 28.2 mg $H_2O/(cm^2 \times d)$ (Muñoz-Garcia and Williams 2005), 2–3 times higher than rates characteristic of mammals. At temperatures above 40°C, birds increase rates of CWL by a factor of 1.5 up to 20-fold, depending on the species (Bernstein 1971*b*; Marder and Ben-Asher 1983; Wolf and Walsberg 1996; Tieleman and Williams 2002). Many species, however, rely on an increase in respiratory water loss (RWL) for evaporative cooling under acute heat stress. In the verdin (*Auriparus flaviceps*) and four species of larks, CWL represented 65% of total evaporative water loss (TEWL) at 25°–30°C, but the contribution of CWL decreased to 13% at temperatures above 45°C (Wolf and Walsberg 1996; Tieleman and Williams 2002). On the other hand, pigeons *Columba livia* chronically exposed to air temperatures (T_a) between 40° and 60°C seem to use CWL to thermoregulate at high temperatures, increasing CWL rates 20 times at T_a's above 50°C (Marder and Ben-Asher 1983). It is not clear whether the differences observed between Columbiformes and Passerines are the result of phylogenetic divergence or the consequence of short-term versus long-term exposures at high T_a. In any case, it appears that the skin of birds serves an important thermoregulatory role by facilitating evaporative water loss when T_a is chronically high and maintenance of body temperature below lethal limits is important.

Species of birds from deserts have lower rates of CWL than species living in mesic environments, which appear to be associated with a high proportion of ceramides and a low proportion of free fatty acids in the SC (Tieleman and Williams 2002; Haugen et al. 2003*b*; Muñoz-Garcia and Williams 2005, 2007). Phenotypic plasticity may account for some variation in CWL among and within species of birds. After acclimation to different temperatures (Haugen et al. 2003*a*), larks increased the proportion of ceramides and decreased the proportion of free fatty acids in their SC, changes presumably correlated with a decrease in CWL. Zebra finches that were water deprived for 5 d (Menon et al. 1989) and pigeons acclimated to high temperatures (Peltonen et al. 1998, 2000) formed intercellular lamellae in the SC, presumably to decrease CWL. Mechanisms that allow individuals to change the structure and lipid composition in the SC as a result of an environmental alteration are, however, not understood. We hypothesized that water stress will change the enzymatic activity in the SC and convert cerebrosides into ceramides, resulting in a tighter permeability barrier and reduced CWL (Muñoz-Garcia and Williams 2005, 2007; Cox et al. 2008). Because of their rodlike shapes and their ability to ionize, free fatty acids interact with ceramides in the upper SC to form a highly ordered lattice impeding water movement. Therefore, the proper ratio of free fatty acids to ceramides may also be an important determinant of CWL. Under chronic heat stress, when evaporative cooling is needed for thermoregulation, inactivation of SC enzymes will maintain a high proportion of cerebrosides, lipids that augment water loss. Cholesterol disrupts this highly ordered orthogonal phase of lipids, increasing the fluidity of the lipid matrix in the SC and consequently making the skin more permeable.

Studies exploring the role of phenotypic flexibility (sensu Piersma and Lindstrom 1997) in the origin and maintenance of variation in CWL are few (Kobayashi et al. 1983; Kattan and Lillywhite 1989; Williams and Tieleman 2000; Tracy and Walsberg 2001; Haugen et al. 2003*a*; Moen et al. 2005). In this study, we experimentally tested the extent to which phenotypic plasticity was responsible for the functional relationship between the lipid composition of the SC and CWL. Humidity has been proposed as one of the factors that may determine CWL in vertebrates, by initiating a cascade of enzymatic activity that may result in a change in the SC permeability (Elias 2004). Therefore, we chose to manipulate this variable in our study.

We exposed house sparrows to two environments, one dry and the other humid, at constant T_a of 30°C, a temperature within their thermoneutral zone (Hudson and Kimzey 1966). We predicted that (1) humid-acclimated sparrows would not change their CWL with respect to nonacclimated birds, (2) dry-acclimated sparrows would reduce their CWL compared with non- and humid-acclimated birds, (3) reduction in CWL in dry-acclimated birds would be associated with an increase in the amounts of ceramides and cerebrosides in the SC and a decrease in the amount of cholesterol, and (4) the free fatty acid : ceramide ratio would decrease in the SC of dry-acclimated birds.

Material and Methods

Humidity Acclimation

We captured adult house sparrows (*Passer domesticus*) in Columbus, Ohio (40°00′N, 83°10′W) using mist nets and Potter traps during February and March 2006. Birds were held in captivity for 1 to 4 d before measurements. Sparrows were fed a mixture of seeds, mealworms, and boiled egg yolk and had unrestricted access to water. Experiments were approved by the Institutional Laboratory Animal Care Use Committee of Ohio State University (protocol 2003-A0072).

We measured CWL, RWL, and oxygen consumption for all house sparrows shortly after capture (see below). After measurements, we randomly assigned each bird to one of two groups: dry acclimated ($n = 14$) and humid acclimated ($n = 9$). We also captured 11 additional sparrows to measure initial lipid composition of the SC before acclimation.

We placed sparrows assigned to humid and dry acclimation groups in wire cages in separate environmental chambers (Percival [Boone, IA], models E-30B and I-30BLL), each containing five or six sparrows, at a constant temperature of 30°C (± 1°C) with a photoperiod of 12L : 12D. A vacuum pump pulled atmospheric air into the chambers at a rate of 75 mL/min. Dry-acclimated sparrows experienced an average relative humidity of 15%–20%, corresponding to a mean absolute humidity of 6.5 g H_2O/m^3. To maintain a low humidity in these chambers, we dried inlet air with two columns of Drierite before it entered the chamber. Inlet air of these chambers had a dewpoint of −38°C. Humid-acclimated sparrows experienced a relative humidity of 90%–95%, corresponding to a mean absolute hu-

midity of 31 g H_2O/m^3. Dewpoint of the air entering those chambers was 28°C, near saturation. To maintain a high humidity in these chambers, before air entered the environmental chamber, we bubbled it through water at 25°C contained in a sealed stainless steel canister. We also placed two pans filled with water beneath the birds's cages inside the environmental chambers. In all chambers, humidity and temperature were monitored continuously using HOBO (Bourne, MA) ProSeries data loggers.

Sparrows in the nonacclimated group were killed by cervical dislocation after measurements of CWL, RWL, and oxygen consumption, and their SCs were isolated for identification and quantification of lipids. Birds assigned to the other groups were acclimated for 3 wk to either a dry or a humid environment. After the acclimation period, we again measured CWL, RWL, and oxygen consumption for birds in the humid- and dry-acclimated groups, killed them, and determined the lipid composition of their SC.

Measurement of Metabolic Rate and Evaporative Water Losses

We measured oxygen consumption, RWL, and CWL using standard flow-through respirometry methods (Gessaman 1987; Tieleman and Williams 2002). Birds were fasted 2–3 h before measurements to ensure postabsorptive conditions, which we later confirmed by examining their digestive tracts. All measurements were made during the inactive phase.

For these measurements, birds were placed in a water-jacketed stainless steel metabolic chamber (29.5 cm × 21.5 cm × 28 cm) with a Plexiglas lid rendered airtight by a rubber gasket. We used a circulating water bath (Isotemp, model 900, Fisher Scientific, Hanover Park, IL) to control T_a in the chamber, set at 30°C. Sparrows stood on a wire mesh platform that allowed feces to fall into a layer of mineral oil, eliminating them as a source of water in our measurements. We quantified CWL and RWL separately using a plastic mask system (Tieleman and Williams 2002). The mask captured all the respiratory gases but did not cover the eyes or most of the head of the bird, so evaporation from these areas contributed to CWL.

We routed atmospheric air through a column of Drierite to remove water and then into the metabolism chamber. Air exited the chamber through two different ports. RWL was measured from air drawn through the mask by a vacuum pump and then routed to a dew point hygrometer (EdgeTech [Marlborough, MA], model 2001-C1-S3), columns of Drierite and Ascarite, and a mass flow controller (Brooks [Hatfield, PA], model 5850) set at 600 mL/min, calibrated with a 1-L bubble meter (Levy 1964). Oxygen concentration of exiting air was measured with an Applied Electrochemisry (Pittsburgh, PA) S3A-II oxygen analyzer. We also directed dry atmospheric air through a different line at a flow rate of 100 mL/min to the oxygen analyzer and used it to calculate ΔO_2. CWL was measured from air drawn from the chamber from a second port and directed through another dew point hygrometer (General Eastern [Woburn, MA], model M4), a column of Drierite, and a calibrated mass flow controller (Brooks, model 5850) set at 400 mL/min. During each measurement we directed air from the CWL port to the oxygen analyzer to verify that the mask captured all respiratory gases; the fraction of O_2 in chamber air was always identical to inlet air (20.95%).

After 2–3 h, when oxygen consumption and dew point temperatures were stable, we recorded concentration of oxygen in the inlet and outlet air, dew point temperatures, and T_a inside the dew point hygrometers. We averaged data that remained stable for at least 10 min. Oxygen consumption was calculated with equation (4a) of Withers (1977) and was converted to kJ/d using 20.08 J/mL O_2 (Schmidt-Nielsen 1997). To estimate RWL, we used the equation $\text{RWL} = (\rho_{\text{mask}} - \rho_{\text{chamber}})(V'_{e1})$, where ρ_{mask} is absolute humidity (g/m^3) of air leaving the mask corrected to standard temperature and pressure (STP), ρ_{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), assuming a respiratory quotient of 0.71 (King and Farner 1961). CWL was determined as $\text{CWL} = (\rho_{\text{chamber}} - \rho_{\text{in}})(V'_{e1} + V'_{e2})$, 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).

Separation and Identification of Skin Lipids

After measuring RWL, CWL, and oxygen consumption, we weighed birds, killed them by cervical dislocation, plucked their feathers, and removed their skin. We pinned the skin to a thin sheet of Teflon, immersed it in a distilled-water bath at 65°C for 3 min, and then gently peeled the epidermis from the dermis (Wertz et al. 1986; Haugen 2003*a*, 2003*b*). We incubated the epidermis at 4°C overnight in a solution of 0.5% trypsin in phosphate-buffered saline (PBS; pH = 7.4, 370 mOsm). The following day we reimmersed the tissue in fresh 0.5% trypsin solution for 3 h at 38°C, a procedure that separates the SC from remaining epidermal cells (Wertz and Downing 1987). We then rinsed the SC with distilled water over fine mesh of polyester cloth to remove any remaining feathers and epidermal tissue, freeze-dried the SC for 12 h, and stored it at −20°C in an atmosphere of nitrogen.

After determining dry mass of the SC (±0.01 mg), we extracted lipids using a series of chloroform : methanol mixtures, 2 : 1, 1 : 1, and 1 : 2 v/v for 2 h each step. The mixtures contained 50 mg/L of the antioxidant butylated hydroxytoluene (Law et al. 1995). We then dried the mixtures using a stream of nitrogen with a gas manifold (N-EVAP, model 11155-O, Organomation, Berlin, MA). To prepare our samples for analytical thin-layer chromatography (TLC), we redissolved the extracted lipids in 200 or 300 μL of chloroform : methanol 2 : 1 containing BHT, depending on the absolute lipid amount extracted from each sample.

We performed analytical TLC to separate lipid classes on 20 × 20-cm glass plates coated with silicic acid (0.25 mm thick; Adsorbosil-Plus 1, Altech, Deerfield, IL). We developed plates with a mixture of chloroform : methanol (2 : 1) to the top to

remove contaminants and thereafter activated plates in an oven at 110°C for 30 min. 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 group), galactocerebrosides, cholesterol, and a mixture of free fatty acids. We dissolved standards in chloroform : methanol (2 : 1) in concentrations ranging from 0.30 to 50 mg/mL, a range that previous work indicated as appropriate for lipids found in the SC of house sparrows (Muñoz-Garcia and Williams 2005). A duplicate series of standards was run on each plate. We pipetted 5 μL of each lipid extract in triplicate in the preadsorbent area of the plates using a Teflon-tipped Hamilton syringe. Two solvent systems were used: one for relatively more polar lipids, such as ceramides and cerebrosides, and another for relatively nonpolar lipids, free fatty acids, and cholesterol. To separate ceramides and cerebrosides, we developed plates twice with a mixture of chloroform : methanol : acetic acid (190 : 9 : 1) to the top, followed by development with hexane : ethyl ether : acetic acid (70 : 30 : 1) run to 12 cm above the preadsorbent zone, and a final development with chloroform : methanol : water (60 : 40 : 5) run 5 cm from the bottom. Cholesterol and free fatty acids were separated using 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 (70 : 30 : 1) 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 × 20-cm aluminum hotplate slowly raised to 180°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 using TN-Image (Nelson 2003). We calculated percentages of lipids as the amount of a given lipid class divided by the sum of the total amount of the four lipid classes analyzed. To validate our ability to quantify lipids, we followed our protocol but used known concentrations of cholesterol as our unknown. The average error, calculated as [(observed − actual)/actual] × 100, was −3.2% ± 3.1% ($n = 20$).

Statistics

We used repeated-measures ANOVA to compare mass, metabolic rates, CWL, and RWL between humid- and dry-acclimated sparrows, with time as a within-subjects effect, treatment as a between-subjects effect, and a time-by-treatment interaction. Nonacclimated sparrows could not be included in our repeated-measures analyses because they were only measured once for each variable. Therefore, we also used one-way ANOVA to compare initial values of each variable, followed by Tukey's post hoc test.

We expressed the composition of each lipid class as both an absolute quantity per unit SC and a percentage of total quantity of lipids in the SC, because the structure and function of the SC permeability barrier may depend on both the amount and the proportion of lipid classes (Haugen et al. 2003*a*, 2003*b*; Muñoz-Garcia and Williams 2005, 2007). Percentages were logit transformed ($\ln[Y/(1 - Y)]$; Zar 1996) before analyses.

Analysis of lipids in the SC is complicated by covariation among lipid classes, probably derived from metabolic sequences within lipids in the SC (Wertz 2000). To detect these interactions, we regressed different lipid classes against each other using general linear models.

We also calculated the ratio of free fatty acids to ceramides because these lipids interact to form the highly ordered lattice that impedes water movement in mammals and because we have found that this ratio correlates with CWL in other avian species (Muñoz-Garcia and Williams 2007). We calculated the ratio of ceramides to cerebrosides because we predicted that an increase in the hydrolysis of cerebrosides to ceramides would occur in response to dry acclimation.

For each of these measures of SC lipid composition, we compared groups using ANOVA with treatment (non-, dry-, and humid-acclimated) as the main effect and subsequently employed a Tukey's post hoc test. To investigate the functional consequences of variation in SC lipid composition, we tested for correlations between individual measures of CWL and these measures of SC lipid composition, both within and among treatments.

All statistical tests were performed with SPSS 14.0 (SPSS, Chicago). Averages are reported ±1 SD. We rejected the null hypothesis at $P > 0.05$.

Results

Body Mass

Before acclimation, we did not detect significant differences in mass, oxygen consumption, RWL, CWL, or TEWL among the three groups of sparrows ($P > 0.19$ for all comparisons). Our analyses revealed a slight decrease in mass during acclimation among groups ($F = 5.57$, $P < 0.03$). However, the magnitude of this decrease did not differ between humid- (2%) and dry- (4%) acclimated groups ($F = 0.09$, $P > 0.77$).

Metabolic Rate

We did not find any difference in pre- and post-acclimation measures of either whole-organism or mass-specific oxygen consumption ($F < 0.07$, $P > 0.20$). We also found no effect of the time-by-treatment interaction on oxygen consumption ($F < 0.07$, $P > 0.80$), indicating that humid- and dry-acclimated sparrows did not significantly differ in postacclimation metabolic rate ($F < 1.12$, $P > 0.47$; Fig. 1*A*).

Respiratory Water Loss

Whole-organism RWL increased significantly following acclimation ($F = 10.45$, $P = 0.004$), a finding that held true when we corrected for body mass ($F = 12.58$, $P = 0.002$). The average magnitude of this increase was 37% in dry-acclimated

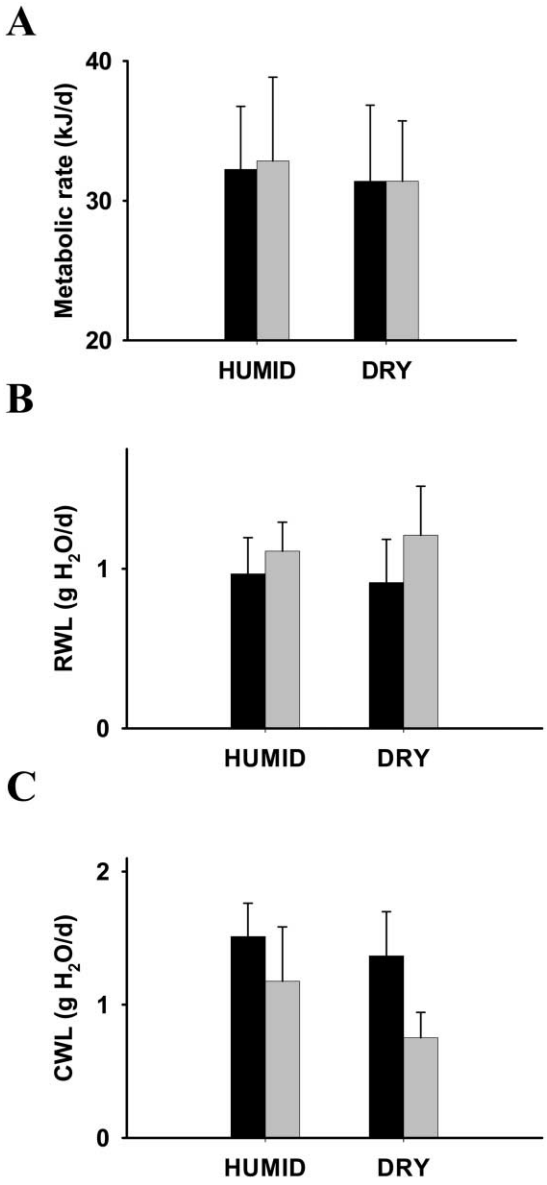

Figure 1. Mean values (±1 SD) for measurements of (*A*) metabolic rate, (*B*) respiratory water loss (RWL), and (*C*) cutaneous water loss (CWL) before acclimation (*black bars*) and after 21-d acclimation (*gray bars*) to humid and dry conditions.

birds and 16% in humid-acclimated birds. However, we did not find a significant time-by-treatment interaction ($F = 1.52$, $P = 0.23$), indicating that neither group differed in post-acclimation RWL ($F = 0.82$, $P = 0.38$; Fig. 1*B*). Because we found no differences in oxygen consumption, apparently oxygen extraction efficiency changed, a phenomenon also observed in four species of larks acclimated to different T_a's (Tieleman and Williams 2002).

Cutaneous Water Loss

Postacclimation CWL was significantly lower in dry- than in humid-acclimated sparrows by 36.1% ($F = 6.73$, $P = 0.02$), even when corrected for surface area ($F = 8.36$, $P = 0.01$; Fig. 1*C*), although the time-by-treatment interaction was not significant ($F = 3.46$, $P = 0.08$). However, we did find a significant time-by-treatment interaction on whole-animal CWL ($F = 4.71$, $P = 0.04$). Both whole-organism and surface-specific CWL decreased significantly during acclimation ($F = 54.50$, $P < 0.001$; $F = 53.15$, $P < 0.001$, respectively; Fig. 1*C*). Compared with initial values, the average magnitude of the decrease in CWL was 45% in the dry group and 23% in the humid group.

Total Evaporative Water Loss

Although RWL increased following acclimation in both treatment groups, this increase was offset by concomitant reductions in CWL such that whole-animal TEWL decreased significantly following acclimation ($F = 7.78$, $P < 0.02$). However, this effect was not significant when we corrected TEWL for body mass ($F = 3.47$, $P > 0.07$). On average, TEWL decreased by 18% in dry-acclimated sparrows and 4% in humid-acclimated sparrows, but we did not find a significant time-by-treatment interaction ($F = 0.47$, $P > 0.50$). At the end of the acclimation period, whole-animal TEWL was not significantly different between dry- and wet-acclimated sparrows ($F = 3.01$, $P > 0.09$),

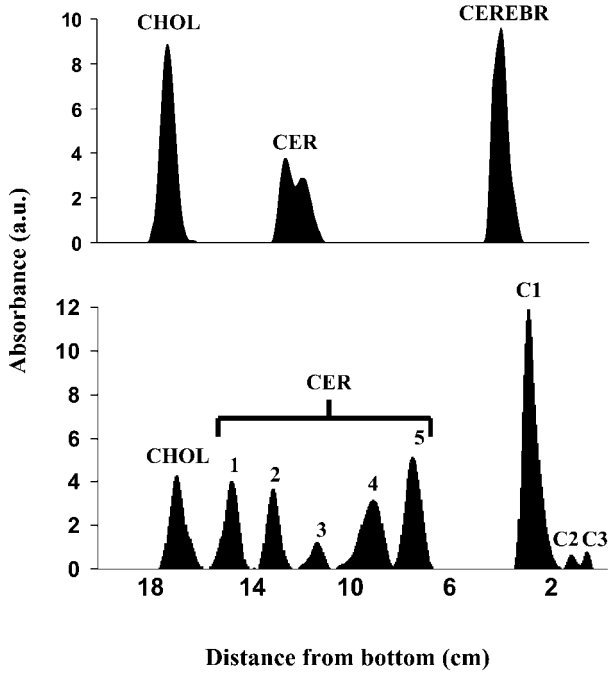

Figure 2. Profile of lipid standards (*upper graph*) and cerebrosides, ceramides, and cholesterol from the extracted lipids in the stratum corneum of house sparrows obtained using photodensitometry (*lower graph*). Ceramides that separated into bands were named in order of increasing polarity, ceramides 1–5, as were the cerebrosides, 1–3. CHOL = cholesterol, CER = ceramide, CEREBR = cerebroside, C1 to C3 = cerebrosides 1 to 3, a.u. = arbitrary units.

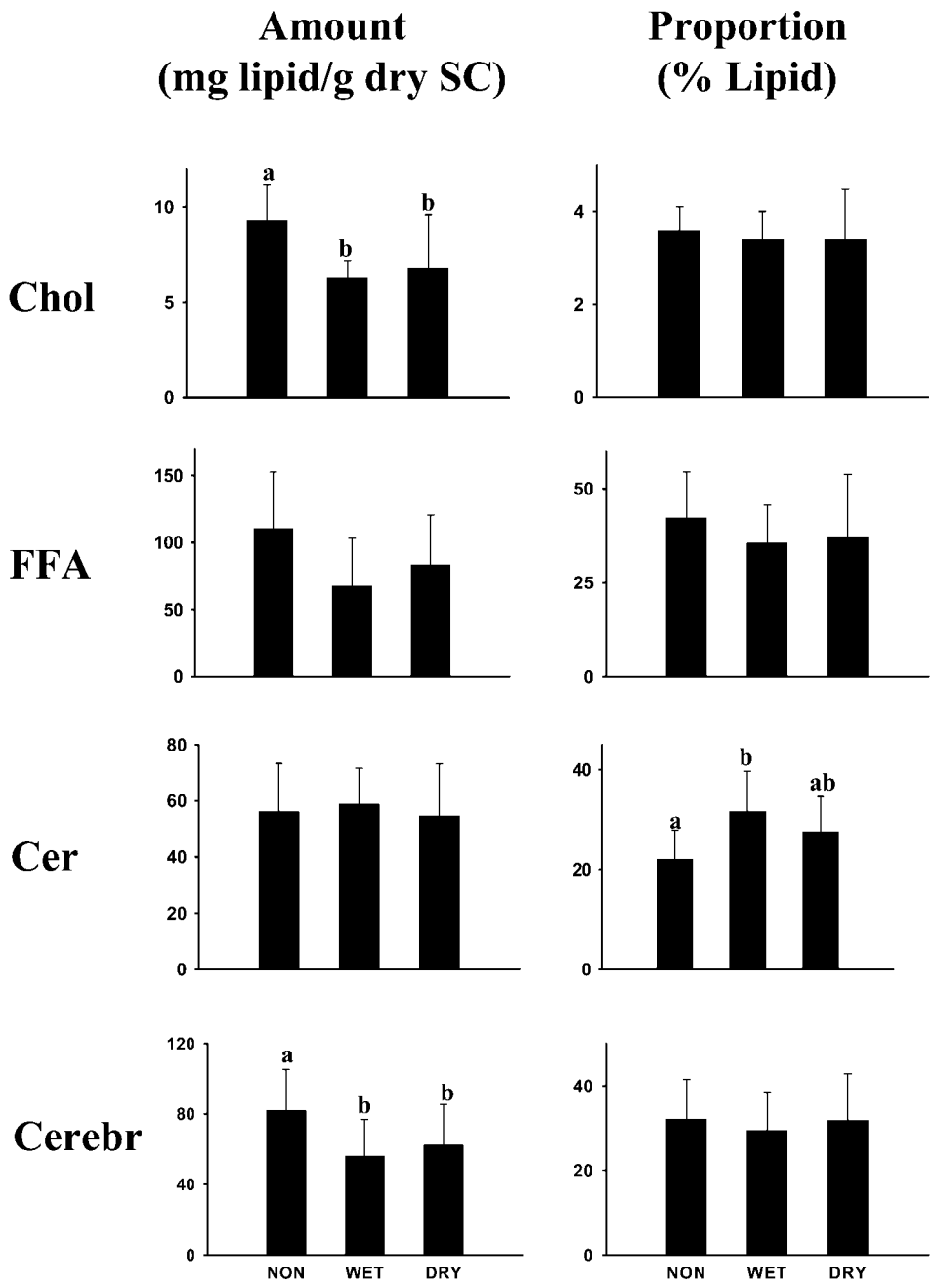

Figure 3. Mean values (± 1 SD) of lipid amounts (*left panels*) and percentages (*right panels*) in the stratum corneum of house sparrows after 21-d acclimation. Lowercase letters above bars indicate significant differences between treatments ($P < 0.05$).

but mass-specific TEWL was 15% lower in dry-acclimated than in humid-acclimated sparrows ($F = 5.09$, $P < 0.04$).

Lipids in the Stratum Corneum

Our chromatographs revealed distinct bands of lipids corresponding to standards of cholesterol, free fatty acids, ceramides, and cerebrosides (Fig. 2). We found significant differences among treatments in the quantity of total lipids ($F = 3.56$, $P = 0.04$), cholesterol ($F = 5.29$, $P = 0.01$), and total cerebrosides ($F = 4.59$, $P = 0.02$; Fig. 3). Tukey's post hoc tests showed that nonacclimated birds had significantly higher amounts of cholesterol and cerebrosides than acclimated birds and that nonacclimated birds had higher quantities of total lipids than humid-acclimated sparrows, with dry-acclimated sparrows exhibiting intermediate values that were not statistically distinct from either group (Fig. 3). Although the amount of free fatty acids followed the same trend among treatments

Table 1: Significant correlations between quantities of the different lipid classes in the stratum corneum of dry-acclimated house sparrows

	b	*a*	R^2	*P*
Free fatty acids vs. total ceramides	.63	.03	.69	.003
Total ceramides vs. cholesterol	.00	.12	.64	.001
Total cerebrosides	.01	.90	.53	.003
Total cerebrosides vs. cholesterol	.01	.07	.38	.019

Note. Equations are of the form $Y = a + b \times X$. There were no significant correlations in non- or humid-acclimated sparrows.

as cholesterol and cerebrosides, differences among groups were not significant ($P > 0.20$).

We also expressed lipid classes as a percentage of the total amount of lipid extracted, because proportions of some lipid classes in the SC are associated with changes in CWL (Haugen et al. 2003*a*, 2003*b*; Muñoz-Garcia and Williams 2005). We found significant differences among our experimental groups in proportions of total ceramides ($F = 4.4$, $P = 0.02$). Tukey's post hoc test revealed that nonacclimated sparrows had lower proportions of total ceramides than humid-acclimated birds, with intermediate values in the dry-acclimated group (Fig. 3).

Correlations among Lipids in the Stratum Corneum

Quantities of some lipid classes were significantly intercorrelated only in dry-acclimated sparrows (Table 1). In this group, ceramides were positively associated with all the other lipid classes in the SC, and cholesterol was positively correlated with total cerebrosides.

Ratios of Lipids in the Stratum Corneum

The ratio of free fatty acids (FFA) : total ceramides was higher in nonacclimated sparrows than in humid- or dry-acclimated birds ($F = 6.52$, $P < 0.01$). The ratio of ceramides to cerebrosides was not different among treatments ($F = 0.99$, $P = 0.38$). However, one datum from each of the three groups was an outlier, defined as a point that differed more than two standard deviations from the mean; when we removed these three outliers, the ratio was significantly lower in nonacclimated sparrows than in acclimated birds ($F = 4.3$, $P < 0.03$).

Cutaneous Water Loss and Lipids

There was a significant negative association between CWL and the quantity of cerebrosides in the SC of dry-acclimated sparrows ($R^2 = 0.70$, $P < 0.001$). Also, in dry-acclimated birds, CWL was positively correlated with the percentage of cholesterol ($R^2 = 0.33$, $P < 0.05$; Fig. 4). In nonacclimated sparrows, CWL was positively associated with quantity of cerebrosides ($R^2 = 0.44$) and percentage of cholesterol ($R^2 = 0.20$), but these correlations were not significant ($P > 0.07$). There were no significant associations between CWL and quantities or percentages of any lipid class in humid-acclimated sparrows.

We also investigated the relationship of the difference in CWL between birds before and after acclimation and the change of the lipid composition in their SC. We could not find any significant correlation between the difference in CWL, expressed as percent change from initial measurements, and the difference in quantities or proportions of any lipid class in humid-acclimated birds. However, in dry-acclimated birds we found a significant negative association between the difference in CWL and the quantities of total lipid, total ceramides, and total cerebrosides ($R^2 = 0.47$, $P < 0.02$; $R^2 = 0.44$, $P < 0.02$; $R^2 = 0.33$, $P < 0.04$, respectively; Fig. 5).

Discussion

Whereas values for CWL in sparrows before acclimation were comparable to those observed previously in the mesic Ohio populations, dry-acclimated birds reduced CWL to values typical of house sparrows from the desert in Saudi Arabia, 12 mg/

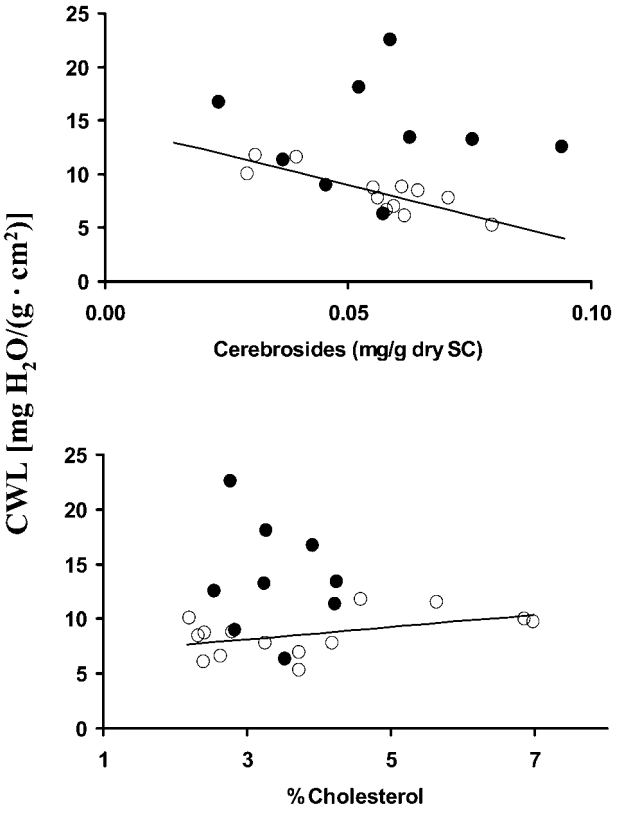

Figure 4. Cutaneous water loss in humid-acclimated (*filled circles*) and dry-acclimated (*open circles*) sparrows as a function of the amount of cerebrosides and the percentage of cholesterol in the stratum corneum. Only correlations for dry-acclimated sparrows were significant.

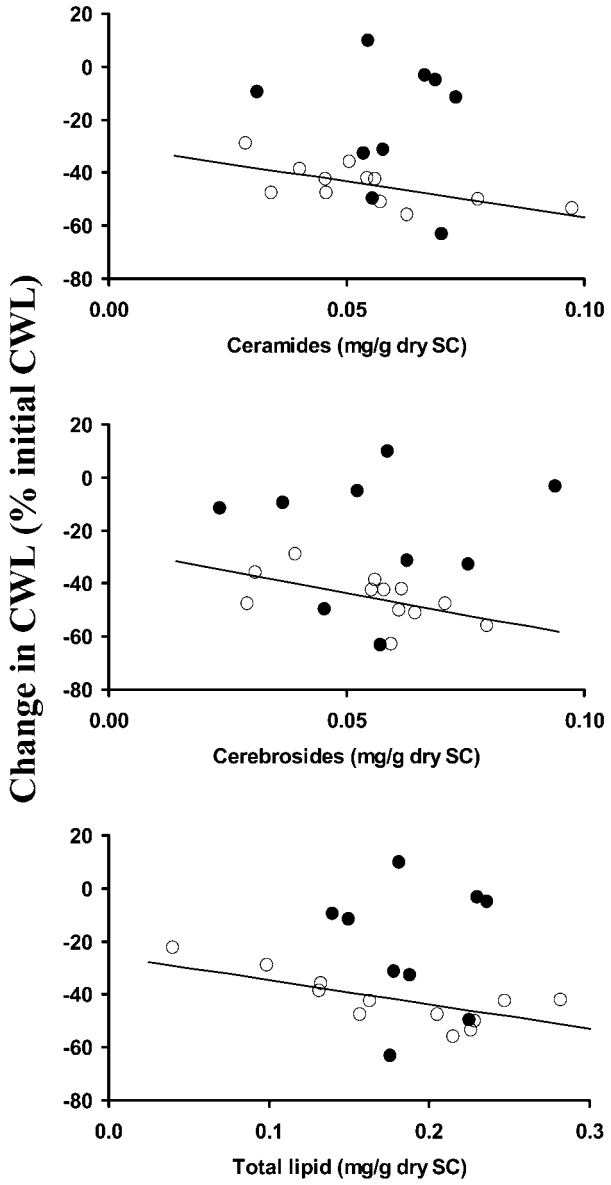

Figure 5. Percent change in cutaneous water loss in acclimated sparrows, calculated as (initial − final) × 100, as a function of the amounts of total ceramides, total cerebrosides, and total lipid in the stratum corneum. Only correlations for dry-acclimated sparrows were significant.

(cm^2 × d) (Muñoz-Garcia and Williams 2005), where humidity is similar to that in our dry experimental chambers. Differences in CWL and the lipid composition of the SC observed between Saudi Arabia and Ohio populations of house sparrows could be a product of acclimation, but we cannot exclude genetic differences or the role of developmental plasticity. In either case, it is clear that sparrows from mesic environments can reduce CWL substantially in response to environmental conditions that favor water conservation.

We could not find any significant difference between dry- and humid-acclimated sparrows in either the amounts or relative proportions of lipid classes in the SC, although CWL was significantly lower in the group acclimated to low humidity. However, restricting our analyses to amounts and proportions of isolated lipid classes may not properly address the complexity of the SC. Therefore, we also examined interactions among the different lipid classes in the SC. We can recognize two kinds of relationships, not necessarily mutually exclusive, among lipids in the SC: (1) functional relationships that make the SC more efficient as a regulator of water vapor diffusion through the skin and (2) potentially nonfunctional relationships resulting from shared metabolic pathways, like those existing between ceramides and cerebrosides or ceramides and some free fatty acids. Creation or degradation of a particular lipid in the SC in response to an environmental stimulus will, therefore, form by-products that are themselves components of the SC, either aiding or interfering as a barrier to water vapor diffusion. In the SC of dry-acclimated birds, four out of the six possible pairs of lipids are significantly intercorrelated. This suggests that the SC of dry-acclimated sparrows has a higher level of organization and a more tightly regulated structure than in humid-acclimated birds, in which we were unable to find any significant correlations. Consistent with this interpretation, we found a negative association between CWL and the quantities of ceramides and cerebrosides in the dry-acclimated group but not in either humid- or nonacclimated sparrows.

Our results also suggest that some lipid ratios are important to control water loss through the skin. We found weak evidence that the ceramide : cerebroside ratio is different among treatments, suggesting that more cerebrosides are metabolized into ceramides in the dry-acclimated sparrows. The ratio of free fatty acids to ceramides was also higher in nonacclimated birds. Dry-acclimated sparrows showed a ratio closer to that of mammals (Menon et al. 1986; Wertz 2000), which might be functionally related to their more competent barrier to water loss.

An unexpected result in our study was the reduction in CWL and concomitant changes in SC lipid composition observed in humid-acclimated birds compared with nonacclimated sparrows. We deliberately exposed house sparrows to 30°C to maintain an environment within their thermoneutral zone and avoid any potential confounding effects of heat or cold stress. At the time of capture, however, sparrows experienced natural environmental temperatures around 0°C, a difference of 30°C with the T_a in our chambers. It is likely, then, that acclimation to a higher temperature may account for the decrease in CWL observed in both dry- and humid-acclimated birds compared with nonacclimated sparrows. Changes in the quantities and proportions of the different lipid classes in the SC between nonacclimated and acclimated sparrows may also indicate an effect of temperature. Haugen et al. (2003*a*) found that proportion of total ceramides in Hoopoe larks (*Alaemon alaudipes*) increased in response to temperature, although proportions did not change in three other species of larks. Therefore, temperature acclimation may have had an overriding effect on CWL and SC lipid composition in our study. However, we also found that dry-acclimated sparrows had lower CWL rates than

humid-acclimated birds, suggesting that this additional reduction can be ascribed to humidity.

In conclusion, we have shown that adult house sparrows acclimated to low humidity reduced rates of CWL by 36.1% compared with birds acclimated to high humidity. We observed that the lipid composition of the SC was correlated with CWL only in dry-acclimated birds, suggesting that the interactions between lipid classes and the organization of the SC are more tightly regulated in birds in response to desiccation. Therefore, structural changes in SC lipid composition may have functional implications for the regulation of CWL after acclimation to low humidity. We found evidence of a high degree of plasticity in both CWL and the lipid composition of the SC in house sparrows from a mesic environment. Comparisons of CWL after acclimation with initial values showed that bringing sparrows into captivity from their winter environment also affected CWL, emphasizing that acclimation to temperature may also significantly affect water loss through skin.

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