COMMENTARY

Climate change and cutaneous water loss of birds

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Summary

There is a crucial need to understand how physiological systems of animals will respond to increases in global air temperature. Water conservation may become more important for some species of birds, especially those living in deserts. Lipids of the stratum corneum (SC), the outer layer of the epidermis, create the barrier to water vapor diffusion, and thus control cutaneous water loss (CWL). An appreciation of the ability of birds to change CWL by altering lipids of the skin will be important to predict responses of birds to global warming. The interactions of these lipids are fundamental to the modulation of water loss through skin. Cerebrosides, with their hexose sugar moiety, are a key component of the SC in birds, but how these lipids interact with other lipids of the SC, or how they form hydrogen bonds with water molecules, to form a barrier to water vapor diffusion remains unknown. An understanding of how cerebrosides interact with other lipids of the SC, and of how the hydroxyl groups of cerebrosides interact with water molecules, may be a key to elucidating the control of CWL by the SC.

Key words: acclimation, ceramides, cerebrosides, cutaneous water loss, global warming, sphingolipids.

Introduction

Climatologists have sounded the clarion call that global warming is causing important changes in climate, and that future increases in ambient air temperature (T_a) pose a danger to the distribution and abundance of animal and plant populations worldwide (Thompson, 2010). Even though some people doubt global warming, the increase in the earth's air temperature over the last 100 years seems incontrovertible, as does the fact that these increases are not a result of natural phenomena (Thompson, 2010; Oerlemans, 2005; Thompson et al., 2009; Briffa et al., 2002; Crowley and Lowery, 2000; Moberg et al., 2005). The melting of glacial ice atop mountains the world over embodies the 'canary in the coal mine', and signals a portent of physiological problems for animal populations as they are faced with the consequences of increases in T_a. In the decades to come, if they are to survive, species will need to alter their distribution patterns, change their behavior patterns and/or make adjustments in their physiology, either by short-term acclimation through phenotypic flexibility or by longer-term evolutionary shifts in physiological phenotype by means of natural selection (Angilletta, 2009; Chown et al., 2010). If we are to predict the consequences of global warming for animals, we will need to understand how their physiological systems will respond to higher temperatures (Pörtner and Farrell, 2008; Somero, 2011).

Birds the world over will be impacted by global warming, but because they are diurnal, and experience scarcity of food and drinking water and, more importantly, temperatures that are already extreme, desert birds may be among the most threatened (Louw and Seeley, 1982; Williams and Tieleman, 2005). In regions of the Middle East where vast deserts occur, simulations for climate change predict that daily T_a will increase by 3–5°C by the end of the 21st century, but rainfall patterns will remain the same (Al Zawad, 2008). Many desert birds are non-migratory, and thus will be forced to cope with increases in T_a if they are to survive. Maximum T_a values of >50°C during heat waves could cause large-scale mortality over

desert regions (McKechnie and Wolf, 2010). In Saudi Arabia, T_a often reaches 45°C during midday in summer and soil surface temperatures now regularly exceed 60°C (Tieleman et al., 2003). Increases in T_a and soil surface temperature associated with climate change will undoubtedly challenge populations of birds because their body temperature (T_b) already reaches 45°C during midday, just a few degrees within the upper lethal limit, widely held to be 47°C for all metazoa (B. I. Tieleman and J.B.W., unpublished) (Pörtner, 2002; Pörtner and Farrell, 2008). Given that these birds already employ various behaviors to regulate their T_b, it is hard to imagine them developing new behavioral repertoires for this purpose. To control T_b below lethal limits, during periods of extreme heat, one might imagine that desert birds will increase their evaporative water losses to control their T_b, but this will impose severe constraints on their water budget. Over the long term, desert birds will likely be forced to further reduce their evaporative water losses, such as cutaneous water loss (CWL). How desert birds will resolve these conflicting demands of water shortage and thermoregulation remains unknown.

Given that it is five times higher than that of fecal water loss, total evaporative water loss – the sum of respiratory water loss (RWL) and CWL – is highly significant in the water economy of birds (Bartholomew, 1972). CWL comprises more than 65% of total evaporative water loss when birds are experiencing normothermic body temperatures, suggesting that CWL is the major avenue of water loss for birds under most circumstances (Fig. 1) (Tieleman and Williams, 2002; Ro and Williams, 2010).

A model that potentially helps us appreciate mechanisms involved in CWL ($g\,H_2O\,cm^{-2}\,s^{-1}$) is: $CWL = (\rho_s - \rho_a)/r_v$, where ρ_s is absolute humidity ($g\,H_2O\,m^{-3}$) below the surface of the skin, assumed to be at saturation at skin temperature, ρ_a is absolute humidity above the skin and r_v is the total resistance to vapor diffusion. Total resistance is a composite of the resistances of the skin, the boundary layer and the feathers, although the contribution of feathers and boundary layer
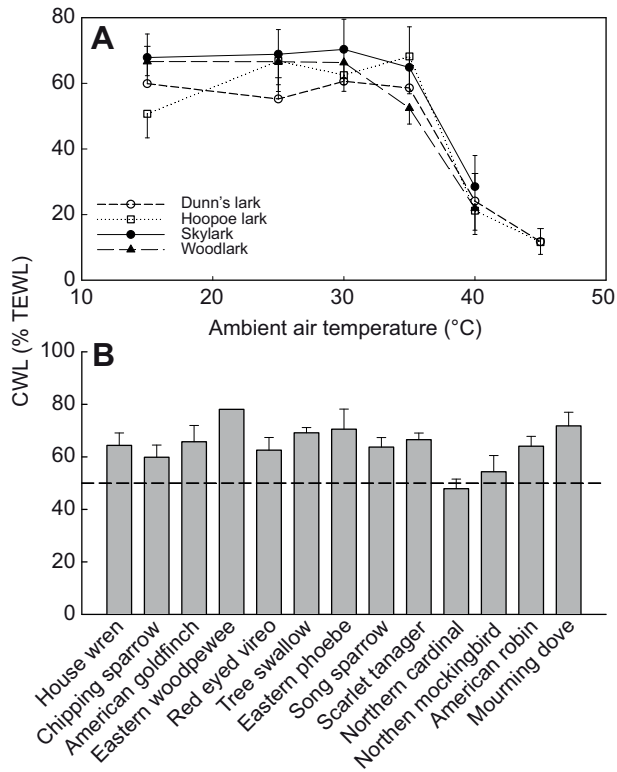
Fig. 1. (A) Cutaneous water loss (CWL) as a percentage of total evaporative water loss (TEWL) as a function of ambient air temperature for four species of larks – skylarks and woodlarks from The Netherlands, and hoopoe larks and Dunn's larks from Saudi Arabia. (B) CWL as a percentage of TEWL in 13 species of temperate birds, all measured at 25°C.
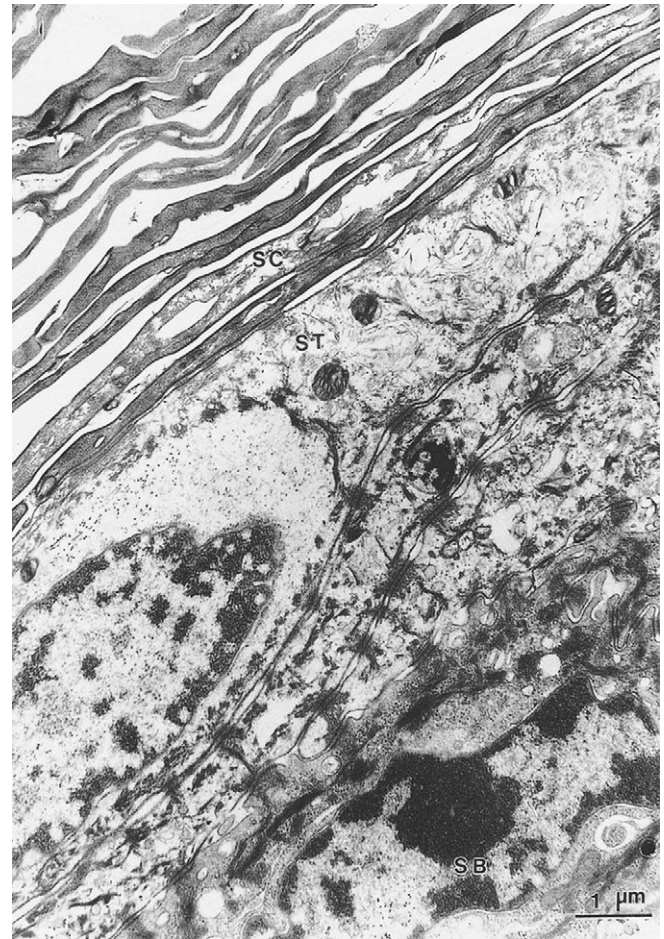

Fig. 2. Electron micrograph of ostrich epidermis, showing the stratum basale (SB), the stratum transitivum (ST) and corneocytes of the stratum corneum (SC) (Menon and Menon, 2000). Reproduced with permission.

to total resistance is thought to be <10% (Tracy, 1982; Marder and Ben-Asher, 1983; Webster et al., 1985) (J.B.W., unpublished). In experiments designed to measure the contribution of feathers to r_v, Williams et al. (J.B.W. and A.M.-G., unpublished) found that CWL was statistically indistinguishable between sparrows with and without body feathers (N=12 each group).

There is extensive evidence that the primary resistance to water loss across the skin, r_v, resides in the stratum corneum (SC), the outermost layer of the epidermis in birds and mammals (Elias et al., 1981; Blank et al., 1984; Menon et al., 1986; Bouwstra, 1997). Resistance across the SC is provided by an intercellular lipid matrix, at least at moderate T_a values (Webster and King, 1987; Wolf and Walsberg, 1996). Extraction of these lipids by organic solvents dramatically decreases r_v and thus increases water permeability, demonstrating that these lipids are the mainstay of the barrier to water vapor diffusion (Berenson and Burch, 1951; Matoltsy et al., 1968; Scheuplein and Ross, 1970; Sweeny and Downing, 1970). How much birds can alter r_v by changing the lipid layers in their SC, through natural selection or phenotypic plasticity, remains unclear for most species.

In this Commentary, we describe the morphological structure of avian skin and its lipid composition, present a molecular model that guides thinking about how lipids within the SC might interact with each other and with water, and examine what we know about variation in CWL and lipids of the SC across environments. Next we discuss short-term phenotypic flexibility of CWL as mediated by changes in the lipid composition of the skin for adults and nestlings. We show that changes in the biochemical properties of lipids of the SC, such as lipid type, hydrocarbon length, degree of

saturation and polarity, influence CWL. Finally, we discuss the importance of understanding how cerebrosides, the most polar lipids in the SC, interact with other lipids and water molecules in order to elucidate the function of specific combinations of intercellular lipids in the SC. We conclude with a lamentation that we have much to learn about how CWL of birds will respond to climate change, and time is short.

The avian skin

The skin of birds is composed of a thicker (120 μm) vascularized dermis and a thin (13–22 μm) nonvascular epidermis, which has two layers, an inner viable layer composed of the mitotically active stratum basale and stratum transitivum, and an outer layer, the SC, consisting of cornified nonliving cells called corneocytes embedded in a lipid matrix (Fig. 2) (Lucas and Stettenheim, 1972; Menon and Menon, 2000). Each corneocyte of the avian SC has a peripheral protein envelope composed of thick structural proteins, mostly involucrin and loricrin (Kalinin et al., 2002). In the lower stratum transitivum, cells contain multigranular bodies (Landmann, 1986), organelles that sequester lipids presumably synthesized in the Golgi apparatus for deposition in the SC along with a battery of lipolytic enzymes (Madison, 2003; Menon and Menon, 2000). Lipids in the multigranular bodies often form bilayers that are stacked in a compact form (Fig. 3). As skin cells migrate upwards, multigranular

Fig. 3. Lipid stacking within a multigranular body within the stratum transitivium of a house sparrow. Magnification=×150,000.

bodies are thought to fuse with cell membranes and extrude their contents into the extracellular domain at the transitivum–corneum interface, although the exact mechanism remains unclear (Menon and Menon, 2000; Groff et al., 2007). Lipids that are exocytosed from the multigranular bodies are acted upon by enzymes, and then form two distinct compartments; some lipids covalently bind to the protein envelope of corneocytes of the SC (covalently bound lipids; CBLs) and others form the intercellular lipid (ICL) compartment. The main lipids of both compartments are ceramides, cerebrosides, a ceramide with a hexose sugar attached to the sphingosine head group, free fatty acids (FFAs), cholesterol and cholesterol esters (Muñoz-Garcia and Williams, 2005; Ro and Williams, 2010; Clement, 2011) (Fig. 4).

Ceramides account for as much as 50% of the total lipids in mammalian SC, but in birds, ceramides constitute approximately

15% of the ICL compartment and cerebrosides 25% (Elias and Friend, 1975; Wertz et al., 1986; Menon and Menon, 2000; Raith and Neubert, 2000). In mammals, the ICLs are organized into layers called lamellae, usually in bilayers (Fig. 5A) (Wertz and Downing, 1982; Madison, 2003) or trilayers (Bouwstra et al., 2000), but the arrangement of ICLs in the avian SC remains unresolved. Some authors have suggested that, under normal circumstances, ICLs are an amorphous mixture without a bilayer structure (Menon and Menon, 2000), whereas others have described lamellar ICLs in avian SC (Groff et al., 2007) (M. J. Haugen, J.B.W., P. Wertz and B. I. Tieleman, unpublished). Moreover, the combination of lipid types that form the intercellular compartment seems to be more important in determining CWL than the total amount of lipid in the SC (Haugen et al., 2003a; Haugen et al., 2003b; Muñoz-Garcia and Williams, 2005; Muñoz-Garcia and Williams, 2008; Muñoz-Garcia et al., 2008a).

When all of the ICLs of the SC are removed by organic solvents, there remain lipids bound to the protein envelope of corneocytes (Fig. 5B). For CBLs, the ω end of each fatty acid chain of the lipid moiety binds to glutamate residues of the protein involucrin on the corneocyte surface (Swartzendruber et al., 1989; Wertz and Downing, 1987; Wertz et al., 1989; Downing, 1992; Stewart and Downing, 2001). Although CBLs are thought to be fundamental in the organization of the intercellular lipid layers by providing a scaffold that orchestrates ICL structure (Wertz and Downing, 1987; Menon and Menon, 2000; Wertz, 2000; Madison, 2003; Gu et al., 2008), in truth, we have studied CBLs in birds so little that it is unclear what role they play in the function of the SC, especially their role in the regulation of CWL.

A remarkable distinction between the lipids of the SC of birds and mammals is that mammals lack cerebrosides in their SC, whereas birds have this lipid class in large concentrations in CBLs and ICLs. In mammals, as lipids are exocytosed to the extracellular space in layers below the SC, cerebrosides are converted to ceramides by the enzyme β-glucocerebrosidase. If mammals lack this enzyme, and cerebrosides are not cleaved to ceramides in their SC, such as in the autosomal recessive disease of humans known as Gaucher's disease, then lamellar layers of lipids in the SC are disrupted, water loss through skin is magnified to pathological levels, and the skin becomes dry and scaly. In knockout mice deficient in β-glucocerebrosidase, water loss through the skin increased 10- to 50-fold over controls because
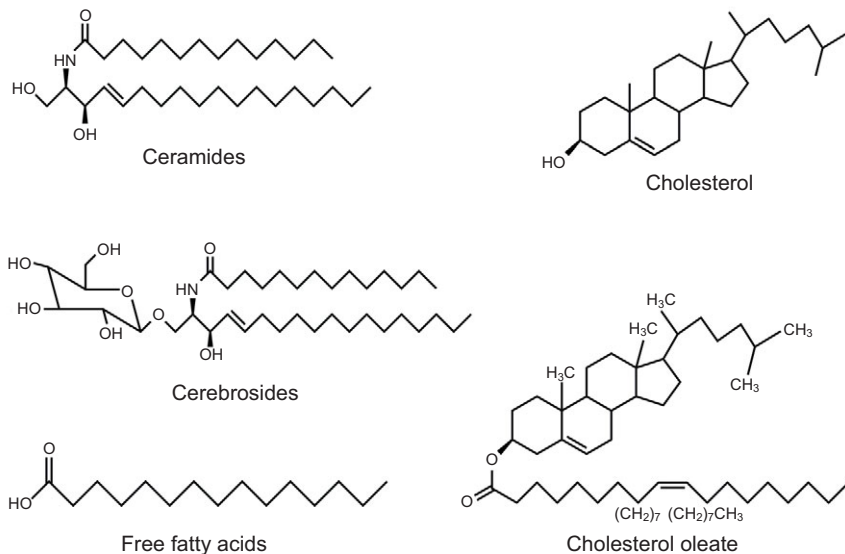

Fig. 4. Diagram showing the major lipids of the stratum corneum.

Ceramides

Cholesterol

Cerebrosides
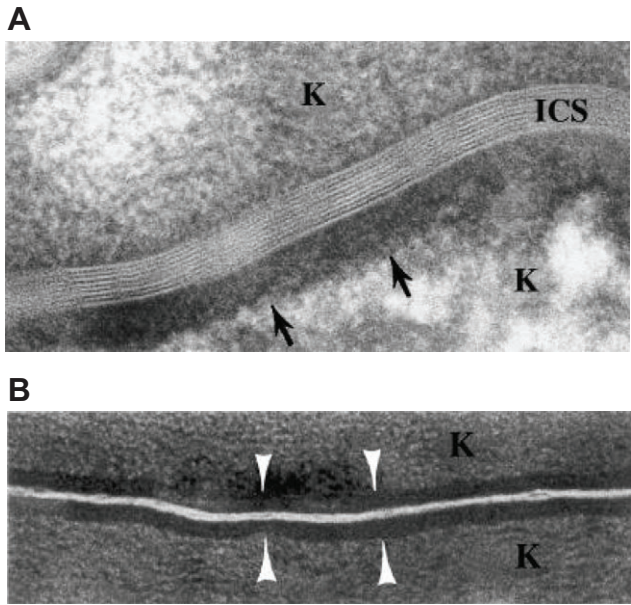
Free fatty acids

Cholesterol oleate

Fig. 5. (A) Electron micrograph showing the lamellar sheets in intercellular space (ICS) in mouse stratum corneum postfixed with ruthenium tetroxide. The cornified envelope of the lower corneocyte is clearly visible (arrows). K, keratin contents of the corneocytes bordering the ICS. Original magnification=×200,000 (Madison, 2003). (B) Electron micrograph of two corneocytes (K) from which the intercellular lipids have been removed. The lipids that remain are covalently bound to the protein envelope. Magnification=×125,000 (Madison, 2003). White arrowheads indicate the cornified envelope of corneocytes, and the lucent layer is the covalently bound lipids. Figure reproduced with permission (Madison, 2003).

the presence of cerebrosides in the SC apparently disrupted lamellae formation in ICLs (Holleran et al., 1993; Holleran et al., 1994). Yet birds have cerebrosides in their SC and do form a competent water barrier. Medical practitioners might consider the avian SC as a model system in their quest to understand the pathology of and remedial measures for Gaucher's disease.

Cerebrosides could have a driving effect on the barrier function of the avian SC and its response to climate change because of the physical and chemical properties of the hexose moiety. The hexose moiety is large compared with other lipid headgroups in the SC, and may play a role in lipid packing and phase transitions of the lipid layers. The hydroxyl groups of the hexose moiety may form strong hydrogen bonds with water molecules, which could contribute to the structure of the lipid layers of the SC. How cerebrosides interact with other lipids and with water in the SC of birds to form a barrier to water vapor diffusion is a fundamental challenge to physiologists. In some studies on birds, high proportions of cerebrosides in the SC have been associated with high rates of CWL (Muñoz-Garcia and Williams, 2007), a finding consistent with the idea that the large sugar moiety of cerebrosides would disrupt tight packing of the fatty acid chains of lipids. But in other studies the opposite has been found (Muñoz-Garcia and Williams, 2005; Muñoz-Garcia et al., 2008b). An understanding of how cerebrosides interact with other lipids of the SC, in the CBL and ICL compartments, and of how the hydroxyl groups of cerebrosides interact with water molecules to form hydrogen bonds in this layer, may be a key to elucidating the control of CWL by the SC.

Molecular models of the SC

To appreciate how the skin functions in controlling water loss to the environment, we will need to understand the molecular interactions that occur within the SC. In a model of the molecular organization of the SC in birds, Muñoz-Garcia et al. (Muñoz-Garcia et al., 2008a) hypothesized that lipid lamellae consist of two outer layers of ceramides in solid crystalline state, with polar heads facing out of the lamellae, and non-polar tails oriented inwards (see also Bouwstra et al., 2000). The central layer of the lamellae was envisioned to consist of FFAs, linoleic acid tails from ceramides in the outer layer, along with ceramides with short fatty acid chains, small amounts of cholesterol and large amounts of cerebrosides (Fig. 6).

Based on this model, one might expect a negative relationship between ceramide content in the SC and CWL, and a positive
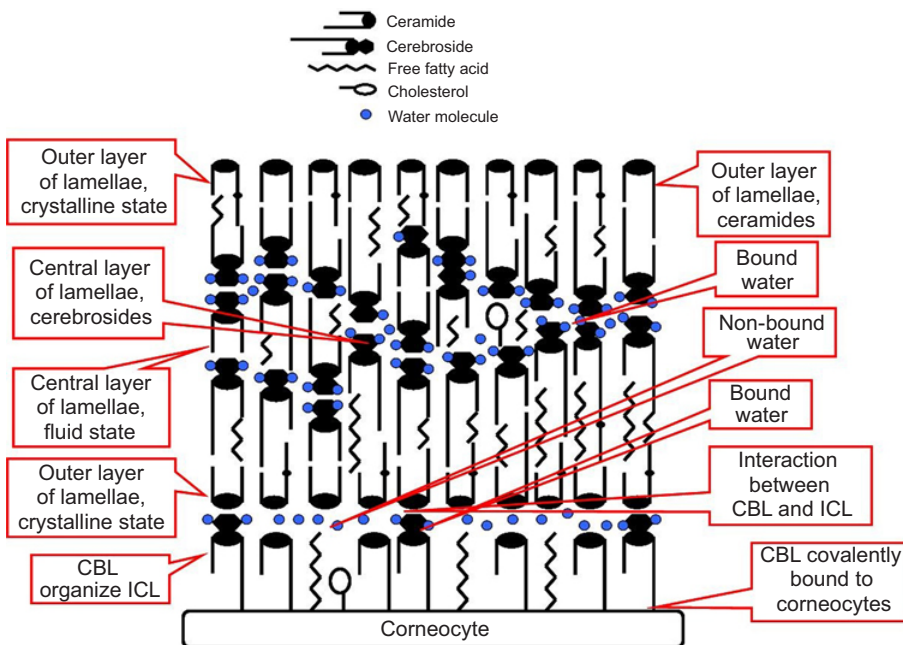
Fig. 6. A molecular model of the lipids within the stratum corneum of birds.

association between CWL and content of FFAs. Increases in the FFA content alter the FFA:ceramide ratio, and affect the formation of the lamellae in the intercellular spaces of the SC (Haugen et al., 2003a; Muñoz-Garcia and Williams, 2005; Muñoz-Garcia et al., 2008a). A consistent pattern that has emerged is that birds with high FFA:ceramide ratios in their SC have higher rates of CWL than those with low ratios (Haugen et al., 2003a; Haugen et al., 2003b) (J. Ro and J.B.W., unpublished).

Variation in CWL and the lipids of the SC across environments

Birds in deserts typically have lower rates of CWL than their cousins that live in more mesic areas, suggesting that natural selection has the potential to modify water loss through the SC (Tieleman et al., 2003; Williams and Tieleman, 2005; Muñoz-Garcia and Williams, 2005; Muñoz-Garcia and Williams, 2007; Ro and Williams, 2010). It is unclear whether these evolutionary changes can be made over relatively short periods of time as envisioned for climate change. In an early study, Haugen et al. (Haugen et al., 2003a) explored the relationship between CWL and ceramides, sterols and FFAs of the SC of species of free-living larks along a temperature–moisture gradient from The Netherlands (mesic) to Iran (semi-arid) and Saudi Arabia (arid). CWL was significantly reduced among larks inhabiting deserts, and they had a higher proportion of ceramides and a smaller proportion of FFAs in their SC (Fig. 7). However, these authors did not measure cerebrosides of the SC. In a follow-up study on seven species of larks from arid and mesic environments, desert larks had a higher concentration of cerebrosides in their SC than mesic larks, even though they had a lower CWL (Fig. 8) (A.C., A.M.-G., T. Shtayyeh, B. I. Tieleman, R. I. Hegemann and J.B.W., unpublished).
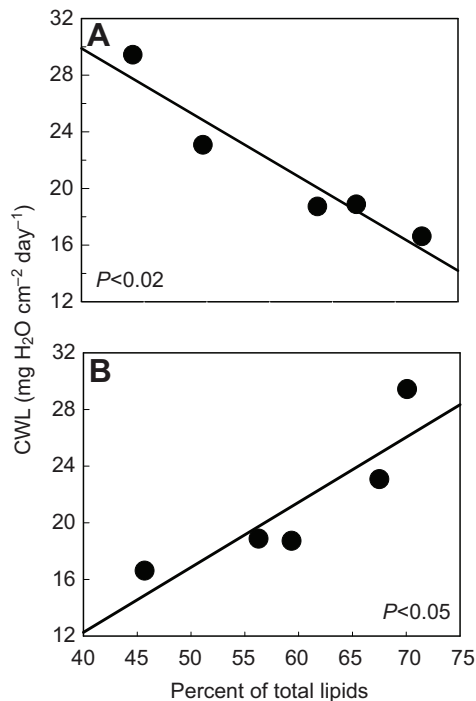

Fig. 7. CWL as a function of the percent ceramides (A) and free fatty acids (B) in the stratum corneum of five species of larks – skylarks and woodlarks from The Netherlands, and Dunn's larks, hoopoe larks and desert larks from Saudi Arabia.

Phenotypic flexibility in CWL and lipids of the SC

When acclimated to 15 and 35°C for 3 weeks, woodlarks (*Lullula arborea*) and skylarks (*Alauda arvensis*), two mesic species, and Dunn's larks (*Eremalauda dunni*), a desert inhabitant, did not change their CWL after acclimation. In contrast, hoopoe larks (*Alaemon alaudipes*) from the deserts of Saudi Arabia had lower rates of CWL when acclimated to 35°C compared with those acclimated to cold (Haugen et al., 2003b). Reduction in CWL at high temperatures in hoopoe larks was associated with an increase in ceramides in the SC, but these authors did not measure cerebrosides. In another study, adult house sparrows from mesic environments acclimated to different humidity regimes but identical T_a showed a reduction of 50% in CWL when acclimated to low humidity (Muñoz-Garcia et al., 2008b). However, changes in CWL were not associated with modifications of the major lipid classes of the SC, either ICLs or CBLs (Muñoz-Garcia et al., 2008b) (Clement et al., 2012). It is unknown whether birds changed other aspects of lipids such as degree of saturation or chain length to modify CWL.

One might imagine that alterations in the concentrations of enzymes within the epidermis, such as transferases, phospholipases or β-glucocerebrosidase, which cleaves the sugar group from a cerebroside forming a ceramide (Coderch et al., 2003), might be a mechanism by which birds can either elevate or decrease their CWL. We have speculated that the increased loss of water molecules through the SC signals genes to alter enzyme activity, but thus far only one study has addressed this issue. β-glucocerebrosidase activity within the SC of mesic house sparrows acclimated to low and high humidity, which would affect $ρ_a$, was negatively correlated with CWL and ceramide content of the SC, but there were no differences in enzyme activity between humid- and dry-acclimated birds (Cox et al., 2008). Sparrows acclimated for 3 weeks to higher temperatures had higher enzyme activity than non-acclimated birds. These results were consistent with the idea that an increase in β-glucocerebrosidase activity leads to alterations in ceramide and cerebroside content of the SC and reduced CWL, but in contradistinction to findings that increases in cerebrosides in the SC of some birds result in a decrease in CWL.

Phenotypic flexibility of nestlings to humidity

Changes in climate will affect not only adults, but also their nestlings, which are typically confined to a fixed location; therefore, avenues of behavioral thermoregulation in nestlings are few. How or whether nestlings will change their CWL in response to higher T_a values remains relatively unstudied. Experiments on nestling house sparrows from desert and mesic habitats acclimated to two humidity regimes, dry and humid, showed that nestlings had the potential to alter CWL (Muñoz-Garcia and Williams, 2008). Rates of CWL of sparrows raised in the humid environment were higher than those of nestlings from the dry environment, consistent with results from adults. CWL of fledglings was higher than that of adults, suggesting that this life-history stage might be particularly vulnerable to environmental warming.

Changes in CWL in nestlings were associated with modifications of the lipid composition of the SC. Nestlings from both habitats reared in a humid environment had a higher FFA:ceramide ratio than nestlings raised in a dry environment. However, the ceramide:cerebroside ratio was constant in both groups of mesic nestlings, but in nestlings from Saudi Arabia this ratio was reduced when raised in a dry environment (Muñoz-Garcia and Williams, 2008). However, CBLs of the SC did not change after acclimation to humidity in nestlings from both habitats, suggesting that these
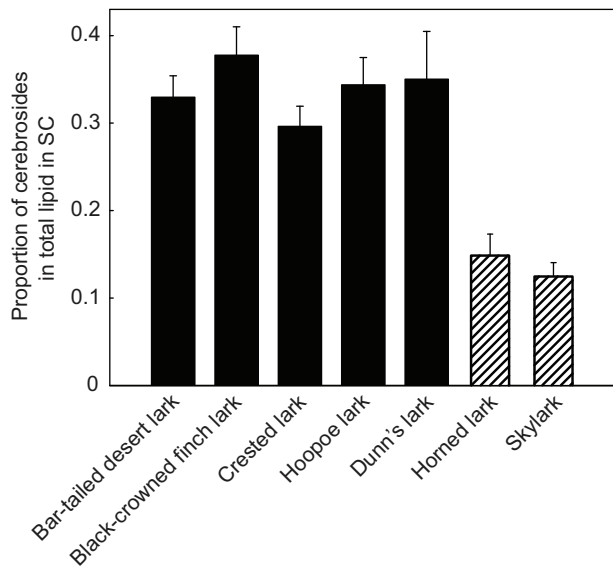
Fig. 8. The proportion of total lipids that are cerebrosides in desert and mesic larks. Desert larks are represented by solid black bars, and mesic larks from The Netherlands are represented by hatched bars (A.C., Hegemann, Tieleman and J.B.W., unpublished).
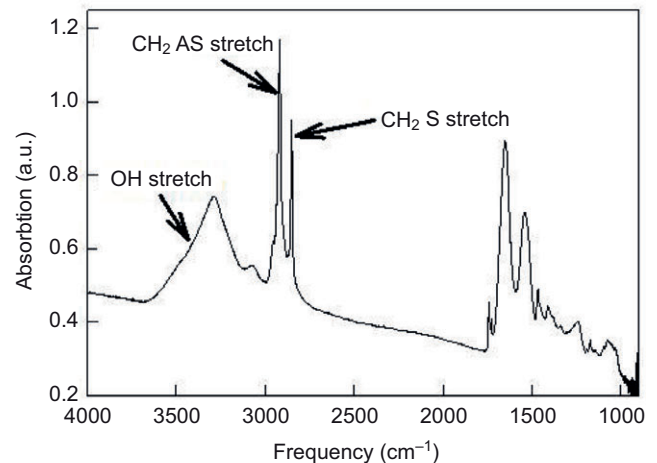

Fig. 9. Infrared absorbance spectrum for the stratum corneum of a house sparrow. AS, asymmetric stretching of hydrogens about carbon; S, symmetric stretching of hydrogens about carbon. a.u., arbitrary units.

lipids serve as a fundamental structural component of the SC regardless of environment (Clement, 2010).

CWL and properties of the lipids of the SC

The biochemical properties of individual lipid molecules thought to be important in determining CWL are length of hydrocarbon chains, degree of unsaturation, and polarity (Lillywhite, 2006). At normal skin temperatures, the ICLs of the SC arrange in a mixture of solid and liquid phases (Friberg et al., 1990). Solid phase lipids exhibit tight alkyl chain packing, whereas liquid phase lipids pack less tightly, creating irregularities in the lipid matrix, where water can potentially permeate more easily through the SC (Potts and Francoeur, 1990). The length of the alkyl chains and the polarity of the head groups are negatively associated with the ability of lipid mixtures to form liquid phases (Gibbs and Pomonis, 1995; Gibbs and Mousseau, 1994). Longer fatty acid chains of sphingolipids presumably favor lipid–lipid interactions and form a more tightly packed SC, creating a tighter barrier to water vapor diffusion (Schaefer and Redelmeier, 1996). Saturated fatty acid moieties will form a straight tail in sphingolipids, whereas double bonds between carbons in the fatty acid chain create kinks that might disrupt the order of the lamellae, increasing CWL.

Interactions of cerebrosides with other lipids and water

Given the abundance of cerebrosides, with their large polar sugar moiety, in the avian SC, an understanding of how this lipid class interacts with other lipids and, just as importantly, with water molecules may lead to breakthrough insights into how lipids form a permeability barrier in the SC of birds, and how changing cerebroside concentrations might affect CWL. One tool that can be used to better understand the role of cerebrosides in the SC is infrared spectroscopy, which passes infrared light at frequencies between 4000 and 400 wavenumbers (cm^{-1}) through a sample to study the characteristic vibrations of bonds between atoms. Chemical bonds within molecules, such as carbon–hydrogen bonds or oxygen–hydrogen bonds, vibrate at unique frequencies and absorb infrared light at those same frequencies. As infrared light is passed

through the SC, an absorption spectrum is produced where peaks can be seen at characteristic absorption frequencies (Fig. 9). The area and positions of these peaks shed light on cerebroside–lipid and cerebroside–water interactions in the SC.

Hydroxyl groups (OH) produce a broad absorbance band between ~3000 and 3600 cm^{-1} as a result of stretching of the bond between oxygen and hydrogen. This band may be divided into two peaks. The first, at 3200 cm^{-1}, signifies the presence of strong hydrogen bonding between water molecules, thought to be facilitated by hygroscopic molecules, such as the sugar moiety of cerebrosides. Hygroscopic molecules order water by binding with a primary layer of water molecules, which then binds with a secondary layer and so on until successive water shells create a sphere of influence around the hygroscopic molecule. Those water molecules not affected by hygroscopic molecules absorb at a second peak (~3400 cm^{-1}), which indicates free water with weak hydrogen bonding (Du et al., 1993). By comparing the ratio of peaks at 3200:3400 cm^{-1}, we can ascertain the degree to which lipids within the SC are interacting with water molecules. When the SC of house sparrows was exposed to 100% relative humidity for 24 h, the ratio of 3200:3400 cm^{-1} decreased, indicating that as water content increased, some water molecules became less ordered. However, at all hydration levels, the ratio of 3200:3400 cm^{-1} was greater than that of pure water, indicating that lipids were interacting with water molecules enough to cause strong hydrogen bonds to form (A.C. et al., unpublished). Water molecules within the spheres of influence of cerebrosides and other polar lipids may form aggregates of water, and thus move more slowly through the lipid layers than individual water molecules while contributing to the structure of lipid layers.

Hydrocarbons produce absorbance peaks at ~2850 and 2910 cm^{-1}, corresponding to symmetric and asymmetric stretching of hydrogen relative to carbon in CH_2 bonds, respectively. Because CH_2 bonds are prevalent in lipid alkyl chains, these peaks provide information about how cerebrosides influence the packing of fatty acid chains in the SC. When lipids have difficulty packing together, their fatty acid chains are more disordered, and the CH_2 stretching peaks absorb at a higher wave number compared with more tightly packed lipids (Golden et al., 1987). One might predict that if cerebrosides are located in the central layers of lamellae, as suggested by the model of Muñoz-Garcia et al. (Muñoz-Garcia et al., 2008a), the sugar moiety and its attendant water molecules would prevent tight packing

among lipid molecules. Thus as cerebroside content of the SC increases, lipid chains become more disordered, shifting the CH_2 stretching peaks to higher wave numbers. However, if intercellular lipids are organized in bilayers (Wertz and Downing, 1982) and cerebrosides are located on the outside of lamellae, increasing cerebroside concentration and hydration should not affect fatty acid chains.

The CH_2 peaks can also measure lipid chain disorder as a function of skin temperature, and thus may resolve the role that lipid phase transitions play in increasing CWL at different T_a values. As the temperature of the SC increases, lipid alkyl chains become more disordered, and absorb at a higher frequency (Prosser and Franses, 2002; Gay, 1994; Gibbs, 2002). In studies of human SC, only a subset of lipids with low melting points become disordered at lower temperatures (<37°C), but as the temperature continues to rise, the entire lipid compartment transitions to a disordered liquid phase (Gay et al., 1994). If lipid phase transitions play a role in increasing CWL at high temperatures, then the SC of mesic birds will show a shift in the CH_2 peaks at lower temperatures than the SC of desert birds.

Conclusions

There is a crucial need to understand how physiological systems of animals will respond to increases in global air temperature, but our knowledge of the ability of birds to alter these systems in response to changes in temperature are rudimentary. Birds in deserts may be among the most threatened species by global warming because their environment is already hot and dry.

Given that water conservation will become increasingly important to birds, there is a distinct need to understand how birds control CWL, the main avenue of water loss. Lipids of the SC, the outer layer of the epidermis, create the main resistance to water vapor diffusion. These lipids form two compartments in the SC, those covalently bound to the protein envelope of the corneocytes (CBLs), and those that form layers in the intercellular spaces (ICLs). Cerebrosides, with their hexose sugar moiety, are a key component of the SC in birds, but how these lipids interact with other lipids of the SC, or with water molecules, to form a barrier to water vapor diffusion remains unknown. An understanding of how cerebrosides interact with other lipids of the SC, and of how the hydroxyl groups of cerebrosides interact with water molecules as they penetrate this layer, may be a key to elucidating the control of CWL by the SC.

To appreciate how the skin functions in controlling water loss to the environment, we will need to understand the molecular interactions that occur within the SC. One model of the avian SC suggested that lipid lamellae consist of two outer layers of ceramides in crystalline state, with polar heads facing out of the lamellae and non-polar tails oriented inwards. The central layer of the lamellae of the avian SC was envisioned to consist of free fatty acids, the linoleic acid moieties of long ceramides from the outer layers, ceramides with short fatty acid chains, small amounts of cholesterol and large amounts of cerebrosides.

Infrared spectroscopy is a powerful technique to examine how cerebrosides affect lipid packing in the SC, and how cerebrosides interact with water molecules. When lipids have difficulty packing together, their fatty acid chains are more disordered, and water is more permeable across the SC. By comparing the ratio of the OH stretching peaks, we can ascertain the degree to which lipids within the SC are interacting with water molecules. When the SC of house sparrows was exposed to a relative humidity of 100%, the ratio of $3200:3400\,cm^{-1}$ decreased, indicating that as water content increased in the SC, the water molecules as a whole become less ordered. More disorder among the fatty acid chains causes the CH_2

asymmetric stretching peak to shift to a higher wave number compared with more tightly packed lipids with more ordered fatty acid chains. When cerebroside content of ICL increases, greater amounts of the sugar moiety might prevent tight packing among lipid molecules, causing the fatty acid chains of the ICL to become more disordered, shifting the CH_2 asymmetric stretching peak to higher wavenumbers, and CWL should increase.

Robert Frost ended his poem 'Stopping by Woods on a Snowy Evening' with the words, 'The woods are lovely dark and deep. But I have promises to keep, and miles to go before I sleep, and miles to go before I sleep'. We end this Commentary in a similar manner. Readers will be dissatisfied with our ability to connect global warming with physiological change because the data are so limited. If we are to be predictive about the impact of global warming on populations of birds and how birds can or cannot alter their physiology in response to temperature change, we truly have 'miles to go before we sleep'.

List of abbreviations

CBL   covalently bound lipid
CWL   cutaneous water loss
FFA   free fatty acid
ICL   intercellular lipid
RWL   respiratory water loss
SC   stratum corneum
T_a ambient air temperature
TEWL   total evaporative water loss

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