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RESEARCH ARTICLE

Presence and persistence of a highly ordered lipid phase state in the avian stratum corneum

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ABSTRACT

To survive high temperatures in a terrestrial environment, animals must effectively balance evaporative heat loss and water conservation. In passerine birds, cutaneous water loss (CWL) is the primary avenue of water loss at thermoneutral temperatures and increases slightly as ambient temperature increases, indicating a change in the permeability of the skin. In the stratum corneum (SC), the outermost layer of the skin, lipids arranged in layers called lamellae serve as the primary barrier to CWL in birds. The permeability of these lamellae depends in large part on the ability of lipid molecules to pack closely together in an ordered orthorhombic phase state. However, as temperature increases, lipids of the SC become more disordered, and may pack in more permeable hexagonal or liquid crystalline phase states. In this study, we used Fourier transform infrared spectroscopy to monitor the phase state of lipids in the SC of house sparrows (*Passer domesticus*) at skin temperatures ranging from 25 to 50°C. As temperature increased, lipids became slightly more disordered, but remained predominantly in the orthorhombic phase, consistent with the small increase in CWL observed in house sparrows as ambient temperature increases. These results differ considerably from studies on mammalian SC, which find a predominantly hexagonal arrangement of lipids at temperatures above 37°C, and the increased order in avian SC may be explained by longer lipid chain length, scarcity of cholesterol and the presence of cerebrosides. Our results lend further insight into the arrangement and packing of individual lipid molecules in avian SC.

KEY WORDS: Bird, Cerebroside, Infrared spectroscopy, Skin, Water loss

INTRODUCTION

The ability of animals to maintain internal water balance is paramount to survival and reproduction in terrestrial environments, especially in the face of climate change (Williams et al., 2012; Collins et al., 2013; Kirtman et al., 2013). In response to increased ambient temperature, birds thermoregulate by increasing evaporative water loss through the skin and across respiratory passages. However, excessive evaporative water loss can result in dehydration and death (McKechnie and Wolf, 2010; Albright et al., 2017). Thus, birds have evolved physiological mechanisms to balance thermoregulation and water conservation depending on the ambient temperature. In most small bird species at an ambient temperature of 25–30°C, cutaneous water loss (CWL) accounts for 65–75% of total evaporative water loss (Tieleman and Williams, 2002; Ro and Williams, 2010). As ambient temperature increases to 40°C, CWL takes on a lesser role as respiratory water loss increases to aid in evaporative heat loss. However, CWL still increases by as much as 115% in mesic species, whereas some desert species exhibit no change in CWL, indicating a possible adaptive ability of birds in hot, dry environments to minimize CWL (Tieleman and Williams, 2002; Champagne et al., 2016). The ability of birds to resist increases in CWL as ambient temperature increases is likely determined by the robustness of their skin barrier to changes in temperature (Champagne et al., 2016).

In birds, the primary barrier to CWL is the stratum corneum (SC), the outermost layer of the skin, composed of flat, dead cells called corneocytes embedded in a matrix of lipids. The majority of these lipids arrange in layers called lamellae, and are composed primarily of cholesterol esters, fatty acid methyl esters, triacylglycerol, free fatty acids, cholesterol, ceramides and cerebrosides (Ro and Williams, 2010; Champagne et al., 2012). In general, the manner in which these lipids are able to pack together laterally within lamellae is dependent on polarity, chain length and bulkiness of head groups (Adams and Allen, 2013; Maggio et al., 2006). Therefore, the composition of lipids within the avian SC is critical in orchestrating the lateral organization of lipids and thus CWL (Champagne et al., 2016).

The lateral organization of a lipid layer is generally expressed in terms of the lipids' phase state, from the highly ordered orthorhombic phase, to the less ordered hexagonal phase, to the disordered liquid crystalline phase (Fig. 1). Lipids in the orthorhombic phase present the greatest barrier to water loss (Damien and Boncheva, 2010), as lipid molecules are packed close together, increasing Van der Waals interactions between alkyl chains and hydrogen bonding between head groups. Lipids in the hexagonal or liquid crystalline phase are spaced further apart, thus increasing the permeability of the lipid matrix to water (Potts and Francoeur, 1990).

At most normothermic skin temperatures (~37°C in birds, ~32°C in humans; Hill et al., 1980; Benedict et al., 1919), lipids form a dynamic mosaic of all three phase states, with one phase state dominating and thus determining the overall permeability of the SC. As the temperature of the SC increases, lamellar lipids gradually become more disordered until they reach a critical temperature and undergo a rapid phase transition to a less ordered phase state (Golden et al., 1987; Gay et al., 1994; Boncheva et al., 2008; Damien and Boncheva, 2010). In human SC, the majority of lipids are thought to be in the orthorhombic phase at normothermic temperatures, and undergo a phase transition to become predominantly hexagonal at ~37°C (Ongpipattanakul et al., 1994; Forslind et al., 1997; Bouwstra and Ponec, 2006; Damien and

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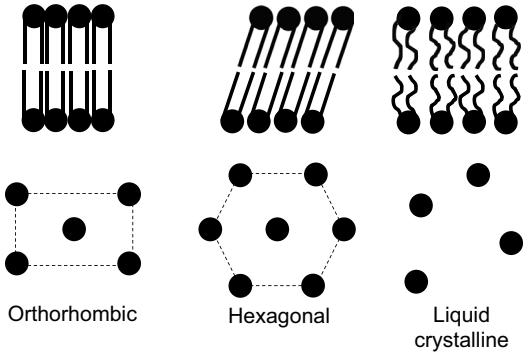

Fig. 1. Chain conformation and lateral packing of SC lipids in the orthorhombic, hexagonal and liquid crystalline phases. Chain conformation (top row) and lateral packing (bottom row) are shown. Black dots represent lipid head groups and lines represent alkyl chains. Dashed lines represent the geometric arrangement of lipid molecules in lateral view.

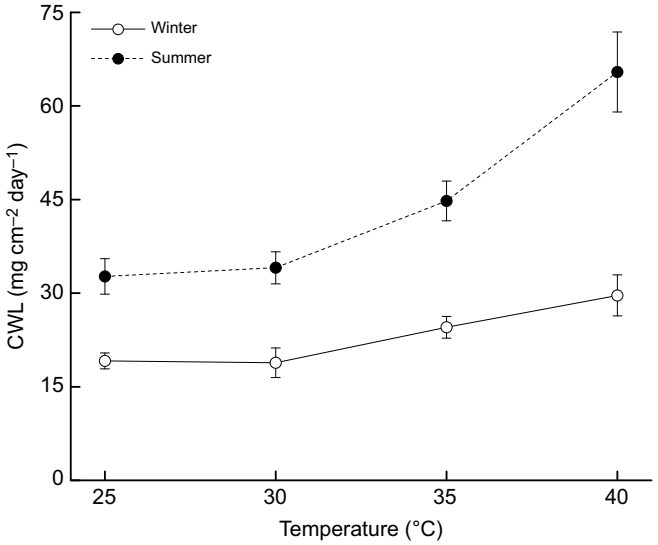

Fig. 2. Cutaneous water loss (CWL) in house sparrows caught in the winter (*n*=11) and summer (*n*=10) at 25, 30, 35 and 40°C. Birds in both seasons increase CWL as ambient temperature increases above 30°C. Error bars represent s.e.m. Data modified from Champagne et al. (2016).

Boncheva, 2010). The arrangement of lipids in avian skin is less certain, but the conformation of alkyl chains in house sparrow SC suggests a mixture of gel and liquid crystalline phases at normothermic temperatures, with no definitive transition temperature between 25 and 50°C (Champagne et al., 2016). However, these data are limited because they reflect only the relative abundance of gauche defects in alkyl chains. Gauche defects appear when carbon–carbon bonds of alkyl chains rotate by approximately 120 deg, thus creating 'kinks' in the chains (Liang et al., 1994). Because these kinks are more prevalent when lipid molecules are spaced further apart, gauche defects are associated with more disordered phase states. However, the number of gauche defects at a given phase state can vary depending on individual lipid properties (Casal and McElhaney, 1990; Casal and Mantsch, 1984). Directly measuring the lateral organization of lipids is more reliable in determining the specific phase state of lipid lamellae at a given temperature (Lewis and McElhaney, 2007).

In this study, we use Fourier transform infrared spectroscopy (FTIR) to identify the predominant phase state of lipids in house sparrow [*Passer domesticus* (Linnaeus 1758)] SC as a function of temperature. Our results offer compelling evidence that avian SC is predominantly orthorhombic, with only minor changes in lipid packing as temperature increases. These results reflect the pattern of CWL observed in passerine birds as ambient temperature increases and reflect a unique lipid composition and placement of lipid molecules in avian SC.

MATERIALS AND METHODS

Capture of birds

We captured adult house sparrows with mist nets in Columbus, OH, USA during August 2010 (n=10) and January 2011 (n=9). CWL was measured within 4 days of capture at ambient temperatures of 25, 30, 35 and 40°C in a repeated measures design. These data have been previously reported within a larger sample and are summarized here (Fig. 2; Champagne et al., 2016). All experiments were approved by the Institutional Animal Care and Use Committee at The Ohio State University (Protocol 2009A0074).

Isolation of SC

To isolate the SC, we killed the birds, plucked feathers, separated the skin from underlying tissue and pinned the skin on a Teflon sheet covered with filter paper. We saturated the paper with a phosphate buffered saline (PBS) solution containing 0.5% trypsin (Fisher Scientific, Pittsburgh, PA, USA; laboratory grade). We then incubated the skin at 4°C for 24-48 h to isolate the epidermis. The epidermis was then submerged in a fresh 0.5% trypsin solution in PBS and incubated for 3 h at 37°C to isolate the SC from the epidermis. The SC was then rinsed with distilled water, placed into a 10 ml vial and freeze dried overnight to remove water. A random sample approximately 5 mm in diameter was chosen from each SC sample to use for infrared (IR) spectroscopy.

FTIR spectroscopy

In FTIR, an IR beam (4000–400 cm^{-1}) passes through a sample and absorbs IR light at unique frequencies associated with the vibrational structure of each molecule. FTIR identifies these absorptions as transmittance ratios, which are translated to absorbance peaks. The frequency of these peaks provide information on the environment surrounding each bond, the ordering of molecules and relative bond strength, whereas the intensity of the peaks provides information on the relative abundance of molecules and bond types.

We acquired IR spectra of the SC by placing freeze-dried pieces of SC between two IR-transparent CaF_2 windows (Pike Technologies, Fitchburg, WI, USA) 25 mm in diameter and 4 mm thick. These windows were secured by a brass sample holder connected to a water bath to regulate temperature (Thermo Scientific, Waltham, MA, USA; Neslab RTE 7). We secured a 36-gauge thermocouple inside one of the CaF_2 windows with epoxy to confirm sample temperature. The sample holder was placed in a PerkinElmer (Waltham, MA, USA) IR spectrometer (Spectrum 100), where a stream of N_2 gas eliminated ambient CO_2 and water vapor. The IR light passed through the sample and windows to a deuterated triglycine sulfate (DTGS) detector at a resolution of 0.25 cm^{-1}, and all spectra were based on an average of 20 scans. We obtained spectra at temperatures ranging from 25 to 50°C at 5°C intervals.

Spectral analysis

The CH_2 scissoring region (~1475–1460 cm^{-1}; Fig. 3A) of the IR spectrum detects bending vibrations in carbon–hydrogen bonds. Although such bonds are not exclusive to lipid molecules, studies on intact and lipid-depleted pig SC demonstrate that intercellular lipids

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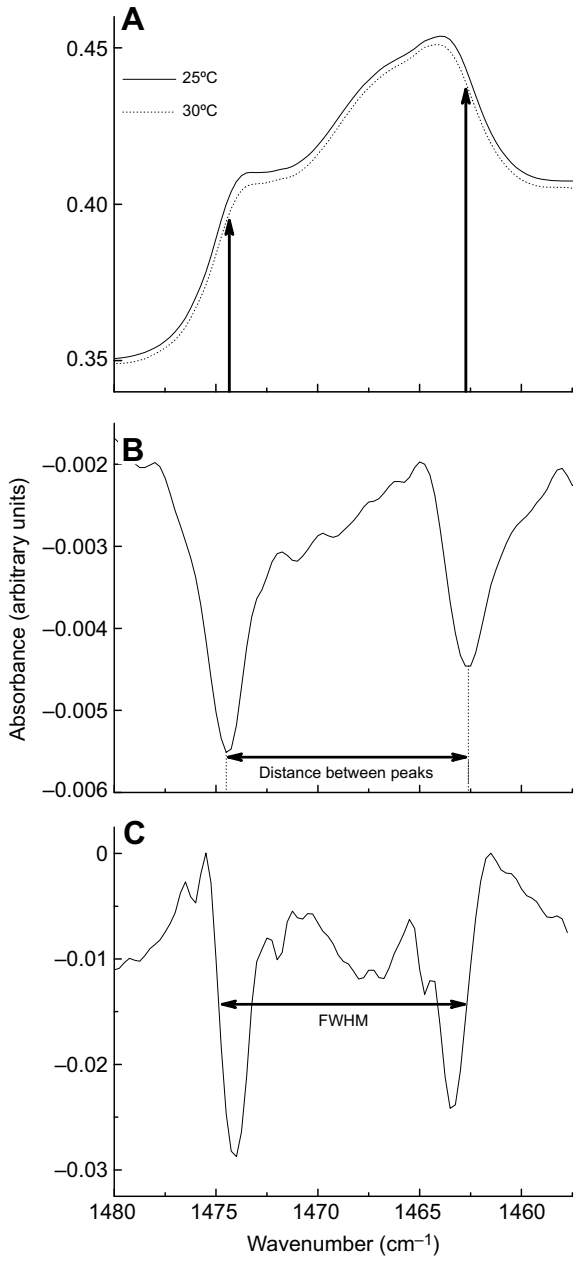

Fig. 3. CH_2 scissoring region of the infrared spectrum and methods for analysis. (A) The CH_2 scissoring region of the infrared spectrum at 25 and 30°C. Arrows demonstrate that the spectra differ most at ∼1474 and ∼1463 cm^{-1}. (B) The resulting difference spectrum when the spectrum at 25°C is subtracted from the spectrum at 30°C to form two distinct negative peaks at ∼1474 and ∼1463 cm^{-1}. Dotted lines indicate peak positions, and the double-headed arrow indicates the distance between the peaks that is measured. (C) The spectrum at 25°C transformed by taking its second derivative, showing a complex of peaks at ∼1474, ∼1468 and ∼1463 cm^{-1}. The double-headed arrow demonstrates the full width half max (FWHM) that is measured.

account for over 93% of the absorbance associated with the CH_2 scissoring region (Ongpipattanakul et al., 1994). Therefore, this region provides information on the predominant phase state of lipid layers because it is sensitive to hydrocarbon chain mobility, the number of gauche conformers, and lateral packing between lipid molecules. When lipids are arranged in predominantly orthorhombic configurations, the CH_2 scissoring region is defined by absorbance bands at ∼1474 and ∼1463 cm^{-1}. However, as the lipids transition to more disordered states, the two bands merge to form a single peak centered at ∼1468 cm^{-1}. As the bands merge, the distance between them decreases in a sigmoidal fashion, with the inflection point representing the transition from predominantly orthorhombic to predominantly hexagonal phases (Gay et al., 1994; Boncheva et al., 2008; Lewis and McElhaney, 2007). We used the peak analyzer function of OriginPro 2016 (OriginLab Corporation, Northampton, MA, USA) software to analyze the CH_2 scissoring region and thus assess the positions and degree of merging between bands via two different methods: (1) difference spectra are calculated by subtracting one spectrum from another to enhance the visibility of peaks of interest (Parry et al., 1991). We obtained difference spectra by subtracting each spectrum of the SC from one taken for the same sample at 5°C higher, resulting in negative peaks at ∼1474 and ∼1463 cm^{-1} (Fig. 3B). After correcting for the baseline of each spectrum, we assessed the positions of these peaks and measured the distance between them. A shorter distance between peaks indicates that the bands at ∼1474 and ∼1463 cm^{-1} are merging, and lipid disorder is increasing. (2) Second derivative spectra calculate the second order derivative of each spectrum and allow the separation of peaks that otherwise overlap or are too small to detect in the original spectrum (Rieppo et al., 2012). The second derivative of the CH_2 scissoring region at each temperature resulted in a complex of peaks at ∼1463, ∼1468 and ∼1474 cm^{-1} (Fig. 3C). To evaluate the presence and extent of orthorhombic phases in the SC, we calculated the full width at half max (FWHM) of this complex of peaks at each temperature after correcting for baseline. As lipid disorder increases, the peaks at ∼1474 and ∼1463 cm^{-1} decrease in size, thus decreasing the FWHM of the entire CH_2 scissoring peak complex. We classified lipid phases from FWHM by using values defined by Damien and Boncheva (2010), where the maximal FWHM value of 12.0±0.1 cm^{-1} indicates that lipids are organized predominantly in the orthorhombic phase. As lipids are heated, FWHM decreases in a sigmoidal fashion to a minimum value of 4.0±0.1 cm^{-1} and the inflection point between these two values, typically around 8.0±0.1 cm^{-1}, represents the transition of the lipids to a predominantly hexagonal phase (Boncheva et al., 2008). In some second derivative spectra, peaks could not be discerned due to a low signal:noise ratio; thus, FWHM was analyzed only in samples with discernible peaks (n=7 summer, n=5 winter).

Statistics

To test for differences in the distance between peaks in difference spectra and FWHM values in second derivative spectra, we used a repeated measures general linear model in SPSS 23.0 (IBM, Armonk, NY, USA) with temperature, season and the interaction as explanatory variables. We report differences between groups and interaction terms only where significant. To elucidate the relationship between CWL and lipid phase state, we correlated CWL and FWHM values at all temperatures in all individuals that had CWL and FWHM data (n=11). To account for repeated measures between individuals, we calculated the repeated measures correlation with the rmcorr package in R (R Core Team, 2017; Bakdash and Marusich, 2017). We did not correlate difference spectra with CWL because of the difficulty in reconciling difference spectra temperature values with the ambient temperatures used for CWL. We set statistical significance at $P \leq 0.05$ for all tests, and all values are presented as means±s.e.m.

RESULTS

Difference spectra

The distance between the peak at ∼1463 cm^{-1} and the peak at ∼1474 cm^{-1} differed as a function of temperature (P<0.001) but not

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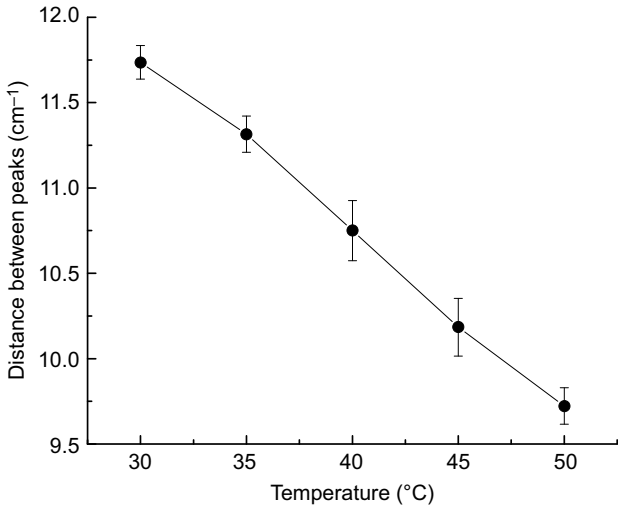

Fig. 4. Distance between negative peaks (~1474 and ~1463 cm^{-1}) taken from difference spectra of the CH_2 scissoring region in house sparrow stratum corneum (SC) as temperature increases. Numbers on the *x*-axis represent the higher of the two temperatures subtracted in the difference spectra (e.g. 30°C represents the spectrum at 25°C – the spectrum at 30°C). The gradual convergence of peaks, with no discernible inflection point as skin temperature increases, indicates a small increase in lipid disorder with no phase transition. Error bars represent s.e.m. (*n*=19).

season (P=0.06). As temperature increased from 25 to 50°C, the distance between the two peaks decreased from 11.73±0.10 to 9.72±0.10 cm^{-1}. However, we could not detect an inflection point indicative of a phase transition (Fig. 4).

Second derivative spectra

The FWHM of the second derivative of the CH_2 scissoring region differed as a function of temperature (P<0.001), but not season (P=0.86). As temperature increased from 25 to 50°C, FWHM decreased from 12.06±0.08 to 10.79±0.09 cm^{-1}, but no inflection point was reached (Fig. 5). Despite the absence of a phase transition, the FWHM exhibited a negative correlation with CWL, indicating

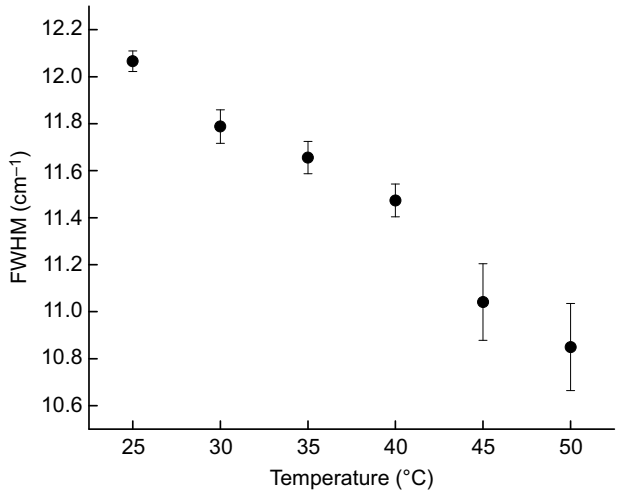

Fig. 5. FWHM values of second derivative spectra of the CH_2 scissoring region in house sparrow SC as temperature increases. The gradual decrease in FWHM, with no discernible inflection point, indicates a small increase in lipid disorder, but no phase transition as skin temperature increases. Error bars represent s.e.m. (*n*=12).

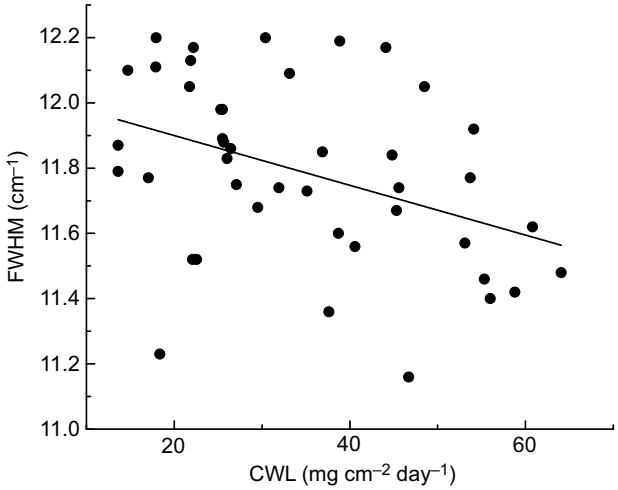

Fig. 6. Correlation between FWHM values of second derivative spectra of the CH_2 scissoring region and CWL. Higher CWL is associated with greater lipid disorder (lower FWHM). *n*=11, 4 temperature treatments for each individual.

that, as lipids became more disordered, CWL increased (r=−0.525; P<0.002; Fig. 6).

DISCUSSION

We have shown that lipids in the SC of house sparrows are organized predominantly in the orthorhombic phase, and remain in this phase up to 50°C. However, small increases in lipid chain disorder are related to small increases in CWL as ambient temperature increases. In addition, the existence and persistence of the orthorhombic phase reflects the properties of individual lipid molecules in avian SC, and provides insights into the arrangement of these lipid molecules within lamellae. This lipid arrangement may have implications for birds' ability to balance evaporative heat loss and water conservation.

The magnitude of the distance between peaks and FWHM in difference and second derivative spectra, respectively, indicate that the orthorhombic phase is the predominant phase at all temperatures in house sparrow SC lipids. Furthermore, the lack of a defined inflection point in both spectra indicate that lipids become slightly more disordered as temperature increases from 25 to 50°C, but do not undergo a major phase transition. Instead, only a small subset of lipids transition from the orthorhombic to the hexagonal phase (Boncheva et al., 2008). These data coincide with the observation that, as skin temperature increases in birds, the prevalence of gauche defects in lipid chains increases slightly, likely causing a small increase in CWL (Tieleman and Williams, 2002; Champagne et al., 2016).

The predominance and persistence of the orthorhombic phase in house sparrow SC may be explained by the length of alkyl chains within lipid lamellae, as longer chains increase Van der Waals interactions between chains to promote the formation of orthorhombic packing (Snyder et al., 1996). Studies using mass spectrometry have found that fatty acid chains of ceramides and cerebrosides in house sparrow SC are commonly over 40 carbons long, and sometimes as long as 74 carbons (Muñoz-Garcia et al., 2008). In contrast, lipids in human SC have a maximum chain length of 36 carbons (Norlen et al., 1998; Masukawa et al., 2009; Van Smeden et al., 2014). Reflecting their reduced chain length compared with house sparrows, lipids in human SC transition from the orthorhombic to the hexagonal phase between 35 and 50°C (Boncheva et al., 2008; Damien and Boncheva, 2010). Systems with shorter lipid chains, such as the SC of pigs or humans with

atopic eczema, exhibit even less orthorhombicity and resistance to phase change as temperature increases (Bouwstra et al., 1995; Wertz and Downing, 1983; Caussin et al., 2008; Janssens et al., 2012).

The high degree of orthorhombicity in house sparrow SC also correlates with its low cholesterol content. In model SC lipid mixtures, the addition of cholesterol lowers lipid transition temperature, increases the abundance of gauche defects and promotes formation of the hexagonal phase (Chen et al., 2001; Höltje et al., 2001). In house sparrows and other birds, cholesterol and its derivatives make up approximately 12% of all SC lipids by mass, with much of it restricted to the outermost layers of the SC (Champagne et al., 2012, 2015, 2016). In human SC, cholesterol accounts for roughly 25% of all lipids, potentially contributing to its lower degree of orthorhombicity (Madison, 2003).

Cohesiveness between lipid head groups may allow lipids in house sparrow SC to remain in the orthorhombic phase despite increases in temperature. In general, polar head groups containing more hydrogen-bonding sites allow for stronger hydrogen bonding between lipids and thus facilitate a greater resistance to lipid disorder (Casel and Mantsch, 1983). In the SC of birds, as much as one third of all lipids are cerebrosides, ceramides with a sugar moiety attached to the head group, whereas healthy humans lack cerebrosides in the SC (Holleran et al., 1994; Champagne et al., 2012). Greater cerebroside content in the avian SC is associated with greater hydrogen-bonding strength and more rigid lipid layers with fewer gauche defects, indicating that cerebrosides increase the ordering of lipid chains (Adams et al., 2017). Although it has been suggested that cerebrosides may facilitate higher CWL at high ambient temperature (Ben-Hamo et al., 2016), the data in this study indicate that cerebrosides likely enhance the ability of lipid molecules to pack in a highly ordered phase and impede water loss even at high ambient temperatures.

The position of cerebrosides within lipid lamellae in avian SC has been the subject of some debate (Muñoz-Garcia et al., 2008; Champagne et al., 2015). However, the demonstration of an orthorhombic packing arrangement in house sparrow SC may clarify the position of cerebrosides within lipid lamellae and refine current models of lipid organization in the avian SC. With their bulky sugar moieties, cerebrosides exhibit steric hindrance when packed too close together, which inhibits their ability to pack into an orthorhombic phase (Maggio et al., 2006). However, the addition of free fatty acids to a pure mixture of cerebrosides reduces steric hindrance and allows molecules to pack closer together. Thus, the ability of cerebrosides to integrate with other lipids to form a homogeneously ordered lipid layer likely reflects a packing arrangement in which small, less polar lipids such as free fatty acids are distributed between cerebroside molecules to fill any gaps caused by steric hindrance (Adams and Allen, 2013). To achieve the orthorhombic phase observed in this study, it is likely that lipids in avian SC arrange themselves in this manner. Furthermore, this arrangement of cerebrosides appears to be robust to small changes in lipid composition, as house sparrows captured in the winter have more cerebrosides than summer-caught birds, yet exhibit no detectable differences in lipid packing arrangement or the prevalence of gauche defects (Champagne et al., 2016).

The tendency of SC lipids to remain in the orthorhombic phase may have an adaptive utility for birds in hot, dry environments. As water is lost through the skin, the skin cools, and thus encourages the flow of ambient heat into the bird, which in many cases must be lost via additional evaporative water loss. In cases in which ambient temperature exceeds body temperature, birds with low CWL gain less heat through dry thermal conductance than birds with high CWL (Maloney and Dawson, 1998; Maloney, 2008). Thus, minimizing CWL may prevent heat gain and thus allow for more efficient heat loss via respiratory water loss or other avenues. However, some bird taxa, such as pigeons and doves (Columbidae), experience greatly elevated CWL at high temperatures; thus, the relationship between CWL, lipid phase state and a bird's thermoregulatory needs should be more extensively studied in these birds (Marder, 1983).

In conclusion, we have found that lipids in house sparrow SC are predominately arranged in the orthorhombic phase, and remain in this phase despite increases in temperature. This persistence of the orthorhombic phase differs from the SC of mammals, and is possibly caused by longer lipid chains, a scarcity of cholesterol and more polar lipid head groups in the SC of birds. Our results lend support to current models for the arrangement of SC lipids, in which the interaction between cerebrosides and small, nonpolar lipids is critical in maintaining a competent, ordered barrier to CWL. The potential adaptive function of the pattern of lipid packing we observed may be clarified by studying lipid behavior in the SC of birds with different relationships between ambient temperature and CWL. As global temperatures continue to rise, understanding the physiological response of different groups of birds to high ambient temperature will be critical in predicting the future distribution of birds (Collins et al., 2013; Kirtman et al., 2013; Albright et al., 2017).

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: A.M.C., H.C.A., J.B.W.; Methodology: A.M.C., H.C.A., J.B.W.; Validation: A.M.C., H.C.A.; Formal analysis: A.M.C.; Investigation: A.M.C., V.A.P.; Resources: H.C.A., J.B.W.; Writing - original draft: A.M.C., V.A.P.; Writing - review & editing: A.M.C., H.C.A., J.B.W.; Supervision: A.M.C., J.B.W.; Project administration: A.M.C., J.B.W.; Funding acquisition: A.M.C., H.C.A., J.B.W.

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References
Adams, E. M. and Allen, H. C. (2013). Palmitic acid on salt subphases and in mixed monolayers of cerebrosides: application to atmospheric aerosol chemistry. *Atmosphere* **4**, 315-336.

Adams, E. M., Champagne, A. M., Williams, J. B. and Allen, H. C. (2017). Interfacial properties of avian stratum corneum monolayers investigated by Brewster angle microscopy and vibrational sum frequency generation. *Chem. Phys. Lipid.* **208**, 1-9.

Albright, T. P., Mutiibwa, D., Gerson, A. R., Smith, E. K., Talbot, W. A., O'Neill, J. J., McKechnie, A. E. and Wolf, B. O. (2017). Mapping evaporative water loss in desert passerines reveals an expanding threat of lethal dehydration. *Proc. Natl. Acad. Sci. USA* **114**, 2283-2288.

Bakdash, J. Z. and Marusich, L. R. (2017). Repeated measures correlation. *Front. Psychol.* **8**, 456.

Ben-Hamo, M., Muñoz-Garcia, A., Larrain, P., Pinshow, B., Korine, C. and Williams, J. B. (2016). The cutaneous lipid composition of bat wing and tail membranes: a case of convergent evolution with birds. *Proc. R. Soc. B* **283**, pii: 20160636.

Benedict, F. G., Miles, W. R. and Johnson, A. (1919). The temperature of the human skin. *Proc. Natl. Acad. Sci. USA* **5**, 218-222.

Boncheva, M., Damien, F. and Normand, V. (2008). Molecular organization of the lipid matrix in intact Stratum corneum using ATR-FTIR spectroscopy. *Biochim. Biophys. Acta* **1778**, 1344-1355.

Bouwstra, J. A. and Ponec, M. (2006). The skin barrier in healthy and diseased state. *Biochim. Biophys. Acta Biomembr.* **1758**, 2080-2095.
Bouwstra, J. A., Gooris, G. S., Bras, W. and Downing, D. T. (1995). Lipid organization in pig stratum corneum. *J. Lipid Res.* **36**, 685-695.
Casel, H. L. and Mantsch, H. H. (1983). The thermotropic phase behavior of N-methylated dipalmitoylphosphatidylethanolamines. *Biochim. Biophys. Acta* **735**, 387-396.
Casal, H. L. and Mantsch, H. H. (1984). Polymorphic phase behaviour of phospholipid membranes studied by infrared spectroscopy. *Biochim. Biophys. Acta* **779**, 381-401.
Casal, H. L. and McElhaney, R. N. (1990). Quantitative determination of hydrocarbon chain conformational order in bilayers of saturated phosphatidylcholines of various chain lengths by Fourier transform infrared spectroscopy. *Biochemistry* **29**, 5423-5427.
Caussin, J., Gooris, G. S., Janssens, M. and Bouwstra, J. A. (2008). Lipid organization in human and porcine stratum corneum differs widely, while lipid mixtures with porcine ceramides model human stratum corneum lipid organization very closely. *Biochim. Biophys. Acta* **1778**, 1472-1482.
Champagne, A. M., Munoz-Garcia, A., Shtayyeh, T., Tieleman, B. I., Hegemann, A., Clement, M. E. and Williams, J. B. (2012). Lipid composition of the stratum corneum and cutaneous water loss in birds along an aridity gradient. *J. Exp. Biol.* **215**, 4299-4307.
Champagne, A. M., Allen, H. C. and Williams, J. B. (2015). Lipid composition and molecular interactions change with depth in the avian stratum corneum to regulate cutaneous water loss. *J. Exp. Biol.* **218**, 3032-3041.
Champagne, A. M., Allen, H. C., Bautista-Jimenez, R. C. and Williams, J. B. (2016). Organization of lipids in avian stratum corneum: changes with temperature and hydration. *Chem. Phys. Lipids* **195**, 47-57.
Chen, H.-C., Mendelsohn, R., Rerek, M. E. and Moore, D. J. (2001). Effect of cholesterol on miscibility and phase behavior in binary mixtures with synthetic ceramide 2 and octadecanoic acid. Infrared Studies. *Biochim. Biophys. Acta* **1512**, 345-356.
Collins, M., Knutti, R., Arblaster, J., Dufresne, J.-L., Fichefet, T., Friedlingstein, P., Gao, X., Gutowski, W. J., Johns, T., Krinner, G., et al. (2013). Long-term climate change: projections, commitments and irreversibility. In *Climate Change 2013: The Physical Science Basis*. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (ed. T. F. Stocker, D. Qin, G.-K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley), pp. 1029-1136. Cambridge: Cambridge University Press.
Damien, F. and Boncheva, M. (2010). The extent of orthorhombic lipid phases in the stratum corneum determines the barrier efficiency of human skin in vivo. *J. Invest. Dermatol.* **130**, 611-614.
Forslind, B., Engström, S., Engblom, J. and Norlén, L. (1997). A novel approach to the understanding of human skin barrier function. *J. Dermatol. Sci.* **14**, 115-125.
Gay, C. L., Guy, R. H., Golden, G. M., Mak, V. H. W. and Francoeur, M. L. (1994). Characterization of low-temperature (i.e. less than 65°C) lipid transitions in human stratum corneum. *J. Invest. Dermatol.* **103**, 233-239.
Golden, G. M., Guzek, D. B., Kennedy, A. E., McKie, J. E. and Potts, R. O. (1987). Stratum corneum lipid phase transitions and water barrier properties. *Biochemistry* **26**, 2382-2388.
Hill, R. W., Beaver, D. L. and Veghte, J. H. (1980). Body surface temperatures and thermoregulation in the black-capped chickadee (*Parus atricapillus*). *Physiol. Zool.* **53**, 305-321.
Holleran, W. M., Ginns, E. I., Menon, G. K., Grundmann, J. U., Fartasch, M., McKinney, C. E., Elias, P. M. and Sidransky, E. (1994). Consequences of beta glucocerebrosidase deficiency in epidermis. Ultrastructure and permeability barrier alterations in Gaucher disease. *J. Clin. Invest.* **93**, 1756-1764.
Holtje, M., Forster, T., Brandt, B., Engels, T., von Rybinski, W. and Holtje, H. (2001). Molecular dynamics simulations of stratum corneum lipid models: fatty acids and cholesterol. *Biochim. Biophys. Acta - Biomembranes* **1511**, 156-167.
Janssens, M., van Smeden, J., Gooris, G. S., Bras, W., Portale, G., Caspers, P. J., Vreeken, R. J., Hankemeier, T., Kezic, S., Wolterbeek, R. et al. (2012). Increase in short-chain ceramides correlates with an altered lipid organization and decreased barrier function in atopic eczema patients. *J. Lipid Res.* **53**, 2755-2766.
Kirtman, B., Power, S. B., Adedoyin, J. A., Boer, G. J., Bojariu, R., Camilloni, I., Doblas-Reyes, F. J., Fiore, A. M., Kimoto, M., Meehl, G. A., et al. (2013). Near-term climate change: projections and predictability. In *Climate Change 2013: The Physical Science Basis*. *Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (ed. T. F. Stocker, D. Qin, G.-K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley), pp. 953-1028. Cambridge: Cambridge University Press.
Lewis, R. N. A. H. and McElhaney, R. N. (2007). Fourier transform infrared spectroscopy in the study of lipid phase transitions in model and biological membranes: Practical consierations. In *Methods in Molecular Biology, Vol. 400: Methods in Membrane Lipids* (ed. A. M. Dopico), pp. 207-226. Totowa, NJ: Humana Press Inc.
Liang, G. L., Noid, D. W., Sumpter, B. G. and Wunderlich, B. (1994). Gauche defects, positional disorder, dislocations, and slip planes in crystals of long methylene sequences. *J. Phys. Chem.* **98**, 11739-11744.
Madison, K. C. (2003). Barrier function of the skin: “La Raison d’Etre” of the epidermis. *J. Invest. Dermatol.* **121**, 231-241.
Maggio, B., Fanani, M. L., Rosetti, C. M. and Wilke, N. (2006). Biophysics of sphingolipids II. Glycosphingolipids: an assortment of multiple structural information transducers at the membrane surface. *Biochim. Biophys. Acta Biomembr.* **1758**, 1922-1944.
Maloney, S. K. (2008). Thermoregulation in ratites: a review. *Aust. J. Exp. Agric.* **48**, 1293-1301.
Maloney, S. K. and Dawson, T. J. (1998). Changes in pattern of heat loss at high ambient temperature caused by water deprivation in a large flightless bird, the emu. *Physiol. Zool.* **71**, 712-719.
Marder, J. (1983). Cutaneous water evaporation 2: survival of birds under extreme thermal stress. *Comp. Biochem. Physiol. A Physiol.* **75**, 433-439.
Masukawa, Y., Narita, H., Sato, H., Naoe, A., Kondo, N., Sugai, Y., Oba, T., Homma, R., Ishikawa, J., Takagi, Y. et al. (2009). Comprehensive quantification of ceramide species in human stratum corneum. *J. Lipid Res.* **50**, 1708-1719.
McKechnie, A. E. and Wolf, B. O. (2010). Climate change increases the likelihood of catastrophic avian mortality events during extreme heat waves. *Biol. Lett.* **6**, 253-256.
Muñoz-Garcia, A., Ro, J., Brown, J. C. and Williams, J. B. (2008). Cutaneous water loss and sphingolipids in the stratum corneum of house sparrows, *Passer domesticus* L., from desert and mesic environments as determined by reversed phase high-performance liquid chromatography coupled with atmospheric pressure photospray ionization mass spectrometry. *J. Exp. Biol.* **211**, 447-458.
Norlen, L., Nicander, I., Lundsjo, A., Cronholm, T. and Forslind, B. (1998). A new HPLC-based method for the quantitative analysis of inner stratum corneum lipids with special reference to the free fatty acid fraction. *Arch. Dermatol. Res.* **290**, 508-516.
Ongpipattanakul, B., Francoeur, M. L. and Potts, R. O. (1994). Polymorphism in stratum corneum lipids. *Biochim. Biophys. Acta* **1190**, 115-122.
Parry, D. B., Samant, M. G. and Melroy, O. R. (1991). Interpreting IR difference spectra. *Appl. Spectroscopy* **45**, 999-1007.
Potts, R. O. and Francoeur, M. L. (1990). Lipid biophysics of water loss through the skin. *Proc. Natl. Acad. Sci. USA* **87**, 3871-3873.
R Core Team (2017). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing. Available online at: https://www.R-project.org/.
Rieppo, L., Saarakkala, S., Närhi, T., Helminen, H. J., Jurvelin, J. S. and Rieppo, J. (2012). Application of second derivative spectroscopy for increasing molecular specificity of Fourier transform infrared spectroscopic imaging of articular cartilage. *Osteoarthritis Cartilage* **20**, 451-459.
Ro, J. and Williams, J. B. (2010). Respiratory and cutaneous water loss of temperate-zone passerine birds. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **156**, 237-246.
Snyder, R. G., Liang, G. L., Strauss, H. L. and Mendelsohn, R. (1996). IR spectroscopic study of the structure and phase behavior of long-chain diacylphosphatidylcholines in the gel state. *Biophys. J.* **71**, 3186-3198.
Tieleman, B. I. and Williams, J. B. (2002). Cutaneous and respiratory water loss in larks from arid and mesic environments. *Physiol. Biochem. Zool.* **75**, 590-599.
Van Smeden, J., Janssens, M., Gooris, G. S. and Bouwstra, J. A. (2014). The important role of stratum corneum lipids for the cutaneous barrier function. *Biochim. Biophys. Acta* **1841**, 295-313.
Wertz, P. W. and Downing, D. T. (1983). Ceramides of pig epidermis: structure determination. *J. Lipid Res.* **24**, 759-765.
Williams, J. B., Munoz-Garcia, A. and Champagne, A. (2012). Climate change and cutaneous water loss of birds. *J. Exp. Biol.* **215**, 1053-1060.