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RESEARCH ARTICLE

Decreased hydrophobicity of iridescent feathers: a potential cost of shiny plumage

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SUMMARY

Honest advertisement models posit that sexually selected traits are costly to produce, maintain or otherwise bear. Brightly coloured feathers are thought to be classic examples of these models, but evidence for a cost in feathers not coloured by carotenoid pigments is scarce. Unlike pigment-based colours, iridescent feather colours are produced by light scattering in modified feather barbules that are characteristically flattened and twisted towards the feather surface. These modifications increase light reflectance, but also expose more surface area for water adhesion, suggesting a potential trade-off between colour and hydrophobicity. Using light microscopy, spectrometry, contact angle goniometry and self-cleaning experiments, we show that iridescent feathers of mallards, *Anas platyrhynchos*, are less hydrophobic than adjacent non-iridescent feathers, and that this is primarily caused by differences in barbule microstructure. Furthermore, as a result of this decreased hydrophobicity, iridescent feathers are less efficient at self-cleaning than non-iridescent feathers. Together, these results suggest a previously unforeseen cost of iridescent plumage traits that may help to explain the evolution and distribution of iridescence in birds.

Supplementary material available online at http://jeb.biologists.org/cgi/content/full/214/13/2157/DC1

Key words: *Anas platyrhynchos*, hydrophobicity, self-cleaning, sexual selection.

INTRODUCTION

The evolution of the bright colours of metazoans has been the subject of intense research ever since Darwin (Darwin, 1871). In addition to their roles in, among other things, flight and thermoregulation, bird feathers are often brightly coloured and important in interspecific (species recognition and predation) (Andersson, 1994) and intraspecific communication [male–male competition (Senar, 2006) and female mate choice (Hill, 2006)]. In birds, male colours may represent a balance between sexual selection for brightness and natural selection for drabness (Andersson, 1994). According to honest advertisement models of sexual selection (Bradbury and Vehrencamp, 1998; Zahavi, 1975), females may gain useful information about male quality based on display traits that confer a differential cost (i.e. handicap) to the male (Andersson, 1994; Zahavi, 1975). Although studies have shown physiological, maintenance or viability costs for red, pigment-based colours (Hill, 2002; Olson and Owens, 1998), few have identified such costs in iridescent feather colours (Hill et al., 2005; McGraw et al., 2002). Further, to understand both the evolutionary trajectories of iridescent traits and whether signal honesty plays a role in the evolution of iridescent plumage coloration, it is necessary to determine the costs and limits of such traits (Meadows et al., 2009).

Feathers in general are hydrophobic (Cassie and Baxter, 1944; Rijke, 1970), and this hydrophobicity is thought to be determined mainly by the width and spacing of barbs and barbules (Rijke, 1970), an arrangement that minimizes contact between water and hydrophilic keratin and maximizes that between water and air. Furthermore, highly hydrophobic surfaces like feathers can frequently self-clean because water droplets bead up and roll off the surface, carrying surface contaminants with them (the so-called ‘lotus effect’) (Zhang et al., 2008).

In contrast to non-iridescent feathers, bright iridescent colours are produced by thin-film interference from organized nanometer-scale layers of keratin, melanin and sometimes air in feather barbules (Prum, 2006) with characteristically and distinctively flat surfaces that are often twisted towards the feather plane along the barbule axis (Chandler, 1916; Doucet et al., 2006; Durrer, 1986; Osorio and Ham, 2002) (see Fig. 1B,C). These modifications maximize the surface area available not only to reflect incident light but also to adhere to and absorb water, and thus may represent a trade-off between display and hydrophobicity. Because the adhesion of water to feathers may have associated metabolic [increased evaporative heat loss (Bakken et al., 2006; Mahoney, 1984)], biomechanical [increased wing loading (Ortega-Jiménez et al., 2010) and decreased tensile strength (Taylor et al., 2004)] or maintenance costs (possibly due to lower self-cleaning efficiency), decreased hydrophobicity may represent a previously unconsidered cost of iridescent feathers. Here, we hypothesized that iridescent feathers are less hydrophobic than non-iridescent feathers, and that this decreased hydrophobicity leads to a lower self-cleaning efficiency. Thus, our objectives were to experimentally determine (a) the hydrophobicity of iridescent and non-iridescent feathers and (b) whether these differences in hydrophobicity impact self-cleaning ability.

MATERIALS AND METHODS

Experimental summary

To test the hypothesis that iridescent feathers are less hydrophobic than non-iridescent ones, we removed patches of iridescent and non-iridescent regions from mallard (*Anas platyrhynchos* L.) secondary feathers and determined the hydrophobicity of each region by measuring the contact angle of a water droplet placed on the feather

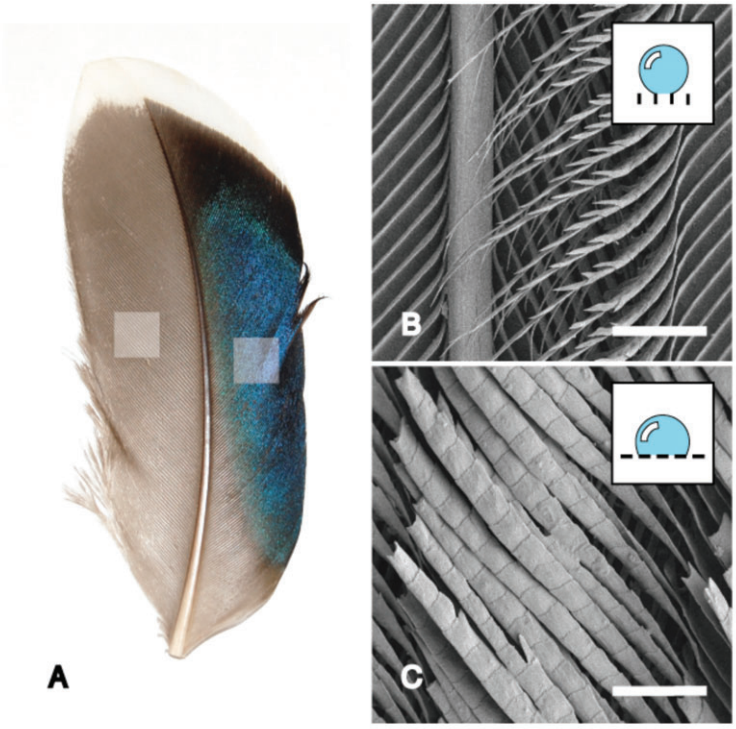

Fig. 1. Secondary flight feather of a male mallard *Anas platyrhynchos* (A). Scanning electron microscope (SEM) images show feather microstructure of non-iridescent (B) and iridescent (C) feather regions highlighted by white boxes in A. Insets are schematic drawings of water droplets (blue) resting on feather barbules (black) in their natural orientations. Scale bars, 100 μm.

surface. We then measured the reflectance of the feathers using spectrophotometry and determined the morphology of barbs and barbules using light microscopy. Next, we repeated this process after washing the feathers to remove preen oils. Finally, to test the hypothesis that the differences in hydrophobicity affect self-cleaning efficiency, we artificially soiled post-wash iridescent and non-iridescent feathers and measured the self-cleaning efficacy of each feather after spraying them with small droplets of water.

Feather collection

We obtained whole, untreated mallard wings from ducks hunted in Arkansas during autumn 2009 (Hareline Dubbin Co., Monroe, OR, USA) and removed secondary flight feathers nos 1 and 5 from the left wing of 10 males using dissection scissors. In mallards, these feathers bear a bright blue, iridescent patch known as the speculum (Humphrey and Clark, 1961). To compare the hydrophobicity of iridescent and non-iridescent feathers, we used dissection scissors to remove 5 cm^2 sections from the iridescent blue and non-iridescent brown regions of secondary feather no. 5 (I-5 and NI-5, respectively; Fig. 1A). Additionally, as NI-5 is often obscured by adjacent feathers in wild birds, and thus may not be directly comparable to an exposed iridescent feather portion, we also removed a section of the naturally exposed region of non-iridescent secondary feather no. 1 (NI-1). We cut all sections (N=30) from the middle of the feather vane near the mid-point of the feather barbs (see Fig. 1A).

To control for the effects of preen oils and surface contaminants, we performed hydrophobicity and feather microstructure measurements (see below) both before and after washing with ethanol to facilitate their removal. Following Lucas and Stettenheim (Lucas and Stettenheim, 1972), after performing the first set of measurements we immersed feathers (attached to slides) in 75% ethanol for 4 min and then rinsed them in de-ionized water for another 4 min, shaking the feathers gently during washing to facilitate the removal of preen oils and contaminants. We then allowed the feathers to air-dry for 4 h before repeating contact angle and microstructure measurements.

Hydrophobicity tests

A droplet of liquid placed onto a surface forms a characteristic angle (contact angle θ_c) between the baseline of the droplet and the tangent line of the droplet at the intersection of the solid (S), liquid (L) and gas (G) phases that is given by Young's equation: $\cos\theta_c=(\gamma_{SG}-\gamma_{SL})/\gamma_{LG}$, where γ represents the interfacial tensions at each interface (Hiemenz and Rajagopalan, 1997). When the liquid is water, a surface is by definition hydrophilic if $\theta_c<90$ deg, hydrophobic if $\theta_c>90$ deg and superhydrophobic if $\theta_c>150$ deg (Zhang et al., 2008).

To compare θ_c in iridescent and non-iridescent feathers, we taped dissected feather sections to clean glass slides and measured the angle formed by a 10 μl droplet of de-ionized water (~2.5 mm diameter) placed on the dorsal surface of each feather. This droplet size was chosen, following Marmur (Marmur, 2006), to be ~2 orders of magnitude greater than the length scale of barbules. We took pictures of the drop profile using a camera attached to a contact angle goniometer (Ramé-Hart, Netcong, NJ, USA) and calculated the mean contact angle of the left and right side of the drop using the DropSnake method (Stalder et al., 2006) in ImageJ (Abramoff et al., 2004). We took all contact angle measurements in the same spot on each feather section to control for variation in a single feather and performed the entire procedure twice (before and after washing to remove preen oils, see above), wearing gloves during testing to ensure that skin oils were not transferred to the feather surface.

Feather microstructure

According to the Cassie–Baxter wetting theory, θ_c of a microstructured or rough surface is a function of the solid area fraction ϕ (the area of the liquid–tissue interface) (Cassie and Baxter, 1944). Thus, to determine ϕ, we used a Leica S8AP0

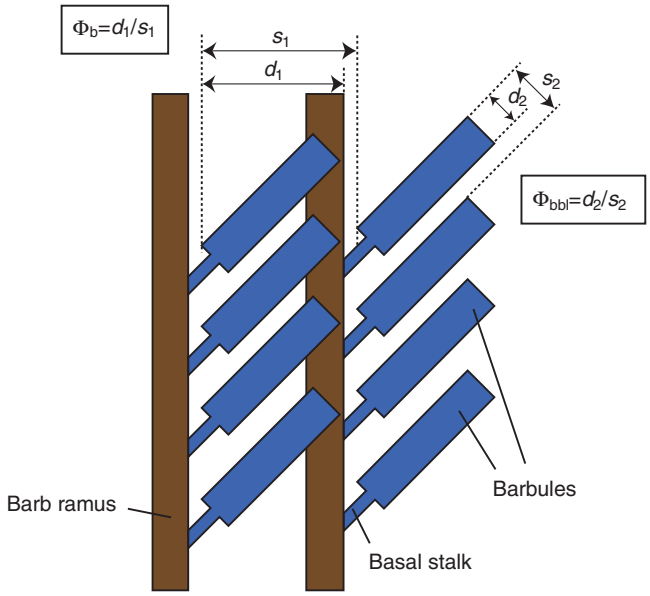

Fig. 2. Schematic drawing of the dorsal surface of an iridescent mallard feather showing iridescent barbules (blue) and barb ramus (brown). The surface fraction of keratin (ϕ) is calculated as width (d) divided by spacing (s) for both barbs and barbules. Note that the surface of the basal stalk is lower (further into the paper) than the surface of the distal region (blue rectangle) of the iridescent barbules. For simplicity, only exposed (distal) barbules are shown.

stereomicroscope (Leica Microsystems GmbH, Wetzlar, Germany) at 80× magnification to obtain images of feather microstructure (barbs and barbules). From the obtained images, we measured the width (d) and spacing (s) at the mid-point of five consecutive barbules (from three different barbs), and also at the mid-point of three separate barbs using ImageJ (Abramoff et al., 2004) (see Fig. 2). We then calculated the solid area fraction as $\phi=d/s$ for barbs (ϕ_b) and barbules (ϕ_{bbl}), and also the combined area fraction ($\phi_{comb}=\phi_b\times\phi_{bbl}$) following Bormashenko and colleagues' recommendation for multi-scale roughness (Bormashenko et al., 2007). Additionally, we used scanning electron microscopy (JSM7401F SEM, JEOL, Tokyo, Japan) to confirm the highly modified iridescent barbules previously described in this species (Chandler, 1916).

Spectrometry

To test for a trade-off between hydrophobicity and iridescent feather colour, we measured the spectral reflectance of these feathers between 300 and 700 nm with a spectrometer and attached deuterium light source (Avantes, Boulder, CO, USA). We removed feather sections from the glass slides, taped them to black velvet cloth, and took the mean of five measurements from each feather (moving the spectrometer 1–2 mm between each reading). We then interpolated the reflectance values into 1 nm bins and calculated the mean brightness (mean integral reflectance), hue (wavelength of peak reflectance) and spectral saturation (see Montgomerie, 2006).

Self-cleaning experiment

To test for differences in self-cleaning ability between iridescent and non-iridescent feathers, we used silica particles 57±14 μm in diameter (approximating natural contamination with dirt or other particulate matter) to artificially soil iridescent (I-5) and non-iridescent (NI-5) regions of the same secondary feather (both N=10; see Fig. 1A). We placed ~10 mg silica particles into a convex dish above the feathers and then forced pressurized air into the dish, evenly depositing the particles onto the feather surface (Bhushan et al., 2009). We then counted the number of silica particles within a 2.28 mm^2 area near the middle of the feather using the particle counting tool in ImageJ (Abramoff et al., 2004).

We then mounted feathers at a 60 deg angle (see below for orientation) and sprayed them with small droplets of de-ionized water at a rate of approximately 34 $\mu l\,cm^{-2}\,min^{-1}$. The sprayer was placed 30 cm above the feather at a distance of 45 cm, allowing water droplets to strike the feather while falling vertically. Additionally, the small size of the droplets (200–250 μm in diameter) ensured low kinetic energy and thus minimal elastic deformation of droplets into spaces between feather barbules upon striking the feather surface (Barthlott and Neinhuis, 1997). After spraying feathers, we determined the number of particles remaining in the same area measured before washing and calculated the self-cleaning efficiency as the percentage of remaining particles, with 0% indicating complete particle removal (complete self-cleaning) and 100% meaning no particle removal (no self-cleaning).

To further verify that self-cleaning was caused by the removal of particles by droplets rolling off the surface (Barthlott and Neinhuis, 1997), we determined tilt angle for iridescent and non-iridescent feathers (each N=3) by placing 10 μl droplets of de-ionized water onto the surface of the feathers and tilting the feathers away from the horizontal until the droplets rolled off the feather surface. We used a goniometer (Ramé-Hart, Netcong, NJ, USA) to tilt the feathers parallel to the barbs, mimicking the way that feathers are oriented on live mallards, and recorded the angle of tilt when the droplets detached from the surface.

Statistics

To determine the effects of feather washing on hydrophobicity (θ_c) and feather microstructure (ϕ), we performed three separate repeated-measures ANOVA for θ_c, ϕ_b and ϕ_{bbl} using the aov function in R (R Development Core Team, 2010), with bird ID as the subject, treatment as a within-subjects factor, and feather region as a between-subjects factor. Because of non-normality of ϕ_b, we squared the data prior to analysis following the recommendation of Sokal and Rolf (Sokal and Rolf, 1995) for left-skewed data. To determine the importance of ϕ_b and ϕ_{bbl} in predicting θ_c, we performed two separate multiple regressions (pre- and post-wash), with ϕ_b and ϕ_{bbl} as predictor variables and θ_c as the response variable. We performed the analysis with both raw data and z-transformed values to allow for meaningful interpretation of estimates (β coefficients) (Quinn and Keough, 2002). To determine the relationship between contact angle and feather colour, we used Pearson correlation tests for brightness and saturation, and Spearman rank correlation tests for feather hue (due to non-normality). We also used Pearson correlation tests to determine the correlation between colour measurements pre- and post-treatment, and performed paired t-tests to analyse the effect of washing on colour properties. For the self-cleaning experiment, we used a paired t-test to compare the initial number, as well as the percentage of remaining silica particles on iridescent (I-5) and non-iridescent feathers (NI-5). We used Pearson's correlation test to determine the relationship between the initial number of particles and the amount removed with misting. We checked all data for normality by inspecting Q–Q plots, and checked for homogeneity of variance by plotting model residuals *versus* fitted values in R (R Development Core Team, 2010). All data values are presented as means ± s.d. unless otherwise noted.

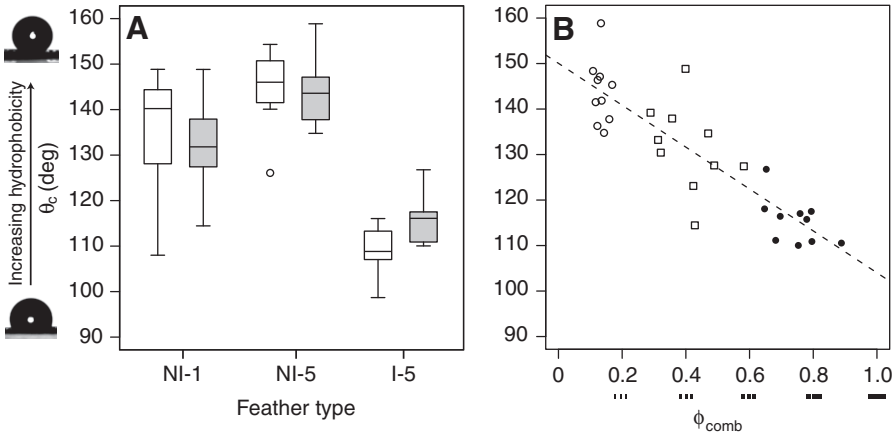

Fig. 3. Effects of washing treatment on hydrophobicity (measured as contact angle, θ_c) in different feathers (A), and the mechanistic basis for differences in θ_c between feathers (B). Boxplots in A (edges, 1st and 3rd quartiles; line, median; and branches, 1.5× interquartile range) show effects of feather type and washing treatment (white: pre-wash, grey: post-wash) on hydrophobicity, with outsets showing droplet profiles on non-iridescent (top) and iridescent (bottom) feathers (NI, non-iridescent; I, iridescent; numbers refer to the location of the secondary feather). Filled circles in B represent iridescent feather regions and open symbols represent non-iridescent regions from secondary feathers nos 5 (circles) and 1 (squares). Results are from post-wash treatment (see Materials and methods for details). Schematic drawings along the *x*-axis depict cross-section through feather barbules for each value of ϕ_{comb} (combined solid area fraction).

RESULTS

Hydrophobicity tests

The difference in hydrophobicity between iridescent and non-iridescent feathers was evident upon examination of the shape of droplets placed onto the feather surface (Fig. 3A, outsets). Iridescent feathers were significantly less hydrophobic than either type of non-iridescent feather (NI-1 and NI-5; Table 1 and Fig. 3A). Furthermore, iridescent feathers were only mildly hydrophobic (θ_c=115±5.11 deg), while non-iridescent feathers approached superhydrophobicity (θ_c=143.83±7.08 deg) (Zhang et al., 2008). The removal of preen oils and other surface contaminants had no significant effect on hydrophobicity (Table 1 and Fig. 3A).

Feather microstructure

The percentage of the total surface area occupied by feather barbules (solid area fraction ϕ_{bbl}, see Materials and methods for details) was noticeably higher in iridescent feathers than in non-iridescent feathers (Fig. 1B,C) and differed significantly between all three types of feather (Table 1 and supplementary material Fig. S1B). There was no significant effect of washing treatment on ϕ_{bbl}; however, the interaction between treatment and feather type was significant, as ϕ_{bbl} decreased in feathers I-5 and NI-5 and increased in NI-1 (Table 1 and supplementary material Fig. S1B). Barb area fraction (ϕ_b), defined analogously to ϕ_{bbl} as the percentage of surface area occupied by feather barbs, differed significantly between non-iridescent and iridescent feathers, but not between the two types of non-iridescent feather (Table 1 and supplementary material Fig. S1A). In addition, ϕ_b decreased significantly after washing, while the interaction between treatment and feather type was not significant (Table 1). Contact angle (θ_c) significantly decreased with ϕ_b and ϕ_{bbl} before and after (Fig. 3B) washing (Table 2), and ϕ_{bbl} had a greater effect on feather hydrophobicity than ϕ_b, as indicated by relatively large β coefficients (Table 2).

Table 1. Results of three separate repeated-measures ANOVA showing effects of treatment and feather region on hydrophobicity (θ_c) and microstructure (ϕ_{bbl} and ϕ_b) of secondary feathers in male mallards, *Anas platyrhynchos*

Variable	d.f.	*F*	*P*
Contact angle (θ_c)			
Feather	2,27	85.81	<0.0001*
Treatment	1,27	0.034	0.86
Feather × treatment	2,27	2.03	0.15
Barb morphology (ϕ_b)			
Feather	2,27	78.88	<0.0001†
Treatment	1,27	6.62	0.016
Feather × treatment	2,27	0.28	0.76
Barbule morphology (ϕ_{bbl})			
Feather	2,27	183.46	<0.0001*
Treatment	1,27	0.33	0.57
Feather × treatment	2,27	4.08	0.028

N=10 individuals, *N*=3 feathers each.
The area fraction ϕ refers to the ratio of keratin to air parallel to the feather surface. *All pairwise comparisons significant. †Pairwise comparisons with iridescent feathers significant, but comparison between non-iridescent feather types not significant (Tukey tests, *P*<0.05).

Spectrometry

Iridescent feathers with low hydrophobicity were noticeably brighter and more saturated than those with high hydrophobicity (Fig. 4A), and indeed all colour variables were significantly and negatively correlated with hydrophobicity after, but not before, washing

Table 2. Results of two separate multiple regressions (pre- and post-wash), with barb and barbule microstructure (ϕ_b and ϕ_{bbl}, respectively) as predictors and θ_c as the response variable

Variable	Estimate	s.e.	*t*	β	*P*
Pre-wash*					
ϕ_b	–71.04	17.51	–4.06	–0.45	<0.0001
ϕ_{bbl}	–33.58	6.90	–4.87	–0.54	<0.0001
Post-wash†					
ϕ_b	–33.83	12.61	–2.68	–0.31	0.012
ϕ_{bbl}	–31.30	5.51	–5.68	–0.66	<0.0001

Standardized partial regression coefficients (β) were obtained from analysing *z*-transformed variables (see Materials and methods for details).
*Model 1: r^2_{adj}=0.81, $F_{2,27}$=62.43, *P*<0.0001. †Model 2: r^2_{adj}=0.74, $F_{2,27}$=42.03, *P*<0.0001.

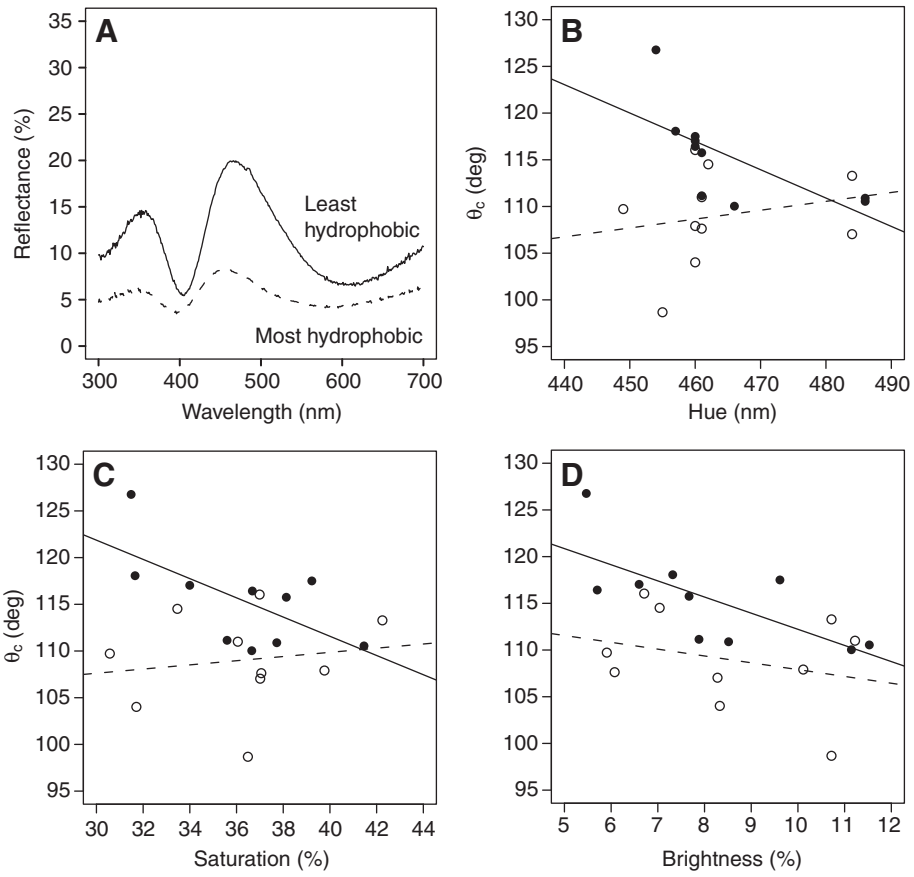

Fig. 4. Relationship between colour and hydrophobicity in mallard secondary feathers. Reflectance curves illustrate the difference between birds with the lowest (solid line) and highest hydrophobicities (dashed line) (A). Scatterplots show relationships between hydrophobicity (θ_c) and colour variables hue (B), saturation (C) and brightness (D) (N=10). Symbols and lines show results before (open circles, dashed lines) and after washing (filled circles, solid lines), with higher values of θ_c indicating greater hydrophobicity.

(Table 3 and Fig. 4B–D). Furthermore, all colour measurements were significantly correlated across treatments (hue: r_8=0.75, P=0.013; brightness r_8=0.97, P<0.0001; saturation: r_8=0.93, P<0.0001). Hue and brightness were not significantly correlated before (Pearson's r_8=0.30, P=0.41) or after washing (Pearson's r_8=0.62, P=0.055), but saturation was significantly correlated with both hue (Pearson's r_8=0.67, P=0.034) and brightness (Pearson's r_8=0.71, P=0.024) after washing. None of the colour variables were significantly affected by preen oil removal (hue: paired t_9=–1.67, P=0.13; brightness: t_9=0.79, P=0.45; saturation: t_9=–0.31, P=0.77).

Self-cleaning experiment

The initial number of deposited silica particles did not differ significantly between feather types (iridescent: 120.3±60.71; non-iridescent: 80.8±13.30; paired t_9=–2.09, P=0.066). After artificially rinsing them, iridescent feathers remained significantly dirtier than non-iridescent feathers (paired t_9=4.25, P=0.0021; Fig. 5) and self-cleaning efficiency (the proportion of removed particles) increased significantly with θ_c (Pearson's r_{18}=0.62, P=0.0033).

Water droplets remained on the surface of iridescent feathers (I-5) even after tilting to 90 deg, while droplets on non-iridescent feathers (NI-5) rolled off the surface at a tilting angle of 39±4.6 deg (both N=3).

Table 3. Correlation coefficients between colour variables and contact angle (hydrophobicity) in iridescent flight feathers of mallards

Treatment	Hue	Brightness	Spectral saturation
Pre-wash	0.27* (0.46)	–0.29 (0.42)	0.15 (0.68)
Post-wash	**–0.94* (<0.0001)**	**–0.71 (0.023)**	**–0.64 (0.046)**

Correlation coefficients marked with an asterisk indicate Spearman rank correlations; all others are Pearson correlation coefficients. Values in bold indicate significant results (P-values in parentheses).

DISCUSSION

Here we have shown that iridescent feathers are less hydrophobic than non-iridescent feathers primarily due to differences in feather microstructure (see Fig. 1B,C). Furthermore, owing to their decreased hydrophobicity, iridescent feathers are less efficient at self-cleaning than non-iridescent feathers (Fig. 5). Together, these findings suggest a previously unforeseen cost to shiny iridescent plumage colours.

Other studies have found a similarly high hydrophobicity (Bormashenko et al., 2007; Elowson, 1984) and low surface area fraction in feathers (Bormashenko et al., 2007; Rijke, 1968; Rijke, 1970; Rijke et al., 2000), but none have explicitly compared hydrophobicity between iridescent and non-iridescent feathers. Interestingly, several studies have shown that non-avian structurally coloured materials, whether artificial (e.g. inverse opals) (Gu et al., 2003) or natural (e.g. the integuments of some snakes, butterflies and beetles) (Doucet and Meadows, 2009; Wagner et al., 1996; Zheng et al., 2007), are more hydrophobic than unstructured materials. These results are opposite to those presented here, probably because the morphologies producing these structural colours differ from those of birds. In contrast to flattened barbules composed of layered materials, the optical effects of some biological structures are achieved by mechanisms that decrease the solid area

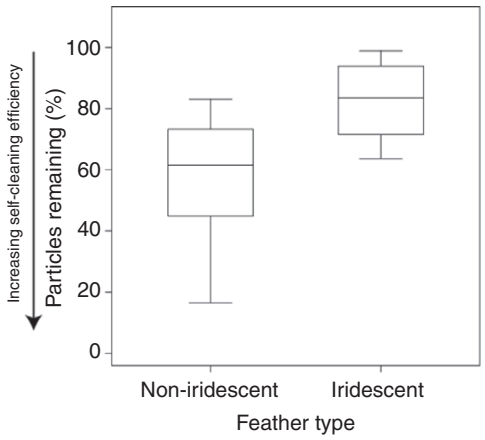

Fig. 5. Self-cleaning efficiency of iridescent and non-iridescent feathers. Boxes show upper and lower quartiles (edges), median (line) and range (branches) of percentage of silica particles remaining on feathers after spraying with a fine mist (see Materials and methods). The difference between the two was highly significant (paired t_9=4.25, P=0.0021).

fraction ϕ. For example, the spacing of chitin and air in the pillar-like structures responsible for colour production in *Morpho* butterfly wings maximizes hydrophobicity by minimizing ϕ (Wagner et al., 1996; Zheng et al., 2007). The cost of becoming waterlogged is presumably greater in small, poikilothermic animals (e.g. spiders, insects), and thus the selection pressures leading to the evolution of colourful iridescent traits in ectothermic *versus* endothermic taxa probably differ (Doucet and Meadows, 2009). These differences should be explored in future research.

Preen oil has been considered a critical determinant of hydrophobicity in feathers (Gill, 1995), but there is surprisingly little data to support this notion. For example, van Rhijn (van Rhijn, 1977) experimentally removed preen oils and found no significant effects on buoyancy or water repellency. Moreover, Giraudeau and colleagues (Giraudeau et al., 2010) showed that blocking access to the preen gland in mallards resulted in decreased feather quality and water repellency, but only after long-term blockage (longer than 3 months). These studies suggest that preen oils may indirectly influence hydrophobicity, perhaps by maintaining an orderly arrangement of barbs and barbules or by increasing their elasticity and flexibility (Rijke, 1970). Similarly, our data show that feather microstructure is likely to be more important than surface composition in determining hydrophobicity, as indicated by a non-significant treatment effect (see Fig. 3A, Table 1), and the agreement of our results with predictions based on the Cassie–Baxter wetting theory [see eqn 1 of Bormashenko (Bormashenko et al., 2007)]. While the interaction between feather type and treatment was not significant (Table 1), the observed increase in hydrophobicity of iridescent feathers after washing compared with decreases in both types of non-iridescent feather (Fig. 3A) suggests some role for preen oil that may vary with feather microstructure. Future studies should seek to decouple these variables, perhaps by chemically modifying feathers (making the keratin either hydrophobic or hydrophilic) while still maintaining their hydrophobic microstructure.

According to honest advertisement models of sexual selection, if iridescent plumage colours are to be reliable indicators of male quality, an associated cost to maintain signal honesty should exist (Andersson, 1994). Some studies have shown evidence for ontogenetic costs (Hill et al., 2005; McGraw et al., 2002), and others have proposed additional costs like enhanced visibility to predators (Andersson, 1994; Promislow et al., 1992). Our results demonstrate for the first time that iridescent plumage colour trades off with hydrophobicity (Fig. 3A, Fig. 4) and therefore self-cleaning efficiency (Fig. 5), potentially explaining why species with ornamental traits spend more time maintaining their plumage (Walther and Clayton, 2005). In addition to this maintenance cost, decreased hydrophobicity may have associated metabolic costs. For example, saturated down feathers in mallard ducklings cause a significant increase in thermal conductance, suggesting greater risks of hypothermia and higher metabolic costs in maintaining homeothermy (Bakken et al., 2006). Future studies should examine hydrophobicity in small iridescent birds (e.g. hummingbirds) with high surface area to volume ratios and high metabolic costs in maintaining body temperature. Biomechanical costs may also be associated with decreased hydrophobicity. For example, low hydrophobicity would increase the contact area between water and keratin, enhancing water absorption and thereby weakening the feather keratin (Taylor et al., 2004). In addition, the added weight due to water can increase wing loading (Ortega-Jiménez et al., 2010). Although decreased hydrophobicity of feathers is probably disadvantageous in other aquatic species, or indeed in any species with feathers whose functions are impaired by wetting (e.g. flight feathers), it may be advantageous in some diving birds (Grémillet et al., 2005). Furthermore, some hydrophobic surfaces may be more susceptible to biofouling than less hydrophobic ones because there is a greater surface area for microorganisms to adhere to (reviewed in Howell and Behrends, 2006). Thus, decreased hydrophobicity may be advantageous in these cases as well.

Iridescent feather barbules are consistently modified in most species studied thus far (Chandler, 1916; Doucet et al., 2006; Durrer, 1986; Osorio and Ham, 2002), suggesting that decreased hydrophobicity is a general characteristic of iridescent plumage traits that may play a role in their evolution across Aves. Iridescent colours are broadly distributed from basal (e.g. Galliformes) to highly derived birds (e.g. Passeriformes), and indeed iridescent nanostructures have recently been discovered in a ~40 million year old fossilized feather (Vinther et al., 2010), suggesting that they are ancient and fundamental components of modern bird plumage. The data presented here provide clues to the factors leading to this broad distribution, and thus open up new avenues of research into the evolution of bright colours.

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REFERENCES

Abramoff, M. D., Magelhae, P. J. and Ram, S. J. (2004). Image processing with ImageJ. *Biophotonics Int.* **11**, 36-42.
Andersson, M. (1994). *Sexual Selection*. Princeton: Princeton University Press.
Bakken, G., Banta, M., Higginbotham, C. and Lynott, A. (2006). It's just ducky to be clean: the water repellency and water penetration resistance of swimming mallard *Anas platyrhynchos* ducklings. *J. Avian Biol.* **37**, 561-571.
Barthlott, W. and Neinhuis, C. (1997). Purity of the sacred lotus, or escape from contamination in biological surfaces. *Planta* **202**, 1-8.
Bhushan, B., Jung, Y. C. and Koch, K. (2009). Self-cleaning efficiency of artificial superhydrophobic surfaces. *Langmuir* **25**, 3240-3248.
Bormashenko, Y., Stein, T., Whyman, G. and Bormashenko, E. (2007). Why do pigeon feathers repel water? Hydrophobicity of pennae, Cassie-Baxter wetting hypothesis and Cassie-Wenzel capillarity-induced wetting transition. *J. Colloid Interface Sci.* **311**, 212-216.
Bradbury, J. W. and Vehrencamp, S. L. (1998). *The Principles of Animal Communication*. Sunderland, MA: Sinauer.

Cassie, A. B. D. and Baxter, S. (1944). Wettability of porous surfaces. *Trans. Faraday Soc.* **40**, 546-551.
Chandler, A. (1916). A study of the structure of feathers: with reference to their taxonomic significance. *Univ. California Publ. Zool.* **13**, 243-447.
Darwin, C. (1871). *The Descent of Man, and Selection in Relation to Sex*. London: Murray.
Doucet, S. M. and Meadows, M. G. (2009). Iridescence: a functional perspective. *J. R. Soc. Interface* **6**, S115-S132.
Doucet, S. M., Shawkey, M., Hill, G. and Montgomerie, R. (2006). Iridescent plumage in satin bowerbirds: structure, mechanisms and nanostructural predictors of individual variation in colour. *J. Exp. Biol.* **209**, 380-390.
Durrer, H. (1986). The skin of birds: colouration. In *Biology of the Integument 2, Vertebrates*, Vol. 2 (ed. J. Bereiter-Hahn, A. G. Matolsky and K. S. Richards), pp. 239-247. Berlin: Springer-Verlag.
Elowson, A. (1984). Spread-wing postures and the water repellency of feathers: a test of Rijke's hypothesis. *Auk* **101**, 371-383.
Gill, F. B. (1995). *Ornithology*. New York: W. H. Freeman and Company.
Giraudeau, M., Duval, C., Guillon, N., Bretagnolle, V., Gutierrez, C. and Heeb, P. (2010). Effects of access to preen gland secretions on mallard plumage. *Naturwissenschaften* **97**, 577-581.
Grémillet, D., Chauvin, C., Wilson, R., Le Maho, Y. and Wanless, S. (2005). Unusual feather structure allows partial plumage wettability in diving great cormorants *Phalacrocorax carbo*. *J. Avian Biol.* **36**, 57-63.
Gu, Z., Uetsuka, H., Takahashi, K., Nakajima, R., Onishi, H., Fujishima, A. and Sato, O. (2003). Structural color and the lotus effect. *Angew. Chem. Int. Ed.* **42**, 894-897.
Hiemenz, P. C. and Rajagopalan, R. (1997). *Principles of Colloid and Surface Chemistry*. New York: CRC Press.
Hill, G. E. (2002). *A Red Bird in a Brown Bag*. New York: Oxford University Press.
Hill, G. E. (2006). Female mate choice for ornamental coloration. In *Bird Coloration Volume II: Function and Evolution*, Vol. 2 (ed. G. Hill and K. McGraw), pp. 137-200. Cambridge, MS: Harvard University Press.
Hill, G. E., Doucet, S. M. and Buchholz, R. (2005). The effect of coccidial infection on iridescent plumage coloration in wild turkeys. *Anim. Behav.* **69**, 387-394.
Howell, D. and Behrends, B. (2006). A review of surface roughness in antifouling coatings illustrating the importance of cutoff length. *Biofouling* **22**, 401-410.
Humphrey, P. S. and Clark, G. A., Jr (1961). Pterylosis of the mallard duck. *Condor* **63**, 365-385.
Lucas, A. M. and Stettenheim, P. R. (1972). *Avian Anatomy. Integument. Part II*. Washington DC: US Department of Agriculture.
Mahoney, S. (1984). Plumage wettability of aquatic birds. *Auk* **101**, 181-185.
Marmur, A. (2006). Soft contact: measurement and interpretation of contact angles. *Soft Matter* **2**, 12-17.
McGraw, K. J., Mackillop, E. A., Dale, J. and Hauber, M. E. (2002). Different colors reveal different information: how nutritional stress affects the expression of melanin- and structurally based ornamental plumage. *J. Exp. Biol.* **205**, 3747-3755.
Meadows, M. G., Butler, M. W. and Morehouse, N. I. (2009). Iridescence: views from many angles. *J. R. Soc. Interface* **6**, S107-S113.
Montgomerie, R. (2006). Analyzing colors. In *Bird Coloration Volume I: Mechanisms and Measurements*, Vol. 1 (ed. G. E. Hill and K. J. McGraw), pp. 90-147. Cambridge, MS: Harvard University Press.
Olson, V. A. and Owens, I. P. F. (1998). Costly sexual signals: are carotenoids rare, risky or required? *Trends Ecol. Evol.* **13**, 510-514.
Ortega-Jiménez, V., Álvarez-Borrego, S., Arriaga-Ramírez, S., Renner, M. and Bridge, E. (2010). Takeoff flight performance and plumage wettability in Cassin's auklet *Ptychoramphus aleuticus*, Xantus's murrelet *Synthliboramphus hypoleucus* and Leach's storm-petrel *Oceanodroma leucorhoa*. *J. Ornithol.* **151**, 169-177.
Osorio, D. and Ham, A. D. (2002). Spectral reflectance and directional properties of structural coloration in bird plumage. *J. Exp. Biol.* **205**, 2017-2027.
Promislow, D., Montgomerie, R. and Martin, T. (1992). Mortality costs of sexual dimorphism in birds. *Proc. R. Soc. Lond. B* **250**, 143-150.
Prum, R. O. (2006). Anatomy, physics, and evolution of structural colors. In *Bird Coloration Volume I: Mechanisms and Measurements*, Vol. 1 (ed. G. E. Hill and K. J. McGraw), pp. 295-353. Cambridge, MS: Harvard University Press.
Quinn, G. P. and Keough, M. J. (2002). *Experimental Design and Data Analysis for Biologists*. Cambridge, MS: Cambridge University Press.
R Development Core Team (2010). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.
Rijke, A. (1968). The water repellency and feather structure of cormorants, Phalacrocoracidae. *J. Exp. Biol.* **48**, 185-189.
Rijke, A. (1970). Wettability and phylogenetic development of feather structure in water birds. *J. Exp. Biol.* **52**, 469-479.
Rijke, A. M., Jesser, W. A., Evans, S. W. and Bouwman, H. (2000). Water repellency and feather structure of the blue swallow *Hirundo atrocaerulea*. *Ostrich* **71**, 143-145.
Senar, J. C. (2006). Color displays as intrasexual signals of aggression and dominance. In *Bird Coloration: Function and Evolution*, Vol. 2 (ed. G. E. Hill and K. J. McGraw), pp. 87-136. Cambridge, MS: Harvard University Press.
Sokal, R. R. and Rolf, F. J. (1995). *Biometry*. New York: W.H. Freeman.
Stalder, A. F., Kulik, G., Sage, D., Barbieri, L. and Hoffmann, P. (2006). A snake-based approach to accurate determination of both contact points and contact angles. *Colloids Surf. A* **286**, 92-103.
Taylor, A., Bonser, R. H. C. and Farrent, J. (2004). The influence of hydration on the tensile and compressive properties of avian keratinous tissues. *J. Mater. Sci.* **39**, 939-942.
van Rhijn, J. G. (1977). Processes in feathers caused by bathing in water. *Ardea* **65**, 126-147.
Vinther, J., Briggs, D. E. G., Clarke, J., Mayr, G. and Prum, R. O. (2010). Structural coloration in a fossil feather. *Biol. Lett.* **6**, 128-131.
Wagner, T., Neinhuis, C. and Barthlott, W. (1996). Wettability and contaminability of insect wings as a function of their surface sculptures. *Acta Zool.* **77**, 213-255.
Walther, B. and Clayton, D. (2005). Elaborate ornaments are costly to maintain: evidence for high maintenance handicaps. *Behav. Ecol.* **16**, 89-95.
Zahavi, A. (1975). Mate selection: a selection for a handicap. *J. Theor. Biol.* **53**, 205-214.
Zhang, X., Shi, F., Niu, J., Jiang, Y. and Wang, Z. (2008). Superhydrophobic surfaces: from structural control to functional application. *J. Mater. Chem.* **18**, 621-633.
Zheng, Y., Gao, X. and Jiang, L. (2007). Directional adhesion of superhydrophobic butterfly wings. *Soft Matter* **3**, 178-182.