

A phylogenetic comparative analysis reveals correlations between body feather structure and habitat

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Summary

1. Body feathers ensure both waterproofing and insulation in waterbirds, but how natural variation in the morphological properties of these appendages relates to environmental constraints remains largely unexplored. Here, we test how habitat and thermal condition affect the morphology of body feathers, using a phylogenetic comparative analysis of five structural traits [i.e., total feather length, the lengths of the pennaceous (distal) and plumulaceous (proximal) sections, barb density, and pennaceous barbule density] from a sample of 194 European bird species.

2. Body feather total length is shorter in aquatic than in terrestrial birds, and this difference between groups is due to the shorter plumulaceous feather section in aquatic birds. Indeed, a reduced plumulaceous section in feather length probably reflects the need to limit air trapped in the plumage to adjust the buoyancy of aquatic birds. In contrast, the high pennaceous barbule density of aquatic birds compared to their terrestrial counterparts reflects water resistance of the plumage in contact with water.

3. Our results show that birds living in environments with low ambient temperature have long plumulaceous feather lengths, low barb density, and low pennaceous barbule density. Data also suggest that plumage probably has limited function in reducing the heat absorption of species living in hot environments.

4. Our results have broad implications for understanding the suite of selection pressures driving the evolution of body feather functional morphology. It remains to be tested, however, how other feather traits, such as the density of plumage (feathers per unit area) and the relative number of different feather types, for example downy feathers, are distributed amongst birds with different water resistance and thermoinsulative needs.

Key-words: body feathers, feather lengths, functional morphology, thermal insulation, vane density, water repellence

Introduction

Body feathers cover most of the body in birds and serve multiple functions, including (but not limited to) waterproofing and insulation. Indeed, the feathers in dinosaurs likely first evolved for one of these functions (Prum & Brush 2002). Body feathers are broadly defined as the stiff outer layer of integument that protects the skin in birds. These feathers are most numerous in terms of their numbers and mass (Wetmore 1936; Davenport

et al. 2009), and are critical for both thermal insulation and waterproofing (Davenport *et al.* 2009; Williams, Hagelin & Kooyman 2015). However, very little is known about how the morphological properties of body feathers relate to function. In the few aquatic bird species studied so far, body feathers provide an impenetrable and rigid waterproof covering over a thick, insulating layer of down (Stephenson & Andrews 1997); in other species, this outer layer is permeable and varies seasonally in structure depending on the changing thermoregulatory needs of a bird (e.g., Middleton 1986; Grémillet *et al.* 2005).

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The basic, non-flight related, functions of bird body feathers are twofold: First, by varying the number of feathers per surface area, the amount of trapped air, and hence water resistance and thermo-conductivity, can be controlled (e.g., Middleton 1986; Swanson 1991; Fernando Novoa, Bozinovic & Rosenmann 1994; Cooper 2002; Williams, Hagelin & Kooyman 2015). Second, by varying the composition, microoptical and microstructural properties of plumage (i.e., color, relative densities of different feather types, morphologies of body feathers), water repellency, and insulatory functions can also be controlled (e.g., Rijke 1970; Middleton 1986; Wolf & Walsberg 2000; Ward *et al.* 2007; Broggi *et al.* 2011; Rijke & Jesser 2011; Pap *et al.* 2015; Williams, Hagelin & Kooyman 2015; Koskenpato *et al.* 2016). These different functions are immediately evident in different species; the Emperor Penguin (*Aptenodytes forsteri*), for example, has a particular spatial pattern and ratio of different feather types, including body and attached after-feathers, filoplumes, and downy feathers that make its body covering uniquely insulative and suited to its lifestyle (Williams, Hagelin & Kooyman 2015), while diving Great Cormorants (*Phalacrocorax carbo*) balance body feather structures between waterproofing and wettability to fulfil requirements of insulation and reduced buoyancy during diving (Rijke 1968; Grémillet *et al.* 2005). Nevertheless, in highly specialized aquatic species it is often difficult to deduce the relative functions of different body feather structures; in terrestrial species that are much less exposed to water penetration, plumage appears adapted to repel, rather than to withstand, infiltration due to high water pressures encountered during diving (Stephenson & Andrews 1997; Rijke & Jesser 2011; Pap *et al.* 2015).

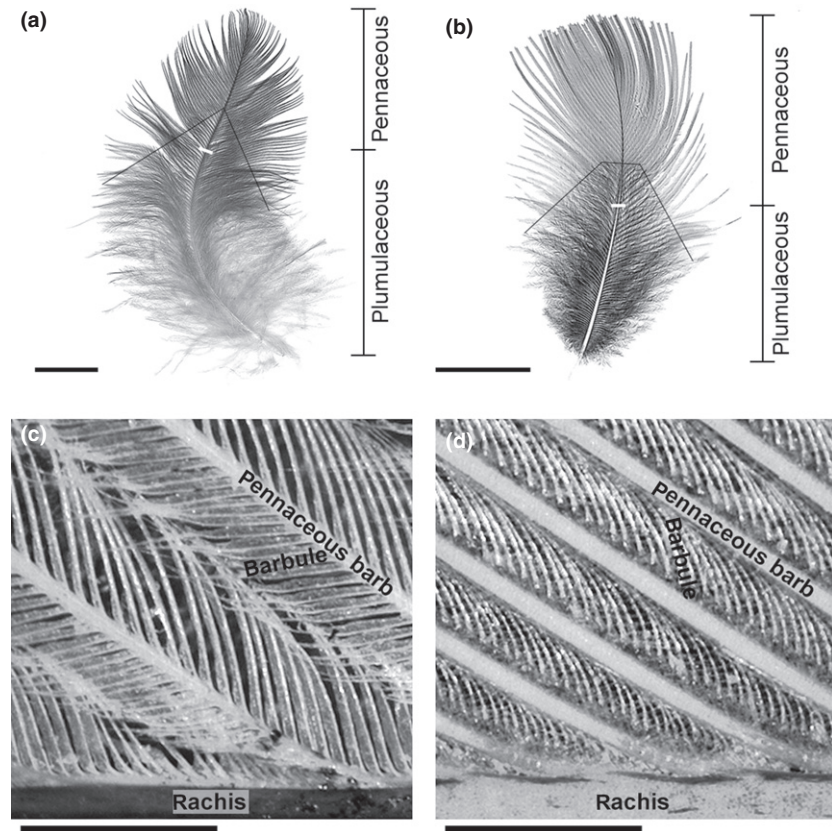
Body feathers are comprised of a shaft with regularly spaced branches (barbs) on either side, which are in turn divided into barbules (e.g., Prum & Brush 2002; Fig. 1). The number of barbs and barbules, and the way they are attached to one another, determines how much air they can trap, their resistance to water pressure, and thus their insulatory and waterproofing properties (e.g., Stettenheim 2000; Rijke & Jesser 2011). Feather vanes can be further divided along their longitudinal axis into distal pennaceous and proximal plumulaceous (downy) sections; the difference between the two is clear from their structure and in the number of connections between their barbs and barbules (Butler, Rohwer & Speidel 2008). Indeed, because the density of barbs and barbules in the pennaceous section of body feathers is tighter and more rigid, it has been suggested that this region plays a role in water repellence and resistance in terrestrial and aquatic birds, respectively (Rijke & Jesser 2011; see also Pap *et al.* 2015). It is thought that a waterproof outer body covering is enabled by a high density of barbs and barbules as well as the low porosity (i.e., a function of width and spacing of barbs and barbules) of the distal, pennaceous vane section (Grémillet *et al.* 2005; Rijke & Jesser 2011). Nevertheless, the relationship between habitat and the vane density of

body feathers has only been tested to date across a narrow range of mainly aquatic species and without taking phylogenetic relationships into account (Rijke 1968, 1970; Rijke & Jesser 2011). In addition, whether an aquatic lifestyle selects for longer body feathers with longer pennaceous sections to provide a thick water resistant external covering built up by overlapping distal vanes, and for a shorter plumulaceous section to limit the amount of air captured in the plumage to reduce buoyancy, particularly in diving birds (see Wilson *et al.* 1992; Grémillet, Tuschy & Kierspel 1998), remains unexplored. A handful of studies, combined with our own observations, indicate that aquatic species are characterized by a dense and short covering of feathers, suggesting that waterproofing is achieved through increasing the density per surface area, but reducing the length of body feathers (Davenport *et al.* 2009; Williams, Hagelin & Kooyman 2015). We also know that thermoregulation is affected by aquatic lifestyle, because contact with water increases thermal conductance (Croll & McLaren 1993; de Vries & van Eerden 1995; Grémillet *et al.* 2001). Water-induced increases in thermal conductance are greatest in diving birds, because in order to reduce buoyancy the volume of air trapped in the plumage is reduced (Wilson *et al.* 1992; Grémillet, Tuschy & Kierspel 1998).

Thermoinsulation is thought to be conferred by different components of the body feathers than those used for a waterproofing function (Middleton 1986; Butler, Rohwer & Speidel 2008; Broggi *et al.* 2011). Feathers may decrease heat flux between the skin and the environment by blocking radiation and trapping air near the body, and they can also increase heat flux by conducting heat to, or from, the body (Walsberg 1988). Thus, the thermoregulatory characteristics of body feathers are probably related to a range of structural traits, including the length of the downy section and the density of barbs and barbules. These traits combine to contribute to the depth and structure of the downy plumage layer (Middleton 1986; Butler, Rohwer & Speidel 2008; Davenport *et al.* 2009; de Zwaan, Greenwood & Martin 2016), and determine the number and size of trapped air spaces near the skin. Long, probably dense, plumulaceous segments help to trap heat close to the body, and a loose plumage surface (i.e., low pennaceous vane density) promotes penetration of radiation towards the body during sunbathing. Birds that live in hot environments reduce heat absorption at the plumage surface to prevent it from reaching the skin (Walsberg & King 1978); thus, birds adapted to warmer climates should have tighter body coverings (i.e., a high pennaceous barb and barbule density) and short and loose downy feather sections (see Butler, Rohwer & Speidel 2008).

Birds vary dramatically in body feather texture depending on species (Butler, Rohwer & Speidel 2008; Pap *et al.* 2013; Fig. 1), likely reflecting functional pressures and trade-offs in integument production (Dawson *et al.* 2000; Butler, Rohwer & Speidel 2008; Pap *et al.* 2008; Vágási *et al.* 2012; Danner *et al.* 2015; Gamero *et al.* 2015). Producing feathers is costly and the development of optimum

Fig. 1. Different parts of a body feather with distal pennaceous and proximal plumulaceous sections delimited by a white stripe across the rachis at the base of the barb in which the length was at least 33% plumulaceous. The black lines define the boundary of the pennaceous and plumulaceous sections of the vane (a,b). Upper figures show two typical species with the ratio of the pennaceous feather section to plumulaceous part. In the Common Raven (*Corvus corax*), a terrestrial species, the plumulaceous part is longer (a), while in the Common Moorhen (*Gallinula chloropus*), an aquatic bird, this feather part is reduced in length (b). Lower figures illustrate a section of pennaceous vane with the rachis and barb to which the barbules attach. The density of pennaceous barbules are lower in terrestrial species, such as the Common Buzzard (*Buteo buteo*) (c) than in aquatic birds, represented here by the Northern Pintail (*Anas acuta*) (d). Scale bars for figures (a) and (b) are 1 cm, and for (c) and (d) are 0.5 mm.



characteristics for each function is likely constrained by limited resources, including available amount and protein content of food (e.g. Pap *et al.* 2008). While there is some consensus on how resource limitation reduces the amounts of keratin invested in feathers (i.e., feather mass vs. feather length), conflicting results have been found with regard to effects on vane barb and barbule density (Pap *et al.* 2008; DesRochers *et al.* 2009; Vágási *et al.* 2012).

In this paper, we use a phylogenetic comparative approach and data from 194 temperate European bird species to test whether habitat, diving behavior, and thermal condition influence the morphology of body feathers. To do this, we compared: (1) Data from species living in different habitats; (2) Diving and non-diving aquatic birds, and; (3) The relationship between feather morphology and maximum breeding temperature and minimum wintering temperature. We hypothesized that the vane barbs and barbules of the distal, pennaceous section of body feathers, the part that forms the outer waterproofing layer, would be denser in aquatic species (see Table 1 for a summary of predictions). Predicting an effect of an aquatic lifestyle on pennaceous feather lengths is more difficult, because both alternatives - shortening and elongation - are plausible. If the length of the plumulaceous section of the feather reflects the volume of air trapped in the coat and hence affects buoyancy, we would expect a noticeable reduction of the downy part in aquatic species, and a further reduction in divers compared to non-divers. We also tested the hypothesis that low ambient temperature selects for a

thermoinsulative coat, manifested as a longer and denser plumulaceous section and a loose surface of body feathers, as suggested by former studies (Middleton 1986; Butler, Rohwer & Speidel 2008; Broggi *et al.* 2011; de Zwaan, Greenwood & Martin 2016; Table 1). Conversely, high ambient temperature is expected to select for a dense pennaceous vane to reduce heat absorption and short, and loose downy feather sections to promote heat loss. Finally, we investigated whether nutrition influences feather structure by comparing species with different amounts of protein in their diets.

Materials and methods

FIELD DATA COLLECTION AND FEATHER MEASUREMENTS

We collected feather samples from 1043 adult birds in 194 species at several locations across Romania between 2009 and 2015. Birds were captured throughout the year, using mist nets and, upon capture, five to ten body feathers were plucked from the abdomen, in the line of the shoulders. In addition, body feathers were collected from birds found dead in Romania and Norway, augmented with museum specimens from the Zoological Museum of Babeş-Bolyai University, Cluj Napoca (Romania). Feather samples were stored dry in small zip-lock plastic bags at room temperature until they were measured. None of the feathers showed any sign of degradation at the time of photographing and measurements.

Digital photographs of feathers placed onto a metric grid background, or stage micrometer, were imported into IMAGEJ version 1.37 (<http://rsb.info.nih.gov/ij/>) to allow feather length as well as

Table 1. Predicted effects of waterproofing (aquatic habitat and diving behavior) and thermoinsulation needs on the morphology of body feathers in birds. Predictions supported by the present study are marked in bold. The direction of the significant effect of minimum winter temperature on plumulaceous barb density, which is contrary to our initial prediction, is shown in bold and parentheses. A dash (–) indicates no *a priori* prediction

	Waterproofing		Thermoinsulation	
	Aquatic life	Diving behavior	Heat retention in cold environment	Reduce heat absorption in hot environment
Feather length				
Pennaceous	–	–	–	–
Plumulaceous	Short	Short	Long	Short
Barb density				
Pennaceous	High	High	Low	High
Plumulaceous	–	–	High (Low)	Low (High)
Barbule density				
Pennaceous	High	High	Low	High

barb and barbule density to be measured. To describe feather structure, the division between pennaceous and plumulaceous sections was determined as follows; we divided the two sections along the rachis at the base of the barb when the length was at least 33% plumulaceous, and measured total feather length including the calamus, pennaceous and plumulaceous sections (Fig. 1). Barbs were identified as pennaceous where the vane structure was tight, enhanced by the hooklets of the barbules on the distal side of the barbs interlocked with the barbules on the proximal side of the adjacent barbs. Conversely, barbs were identified as plumulaceous when numerous non-interlocked barbs were present which made the vane appearance puffy (see also Fig. 1 in Broggi *et al.* 2011). Barb density was measured along the rachis, calculated as the number of branching events along the plumulaceous and pennaceous parts of the rachis expressed as a unit of length (per mm). Barbule density was measured near the rachis and in the middle of the pennaceous vane along a 1 mm barb length section (see Appendix S1 in Supporting Information). In order to reduce the error due to multi-person measurements, only BW marked on pictures the border between the pennaceous and plumulaceous sections. Conversely, both BW and TD measured the feather traits, and this may have introduced some measurement error and increased variance in the dataset. Feather samples were measured (Pap *et al.* 2015) randomly, however, and repeatability between the measurers was high for all feather parameters [total length: intraclass correlation coefficient (ICC) = 0.81, 95% CI 0.59–0.88; pennaceous length: ICC = 0.96, 0.94–0.98; plumulaceous length: ICC = 0.71, 0.63–0.88; total barb density: ICC = 0.83, 0.68–0.88; pennaceous barb density: ICC = 0.79, 0.64–0.88; plumulaceous barb density: ICC = 0.79, 0.62–0.86; pennaceous barbule density: ICC = 0.94, 0.85–0.97; all $F > 5.83$, $N = 16$, all $P < 0.001$].

BODY MASS, ECOLOGICAL TRAITS, AND HABITAT

We obtained approximate body masses for the species in our sample using the compilations of Dunning (2007) and del Hoyo, Elliot & Sargatal (1992–2013), distilling data to include just European populations and subspecies with European distributions in cases where data for several subspecies or populations are reported. We classified species into one of three habitat groups (Cramp & Perrins 1977–1994); terrestrial (i.e., rarely encountering water), riparian (i.e., living in wetland habitats like marshes and sedges), or aquatic (i.e., species that move around on the surface of, or in, water). Aquatic species were further categorized as either divers or non-divers. We extracted ambient temperature data from the University of East Anglia Climate Research Unit database (<http://www.cru.uea.ac.uk/>; version 3.10.01) (Mitchell & Jones 2005), a

global dataset containing interpolated monthly average land temperatures (°C) in a grid of spatial coordinates (0.5×0.5 degrees). For this study, we used the most recent temperature data (“cru_ts_3.23.2011.2014”, downloaded on 26 September 2015) containing monthly temperature means from 2011 to 2014, and averaged over 4 years to calculate average monthly temperatures for each spatial grid cell. From the resultant data file, we created a 12-layer shape file with each layer containing average temperatures for a separate month in each cell. Thus, by intersecting temperature and species distribution shape files, we were able to calculate monthly temperatures for each species separately for their wintering and breeding grounds. Distribution maps (shape files) were retrieved from <http://www.birdlife.org/datazone/info/spcdownload> (BirdLife International & NatureServe 2012). For breeding areas, we extracted highest monthly mean (‘maximum breeding temperature’) for the period April to August as a proxy for breeding heat stress, while for wintering grounds, we extracted lowest monthly mean (‘minimum winter temperature’) for the period December to February as a proxy for winter harshness. Monthly temperatures were calculated for breeding and wintering distribution areas for species between longitudes 20° W and 60° E, although, in some cases, where breeding or wintering areas are situated out of this range, we used global ranges. Because African and Arabian resident populations of several species are clearly separated from European migratory populations (with likely little gene flow between them), they were not included in our analyses (see Appendix S2). Because the protein content of the food may limit the feather synthesis (Pap *et al.* 2008), we included in our analyses the quality of the diet as a factor. Diet during molt was assigned to one of two categories, either ‘high’, or ‘low’ protein content (see Pap *et al.* 2015). A high protein content is defined as exclusive feeding on invertebrates and/or vertebrates, while low is either omnivorous and/or species feeding on plants. Dietary information for each species was obtained from Cramp & Perrins (1977–1994), and all variables are reported for each species in Appendices S1 and S2 of the Supporting Information.

STATISTICAL ANALYSES

We investigated the relationship between feather morphological variables and body mass, diet, habitat, breeding maximum and wintering minimum temperatures across birds. It is well-known that large within-group (i.e. within-species) variation in studied traits can cause significant bias in phylogenetic comparative analyses (Freckleton, Harvey & Pagel 2002). Thus, in order to check whether our traits for feather quality are species-specific and suitable for multispecies comparisons, we tested their repeatability by

assessing the importance of between-species compared to within-species variance (i.e., using 'ICCest' function in the R package 'ICC'; Wolak, Fairbairn & Paulsen 2012).

To account for phylogenetic non-independence, we used trees from <http://birdtree.org> (Jetz *et al.* 2012). We downloaded 1000 random trees using the Hackett backbone tree (Hackett *et al.* 2008), and calculated a rooted, ultrametric consensus tree, using the SUMTREES software (Sukumaran & Holder 2010). We used a phylogenetic generalized least squares (PGLS) model (Pagel 1997, 1999) employing backward-stepwise deletion of non-significant predictors from the full model based on the largest *P*-value. This PGLS approach controls for non-independence among species by incorporating a matrix of covariances based on phylogenetic relationship (Martins & Hansen 1997; Pagel 1997, 1999), and estimates the importance of phylogenetic corrections in analyses (Freckleton, Harvey & Pagel 2002). In all analyses, we set the degree of phylogenetic dependence (Pagel's λ) to the most appropriate value evaluated for each model by likelihood ratio statistics; thus, a value of λ close to zero indicates phylogenetic independence, while larger values indicate that closely-related species are more similar to each other than would be expected by chance (Pagel 1997, 1999). Species-specific feather lengths, barb and barbule density, and body mass were log-transformed in all statistical models to ensure model residual normality. Habitat and diet were entered into the models as fixed factors, while all other (continuous) variables were included as covariates.

Species in our dataset were subject to various levels of sampling effort, ranging from one to 24 individuals. Such differences in sampling can be sources of bias as different resultant estimates cannot be predicted with similar precision (Garamszegi & Møller 2010, 2011). However, if within species variance is particularly small compared to between species variance, ignoring this measurement error has no effect on type I error in phylogenetic analyses (Harmon & Losos 2005). Our body feather structure variables meet this requirement because conspecific feather quality measures were highly similar within species in our dataset (Results), a finding congruent with previous work on a different dataset (Butler, Rohwer & Speidel 2008). The likelihood-ratio statistics indicate that weighting phylogenetic models by log-transformed within species sample size significantly increases the model fit, but because our subsequent statistical procedure further decreases the bias caused by differential sampling effort across species, we present here just the results of our weighted minimal models.

All statistical analyses were conducted using the R statistical computing environment, version 3.2.2 (R Development Core Team 2015), and PGLS models were built as implemented in the 'nlme' package (Pinheiro *et al.* 2015). For some species, we were able to determine the age of birds as either first year or adults based on their plumage traits, and phylogenetic-paired *t*-tests (Lindénfors, Revell & Nunn 2010) show that feather parameters are very similar between the two age classes (in all cases $t < 0.01$, $N_{\text{barb}} = 88$, $N_{\text{barbule}} = 87$, $\lambda > 0.92$, $P > 0.9952$). Thus, it is unlikely that our results are affected by age specific changes in feather traits. Feather quality parameters were also statistically not significantly different between live and museum birds (Wilcoxon matched pairs test: total length: $Z = 1.06$, $N = 24$, $P = 0.2905$; pennaceous length: $Z = 0.60$, $N = 24$, $P = 0.5485$; plumulaceous length: $Z = 1.34$, $N = 24$, $P = 0.1739$; total barb density: $Z = 1.20$, $N = 24$, $P = 0.2301$; pennaceous barb density: $Z = 1.74$, $N = 24$, $P = 0.0814$; plumulaceous barb density: $Z = 0.26$, $N = 24$, $P = 0.7971$; pennaceous barbule density: $Z = 0.77$, $N = 24$, $P = 0.4445$). Therefore, our results are not biased by degradation of feathers collected from either recently dead or museum specimens. Means $\pm$ standard errors are reported throughout, and model predictions and associated standard errors for graphical representations of data were obtained using the 'lsmeans' function of the 'lsmeans' R package (Lenth 2016).

Results

BODY FEATHER MORPHOLOGY, REPEATABILITY, AND PHYLOGENETIC SIGNAL

Pennaceous feather length is similar to the length of the plumulaceous feather section (phylogenetic paired *t*-test: $t = 0.70$, $N = 194$, $P = 0.4824$), but pennaceous barb density was lower than plumulaceous barb density ($t = 6.92$, $N = 194$, $P < 0.0001$).

Our analyses show that traits for feather quality are highly repeatable and have narrow confidence intervals, which indicates that they are species-specific (i.e., total length: ICC = 0.90, 95% CI 0.88–0.92; pennaceous length: ICC = 0.85, 0.82–0.88; plumulaceous length: ICC = 0.77, 0.73–0.81; total barb density: ICC = 0.52, 0.45–0.49; pennaceous barb density: ICC = 0.80, 0.77–0.84; plumulaceous barb density: ICC = 0.59, 0.53–0.65; pennaceous barbule density: ICC = 0.62, 0.55–0.68). Results show that the species in our dataset can be adequately characterized in terms of feather quality traits.

Results show that body feather morphology is highly dependent on phylogeny; Pagel's λ varies between 0.66 and 0.91 in our sample, indicating a high degree of phylogenetic dependence (total length: $\lambda = 0.91$, $P < 0.0001$; pennaceous length: $\lambda = 0.76$, $P < 0.0001$; plumulaceous length: $\lambda = 0.75$, $P < 0.0001$; total barb density: $\lambda = 0.66$, $P < 0.0001$; pennaceous barb density: $\lambda = 0.70$, $P < 0.0001$; plumulaceous barb density: $\lambda = 0.67$, $P < 0.0001$; pennaceous barbule density: $\lambda = 0.91$, $P < 0.0001$).

BODY FEATHER MORPHOLOGY, HABITAT, AND TEMPERATURE

In general, feather lengths differ between habitat categories, explained by wintering minimum temperature. In contrast, barb density is only explained by wintering minimum temperature and does not differ between habitats (Table 2). Pennaceous barbule density is explained by habitat and wintering minimum temperature, while no other life-history or ecological trait (i.e., diet or breeding maximum temperature) had any effect on feather traits.

The total length of body feathers differs among species from different habitats. Body feathers are significantly longer in terrestrial than in aquatic species, while those of species that live in riparian habitats are intermediate in length (Table 2; Fig. 2a). The effect of habitat on total body feather length can be explained by the fact that the distinct plumulaceous feather section in terrestrial species is significantly longer than that measured in either riparian or aquatic species (Fig. 2b). In contrast, pennaceous feather lengths do not differ among species from different habitats; the length of the plumulaceous section is negatively correlated with wintering minimum temperature (Table 2; Fig. 2c), suggesting that species wintering in colder areas have longer plumulaceous sections. The

Table 2. Minimum adequate phylogenetic generalized least squares models investigating the relationships between body feather morphological parameters, body mass, life-history, and ecological traits. Minimal models were obtained by eliminating non-significant predictors from full models in a backward stepwise manner based on the largest *P*-value

	Feather length				Feather density		
	d.f.	<i>F</i>	<i>P</i>		d.f.	<i>F</i>	<i>P</i>
Body feather total length (Pagel's $\lambda = 0.77$, $N = 194$)				Total barb density (whole feather) (Pagel's $\lambda = 0.81$, $N = 194$)			
Body mass	1	428.54	<0.0001	Body mass	1	8.75	0.0035
Habitat	2	6.51	0.0018	-			
Pennaceous length (Pagel's $\lambda = 0.82$, $N = 194$)				Pennaceous barb density (Pagel's $\lambda = 0.78$, $N = 194$)			
Body mass	1	241.21	<0.0001	Body mass	1	5.4	0.0211
Plumulaceous length (Pagel's $\lambda = 0.66$, $N = 194$)				Plumulaceous barb density (Pagel's $\lambda = 0.75$, $N = 194$)			
Body mass	1	140.46	<0.0001	Body mass	1	0.11	0.7355
Habitat	2	5.17	0.0062	-			
Min. winter temp.	1	10.85	0.0012	Min. winter temp.	1	6.80	0.0098
				Density of pennaceous barbules (Pagel's $\lambda = 0.84$, $N = 194$)			
				Body mass	1	1.80	0.1813
				Habitat	2	4.03	0.0194
				Min. winter temp.	1	6.04	0.0149

interaction between habitat and wintering minimum temperature is not significant ($F = 1.47$, d.f. = 2, $P = 0.2325$) showing that the effect of temperature on feather length was similar in each habitat.

Plumulaceous barb density is positively correlated with wintering minimum temperature (Table 2; Fig. 2d), demonstrating that species that winter in colder areas are characterized by less dense, downy body feathers. Interestingly, however, both total feather density and pennaceous barb density are unaffected by temperature. Pennaceous barbule density is explained significantly by habitat and is higher in aquatic species than in terrestrial ones, with an intermediate value recorded for riparian birds (Table 2; Fig. 2e). Barbule density is positively correlated with wintering minimum temperature (Table 2; Fig. 2f). The lack of a significant interaction between habitat and wintering minimum temperature ($F = 0.55$, d.f. = 2, $P = 0.5775$) showed that the effect of temperature on barbule density is similar in each habitat. Finally, we found no difference between diving and non-diving birds with respect to any feather traits (Table 3).

Discussion

GENERAL DESCRIPTION OF FEATHER MORPHOLOGY

Our results show that the plumulaceous section of body feathers is similar in length to that of the pennaceous region. However, vane barb density is higher in the downy part of the feather than it is in the pennaceous vane, corroborating Butler, Rohwer & Speidel's (2008) comparative study, but using a much larger species sample size and controlling for phylogeny.

The denser downy (plumulaceous) section compared with the pennaceous region of the body feather vanes implies that the former is important in thermoregulation. Indeed, there may be a trade-off in terms of increased vane

density in response to a lower density of feathers. Aquatic species, especially penguins, are known for their high number of down feathers, yet anecdotal data and our observations suggest a low number of these feathers are present generally in birds (pers. obs.), especially terrestrial and riparian species (Wetmore 1936; Davenport *et al.* 2009). Because comparative data on the relative numbers and density of body and downy feathers found within different species is still not available, the relative importance of body feather density in thermo-insulation is unknown.

FEATHER MORPHOLOGY AND HABITAT

Our first hypothesis was only partially supported by our results because aquatic species do have body feathers with a higher density of pennaceous barbules than terrestrial ones, yet barb density along the vane does not change (Table 1). Within aquatic birds, the density of barbs and barbules is similar between divers and non-divers, suggesting that an aquatic habitat in general affects pennaceous barbule density and not aquatic foraging mode. The barb density of the plumulaceous part of the feather, not at the interface between the plumage and water, is also not influenced by habitat. The absence of an effect of aquatic habitat on the barb density of the pennaceous vane is in contrast to existing studies on body and flight feathers, where higher barb density and lower porosity was found in aquatic compared to terrestrial species, and in divers vs. non-divers (Rijke 1968, 1970; Rijke & Jesser 2011; Pap *et al.* 2015). One possible explanation for this difference between our study and earlier ones (Rijke 1968, 1970; Rijke & Jesser 2011) may be rooted in methodology; here, we measured barb density while earlier authors measured porosity, which is a function of barb width and spacing. However, spacing explains most of the variance in porosity because inter-barb distances are considerably longer than the diameter of the barbs (Pap *et al.* 2015). Therefore, we

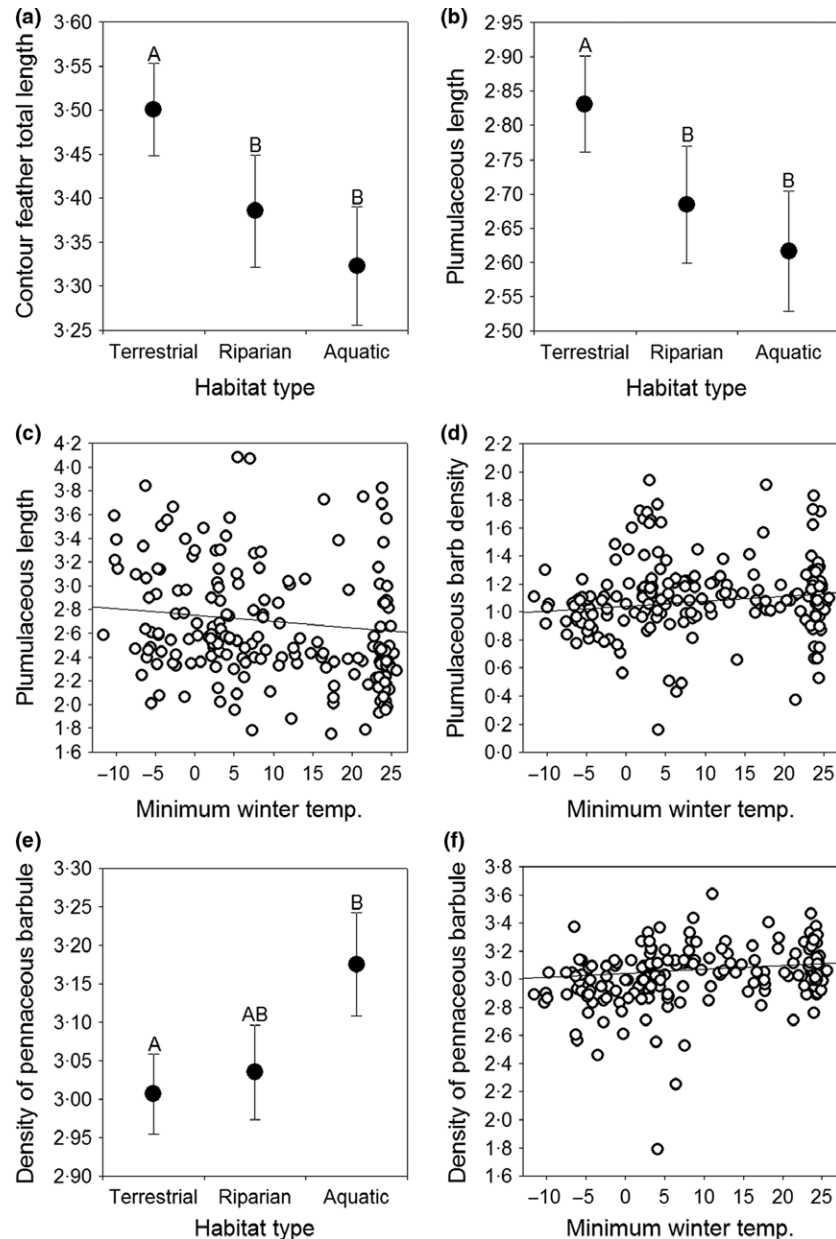


Fig. 2. The relationship between habitat type, minimum wintering temperature, and feather morphological variables. Different letters denote significant differences at $P < 0.05$, as indicated by the phylogenetic generalized least squares models. The sample size for the terrestrial, riparian, and aquatic groups are 116, 22 and 56, respectively. The values from figures a and b are shown as mean $\pm$ SE predicted from the models presented in Table 2. The slopes on figures c, d and f are extracted from models presented in Table 2.

expect barb density to relate closely to porosity. Barb width, and in particular spacing may vary along the vane (Butler, Rohwer & Speidel 2008) and, therefore, porosity values change as a function of the position along the vertical and horizontal axes of the vane. Pertinently, measurement positions along the vane were not the same in the present and Rijke's (1968, 1970) studies, which may offer an explanation for the difference in results. More importantly, earlier studies were conducted on a restricted number of species and without phylogenetic control, which makes the results sensitive to outlying data points and traits with a strong phylogenetic signal. This effect can be particularly strong in this case, because the phylogenetic signal in feather morphology is strong. In a previous study we found that the barb and barbule density of the vanes of primary feathers were significantly higher in aquatic than in terrestrial species, and in divers than in non-divers (Pap

et al. 2015). The divergent responses of body and flight feathers to aquatic life can be explained by the function of these feathers. The wetting of flight feathers has a direct effect on flight and escape performance (see Swaddle *et al.* 1996), and the effect of aquatic habitats on the morphology of flight feathers with respect to water repellency is high. The loosely structured and probably less resistant to water penetration body feathers apparently is non-adaptive, however, this can be valid only if the inner downy layer is affected by water infiltration. Diving behaviour did not influence the vane density of body feathers, and this finding is also inconsistent to that found for flight feathers (Pap *et al.* 2015). Among aquatic birds the density of primary feather barbs was significantly higher in divers than in non-divers, whereas no difference was found for barbule density. The absence of an effect of diving behaviour on body feather barb density can be explained by the ventral

Table 3. Results of phylogenetic generalized least squares models investigating whether morphological parameters of body feathers differ between diving and non-diving bird species

		Feather length		Feather density	
	d.f.	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
		Body feather total length (Pagel's $\lambda = 1.00$, $N = 56$)		Total barb density (whole feather) (Pagel's $\lambda = 0.93$, $N = 56$)	
Body mass	1	83.50	<0.0001	2.07	0.1561
Diving behavior	1	0.47	0.4961	0.16	0.6898
Min. winter temp.	1	3.45	0.0687	1.39	0.2432
		Pennaceous length (Pagel's $\lambda = 0.99$, $N = 56$)		Pennaceous barb density (Pagel's $\lambda = 0.93$, $N = 56$)	
Body mass	1	41.77	<0.0001	5.67	0.0209
Diving behavior	1	0.13	0.7242	0.81	0.3736
Min. winter temp.	1	5.05	0.0289	0.09	0.7604
		Plumulaceous length (Pagel's $\lambda = 0.88$, $N = 56$)		Density of pennaceous barbules (Pagel's $\lambda = 0.78$, $N = 56$)	
Body mass	1	20.36	<0.0001	0.12	0.7326
Diving behavior	1	0.28	0.6008	0.84	0.3635
Min. winter temp.	1	0.43	0.5127	0.12	0.7305
				Pennaceous barbule density (Pagel's $\lambda = 0.96$, $N = 56$)	
Body mass	1			0.01	0.9091
Diving behaviour	1			0.33	0.5698
Min. winter temp.	1			0.00	0.9799

position of the feathers. Because abdominal feathers are in permanent contact with the water and are under permanent water pressure, the resistance of the plumage is likely more similar in divers and non-divers. Furthermore, in order to reduce buoyancy in divers due to high air volume trapped in the feather vane, we might expect the evolution of feathers that do not trap large volumes of air. For example, in the great cormorant, it was found that body feathers have a loose, instantaneously wet, outer section and a highly waterproof central area (Grémillet *et al.* 2005). This indicates that the plumage of great cormorants is only partly wettable, and that birds maintain only a thin layer of air in their plumage. This structure suggests a morphological-functional adaptation to diving, which balances the need for thermoregulation and buoyancy reduction (Grémillet *et al.* 2005).

Body feather total length is shorter in aquatic than in terrestrial birds, and the difference between groups was due to the shorter plumulaceous feather section in aquatic species. Pennaceous feather section length was not affected by habitat, perhaps reflecting the importance of the distal part of body feathers in the formation of the outer, waterproof layer in all birds. The reduced length of the plumulaceous feather in aquatic birds may be explained by a general need to adjust buoyancy (Wilson *et al.* 1992; Grémillet, Tuschy & Kierspel 1998), because the air trapped in the plumage may be determined by the thickness of the downy feathers (Middleton 1986; Walsberg 1988; Swanson 1991; Butler, Rohwer & Speidel 2008). It may be that too much buoyancy results in a higher body position in the water column (less body submerged), which increases the height of the centre of gravity above the centre of buoyancy resulting in a less stable position whilst

swimming upon the water surface. Interestingly we found no effect of diving behaviour on feather length, suggesting that the need for reduced buoyancy in aquatic birds can be attained in multiple ways (see for example Grémillet *et al.* 2005), and the density and length of the downy feather section are not similarly affected between species. Different feather and body density combinations could adjust buoyancy, and these traits may vary between species with varying life history and habitat (Wilson *et al.* 1992; Davenport *et al.* 2009; Williams, Hagelin & Kooyman 2015).

FEATHER MORPHOLOGY AND TEMPERATURE

Currently, variation in plumage morphology and properties, and their effects on thermal insulation in birds living in complex natural environments remains largely unexplored. The non-radiative heat flow through avian plumage is dominant and results from approximately equal contributions of thermal conduction through the feathers and the sum of conductive and convective heat transfer through the air contained within the plumage (Walsberg 1988). Therefore, in birds, insulation through the plumage has an important role in thermoregulation, which can be fulfilled by varying the number and density of body feathers and the morphology of those feathers. Our results show that a cold wintering habitat selects for a longer plumulaceous section, lower plumulaceous barb density, and lower pennaceous barbule density. The longer and less dense plumulaceous part of the body feather in cold dwelling species makes sense, because both features increase the volume available to trap air. The former through increasing layer depth and the latter by increasing the empty space within that layer. A thicker downy coat probably captures more

air, which ensures effective thermo-insulation in birds (Wilson *et al.* 1992). However, the low plumulaceous barb density in cold dwelling species is contrary to what was suggested by former studies (Middleton 1986; Butler, Rohwer & Speidel 2008; Broggi *et al.* 2011; de Zwaan, Greenwood & Martin 2016). Thus, our results show that rather than the high barb density, it is the loose plumulaceous structure that provides the insulative property of the feather. The reason for a lower barbule density in the pennaceous vane of species wintering in cold areas, is less intuitive, but perhaps increasing the air spaces for trapping air in the outer part of the plumage is also insulative. The higher plumulaceous barb and pennaceous barbule densities in species wintering in warm areas suggest a function for a denser feather vane in insulating against excessive heat. These results are contrary to Butler, Rohwer & Speidel (2008) who hypothesize that low pennaceous barb density promotes penetration of the sun's rays onto the underlying body in cold weather species. We found little support for the hypothesis that birds in hot environments can reduce heat absorption at the plumage surface to prevent it from reaching the skin (see Walsberg & King 1978). Our results do not support the idea that birds living in warm areas (characterized by maximum breeding temperature) have more continuous feather surfaces (high pennaceous barb density) and short down ensuring reduced heat absorption (Butler, Rohwer & Speidel 2008). The reason for this may be that radiative heat transfer accounts for <5% of total heat flow (Walsberg 1988). It is interesting to note, that we found no significant interaction between winter minimum temperature and habitat, indicating that the thermo-insulation needs, reflected in feather morphology, are similar between species living in different habitats. Thermo-conductance should increase in aquatic birds because water conducts heat at least one order of magnitude faster than air, which makes heat loss particularly high in aquatic and in submerged birds (Grémillet, Tuschy & Kierspel 1998). Therefore, we might expect the plumage of aquatic birds, such as the penguins, petrels, fulmars and several gull species, to have features that both confer buoyancy and thermo-isolation (see Williams, Hagelin & Kooyman 2015). However, because of the high phylogenetic inertia of feather traits, it is likely that adaptations (behavioural and physiological) other than feather morphology could provide increases in thermo-insulation.

FEATHER MORPHOLOGY AND DIET

Contrary to our expectation, we found no difference in the morphology of body feathers between birds feeding mainly on plants and those feeding on insects and vertebrates, corroborating our former comparative study on flight feathers (Pap *et al.* 2015). Intraspecific studies indicate a protein limitation on feather synthesis during growth, which shows that within the range of food composition naturally occurring in the diet, feather structure largely depends on the quality of ingested food (Pap *et al.* 2008).

The findings of the present study, however, suggest that across species the structure of feathers is not limited by food type, possibly indicating a species level adaptation to the protein content of the food upon which keratin synthesis depends during feather growth.

It is clear that our present understanding of the selection pressures that drive body feather morphology is limited. Based on previous observational and experimental studies, several predictions were formulated about how habitat and thermoinsulation needs may affect the morphology of body feathers (see Table 1). Several of these predictions were confirmed in this comparative study. Our study shows that aquatic species have body feathers with a short plumulaceous section length and a high pennaceous section barbule density, possibly reflecting the needs to adjust buoyancy (through reducing the amount of air trapped in the plumage) and water resistance of the plumage. Low ambient temperature is correlated with a long plumulaceous section length and low barb density, and a low pennaceous section barbule density. In contrast, the plumage may have a limited function in reducing the heat absorption in species living in hot environments. It remains to be tested, however, how whole plumage traits (such as feather density), perhaps in interaction with the individual feather traits, affect the water resistance and insulative functions of the coat in birds.

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Data accessibility

Data associated with this study are provided in the online Supporting Information.

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Supporting Information

Details of electronic Supporting Information are provided below.

Appendix S1. Morphological parameters for the body feathers. *N* is the sample size for each parameter. See Material and methods for sources.

Appendix S2. Summary of data showing species-specific body mass, habitat (T – terrestrial, R – riparian, A – aquatic), diving behaviour (D – diving, ND – non-diving), food type (1 – food composed mainly of insects and vertebrates, 2 – large proportion of the food composed of plant materials) and the maximum and minimum monthly temperature on the breeding and wintering areas, respectively. See Material and methods for sources.