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VARIATIONS IN PLUMAGE STRUCTURE AS A FUNCTION OF THE ENVIRONMENTAL CONDITIONS AND FLIGHT STYLE OF BIRDS

Thesis for the Degree of Doctor of Philosophy (PhD)

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Table of contents

ESSAY SECTION

1. Introduction and objectives

1.1. The plumage of birds

Feathers are the most numerous, elaborate and diverse derivatives of the avian integument, making birds unique and spectacular in the animal kingdom. Feathers, collectively referred to as plumage (Stettenheim, 2000), cover almost the entire surface of the body, serving a wide variety of functions. The main roles of plumage include the following: (1) it provides insulation from water and cold temperatures, allowing birds to live in many different types of environments; (2) it enables flight under a wide range of conditions; (3) it provides camouflage in different habitats; and (4) it plays a crucial role in avian communication (Lombardo *et al.*, 1995; Lovette & Fitzpatrick, 2016).

To fulfil their complex roles, body feathers have evolved a great variety of shapes, sizes and structures. However, they can still be divided into two basic groups: contour feathers (vaned or pennaceous) and down feathers (plumulaceous) (Gill, Prum, & Robinson, 2019). The contour body feathers constitute the outline or contour of the body, setting up the outer protective/waterproofing cover, over the inner insulative downy layer of the plumage (Figure 1) (Walsberg, 1988; Wolf & Walsberg, 2000; Williams, Hagelin, & Kooyman, 2015). Flight feathers are all pennaceous and serve to create the wing surface that is essential for flying. They have to be light and flexible, as well as adequately strong to be able to withstand the aerodynamic force they are exposed to (Videler, 2005; Pennycuick, 2008).

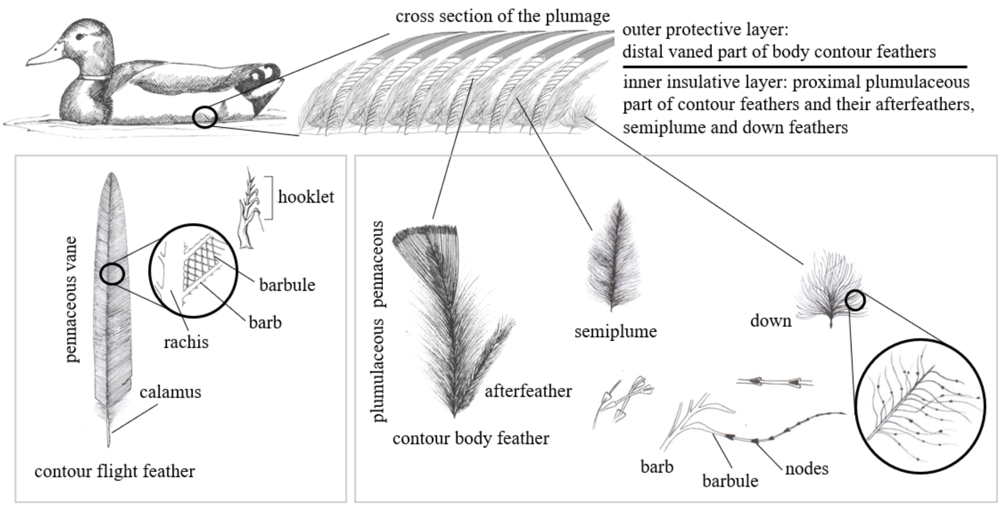

Figure 1. The basic structure of the plumage and main feather types (drawings courtesy of Kinga Kelemen)

1.2. Adaptations of body feathers to insulation and waterproofing

Thanks to its unique structural features, body plumage regulates heat transfer and prevents water penetration, forming an excellent barrier between the bird's body and the environment (Handrich *et al.*, 1997; Ponganis *et al.*, 2003). In the case of species living in extreme environments, it can retain as much as 90% of the heat leaving the body (McCafferty *et al.*, 2013). Body feathers insulate birds from cold temperatures and water by being able to trap air in an insulating layer next to the skin, while the arrangement and structure of the feathers prevent water penetration (Cooper, 2002; Williams *et al.*, 2015).

Bird plumage comprises different feather types that all have subtly altered structures and roles (Figure 1) (Prum & Brush, 2002). Thus, quantifying the densities (e.g. feathers/cm^2) of different feather types is crucial if we are to comprehend how plumage performs its unique thermo-insulatory and water-repellent functions. The most numerous components of avian plumage are contour body feathers, which have multiple functions due to their dual structure (Butler, Rohwer, & Speidel, 2008). The pennaceous distal vaned part of contour body feathers is created by the zippering interaction between hooklets and barbules aligned along the barbs. These sections are arranged in an overlapping pattern across the entire plumage, like shingles on a roof, and form an external water-repellent coat over the inner insulative cover of the plumage, built up by the proximal plumulaceous feather section of contour feathers, semiplumes and down feathers (Rijke, 1970; Srinivasan *et al.*, 2014) (Figure 1). The structure of these feathers or feather parts is optimized to trap a layer of air next to the skin, thus providing insulation. Semiplumes have a central shaft with lateral barbs, bearing barbules but with the difference that they are entirely plumulaceous i.e. all the barbs are downy and lack hamuli so that they are free of attachment to each other, and there is consequently no vane. Down feathers are constructed entirely of down, with little or no rachis. (Stettenheim, 2000). In addition, the contour feathers of many birds have an auxiliary shaft attached to their calamus, called afterfeather. The afterfeather typically resembles a smaller, downier feather with plumulaceous barb and barbule structure and its primary role is assumed to be enhancing insulation (Williams *et al.*, 2015).

Environmental conditions are key determinants of heat loss, so they may facilitate the evolution of various features in the optimal plumage structure and density (Wolf & Walsberg, 2000). For instance, plumage that has been optimized to trap a large amount of air can be advantageous in aquatic environments due to the 23 times greater thermal conductivity of this medium; however, aquatic birds, especially diving species, have to overcome the buoyancy of the water when they dive, which could be made more difficult by the large amount of insulative air stored in the plumage (Croll & McLaren, 1993; de Vries & van Eerden, 1995; Grémillet *et al.*, 2001). Nevertheless, to date, no comparative study has investigated the diversity in feather density among bird species or looked at how these differences correspond to the different lifestyles and environmental requirements of birds. There can be a huge difference among species in the total number of feathers found in their plumage (Wetmore, 1936; Hutt, 1938; Cooper, 2002; Davenport *et al.*, 2009; Williams *et al.*, 2015), and this variation has previously been attributed to the different environmental conditions and lifestyle of birds (Dawson *et al.*, 1999; Williams *et al.*, 2015). However, these conclusions have generally been based on single species that mostly live in extreme conditions and are therefore inadequate for formulating a general idea of plumage adaptations.

In light of the above, our comparative study involving 152 bird species (study I) investigates how the habitat, the lifestyle of the species and the thermal environmental conditions may explain the differences in feather density in different plumage areas. We predicted a greater feather density in aquatic and especially in diving species than in their non-diving and terrestrial counterparts, respectively. In the case of terrestrial species, we expected to find similar feather densities on the ventral and dorsal sides, as they are exposed to almost the same degree of environmental factors (water, temperature), while we assumed that there would be a greater feather density on the ventral side of aquatic species (particularly in floaters) due to their constant exposure to the water and hydrostatic pressure. We predicted denser plumage in species living in colder habitats, and in the case of birds living in warmer climates, we expected to find lower feather densities, ensuring a looser structure for the plumage, which can help ventilation, facilitating heat dissipation.

Besides density, the insulating capacity of the plumage can also be significantly determined by the microstructure of the feather types it consists of (Rijke, 1968; Wolf & Walsberg, 2000; Ward *et al.*, 2007; Rijke & Jesser, 2011; Broggi *et al.*, 2011; Pap *et al.*, 2015; Williams *et al.*, 2015; Koskenpato *et al.*, 2016). The structure of contour feathers is especially important, as these feathers make up the largest part of the plumage (Wetmore, 1936), and due to their dual structure, they play multiple roles of high importance in insulating against the unfavourable temperatures and waterproofing (Davenport *et al.*, 2009; Williams *et al.*, 2015). However, we do not have much information on how the morphological features of these feathers have adapted to these functions. In some aquatic birds, it has been found that the distal pennaceous parts of contour feathers, which form the outer protective layer of the plumage, act as an excellent watertight barrier, preventing water from getting into the proximal, downy layer responsible for thermal insulation (Stephenson & Andrews, 1997). In other cases, it was found that the distal part is partially permeable, or that its structure changes according to the given habitat's temperature and the thermoregulatory needs of the bird (Middleton, 1986; Grémillet *et al.*, 2005). The waterproofing properties of the distal pennaceous section is decisively determined by their barb and barbule density and it is a widely held view that a waterproof outer body covering is ensured by a high density of barbs and barbules (Grémillet *et al.*, 2005; Rijke & Jesser, 2011), although the relation between vane structure and the bird's habitat has only been investigated in a few species, and these were mostly aquatic birds (Rijke, 1968; Rijke & Jesser, 2011). Furthermore, the length of the vane is also important in terms of protection, as larger vanes with better coverage mean that the outer protective layer is thicker and may confer better protection from water penetration (Grémillet, Tuschy, & Kierspel, 1998).

The thermoregulatory characteristics of contour feathers may depend on several factors. The length of the proximal, downy part, as well as its barb and barbule density, determine the thickness and texture of the downy insulating layer of the plumage and the number and size of spaces with trapped air near the skin. It is likely that a longer plumulaceous downy part with higher barb and barbule density, and a loose plumage surface (i.e., low pennaceous vane density) can help sunlight penetrate the inner layers. On the other hand, species living in warm conditions can reduce heat absorption on the surface of the feathers by preventing heat from reaching the skin (Walsberg & King, 1978), so birds adapting to hotter environments need a more sealed protective layer (i.e. the vanes need to have higher barb and barbule density) and thinner insulation (Butler *et al.*, 2008). However, there are no cross-species studies that would make it possible to produce a generalization on the functional aspects of feather morphology.

Therefore, our comparative study involving 194 bird species (study II) investigates how the structure of contour feathers changes depending on the habitat (terrestrial, riparian or aquatic), the temperature of breeding ranges and wintering grounds, and the lifestyle of the birds (floating or diving). We predicted that the barb and barbule density of the vanes of contour feathers (the outer protective layer of the plumage) is higher in aquatic birds than in their terrestrial and riparian counterparts, respectively. The lengths of the proximal, downy part and the distal part, the vane, are more difficult to predict based on the habitat type. If the length of the downy part is related to the amount of air deposited in the plumage, then a longer downy part may be more advantageous for aquatic species, although it can affect diving ability by increasing buoyancy. We also tested the hypothesis that the contour feathers of species living in low temperature environments would have longer and denser (higher barb density) downy parts and more loosely structured vanes (lower barbule density) (Middleton, 1986; Butler *et al.*, 2008; Broggi *et al.*, 2011). As opposed to this, in species living in areas with higher temperatures, we expected the vane part to be denser and thus to close better and prevent heat absorption, and the downy part to be shorter and to have a looser structure and thus retain less warm air in the plumage.

In terms of thermal insulation, morphological adaptations can also be predicted by down feathers, the other type of feather in the plumage. These feathers provide the best insulation out of all natural materials in terms of their warmth-to-weight ratio, as they can trap large amounts of air, i.e. they have high fill power (Fuller, 2015). Another important quality of this part of the plumage is compressibility – it can recover its original volume, for example, after being submerged in water and compressed by hydrostatic pressure (Fuller, 2015). These outstanding physical qualities of down feathers are related to their unique structure. As opposed to contour feathers, the rachis of down feathers is short, and the barbs and barbules are long and not connected (Figure 1), so they have a downy, loose structure (Stettenheim, 2000; Prum & Brush, 2002). Another characteristic feature of the barbules of down feathers is that there are nodes along their longitudinal axis (Figure 1). The length of the barbules and the density of the nodes determine the cohesion between the down feathers and also their fill power, and high node density increases their compression resistance (Fuller, 2015; D'alba *et al.*, 2017). Previous studies have described a great variation in the structure of down feathers among species, which can be linked to the different thermoregulatory needs of the species. (Dove, 2000; Robertson, 2002; Dove & Agreda, 2007; Lee *et al.*, 2016; Cheek, Alza, & McCracken, 2018). However, we still do not know how the structure of down feathers adapts to the diverse environmental conditions experienced by birds, as studies so far have only focused on a few species. **To explore these correlations, we conducted a study (study III) in which we collected feathers from 154 species and investigated how the type and temperature of the habitat might explain the structural characteristics of down feathers.**

Previous studies have indicated that down with a looser structure may result in better insulation as it can trap more air in the plumage, i.e. it has a higher fill power (Fuller, 2015; D'alba *et al.*, 2017). According to this, we predicted longer down feathers with shorter and less dense barbules in species living in harsh environments. The higher number of nodes on barbules may increase the cohesion between downy elements, but decrease the fill power of the plumage (D'alba *et al.*, 2017), thus it is difficult to make predictions on node density in function of environmental conditions (wind speed, temperature). Previous research has indicated that aquatic life is associated with smaller down feathers with longer and denser barbules, as well as a reduced number of nodes on barbules (Dove and Agreda 2007; Fuller 2015; D'alba et al. 2017). Nevertheless, shorter and less dense down feathers (i.e. lower barbule density), with a lower number of nodes are also plausible and were also predicted for aquatic birds because such a structure may help submersion, since the plumage is more compressible and air can escape more easily (Fuller, 2015; D'alba *et al.*, 2017).

1.3. Feather structure and flight

In order to fly, birds need to generate lift and propulsive thrust, putting considerable load on remiges, i.e. the flight feathers which make up the wing (Lindhe Norberg, 2002; Pennycuick, 2015).

The aerodynamic forces are transmitted to the body via the rachis (the central shaft of the feathers). Therefore, the rachis has to be stiff enough to resist bending (Bonser, 1996). The bending stiffness of the rachis is primarily determined by the quantity and distribution of the material (keratin) in the cross-section (i.e. second moment of area), and changing the cross-sectional shape is an effective solution for adapting the bending stiffness of the different rachis segments to resist differential aerodynamic forces (Purslow & Vincent, 1978; Weber *et al.*, 2010). Accordingly, it has been found that the cross-sectional shape of the rachis changes along the longitudinal axis of feathers (Bachmann *et al.*, 2012; Sullivan *et al.*, 2017), with the largest diameters and stiffnesses in the planes with the largest bending forces, thereby keeping the weight low while still possessing rigidity in the important directions (Norberg, 1972; Purslow & Vincent, 1978).

The magnitude and the direction of the aerodynamic forces varies across the wing span, with outer primaries need to resist higher out-of-plane forces than inner primaries or secondary feathers, particularly during downstrokes in flapping flight (Ennos, Hickson, & Roberts, 1995; Pennycuick, 2008). Feather position along the wing might ultimately result in differentiation in the morphological properties of the rachis (Pap *et al.*, 2015). Furthermore, the distribution of aerodynamic forces along the wing is influenced by the flight type of birds, namely whether a bird flies with continuous and high frequency wing flapping or has intermittent flight (flapping phases are interrupted by flexed-wing bounds) and whether it flaps less with lower frequency and soars or glides more. Hence, we might expect the cross-sectional morphology of the rachis to change with the main flight type among species. However, to date, morphological studies have mostly focused on primary feathers, or on a single feather of the wing. It is still unknown how shape varies along the rachis within individual feathers and among the remiges of the entire wing, and how the pattern of variation differs among species. **Therefore, in our fourth study (study IV), we investigated how the cross-sectional shape of the rachis changes (1) along the longitudinal axis of individual flight feathers, (2) across the wing and (3) among species characterized by different flight types.**

For flight, not only the morphology of the rachis of flight feathers is crucial, but also the structure of the vane (Figure 1), as it makes up most of the lifting surface of the wing, which is essential for the generation of aerodynamical forces (Lindhe Norberg, 2002). Both the leading (outer) and the trailing (inner) vanes of the feathers are created by the interconnection of barbs and barbules. The barbs are parallel branches, which are aligned on two sides of the rachis. Each barb has small barbules and even smaller hooks (barbicels). The zippering interaction between hooklets and barbules creates the smooth planar surface, the pennaceous feather vane. (Stettenheim, 2000) (Figure 1). Previous studies have shown that these structural elements are highly important. The barb and barbule density defines the air transmissivity of the vane (Müller & Patone, 1998; Dial, Heers, & Tobalske, 2012), while the branching angle of the barbs determines the resistance of the vane against the forces from different directions that are generated during the wingbeat cycle (Ennos *et al.*, 1995). However, the magnitude and direction of the aerodynamic load acting upon the different segments of flight feathers may vary along the wing and in function of flight style (Pennycuick, 2008; Pap *et al.*, 2015). In view of this information, it can be assumed that these structural elements adapt to the different aerodynamical forces, and the density and branching angle of the barbs change (1) along the longitudinal axis of the vanes, (2) from the outer to the inner vanes, and (3) from the distal to the proximal feathers on the wings. Furthermore (4), this variation in structural elements is presumably connected to the flight style of the birds. These assumptions are also partly substantiated by previous studies. For example, in the domestic pigeon, a difference has been shown between the outer and inner vanes of the flight feathers in terms of barb density and air transmissivity, which is important for creating of a smooth uniform wing surface (Müller & Patone, 1998). In addition, the barb angle also changes decreasing from the base of the vane towards the tip, resulting in higher resistance against forces perpendicular to its plane (dorso-ventral forces) (Ennos *et al.,* 1995; Bachmann *et al.*, 2007).

However, very few studies have focused on more than one species and on the variation of vane structures by examining several feathers and measurement points, yet the different flight styles of bird species may be crucial in the development of feather structure (Pap *et al.*, 2015). **In our fifth study (study V), we conducted a comprehensive comparative analysis with 178 species, and examined differences in the density and branching angle of the barbs along the longitudinal axis of the outer and inner vanes of the distal (P8) and proximal (P1) primary flight feathers, and to what extent this could be explained by the different flight style of the birds.**

In summary, even though there have been many studies on the growth and origin of feathers, we have little information on the variation of the structural properties of the feathers among species and the aerodynamical, ecological and evolutionary background of this variation. Understanding how the structure of the plumage and different feather types varies among birds is essential information for answering the question of how feathers have evolved to fulfil the requirements imposed by the different environmental conditions and flight style of species. Therefore, the aim of the studies presented in my dissertation was to explore the structural variation of the plumage of birds and the connection between this variation and the different ecological and lifestyle features to find potential adaptations.

2. Materials and methods

2.1. Study I

We used 337 cadavers of 152 bird species for the study, quantifying plumage density (number of feathers/unit of area) in two parts of the body (ventral and dorsal). We removed all feathers along specific anatomical structures of the specimens, took photos of the bare parts and used the ImageJ software to measure their area (cm^2). We counted the feathers collected from the specific areas and sorted them according to feather type. Then we calculated the total feather density of the ventral and dorsal sides, and the density of the specific feather types (contour feathers, semiplume + down feathers). The species involved in the study were classified into five categories according to their lifestyle and habitat: terrestrial, riparian, waders, floating and diving aquatic birds. We used multivariate phylogenetic generalised least squares (PGLS) models, incorporating phylogenetic relatedness across species. The feather density of the ventral and dorsal side, and the density of certain feather types were used as dependent variables in separate models, in which body weight, the type of habitat and the minimum and maximum mean temperature of the wintering and breeding grounds were also included as explanatory variables.

2.2. Study II

We collected feathers from the ventral side of 1043 birds of 194 different species. We measured the following structural parameters from all feathers: length of the feathers, length of the pennaceous (distal) part, length of the plumulaceous (proximal) region, barb density of the plumulaceous and pennaceous sections, barbule density of the pennaceous section (Figure 1). Species involved in the study were classified into four categories: terrestrial, riparian, floating and diving aquatic birds (wading species were included in the group of riparian species). Thermal conditions were characterized by the maximum mean temperature of the breeding range and minimum mean temperature of the wintering grounds of the species. In order to test the effect of ecological variables on feather traits, we performed PGLS models again, incorporating phylogenetic relatedness across species. Feather trait values were used as dependent variables in the model that also included body mass, habitat type and ambient temperature as covariates. Interactions among covariates were also tested.

2.3. Study III

We collected down feathers from 370 individuals of 156 species (Figure 1). We imported photographs of the feather samples into the ImageJ software to measure the following structural parameters: length and area of feathers, length of the barbs and barbules, density of barbules, and node density on barbules.

Species were classified into five habitat categories: terrestrial, riparian, waders, floating and diving aquatic species. Temperature loss of the body is affected by several environmental parameters; therefore, we calculated the thermal sensation index (wind chill index) using the minimum temperatures and winter maximum wind speeds of the wintering ground.

We built PGLS models where feather traits were used as a dependent variable, while body mass, habitat type and wind chill index were included as covariates.

2.4. Study IV

We collected wing feathers from eleven individuals of white stork (*Ciconia ciconia*) and ten carcasses of common buzzard (*Buteo buteo*), pygmy house sparrow (*Passer domesticus*), and pygmy cormorant (*Microcarbo pygmaeus*), representing the four main flight styles: flapping and soaring (white stork), flapping and gliding (common buzzard), continuous flapping (pygmy cormorant), and passerine-type flight (house sparrow). We plucked all remiges from one of the two wings from each individual. For each feather collected we measured the dorso-ventral and lateral width at four positions along the rachis (Figure 2.). In order to describe the variation in the shape of the rachis, we calculated the dorso-ventral/lateral width ratio (i.e. height/width ratio) for each measurement position. A width ratio value higher than one indicates a higher rather than wider rachis section (‘compressed’ shape), and a value lower than one indicates a wider rather than higher rachis section (‘depressed’ shape) (Figure 2, B1-B2). We also measured the second moment of area of the rachis cross-sections (I, mm^4) along two different axes (x, y). These indices reflect the capacity of a cross-section to resist bending in dorso-ventral (I_x) and lateral directions (I_y). We also calculated the stiffness ratio using these values (Figure 2, C1-C2).

In order to examine the variation in rachis width ratio (rachis shape) among the four measurement points of wing feathers and among the four bird species, we built GAMs (General Additive Models).

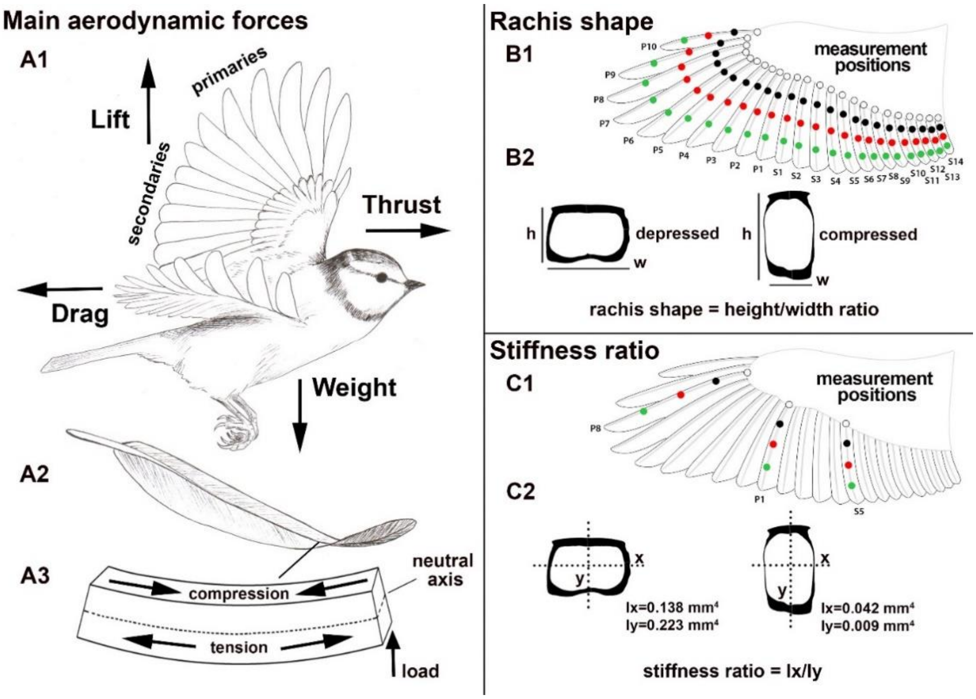

Figure 2. A1-3: The fundamental loads acting on the wing and the bending of the rachis; B1-2, C1-2: The examined morphological parameters across the wing (**study IV**).

2.5. Study V

We collected distal (P8) and proximal (P1) primary flight feathers from 178 species. The leading (outer) and the trailing (inner) vanes of the two feather types were marked at three positions: 25% (base), 50% (middle) and 75% (tip) along the length of the vane. We took photographs of the feathers at each measurement position, and measured the density and branching angle of barbs with the ImageJ software.

Species were classified into four categories based on their flight style (Pennycuick, 2008; Bruderer *et al.*, 2010; Pap *et al.*, 2015): flapping-soaring, flapping-gliding, continuous flapping and passerine-type fliers.

To investigate the differences in structural parameters (barb angle and barb density) between distal (P8) and proximal (P1) primary feathers, we used phylogenetic paired t-test separately at each segment along the vane (base, middle, tip) for both the outer and inner vanes. We performed similar tests to examine whether the density and branching angle of barbs changed between the outer and inner vanes and between different segments along the shaft of both P8 and P1. To investigate the effect of flight style on the variation of the structural parameters of P8 and P1, we built generalized least squares (PGLS) models.

3. Results and discussion

3.1. Adaptations in feather density to different environmental conditions

We found (**study I**) that habitat type and temperature of the wintering ground are related to the feather density, while the temperature of the breeding range does not affect the number of feathers per unit of surface area, or only to a lesser extent. On both sides of the body, the density of the examined types of feathers (contour feathers and down feathers) is greater in floating and diving birds than in terrestrial, riparian and wading birds. Furthermore, the down feather to contour feather ratio is also significantly higher in aquatic species than in their non-aquatic counterparts. We did not find any differences in feather density parameters between the two groups of aquatic species (floating and diving).

Aquatic species, compared to other groups, generally had higher feather density values, which is not surprising if we consider the water protective role of contour feathers (Grémillet *et al.*, 2005; Rijke & Jesser, 2011); the higher number of contour feathers can presumably provide a high protection level against the penetration of water, while the higher number of down feathers can provide better thermal insulation in aquatic environments. Despite the exposure to different thermal loss and buoyancy (de Vries & van Eerden, 1995; Grémillet *et al.*, 2001), diving and swimming birds had similar feather density, suggesting that the adaptation to the diving way of life manifests at the a microscopic level of feathers rather than in their density (Dove & Agreda, 2007). Our results highlighted the importance of down feathers in thermal insulation because we found that birds wintering in colder habitats have a higher density of these feathers, thus more insulating air can be stored in their plumage in harsh conditions (Williams *et al.*, 2015).

Temperature had a quadratic effect on the density of the contour feathers on the ventral side, the total density of feathers and the minimum winter temperature, thus indicating a higher density of feathers among the species that winter in the intermediate temperature range, and a lower density among those in cold or warm habitats. For species living in warmer habitats, low feather density can be beneficial as it can facilitate air flow, thereby facilitating heat dissipation. In contrast, we cannot provide a clear explanation for the low feather density of species living in colder areas. Our results suggest that an adequate heat retention can be achieved even at lower feather densities, presumably due to the structural adaptation of the feathers. However, further studies are needed to confirm this. Our research is the first study that introduces and discusses diversity in the density of the plumage and considers the different environmental conditions of the species at the same time across species.

3.2. Structural adaptations of contour body feathers to different environmental conditions

Our findings showed (**study II**) that the structure of a bird’s contour feathers is affected by the type of habitat and the temperature of its wintering ground, while it is not affected by the temperature of its breeding range. We found no differences between the pennaceous vane length of aquatic and terrestrial birds, although the downy part of the contour feathers is shorter in aquatic birds than in terrestrial birds, and the length of the contour feathers is thus also shorter in the former than in the latter. The shorter plumulaceous section in the feather length of aquatic birds suggests the need to reduce the quantity of the air trapped in the plumage to optimize the buoyancy of aquatic birds (Grémillet *et al.*, 2005). We did not find any differences in any parameter between the two types of aquatic lifestyles (floating and diving). The barbule density of the vane part was higher in aquatic than in terrestrial species; this is presumably the result of their adaptation to the aquatic lifestyle, since the contour feathers with higher barbule density may be more water resistant (Rijke & Jesser, 2011; Pap *et al.*, 2015).

The plumulaceous (downy) part of the contour feathers was longer and the barbule density was lower in the birds that winter in colder conditions than in those that winter in warmer conditions. Moreover, the barbule density of the vane part positively correlated with the minimum mean temperature of the wintering ground, indicating lower barbule density in the vaned pennaceous part of the contour feathers of the species that winter in harsh conditions. Overall, these structural characteristics refer to plumage that insulates better in cold environments, since the longer downy part shows that the insulating layer is thicker in these species, thus enhancing the air storage capacity of the plumage (Wilson *et al.*, 1992).

The positive correlation between the barbule density of the plumulaceous part and the temperature of the wintering ground seemingly contradicts former findings (Butler *et al.*, 2008; Broggi *et al.*, 2011). Our results suggest that from the point of view of thermal insulation, a looser feather type with lower barbule density is more beneficial, since it allows more air storage. Presumably, this explains why the barbule density of the vane was lower. However, further studies are needed to confirm this link in order to reveal the correlation between the extent of heat transfer through the plumage and the feather structural parameters.

3.3. Structural adaptations of down feathers to different environmental conditions

Results showed (**study III**) that aquatic birds had down feathers with smaller and shorter barbules, and a lower node density than non-aquatic species (terrestrial, riparian and wading birds). This pattern evolved similarly on both the ventral and the dorsal side. Accordingly, aquatic habitats seem to select for a smaller down feather-structure, which resists compression to a lesser degree. The smaller down feathers show that the insulating layer they form in aquatic birds is thinner, which apparently contradicts the predicted adaptation to the higher heat loss associated with aquatic environments (Croll & McLaren, 1993; de Vries & van Eerden, 1995; Grémillet *et al.,* 2001). However, aquatic species, particularly diving birds that feed underwater, also have to face higher buoyancy if a large amount of insulating air is stored in their plumage. Presumably, adaptation to this environment may explain the thinner down layer, shorter barbules, and low node density, which may reduce the amount of air stored in the plumage (Grémillet *et al.*, 2005; D'alba *et al.*, 2017). With this structure, the plumage is less resistant to the hydrostatic pressure exerted on it during diving, and its compression facilitates the escape of air, and subsequently submerging. In addition, released air bubbles reduce friction between the body and the water, which can further facilitate the submerging of birds (Davenport *et al.*, 2011).

Wind chill index only had an effect on the node density of the dorsal part and was higher in species that live in harsher weather conditions. Higher node density in cold and windy habitats can be explained by the need for stronger cohesion of the down feathers (Fuller, 2015; D'alba *et al.*, 2017). This is one of the main structural features for providing better air trapping capability (Fuller, 2015; D'alba *et al.*, 2017) and, hence, insulation in strong winds. Furthermore, it is plausible that the effect of habitat type and thermal condition on feather traits are not independent from each other. However, except for study II on body feather structure, we were not able to test the interaction between habitat type and ambient temperature on feather density (study I) and down feather morphology (study III). We therefore propose further studies with larger sample sizes.

3.4. Variation in rachis morphology across the wingspan of different species

We found (**study IV**) that the cross-sectional shape of the rachis, as well as its bending resistance (indicated by the second moment of area of cross-sections) to forces acting from different directions, vary along the longitudinal axis of each flight feather and across the wing. Furthermore, the pattern of change of rachis shape varied among the four species studied. In general, the calamus of flight feathers was oval in shape (slightly higher than wide) and more resistant in dorso-ventral direction for all four birds. A dorso-ventrally oriented rachis is expected, because the large forces acting on the feathers during flight are redirected towards the bones through the calamus (Bachmann *et al.*, 2012; Sullivan *et al.*, 2017). In addition, the relatively uniform shape of the calamus across the wing suggests that the forces are distributed more evenly at the base of the wing, compared to the more distal feather segments.

In contrast to the calamus, other parts of the feathers showed larger variation in the shape of the rachis across the wing. In the outer primaries of the white stork, common buzzard and house sparrow, both the width and stiffness ratio decreased along the feather shaft (i.e. the rachis became wider and stiffer laterally than dorso-ventrally towards the feather tip), while in inner primaries and secondaries, the dorso-ventral dimeter and stiffness exceeds the lateral rachis thickness. This indicates that the rachis of outer primaries is relatively more resistant to in-plane forces, while the inner primaries and secondaries are stiffer in dorso-ventral than in lateral flexion. A generally greater dorso-ventral bending resistance is expected because the wing feathers are exposed to greater out-of-plane forces (Heers, Tobalske, & Dial, 2011; Dial *et al.*, 2012), particularly during downstrokes in flapping flight (Corning & Biewener, 1998), therefore the rachises should be stiffer in the dorso-ventral direction. However, the rachis of the outer primaries is oriented perpendicularly to the direction of the wind, hence the rachis of these feathers has to be stiff enough to withstand lateral aerodynamic forces. This interpretation of our findings corroborates the result of the study by Purslow and Vincent (1978), showing that the outermost primary of the pigeon is equally stiff laterally and dorso-ventrally, whereas the inner to outermost primaries have a higher dorso-ventral than lateral stiffness.

In the white stork and common buzzard, both the rachis of the outermost and the rachis of all emarginated distal primaries were wider laterally than dorso-ventrally. In these soaring and gliding species, the emarginated outermost primaries bend upwards and separate vertically to form the slotted wing tip on the extended wing, which confer multiple aerodynamic advantages during flight (Tucker, 1993, 1995; Palmer, 2014). In this wing type, both the outermost primary, which constitutes the leading edge, and each emarginated primary are loaded heavily in the lateral direction because these feathers are separated and each one acts like an individual airfoil, while cutting the air and overcoming air resistance (Tucker, 1995). Hence, the distal part of these feathers has to be stiff enough laterally to resist the aerodynamic forces that act in a lateral direction. Furthermore, among the outer emarginated primaries of the white stork and common buzzard, the width ratio gradually increased from the outermost towards the inner primaries, and from the tip to the base of individual feathers. This change in orientation reveals that the width in the lateral direction is most pronounced at the distal segment of the outermost primary and starts to transform wider dorso-ventrally than laterally, progressively from the outermost towards the inner primary feathers. This result supports the idea of Pennycuick (2008), and shows that the flexibility of the rachis in outer primaries is graduated, which allows them to form the slotted wingtip passively by variations in the bending stiffness of the rachises.

In the pygmy cormorant, the lateral rachis thickness exceeds the dorsoventral width at each measurement position except at the calamus, representing an unexpected result in the case of a species characterized by high wing-beat frequencies, and therefore high aerodynamic load on the wing's feathers from the dorso-ventral direction (Usherwood 2003, Pap et al. 2015). We speculate that the rachis morphology of pygmy cormorants might be determined primarily by their aquatic lifestyle, rather than their flight style. We propose further investigations in order to analyse the effect of an aquatic lifestyle on the morphology and flexural stiffness of the rachis on a larger sample size including diving and non-diving aquatic species.

3.5. Vane density, barb angle and flight in birds

We found (**study V**) that barb density and barb angle vary along both sides of the (leading and trailing) vane and across distal (P8) and proximal (P1) flight feathers. Barb density at all measurement positions (base, middle, tip) of the leading vane of P8 was lower than that on P1. In the case of the trailing vanes, only the middle part of the vane differed, showing a higher barb density on P8 feathers. Regarding the change along the rachis, we found that both the barb density and the barb angle decreased from the base towards tip on the leading and trailing vane of both feathers. As for the differences between the leading and trailing vanes, we found that the density of barbs was higher at each position of the outer vane for both P8 and P1 feathers. Branching angle of the barbs was smaller at all measurement points of the outer vane of the P8 and P1 feathers, except the base of P1.

In general, the variation seen in the structural elements reflects the need for feathers and feather parts of different air transmissivity and resistance. In flight, the lift generation is highest at the base of flight feathers (Müller & Patone, 1998). The uniformly higher density of barbs shown at the base of the inner vanes reflects the need for low air transmissivity in this part of the wing. Distal wing feathers are exposed to a higher load, especially their tip, which is also exposed to aerodynamic forces acting from different directions (Norberg, 1990). The tip of these can twist up to 180 degrees at certain stages of wing-beat cycles (Crandell & Tobalske, 2011). Accordingly, the barb angle decreased toward the tip of the P8 feathers, providing greater resistance to the vane and helping to avoid the detachment of barbs when the vane of the feathers is loaded heavily by aerodynamic forces (Ennos *et al.*, 1995).

In line with our predictions, the flight styles associated with different aerodynamic costs significantly influence the density and branching angle of the barbs of the flight feather vanes. Similarly to the results of our previous study (Pap *et al.*, 2015), the barb density of the vane is higher in the species that fly at a high wing-beat frequency (continuous flapping and passerine type flight) than in species characterized by more passive flight styles (soaring and gliding). In the case of the flight types, there is a higher load on flight feathers compared to the latter two types (Corning & Biewener, 1998; Usherwood, 2003; Pap *et al.*, 2015), so an adaptation is expected in terms of the high barb density in vanes, which reduces air transmissivity and increases the resistance of the vane (Ennos *et al.*, 1995). Contrary to our expectations, we did not find a consistent barb angle variation pattern among flight styles, which suggests that other factors, such as energetically and aerodynamically costly take-off and landing may also shape the vane structure, masking any possible effect of flight style.

4. Conclusions

The plumage plays various roles in the life of birds, providing insulation and enabling flight. The particularities of feather types covering the body are decisive for insulation, while the structural characteristics of the flight feathers are determinative for flight.

Based on our results, the challenges of the aquatic lifestyle, especially managing the upthrust forces that act during submersion in water, have led to a number of adaptations to the structure of the plumage. The pennaceous vane of the contour body feathers, forming the outer layer of aquatic birds' plumages, is characterized by a high barbule density, which is more resistant to water penetration. Their insulating layer consists of densely spaced down feathers; however, this layer is relatively thin and easy to compress, which is promoted by shorter barbules with rarer nodes. On the contrary, the down feather layer of terrestrial species has a looser structure, capable of storing larger amounts of air. Presumably, the characteristic structure of aquatic birds – a dense layer of down feathers with a high keratin content – has the advantage of providing good insulation above water, but being easily compressed when submerging, decreasing the quantity of air deposited in the plumage and thus buoyancy that acts against submerging. Our studies show that habitat temperature has a significant effect on the structure and number of different feather types. Cold living birds have denser plumage that consists of longer contour feathers with a longer and more loosely structured plumulaceous downy section compared to species living in warmer environments. However, contrary to our predictions, the structure of down feathers, which provide the most effective insulation among feather types, was only negligibly affected by ambient temperature. This suggests that the selection pressure exerted by ambient temperature affects the composition of the entire plumage as well as the feather density, but not the structure of this feather type. The thermal insulation of the plumage is presumably affected by the change in the density of down feathers rather than by the differences visible in their microstructure. Furthermore, it is plausible that the effects of habitat type and temperature are not independent from each other, but due to limitation of sample size the effect of interaction between habitat type and ambient temperature could only be tested on the structure of contour body feathers. Therefore, the common effect of environmental factors on feather density and down feather structure remains to be tested on a larger sample size.

The aerodynamic pressure on the flight feathers of birds is decisive in shaping the morphology of the feathers. Our comparative study revealed that the structure of the vane varied both along the axis of each feather and among different parts of the wing. Overall, the patterns of variation found in the density and branching angle of the barbs of the vane reflect the different demands on the air transmissivity and resistance of vanes which have different roles in flight. Furthermore, structural differences are reflected differently in species with different flight types. Based on our research, the flight habits and lifestyle characteristics of species play an important role in the evolution of the diversity of feather structure seen in various species. The vanes of species flying at high wing-beat frequencies can be characterized by higher barb densities than those of soaring species; however, the barb angles of both distal and proximal flight feathers are significantly affected by flight style. Similarly to vanes, the morphology of the rachis and its resistance to bending varies along the axis of each flight feather and across remiges of the four bird species studied. Our results thus indicate that the cross-sectional morphology of the rachis appears to be driven by the direction of in situ aerodynamic loads and the necessity to ensure appropriate stiffness to resist them. Nevertheless, because of the reduced sample size of the study, further comparative investigations are needed in order to explore the adaptation of the rachis morphology to the different flight style of birds. In addition, the results of our study provide a good basis for further comparative studies. It points out that the cross-section of the rachis varies along the wing in function of the measurement position. Thus, if the goal is to explore adaptation to flight style, it is important to select the appropriate feather and measurement position. In closing, our series of studies suggests that there is a high degree of variation in the morphology of bird plumage, which is actually the result of adaptation to the challenges imposed by different environmental conditions and flight styles.

5. Összefoglaló

A tollazat a madarak egyedülálló jellege, melynek a kommunikációban, a hővel és vízzel szembeni szigetelésben, valamint a repülésben egyaránt fontos szerep jut. Alkotásában változatos méretű, alakú és szerkezetű tollak vesznek részt, melyek különböző funkciók betöltésére specializálódtak. A disszertációmban bemutatott vizsgálatok célja az volt, hogy feltárja a madarak tollazatának bizonyos jellegzetességeiben megfigyelhető strukturális változatosságot, és a tollazati jellegzetességek kapcsolatát a madarak eltérő ökológiai- és életmenet-jellegeivel, lehetséges adaptációk után kutatva.

I. vizsgálat: A tollazat sűrűsége a környezeti feltételek függvényében

A tollazat szigetelő-képességét nagymértékben befolyásolhatja az alkotásában részt vevő tolltípusok egységnyi felületre eső száma, azonban eddig a különböző madárfajokra jellemző tollsűrűségről nagyon kevés információ állt rendelkezésünkre. A tollazatot alkotó, különböző szerkezetű tolltípusok eltérő jelentőséggel bírnak. A kontúrtollaknak például a külső zászlós részei alkotják a tollazat külső védőrétegét, míg a testhez közelebb elhelyezkedő pelyhes részek inkább a hőszigetelésben játszanak szerepet.

Kutatásunkban, tolltípusokra lebontva (tollszám/cm^2), összesen 152 faj 337 egyedénél mértük meg a tollazat sűrűségét (tollszám/cm^2), a has- és hátoldalon egyaránt, és megvizsgáltuk, hogy ennek változatosságát hogyan magyarázzák a fajok eltérő ökológiai sajátosságai (élőhely típusa, környezet hőmérséklete).

Eredményeink alapján az élőhely jellege és a telelőterület hőmérséklete egyaránt összefüggött a testtollak egységnyi felületre eső számával, míg a költőterület hőmérséklete nem, vagy csak kis mértékben. Az úszó és bukó vízimadaraknál egyaránt mindkét vizsgált tolltípusnak (kontúr testtoll, pehelytoll) nagyobb volt a hasoldalon és a hátoldalon található sűrűsége (külön-külön nézve és egyben is), mint a szárazföldi, vízközeli vagy vízparti madarak esetében. Továbbá a hátoldali tollazatban a pehelytollak és kontúrtollak sűrűségi aránya is szignifikánsan nagyobb volt a vízimadaraknál a nem vízimadarakéhoz képest. Vízimadarakon belül azonban (úszó és bukó fajok között) nem találtunk különbséget egyik vizsgált tollsűrűségi paraméter esetében sem.

A vízimadarak a többi csoporthoz viszonyítva általánosságban nagyobb tollsűrűségi értékkel rendelkeztek, ami nem meglepő, ha figyelembe vesszük a kontúrtollak vízzáró szerepét. Vélhetően a nagyobb számú fedőtoll magasabb védelmet nyújt a víz bejutásával szemben, a pehelytoll pedig jobb hőszigetelést biztosít a vizes közegben. Az eltérő hővesztésnek és felhajtóerőnek való kitettség ellenére a bukó és úszó madarak hasonló tollsűrűséggel rendelkeztek, ami arra utal, hogy a bukó életmódhoz való adaptáció inkább az egyes tolltípusok mikrostruktúrájában keresendő, és nem a tollazat denzitásában. Hogy milyen fontosak a pehelytollak a hőszigetelésben azt megmutatja azon eredményünk, hogy a hidegebb élőhelyen telelő madaraknál nagyobb ezeknek a tollaknak a sűrűsége, így több szigetelő szerepű hatású levegő tárolása lehetséges a zord körülmények között.

A hasoldal kontúr testtollainak sűrűsége, a teljes tollsűrűség és a téli hőmérséklet között másodfokú összefüggést találtunk. Nagyobb tollsűrűséget láthatunk a közepes hőmérsékleti tartományban élő, alacsonyabbat a hideg és meleg élőhelyeken található fajoknál. Ez az eredmény arra utal, hogy a hőmegtartás és a hőleadás szempontjából is előnyös lehet az alacsony tollsűrűség. Ennek tisztázásához azonban további vizsgálatok szükségesek. Kutatásunk az első, amely nagyszámú madárfaj esetében mutatja be és tárgyalja a tollazat sűrűségében megfigyelhető változatosságot, figyelembe véve a fajok eltérő környezeti feltételeit is.

II. vizsgálat: A kontúr testtollak szerkezete eltérő környezeti feltételek függvényében

A madarak majdnem egész testét beborító kontúr testtollak elsődleges szerepe a vízzel és hővel szembeni szigetelés. Bár szerkezetük meghatározó e szerepek betöltésében, nagyon kevés információ áll rendelkezésünkre a különböző madárfajok tollstruktúrájában megfigyelhető változatosságról, és az ezt meghatározó sajátosságokkal való kapcsolatról. 194 madárfajt érintő komparatív vizsgálatunkban a hasi kontúrtollak szerkezetét vizsgáltuk, lemérve a tollak teljes hosszát, a pihés, illetve pihe nélküli részek hosszát és arányát, valamint az ágak és ágacskák sűrűségét.

Azt találtuk, hogy az élőhely típusa (vízi, vízközeli, szárazföldi), illetve a telelőterület minimum hőmérséklete összefügg a testtollak szerkezetével. A tollak teljes, valamint pelyhes részének hossza rövidebb, a pihe nélküli rész ágacska-sűrűsége pedig nagyobb volt a vízi fajoknál. Ez vélhetően azzal magyarázható, hogy a vízimadaraknál előnyösebb a rövidebb, így kevesebb levegőt tároló pihés rész, mely limitáló tényező lehet alámerüléskor. Viszont fontos a víz bejutását megakadályozó, nagy ágacska-sűrűségű külső zászlós rész, amely vízzáró rétegként szolgálhat. Emellett, a hidegebb területeken áttelelő madaraknál a pelyhes rész hosszabb, a tollak pedig lazább szerkezetűek (vagyis kisebb ág- és ágacska-sűrűség jellemző). Ez a szerkezet nagyobb mennyiségű levegő benntartását teszi lehetővé, így hatékonyabb lehet a hőszigetelés. Eredményeink alapján a fajra jellemző ökológiai körülmények fontos szerepet játszhattak a testtoll-struktúra evolúciójában.

III. vizsgálat: A pehelytollak szerkezete eltérő környezeti feltételek függvényében

A természetes anyagok közül, a hőtartás és a súly arányát tekintve, a pehelytollak rendelkeznek a legjobb szigetelőértékkel, köszönhetően annak, hogy nagy mennyiségű levegőt képesek megkötni, vagyis magas töltőértékkel bírnak. Egy másik fontos tulajdonságuk az összenyomhatóság, mely lehetővé teszi a tollrétegek eredeti térfogatának visszanyerését, például víz alá merülést követően, amikor a tollazat a hidrosztatikus nyomás következtében kompressziónak van kitéve. A pehelytollak ezen kiemelkedő fizikai jellemzői a sajátos egyedi struktúrájukkal hozhatók összefüggésbe. A kontúr testtollaktól eltérően a pehelytollak tollszára rövid, az ágaik és ágacskáik hosszúak, és nem kapcsolódnak össze, így pelyhes, laza szerkezetűek. Továbbá, a pehelytollak ágacskáinak egyik jellegzetessége, hogy hosszanti tengelyük mentén csomók (nóduszok) figyelhetőek meg. Korábbi vizsgálatok azt mutatják, hogy a pehelytollak szerkezetére nagy változatosság jellemző. Ágacskáik hossza, valamint az ezeken lévő nóduszok sűrűsége meghatározza a tollak közötti kohéziós erőt és a pehelytollak töltőértékét, továbbá magas nóduszsűrűségük növeli a kompresszióval szembeni ellenállóságukat is.

Az eddigi vizsgálatok tehát azt mutatják, hogy a pehelytollak szerkezeti változatossága összefüggésbe hozható a fajok eltérő termoregulációs igényeivel. A lazább szerkezetű tollazat, hosszabb tollakkal, valamint rövidebb és kevésbé sűrű ágacskákkal, jobb hőszigetelést tesz lehetővé azáltal, hogy több levegőt képes a tollazatban benntartani, vagyis magasabb töltőértékkel rendelkezik. Másrészről, a pehelytollak közötti kisebb kohéziós erő elősegítheti az alámerülést, mivel a tollazat jobban összepréselhető, így a levegő könnyebben távozik. Egyelőre azonban nem ismeretes, hogy a pehelytollak szerkezete hogyan igazodik a madarak változatos környezeti feltételeihez, ugyanis az eddigi vizsgálatok csupán néhány fajjal foglalkoztak.

Kutatásunk során 156 fajtól gyűjtöttünk pehelytollakat, has- és hátoldalról egyaránt, és megvizsgáltuk, hogy a szerkezeti paramétereikben megfigyelhető változatosság (tollak teljes hossza és felszíni területe, ágak és ágacskák hossza, ágacskák sűrűsége és nóduszok sűrűsége az ágacskák mentén), hogyan igazodik a fajok eltérő ökológiai jellegei (élőhely típusa, környezet hőmérséklete) által támasztott igényekhez.

Eredményeink azt mutatják, hogy a vízimadarak kisebb és rövidebb ágacskákból felépülő pehelytollakkal rendelkeznek, melyek nóduszsűrűsége is alacsonyabb, mint a nem vízi (szárazföldi, vízközeli, vízparti) fajok esetében. Ez a mintázat hasonló volt a hasoldalon és ahátoldalon. Mindezek alapján tehát a vízi élőhelyen a kis méretű, és a kompressziónak kevésbé ellenálló pehelytoll-szerkezet részesül előnyben. A pehelytollak kisebb mérete azt mutatja, hogy vízimadaraknál az ezek által alkotott szigetelőréteg vékonyabb, ami látszólag ellentmond a vízi környezettel járó magas hővesztéshez való alkalmazkodás követelményeinek. Azonban ha a vízimadarak, főképp a víz alatt táplálkozó bukó fajok, nagy mennyiségű szigetelő levegőt tárolnak a tollazatban, akkor a nagyobb felhajtóerővel is szembe kellene nézniük. Vélhetően az ehhez való alkalmazkodással magyarázható a vékonyabb pehelyréteg, a rövidebb ágacskák és az alacsony nóduszsűrűség, mely csökkentheti a tollazatban tárolt levegő mennyiségét. Ezen struktúra által a tollazat kevésbé áll ellen merüléskor a ránehezedő hidrosztatikai nyomásnak, összepréselődése pedig elősegíti a levegő távozását, így megkönnyíti a merülést. Emellett a távozó levegőbuborékok csökkentik a súrlódást a test és a víz között, ami tovább könnyítheti a madarak alámerülését.

A vizsgált paraméterek közül a telelőterület hőérzet-indexe (hőmérsékletből és szélerősségből kifejezett érték) csak a hátoldal nóduszsűrűségére volt hatással, ami magasabb volt a zordabb körülmények között élő fajoknál. Ez azzal magyarázható, hogy a nagyobb nóduszsűrűség erősíti a pehelytollak közötti kohéziót, egy egységesebb szigetelőréteg pedig jobb védelmet biztosíthat szeles és hideg élőhelyen.

IV. vizsgálat: A tollszár morfológiájának változása a szárny mentén

Repüléskor a levegőbe való felemelkedéshez és az előrehaladáshoz felhajtó- és tolóerő generálása is szükséges, így a madarak szárnyát alkotó evezőtollak jelentős terhelésnek vannak kitéve. Az evezőtollak fő szerkezeti eleme a tollszár vagy tollgerinc, melynek morfológiai jellemzői meghatározzák a tollak elhajlását, terheléssel szembeni rezisztenciáját.

Az aerodinamikai erők eloszlása a szárny mentén nem egyenletes, és több tényező is befolyásolhatja, melyek közül meghatározó az egyes tollak szárnyon elfoglalt helyzete, valamint a madarak repülési stílusa. A disztális kézevezőket nagyobb terhelés éri a proximális evezőkhöz képest, különösen fel- és leszálláskor, vagy aktív, nagy szárnycsapás-frekvenciával jellemezhető repülési stílus esetében, mely következtében a disztális kézevezőknek nagyobb, főleg a tollzászló síkjára merőlegesen ható (hát-hasi irányú) terhelésnek kell ellenállni. Míg a felhajtóerő képzésében szerepet játszó belső kéz- és karevezők egyöntetűen főként a hát-hasi irányú erőkkel kell szembenézzenek, addig a tolóerő képzésében is szerepet játszó külső tollakra jelentős oldalirányú és torziós erők is hatnak, különösen a tollcsúcs közelében. A terhelésben megfigyelhető különbségek eltérésekhez vezethetnek az evezőtollak tollszár-morfológiájában. Korábbi vizsgálatokból kiderült, hogy a tollak tömegének alacsonyan tartása mellett a tollszár keresztmetszeti alakjának változása is fontos optimalizáció a megfelelő rezisztencia elérése érdekében. Ennek megfelelően azt találták, hogy a tollszár keresztmetszeti alakja változik a csévétől a tollcsúcs irányába, valamint a szárny külső és belső tollai között, melynek eredményeképpen a szárnytollak eltérő elhajlással szembeni ellenállást mutatnak a hát-hasi és oldalirányú erőkkel szemben. Az erre vonatkozó eddigi vizsgálatok során azonban vagy egy fajt, vagy egyetlen szárnytollat vizsgáltak, főként a kézevezőkre fókuszálva. Ezek alapján a disszertáció negyedik (IV. vizsgálat) vizsgálatában arra kerestük a választ, hogy hogyan változik a tollszár keresztmetszeti alakja az (1) egyes evezőtollak tengelye mentén és a (2) teljes szárny mentén, (3) valamint a változás mintázata eltérő-e a különböző repülési stílussal jellemezhető fajok között. A tollszár keresztmetszeti alakjának kifejezéséhez a hát-hasi és az oldalirányú vastagságának arányát használtuk, feltételezve, hogy a szárny különböző tollai adaptálódtak az eltérő irányú terheléshez, így olyan irányba szélesedtek (hát-hasi irány, oldalirány), mely irányból nagyobb terhelés hat rájuk.

Kutatásunk során négy, eltérő repülési kategóriához tartozó madárfaj 10-10 elpusztult egyedétől begyűjtöttünk minden egyes evezőtollat [fehér gólya (vitorlázó), egerészölyv (sikló), házi veréb (énekesmadár-típusú), kis kárókatona (folyamatosan csapkodó)]. A tollszár alakjának jellemzéséhez a magasság/szélesség arányának értékét használtuk, melyet minden egyes evezőtoll négy pontján mértünk [0% (cséve), 25% (alap), 50% (közép) és 75% (csúcs)] (2. ábra, B1-B2). Ugyanezekben a mérési pontokban, az oldalirányú (I_x) és a hát-hasi (I_y) tengelyek mentén, meghatároztuk a tollszárkeresztmetszetek másodrendű nyomatékát is (I, mm^4), mely jó mutatója az elhajlással szembeni rezisztenciának az adott mérési pontban.

Eredményeink azt jelzik, hogy a tollszár keresztmetszeti alakja, valamint a különböző irányú erőkkel szembeni ellenállása az egyes tollak hosszanti tengelye és a teljes szárny mentén is változik. A szárnymenti változás mintázata a négy vizsgált fajnál eltérően alakult. A tollszár alapi része, a cséve, hát-hasi irányban vastagabb és egységesen ovális alakú a négy madár esetében. Ez az alak képes optimálisan ellenállni a szárny ezen részére jellemző, hát-hasi irányból ható terhelésnek.

A fehér gólya, az egerészölyv és a házi veréb esetében a szárny csúcsát alkotó külső kézevezők disztális részén a tollszár vastagabb oldalirányban, mint hát-hasi irányban, és ellenállóbb az oldalirányú erőkkel szemben, míg a szárny többi részén a tollszár vastagabb hát-hasi, mint oldalirányban, és rezisztensebb is az ebben a síkban ható erőkkel szemben.

A fehér gólya és az egerészölyv külső szűkített és bemetszett evezőtollai esetében a belső kézevezők felé haladva fokozatos növekedés volt megfigyelhető a hát-hasi/oldalirányú vastagság arányában. Úgy véljük, hogy ezeknél a vitorlázó és sikló fajoknál e morfológiai mintázat teszi lehetővé a külső kézevezőknél az ‘ujjas’ szárnycsúcs passzív kialakulását vitorlázó és sikló repülés közben.

Predikcióinkkal ellentétben, a nagy szárnycsapás-frekvenciával repülő kis kárókatonánál, az alapi rész kivételével az összes toll tollszárának minden egyes mérési pontjában nagyobb volt az oldalirányú, mint a hát-hasi irányú vastagság. Ez a mintázat összefüggésben állhat a vízi-bukó életmóddal, azonban további sokfajos vizsgálat szükséges a tollszár morfológiájában megfigyelhető adaptációk pontosabb feltárásához.

V. vizsgálat: Az evezőtollak zászlójának szerkezete a repülési mód függvényében

Repülés szempontjából a szárnytollak tollzászlói kiemelten fontosak, hiszen ezek alkotják a toló- és felhajtóerők képzéséhez elengedhetetlen szárnyfelszín nagy részét. Az egyes tollakra ható aerodinamikai terhelés iránya és mértéke változik a szárny mentén és ezt döntő mértékben meghatározza a madarak repülési stílusa is, így strukturális adaptációkra lehet számítani.

A tollak külső és belső zászlói a tollszár két oldalából kiinduló ágak és ágacskák összekapcsolódásából jönnek létre. Az eddigi vizsgálatok azt mutatják, hogy ezek a szerkezeti elemek kiemelten fontosak, ugyanis az ágak és ágacskák sűrűsége határozza meg a tollzászló légáteresztő-képességét, míg az ágak tollszárhoz való kapcsolódási szöge megszabja a tollzászló rezisztenciáját a szárnycsapásokkor létrejövő, különböző irányból ható erőkkel szemben. Feltételezhető tehát, hogy ezen szerkezeti elemek adaptálódtak az eltérő aerodinamikai erőkhöz, ezt megerősítendően azonban elenyésző számú vizsgálat született eddig összehasonlító szinten, több szárnytollat is figyelembe véve.

Mindezek alapján jelen munkánk során arra kerestük a választ, hogy az ágak sűrűsége és kapcsolódási szöge hogyan változik (1) a tollzászlók hosszanti tengelye mentén, (2) a külső és belső zászlók között, (3) valamint a szárny disztális és proximális evezőtollai között, továbbá, (4) a strukturális elemekben megfigyelhető változatosság összefügg-e a madarak repülési stílusával.

Vizsgálatunk során 178 madárfajtól gyűjtöttünk külső (P8) és belső (P1) evezőtollakat. A két tolltípus külső és belső zászlóját három pontban jelöltük meg: a tollzászló hosszának 25%-án (alap), 50%-án (közép) és 75%-

án (csúcs). A tollakat lefotóztuk, majd az ImageJ program segítségével mindhárom pontban megmértük az ágak tollszárhoz való kapcsolódási szögét és sűrűségét. A vizsgálatban szereplő fajokat repülési stílusuk alapján négy kategóriába soroltuk: vitorlázó, sikló, folyamatosan csapkodó és énekesmadár-típusú. A repülési stílus hatását az egyes mérési pozíciók tollszerkezeti paramétereire, általánosított legkisebb négyzetek módszerével teszteltük (PGLS) és az elemzések során kontrolláltunk a fajok rokonsági kapcsolataira és a testtömegükre.

Eredményeink azt mutatták, hogy az ágsűrűség és az ágszög a tollzászló és a szárny mentén [külső disztális kézevező (P8) és belső proximális kézevező (P1) között] egyaránt változott. A P8 külső zászlójának minden mérési pontjában alacsonyabb volt az ágsűrűség, mint a P1 esetében. A belső zászlók esetében csak a tollzászló középső része különbözött, nagyobb ágsűrűséget mutatva a P8 tollaknál. A tollszár menti változást illetően azt találtuk, hogy az ágsűrűség és az ágszög is csökken a zászló alapjától a tollcsúcs irányába, mindkét toll külső és belső zászlóján egyaránt. A külső és belső zászlók közötti különbségeket illetően, minden mérési pozícióban és mindkét tollnál (P8, P1) nagyobb volt a külső zászló ágsűrűsége mint a belső zászlóé. Továbbá az ágszögek kisebbek voltak a külső zászlón, mint a belsőn és a P1 tollak alapi részének kivételével, ez igaz volt a mindkét toll minden mérési pontjára.

Általánosságban elmondható, hogy a szerkezeti elemekben megfigyelt változatosság tükrözi a tollak és tollrészek eltérő légáteresztő ésellenálló képességgel kapcsolatos eltérő igényeit. A tollak alapi része által alkotott szárnyrésznél képződik a felhajtóerő nagy része. A belső zászlók alapi részénél egységesen kimutatott nagyobb ágsűrűség tükrözi az alacsony légáteresztő-képességre irányuló igényt a szárny ezen részénél. A disztális tollak nagyobb terhelésnek vannak kitéve, különösen csúcsi részük, melyet oldalirányú aerodinamikai erők is érnek. Ezen tollak csúcsi része akár 180 fokban is elcsavarodhat a szárnycsapások bizonyos szakaszaiban. Ezzel összhangban az ágak kapcsolódási szöge csökkent a P8 tollak csúcsának irányába, mely nagyobb rezisztenciát biztosít a tollzászlónak, csökkentve a szerkezeti elemek szétkapcsolódásának esélyét.

Predikcióinknak megfelelően az eltérő aerodinamikai költségekkel járó repülési stílus szignifikánsan befolyásolja a tollzászló ágsűrűségét és ágszögét. Egy korábbi vizsgálatunk eredményeihez hasonlóan, a nagy szárnycsapás-frekvenciával repülő fajoknál (folyamatosan csapkodó, énekesmadár-típusú) a tollzászló ágsűrűsége nagyobb, mint a passzívan (sikló, vitorlázó) repülő fajoknál. Előbbi stílus esetében nagyobb az evezőtollak terhelése, mint az utóbbi két típus esetében, így várható adaptáció a tollzászlók nagy ágsűrűsége, mely csökkenti a tollzászló légáteresztő-képességét és növeli ennek rezisztenciáját. A vártakkal ellentétben, az ágszög esetében nem találtunk konzekvens mintázatot a repülési stílusok között, ami arra utal, hogy más tényezők, mint például az energetikailag és aerodinamikailag költséges fel- és leszállás, ugyancsak alakíthatják a tollzászló szerkezetét, elfedve a repülési stílus esetleges hatását.

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COMPILATION SECTION

1. List of publications related to the dissertation

I. **Osváth G**, Daubner T, Dyke GJ, Fuisz TI, Nord A, Pénzes J, Vargancsik D, Vágási CI, Vincze O and Pap PL (2018). How feathered are birds? Environment predicts both the mass and density of body feathers. *Functional Ecology* 32: 701-712

II. Pap PL, Vincze O, Wekerle B, Daubner T, Vágási CI, Nudds RL, Dyke GJ and **Osváth G** (2017). A phylogenetic comparative analysis reveals correlations between body feather structure and habitat. *Functional Ecology* 31: 1241–1251.

III. Pap PL, **Osváth G**, Daubner T, Nord A, and Vincze, O. (2020). Down feather morphology reflects adaptation to habitat and thermal conditions across the avian phylogeny. *Evolution*, 74(10): 2365-2376.

IV. **Osváth G**, Vincze O, David DC, Nagy LJ, Lendvai ÁZ, Nudds RL, and Pap PL (2020). Morphological characterization of flight feather shafts in four bird species with different flight styles. *Biological Journal of the Linnean Society*, 131(1): 192-202.

V. Pap PL, Vincze O, Vágási CI, Salamon Z, Pándi A, Bálint B, Nord A, Nudds RL and **Osváth G** (2019). Vane macrostructure of primary feathers and its adaptations to flight in birds. *Biological Journal of the Linnean Society*, 126(2): 256-267.

2. Research items

Study I: *How feathered are birds? Environment predicts both the mass and density of body feathers*

Study II: *A phylogenetic comparative analysis reveals correlations between body feather structure and habitat*

Study III: *Down feather morphology reflects adaptation to habitat and thermal conditions across the avian phylogeny*

Study IV: *Morphological characterization of flight feather shafts in four bird species with different flight styles*

Study V: *Vane macrostructure of primary feathers and its adaptations to flight in birds*

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RESEARCH ARTICLE

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How feathered are birds? Environment predicts both the mass and density of body feathers

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Abstract

1. Studies modelling heat transfer of bird plumage design suggest that insulative properties can be attributed to the density and structure of the downy layer, whereas waterproofing is the result of the outer layer, comprised of contour feathers. In this study, we test how habitat and thermal condition affect feather mass and density of body feathers (contour, semiplume and downy feathers) measured on the ventral and dorsal sides of the body, using a phylogenetic comparative analysis of 152 bird species.
2. Our results demonstrate that feather mass and the density of downy feathers are higher in species that inhabit colder environments, whereas total feather density is higher of species breeding under intermediate temperatures compared to the ones breeding under more extreme conditions. The density of contour feathers, depending on the body region, is either quadratically related or negatively correlated with minimum winter temperature.
3. The density of contour and downy feathers, measured on both sides of the body, is higher in aquatic than in terrestrial birds. However, among the former, diving behaviour does not select for further increases in body feather mass or density.
4. The results of this study provides key insights into how the plumage of birds is adapted to different environments and lifestyles and provides a basis for understanding the diverse range and the evolution of variation in these characteristics.

KEYWORDS

birds, comparative analysis, feather density, feather mass, thermoinsulation, waterproofing

1 | INTRODUCTION

The plumage of birds functions as a critical barrier between the skin and the environment by controlling heat transfer and preventing the penetration of water, providing up to 90% of the insulation requirements of an individual (Jarman, 1973; McCafferty et al., 2013). The high thermoinsulatory capacity of the plumage enables birds to maintain a core body temperature surpassing 40°C (Prinzinger, Preßmar, & Schleucher, 1991) over a wide range of environmental temperatures (Handrich et al., 1997; Ponganis et al., 2003). While plumage can function to decrease heat flux between the skin and the environment by blocking radiation and trapping air near the skin, it can also increase heat flux via conduction backwards and forwards to the body (Walsberg, 1988). The thermoregulatory characteristics of body feathers are probably related to a range of attributes, including plumage depth, density and morphology (Prum & Brush, 2002; Srinivasan et al., 2014). Indeed, multiple traits of the plumage, especially body feathers, define the density and morphology of the downy plumage layer (Butler, Rohwer, & Speidel, 2008; Davenport, O'Halloran, Hannah, McLaughlin, & Smiddy, 2009; Dove & Agreda, 2007; Du, Fan, Wu, Chen, & Liu, 2007; Eadie & Ghosh, 2011; Pap et al., 2017; Wilson, Hustler, Ryan, Burger, & Noldeke, 1992), as well as the number and size of spaces that trap air adjacent to the skin (Dawson, Vincent, Jeronimidis, Rice, & Forshaw, 1999; Wilson et al., 1992). Plumage water repellence is also thought to be conferred by a layer of contour feathers that form an external barrier, whereas water resistance is further determined by plumage density. Thus, the amount of keratin, the number of feathers per unit surface and the air spaces trapped within them, comprise a multiscale air cushion at the feather–water interface. This cushion functions to prevent feathers from getting wet (Davenport et al., 2009; Eadie & Ghosh, 2011; Grémillet, Chauvin, Wilson, Le Maho, & Wanless, 2005; Grémillet et al., 2001; Kooyman, Gentry, Bergman, & Hammel, 1976; Pap et al., 2017; Rijke, 1970; Williams, Hagelin, & Kooyman, 2015). In water birds, however, alterations in insulation must occur because the thermal conductivity of this medium is about 23 times that of air, whereas further modifications are required in diving birds because increased hydrostatic pressure impinges on the plumage and compresses the air trapped among the feathers (Grémillet, Tuschy, & Kierspel, 1998; Grémillet et al., 2001; Wilson et al., 1992). Thus, in diving birds, the reduced volume of trapped air is likely partially compensated for by denser plumage (Walsberg, 1988).

Plumage in birds comprises different feather types that all have subtly different functions (Prum & Brush, 2002). This means that quantifying the densities of different feather types is essential if we are to understand how avian plumage achieves its unique thermoinsulatory and water-repellent functions. The most numerous components of avian plumage are contour feathers which cover most of the body and have multiple functions (Butler et al., 2008; Pap et al., 2017); the pennaceous distal region of these feathers form an outer water-repellent cover over the inner thermoinsulatory layer, defined by the proximal plumulaceous feather section (Pap et al., 2017; Prum & Brush, 2002; Rijke, 1970; Srinivasan et al., 2014; Stephenson & Andrews, 1997). The contour feathers of some species also have an auxiliary shaft called the afterfeather; it has been assumed that this shaft has a function in thermoinsulation, although its role in thermoregulation remains unclear (Williams et al., 2015). In addition, the plumulaceous part of contour feathers, semiplumes and downy feathers also contribute significantly to the insulating layer. Semiplumes have a central shaft which is longer than their longest barb, whereas lateral plumulaceous barbs radiate from here with barbules that do not attach to one another. Downy feathers are fluffy and have short rachises which are shorter than the longest barbs (Lucas & Stettenheim, 1972).

The mass and the density of different body feather types vary dramatically among bird species (Cooper, 2002; Davenport et al., 2009; Hutt & Ball, 1938; Swanson, 1993; Wetmore, 1936). This variation has previously been attributed to thermal conditions as well as water repellency in aquatic birds (Dawson et al., 1999; Williams et al., 2015). However, these conclusions have been based mostly on single species, and no systematic analyses of these variations have been undertaken. Most studies completed thus far on plumage density as an adaptation to cold environments and an aquatic lifestyle have been carried out on highly specialized species which means it has been difficult to deduce the specific functions of different body feathers. For example in terrestrial species that are much less exposed to water, body plumage appears adapted to repel, rather than to withstand, water infiltration (Rijke, 1970; Srinivasan et al., 2014).

Variation in the density of avian body feathers is likely subject to a trade-off between the benefits of the plumage and costs of integument production (Butler et al., 2008; Danner, Greenberg, Danner, & Walters, 2015; Dawson, Hinsley, Ferns, Bonser, & Eccleston, 2000; Pap, Vágási, Czirják, & Barta, 2008). Producing feathers is likely constrained by limited resources, including available protein content of food (Pap et al., 2008). While there is some consensus on how resource limitation reduces the amount of keratin invested in feathers, no studies to date have considered its effects on plumage mass and density.

We employ a phylogenetic comparative approach to investigate how habitat, diving behaviour, thermal environment and diet influence the mass and density of feathers in a dataset that incorporates dorsal and ventral plumage samples from 152 bird species. Our prediction is that body feather mass and density will be higher in aquatic birds and divers than in their terrestrial and non-diving counterparts respectively. We predict that dorsal and ventral plumage traits are variously affected by habitat because sides of the body are similarly exposed to rain and water in terrestrial species, whereas the plumage on the ventral side of aquatic birds (particularly in floaters) is subject to higher water pressure than on the dorsal side. We also predict that low ambient temperature will select for a more thermoinsulatory plumage, manifested in increased body feather mass and feather density (Dawson et al., 1999; Williams et al., 2015). Plumage may also function to facilitate heat loss (Butler et al., 2008); it is likely that looser plumage is correlated with enhanced heat loss in birds that live in hot environments. Therefore, we expect that birds that are adapted to warmer climates will have a lower mass and density of body feathers (Ward, Ruxton, Houston, & McCafferty, 2007). Heat retention and heat transfer can be ensured by low or high feather mass and density, respectively, because the number of air vacuoles vary in function of these feather traits, whereas the amount of air trapped in the plumage may remain unchanged. As a result, species living in warm or cold environments may show the same adaptation in plumage traits. Therefore, we assumed in models both linear and quadratic effect of temperature on plumage traits.

2 | MATERIALS AND METHODS

2.1 | Field data collection and feather measurements

We collected feather samples from 337 birds encompassing 152 neognath species at several locations across Romania, Hungary, Norway, Sweden and Scotland, as well as the sub-Antarctic Crozet Islands archipelago (king penguin, *Aptenodytes patagonicus*) between 2012 and 2016. Most individuals died due to natural causes (e.g. road kill, building collision, electrocution, starvation) or were procured from authorized hunters. All samples were preserved at −20°C in a freezer until examination in 2016. The age of all specimens was recorded upon investigation that was determined based on plumage characteristics, degree of skull pneumatization and by examining flight feather moult patterns. Feathers were collected from the ventral and dorsal sides of the body, with samples used including just non-moulting individuals in good condition with clean plumage. We collected samples of ventral feathers from an area delimited by the breastbone, the contact point of the wing and leg to the body and side, including one ventral feather tract (see Appendix S1). The rectangular-shaped dorsal surface of the bird was defined as the area between the shoulder, the tail end of the synsacrum and the side. King penguin feathers were collected from a smaller area (*c*. 20 cm^2) on ventral and dorsal sides; however, because of the uniform distribution of feathers across the body in these birds (Dawson et al., 1999; Williams et al., 2015), this smaller sampling area probably had little effect on feather mass and density estimates of this species. Subsequent to feather collection, we photographed bare skin areas in front of a metric grid (1 × 1 mm) background and measured the surface using the ImageJ software, version 1.51 (http://rsb.info.nih.gov/ij/). Body feathers were then weighed and desiccated for 24 hr at 37°C to eliminate any moisture captured in the plumage. We weighed all body feathers (using a professional digital analytical balance Axis AGN200, accuracy class I, $e = 0.001g$; $d = 0.0001g$) from ventral and dorsal sides separately, and recorded the total feather mass per unit area (g/cm^2), presenting just the mass of total feathers because the weights of different types were too low to be measured with precision. We then separated and counted different feather types in each sample to record the numbers of contour feathers, semiplumes and downy feathers per unit area (number/cm^2). Given that the thermoinsulatory function of semiplumes and downy feathers are probably similar, and because the number of the former in most species was very low, we pooled our data for these two feather types in all analyses. We measured the length of one contour feather from each individual on both sides of the body, collected from the middle of the breastbone in case of the ventral part and from the middle of the backbone in case of dorsal part, using a ruler, and these data were completed with additional measurements from an earlier work (Pap et al., 2017); thus, our sample sizes for feather mass and density, as well as contour feather length differ slightly (Osváth et al., 2017). Feather samples from different species and individuals were randomly assigned to one of the five investigators (JP, CIV, GO, PLP and TD). Repeatability between observers was high for all parameters (total feather density: ICC (Intra-class Correlation Coefficients) = 0.95, 95% CI 0.88–0.98; contour feather density: ICC = 0.96, 95% CI 0.91–0.99; down feather density: ICC = 0.94, 95% CI 0.85–0.98; semiplume density: ICC = 0.78, 95% CI 0.55–0.91; all $F > 11.34$, $n = 14$, all $p < .0001$).

2.2 | Body mass, ecological traits and habitat

We obtained body mass data from (Del Hoyo, Elliot, & Sargatal, 1992–2013; Dunning, 2007). Where mass data were available for multiple regions, we selected the one for European populations and subspecies. We then classified species into one of five habitat groups Cramp and Perrins (1977–1994); terrestrial (i.e. rarely encountering water), riparian (i.e. living in wetland habitats like marshes and sedges), waders (i.e. species that move above, and around water surface), floaters (i.e. aquatic species that float on the water but do not dive) and 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). We used the most recent 10 years of temperature data for this study (encompassing the period between 2006 and 2015, downloaded on 3rd November 2016), and calculated average monthly mean temperature over this period for each spatial grid cell. From these monthly mean data, we created a 12-layer shape file for each grid cell, and by intersecting temperature and species distribution files, we calculated monthly temperatures for each species separately for wintering and breeding grounds (Pap et al., 2015, 2017; Vincze, 2016). Distribution maps (shape files) were retrieved from http://www.birdlife.org/datazone/info/spcdownload (BirdLife International & NatureServe, 2015, accessed on 27th October 2016). For breeding areas, we extracted the highest monthly mean ("maximum breeding temperature") for the period April to August as a proxy for breeding heat stress, whereas for wintering grounds we extracted the 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 were situated outside this range, we used global ranges or other ranges specific for the sampled population (e.g. king penguin; see Osváth et al., 2017). Because African and Arabian resident populations of several species are clearly separated from their European migratory counterparts (with likely little gene flow between them), these populations and areas were not included in our analyses (Osváth et al., 2017). Furthermore, because the protein content of food can limit feather synthesis (Pap et al., 2008), we included diet type as a factor in our analyses. Diet was assigned to one of two categories, "high," or "low" protein content (Pap et al., 2015); a high protein content was defined as exclusively feeding on invertebrates and/or vertebrates, whereas low was used for species that are either omnivorous and/or species feeding on plants. Dietary information for each species was obtained from Cramp and Perrins (1977–1994). All variables for all species can be found at Osváth et al. (2017).

2.3 | Statistical analyses

Large within-group (i.e. within-species) variation in traits can lead to significant bias in phylogenetic comparative analyses (Freckleton, Harvey, & Pagel, 2002). Thus, in order to determine whether, or not, our feather parameters were species-specific and suitable for multi-taxon comparisons, we tested repeatability by assessing the importance of "between-species" compared to "within-species" variance using the "icc" function in the R package "irr" (Gamer, Lemon, Fellows, & Sing, 2010). As within-species sample size varies, we calculated repeatability using two randomly selected individuals per species; this test was repeated 1,000 times and ICCs and corresponding confidence intervals (CIs) were calculated as the means of 1,000 estimates (Pap et al., 2015). Repeatability was tested using raw, non-transformed values. Feather traits are highly species-specific for both ventral (total feather mass: ICC = 0.75, 95% CI 0.65–0.83; total feather density: ICC = 0.85, 95% CI 0.79–0.90; contour feather density: ICC = 0.84, 95% CI 0.77–0.89; downy + semiplume feather density: ICC = 0.58, 95% CI 0.42–0.70) and dorsal sides of the body (total feather mass: ICC = 0.71, 95% CI 0.59–0.80; total feather density: ICC = 0.84, 95% CI 0.77–0.89; contour feather density: ICC = 0.81, 95% CI 0.73–0.87; downy + semiplume feather density: ICC = 0.60, 95% CI 0.45–0.71; all $F > 4.19$, all $p < .0001$). We therefore conclude that the species included in our datasets are adequately characterized in terms of their feather traits, and that small sample sizes in some species are unlikely to bias our results. Because feather traits can also vary over the course of a year due to changes in temperature (Cooper, 2002; Middleton, 1986; Novoa, Bozinovic, & Rosenmann, 1994; Swanson, 1991), results could also be affected by systematic bias in sampling dates among species. Thus, to test whether seasonality affects our feather traits, we compared data from birds collected in the winter (October to March) and summer (April to September) using phylogenetic paired *t* tests (R package "phytools") (Revell, 2012). Note that only a subsample of the species could be used for these tests, those for which at least one individual was measured in both periods. Feather parameters were similar between the two periods (in all cases $t < 1.87$, $N_{\text{ventral side}} = 25$, $N_{\text{dorsal side}} = 24$, $p > .0753$). Furthermore, because feather traits can vary depending on the age of a bird (Butler et al., 2008), and because our sample includes birds in both immature and adult plumages, we tested differences in feather traits between age classes using phylogenetic paired *t* tests. All parameters remained similar between age classes (in all cases $t < 1.62$, $N_{\text{ventral side}} = 26$, $N_{\text{dorsal side}} = 24$, $p > .1194$), therefore, it is unlikely that our results are affected by either within-species seasonal differences or age-specific changes in feather traits.

We used phylogenetic paired *t* tests to test whether the feather parameters (mass and density) differed between ventral and dorsal sides of the body. Differences between ventral and dorsal sides of the body (Δ = ventral minus dorsal) were analysed as response variables in multivariate models separately to test whether these differences were influenced by ecology and diet. To investigate the relationship between feather density or feather mass and body mass, diet, habitat, breeding maximum and winter minimum temperatures across birds, we built phylogenetic generalized least squares (PGLS) models using ventral, dorsal and Δ feather masses and feather densities as response variables. Because feather mass is largely determined by the length of individual body feathers, we additionally carried out separate analyses for the ventral and dorsal sides in which contour feather length of the respective body side was also added as an explanatory variable in models of feather mass. Total feather density and density of different feather types may also be influenced by the presence of afterfeathers on contour feathers; we therefore included this factor as an explanatory variable in feather density models. Sampling effort in our dataset differs among species, ranging between one and five individuals. Sampling inequality can cause bias in the data as measured parameters cannot be estimated with similar precision for each species (Garamszegi & Møller, 2010, 2011). Accordingly, likelihood-ratio statistics indicated that weighting phylogenetic models by log-transformed, within-species sample size significantly increases the model fit. Therefore, we present the results of models weighted by log-transformed within-species sample size in the case of each feather parameter investigated.

We accounted for phylogenetic non-independence using trees from http://birdtree.org (Jetz, Thomas, Joy, Hartmann, & Mooers, 2012). We downloaded 100 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 this consensus tree in the PGLS model (Pagel, 1997, 1999) and we set the degree of phylogenetic dependence (Pagel's λ) to the most appropriate value estimated by maximum likelihood in each model separately (Freckleton et al., 2002). We employed backward-stepwise elimination of non-significant ($p > .05$) predictors from the full model. Habitat, the presence of afterfeathers and diet, were all entered into models as fixed factors, whereas all other (continuous) variables were included as covariates. Temperature variables were included in the model as second-order orthogonal polynomials in order to test for possible quadratic effects on feather traits. The only flightless species in our study was the king penguin. For this species, breeding temperature as well as a number of other feather traits represented outliers in our datasets (see Figure S1). We therefore re-ran all PGLS models excluding this species, but we present both model sets in the Results. Although we present the results of our models incorporating a consensus tree, we re-ran both full and minimal PGLS models using all 100 phylogenies to control for phylogenetic uncertainty. All models yielded highly consistent results across the 100 phylogenetic trees tested. *p*-values from these models were plotted as histograms (Figure S2).

All statistical analyses were conducted using the R statistical computing environment, version 3.3.2 (R Development Core Team, 2016), and all PGLS models were built using the "nlme" package (Pinheiro, Bates, DebRoy, & Sarkar, 2015). Sample sizes vary across models depending on the body side (i.e. ventral versus dorsal) as measurements were unobtainable for some individuals from one side of the body. Finally, all feather traits and sample sizes were $\log_e(x + 1)$-transformed in all statistical models to ensure model residual normality. Means ± *SE* are reported throughout the text and tables. Model predictions and associated *SE* for graphical representations of data were obtained using the "lsmeans" package in R (Lenth, 2016).

3 | RESULTS

3.1 | Feather mass and density on the ventral and dorsal sides

Winter minimum temperature is significantly negatively correlated with feather mass on the ventral and dorsal sides of the body (Table 1a,b), indicating larger feather masses in colder than in warmer climates. This correlation remains significant after controlling for the confounding effect of feather lengths on plumage weight (Table S1). Excluding the king penguin did not change this relationship (Table 1a; Figure 1). Habitat does not affect the mass of feathers on either side of the body (Table 1a). Nonetheless, after controlling for the confounding effects of feather length on plumage weight, the effect of habitat on ventral feather mass became significant (Table S1), although there was no clear trend between groups (contrasts between groups: W < F and W < D at $p < .01$, all other contrasts at $p > .05$). Excluding the king penguin does not affect these results (Table 1a).

Depending on feather types and body region, feather density parameters are either quadratically related or negatively correlated with maximum breeding temperature (Table 1b), indicating higher feather densities in species breeding under intermediate temperatures compared to the ones breeding under more extreme conditions, or lower feather densities in hotter compared to colder breeding climates. The latter effect holds even when the presence of afterfeathers on contour feathers is controlled (Table S1). However, the effect of breeding maximum temperature disappears in most cases, when data for the king penguin are excluded from the analyses, only showing a relatively weak but significant negative linear association with total feather density on dorsal and contour feather density on ventral sides (Table 1b). In general, feather density and winter minimum temperature are quadratically related (Table 1b), with higher feather densities in species wintering under intermediate climates compared to species wintering in colder or hotter areas. Moreover, this effect is consistent even after controlling for the presence of afterfeathers on contour feathers (Table S1). The quadratic effect of minimum winter temperature on feather density is also significant for total feather density and contour feather density measured on the ventral side after excluding the king penguin (Table 1b; Figure 2), whereas the effect turns negative and linear for contour feather density on dorsal side and downy + semiplume density on both sides of the body, indicating higher densities of these feather types in colder climates. Finally, we calculated the density of downy + semiplumes compared to total feather density; this trait measured on the ventral side of the body is significantly negatively and linearly correlated with winter minimum temperature, indicating proportionally higher number of purely insulatory than contour feathers in species inhabiting colder climates; this effect was significant both with and without the king penguin's data (data of the king penguin included: $F = 6.61$, $p = .0112$, $\beta(SE) = -2.32 \times 10^{-3} (9.06 \times 10^{-4})$; excluded: $F = 6.56$, $p = .0114$, $\beta(SE) = -2.17 \times 10^{-3} (9.92 \times 10^{-4})$.

Habitat affects feather density parameters, with the exception of the density of contour feathers on the dorsal side of the body, and this effect is significant both with and without the king penguin's data (Table 1b; Figure 3). Results show that the total feather density, and density of different types of body feathers increased from terrestrial to aquatic birds, with intermediate values seen for riparian and wading taxa. This change in feather density between habitat groups varied, however, as terrestrial, riparian and wading birds were similar to one another, as well as floaters and divers were similar to one another, whereas the difference between the first three and last two groups was significant (Figure 3). The effect of habitat on feather density remained significant even after we controlled for the presence of afterfeathers on contour feathers (Table S1). The density of downy + semiplume to total feather density, measured on the dorsal side of the body is also significantly related to habitat, as terrestrial, riparian and wader birds formed one cluster and floaters and divers another, whereas the difference between the two clusters was significant in the models both with and without the king penguin (with king penguin: $F = 3.94$, $p = .0046$; without: $F = 3.97$, $p = .0044$; contrasts between groups: T = R = W at $p > .9838$, F = D at $p = .6222$, F + D > T + R + W at $p < .0003$).

Food category affects the total feather density as well as the downy + semiplume density measured on the ventral side with, or without, the king penguin data, as species with high food protein content had higher densities of these feather types than species with low food protein content (Table 1b). The effect of food on feather density remains consistent after controlling for the presence of afterfeathers on contour feathers (Table S1).

3.2 | Effects of ecological traits on $\Delta_{\text{feather mass}}$ and $\Delta_{\text{feather density}}$

Overall differences in all feather traits between ventral and dorsal sides of the body are non-significant (phylogenetic paired t tests, feather mass: $t = 0.00$, $p = 1.0000$; total feather density: $t = 0.00$, $p = 1.0000$; contour feather density: $t = 0.00$, $p = 1.0000$; downy + semiplume density: $t = 0.64$, $p = .5243$; $N = 147$), indicating no general cross-species dorsoventral difference in feather traits. Habitat influences $\Delta_{\text{feather mass}}$ in the models with and without the king penguin, although there was no clear trend between groups (contrasts between groups: W < F and D at $p < .01$, all other contrasts at $p > .05$). No other dorsal-to-ventral differences are influenced by habitat. $\Delta_{\text{feather mass}}$ is related to food in the models with and without the data of the king penguin (Table 2a), indicating that species with higher protein content in their diet have higher feather mass on their dorsal than on their ventral side. $\Delta_{\text{feather density}}$ is not influences by any predictors (Table 2b).

4 | DISCUSSION

4.1 | Feather traits and habitat

We found that habitat does not affect feather mass per unit area across species. This result is counter-intuitive as our initial

TABLE 1 Minimum adequate PGLS models used to investigate relationships between feather mass (a), total feather density and density of different body feathers (b) on the ventral and dorsal sides of the body, in relation to 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 (see text for details). Temperature variables are included as second-order orthogonal polynomials. Exponent at the *F* value of these two variables indicate whether both the linear and quadratic (l + q) or only the linear (l) component of polynomial is retained in the minimal model after model selection

		Models including king penguin				**Models without king penguin**			
		Ventral		**Dorsal**		**Ventral**		**Dorsal**	
	df	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***
(a)									
Feather mass		Pagel's λ = 0.50, *N* = 148		Pagel's λ = 0.60, *N* = 151		Pagel's λ = 0.49, *N* = 147		Pagel's λ = 0.54, *N* = 150	
Body mass	1	37.80	<.0001	2.17	.1432	32.38	<.0001	0.43	.5123
Minimum winter temperature	1	20.62	<.0001	22.30	<.0001	20.81	<.0001	23.92	<.0001
(b)									
Total feather density		Pagel's λ = 0.91, *N* = 148		Pagel's λ = 0.60, *N* = 151		Pagel's λ = 0.82, *N* = 147		Pagel's λ = 0.61, *N* = 150	
Body mass	1	198.79	<.0001	207.17	<.0001	211.75	<.0001	223.20	<.0001
Habitat	4	7.91	<.0001	6.82	<.0001	10.18	<.0001	7.36	<.0001
Maximum summer temperature (linear + quadratic effects)	1 or 2	10.27^{l}	.0017	13.12^{l+q}	<.0001	–		6.66^{l}	.0109
Minimum winter temperature (linear + quadratic effects)	1 or 2	7.00^{l+q}	.0013	4.35^{l+q}	.0146	7.81^{l+q}	.0006	4.75^{l+q}	.0100
Food	1	6.29	.0133	–		6.01	.0155	–	
Contour feather density		Pagel's λ = 0.91, *N* = 148		Pagel's λ = 0.77, *N* = 151		Pagel's λ = 0.90, *N* = 147		Pagel's λ = 0.79, *N* = 150	
Body mass	1	296.85	<.0001	242.64	<.0001	322.72	<.0001	268.86	<.0001
Habitat	4	4.51	.0019	–		4.14	.0034	–	
Maximum summer temperature (linear + quadratic effects)	1 or 2	11.29^{l}	.001	11.99^{l+q}	<.0001	4.77^{l}	.0306	–	
Minimum winter temperature (linear + quadratic effects)	1 or 2	4.27^{l+q}	.0158	–		4.37^{l+q}	.0145	8.74^{l}	.0036
Downy + semiplume feather density		Pagel's λ = 0.60, *N* = 148		Pagel's λ = 0.72, *N* = 151		Pagel's λ = 0.61, *N* = 147		Pagel's λ = 0.69, *N* = 150	
Body mass	1	41.56	<.0001	45.49	<.0001	46.22	<.0001	50.65	<.0001
Habitat	4	6.67	.0001	5.18	.0006	8.62	<.0001	7.13	<.0001
Maximum summer temperature	1 or 2	7.94^{l+q}	.0056	9.30^{l}	.0027	–		–	
Minimum winter temperature (linear + quadratic effects)	1 or 2	7.3^{l+q}	.001	3.6^{l+q}	.0298	15.47^{l}	.0001	7.56^{l}	.0067
Food	1	16.63	.0001	–		14.38	.0002	–	

prediction was that plumage should be heavier in aquatic birds, particularly in divers, because of enhanced insulatory and water resistance capabilities (Davenport et al., 2009; Williams et al., 2015). The density of all feather types was generally higher in aquatic compared to terrestrial birds, which indicates that habitat affects not only the density of water-repellent contour feathers, but also the thermoinsulative downy layer. The water resistant outer plumage layer is composed of short, overlapping contour feathers in aquatic birds (Eadie & Ghosh, 2011; Grémillet et al., 2005; Pap et al., 2017; Rijke & Jesser, 2011), and the resistance of this layer is further

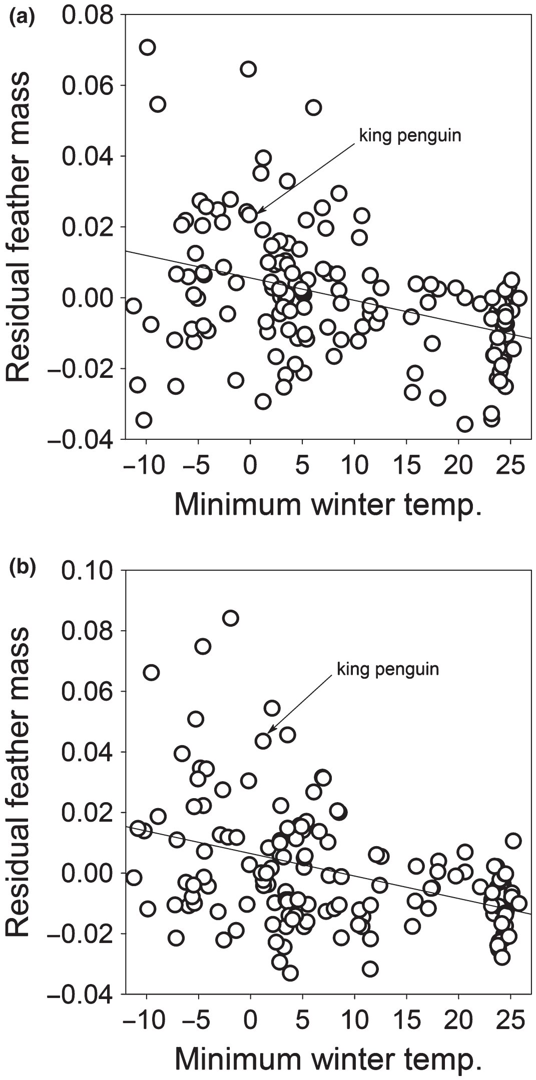

FIGURE 1 The relationship between residual feather mass corrected for body mass and minimum winter temperature, measured on ventral (a) and dorsal (b) sides of the body. Lines are linear regressions without the king penguin's data with the equations $y = 0.0056 - 0.0006x$ (a), and $y = 0.0065 - 0.0007x$ (b)

enhanced by the high vane density of the distal pennaceous parts of contour feathers, which prevents the infiltration of water into deeper plumage layers (Pap et al., 2017; Rijke, 1970; Rijke & Jesser, 2011). This result, that aquatic birds possess a higher density of contour feathers compared with their terrestrial counterparts, corroborates the earlier finding of Pap et al. (2017) regarding structural modifications of the vane of contour feathers, and further demonstrates the presence of a complex set of plumage adaptations correlated with aquatic lifestyle in birds. In line with this, we found that the density of downy feathers on both body sides, and the ratio of downy + semiplume to total feather density on the dorsal side of the body was higher in aquatic than in terrestrial species, which is also expected if we consider the high thermal conductivity of water. The high density of downy feathers and semiplumes may also enhance compression resistance of this layer during swimming or diving (Kooyman et al., 1976), although these characteristics in aquatic birds probably replace air-filled spaces in the plumage by a keratin matrix. Because heat flow through avian plumage is the result of approximately equal contributions of thermal conduction through the feather elements and the sum of conductive and convective heat transfer through encapsulated air (Walsberg, 1988), insulating properties are likely not compromised. Furthermore, because the density of keratin is much higher than air, it follows that aquatic birds are able to minimize upthrust whilst keeping heat loss at a minimum (Grémillet et al., 1998).

Our results did not reveal any differences in feather density between divers and non-diving birds, a surprising outcome if we consider the higher thermal stress and plumage compression that is encountered when swimming underwater compared to surface feeding (De Vries & van Eerden, 1995; Grémillet et al., 2001; Wilson et al., 1992). This result implies that floaters and divers are subject to the same thermal stresses and water pressure as the functional morphology of their plumage remains unchanged irrespective of environment, and is further supported by the fact that there are no differences between the contour feather morphology of diving and non-diving birds (Pap et al., 2017). In addition, we show that there is a similar density of body feathers on the ventral and dorsal body surfaces of all species irrespective of habitat categories. However, the adaptation to diving exists at least at microscopic level of the downy feathers, in the barbule morphology, indicating that feather morphology, and less the density of the plumage ensure the enhanced repellency and insulation capacity of the diving birds (Dove & Agreda, 2007).

4.2 | Feather traits and temperature

Our results show that feather mass and density of downy + semiplume feathers, and the ratio of downy + semiplume to total feather density on the ventral side of the body, were all negatively correlated with minimum winter temperature, corroborating the prediction that a dense plumage plays a role in thermal insulation (Cooper, 2002; Middleton, 1986; Novoa et al., 1994; Swanson, 1991; Williams et al., 2015). However, because we were unable to weigh different types of body feathers separately, we could not determine the specific effects of temperature. It therefore remains to be seen which specific feather types evolved to enhanced thermal insulation. The high density of downy feathers of species wintering under cold conditions support our prediction that the downy plumage layer has high insulating properties (Williams et al., 2015). The high density of contour feathers on the dorsal side of the body in species living in cold areas is also intriguing and shows that, in addition to the downy plumage layer, these may serve a similar function in thermoinsulation (Butler et al., 2008; Pap et al., 2017). Morphological adaptation of the contour feathers include the proximal plumulaceous part of the vane, in addition to a well-developed afterfeather (Osváth et al., 2017) in some species. It is thus not surprising that our results show that the density of contour feathers is higher in species that winter in colder areas; to the

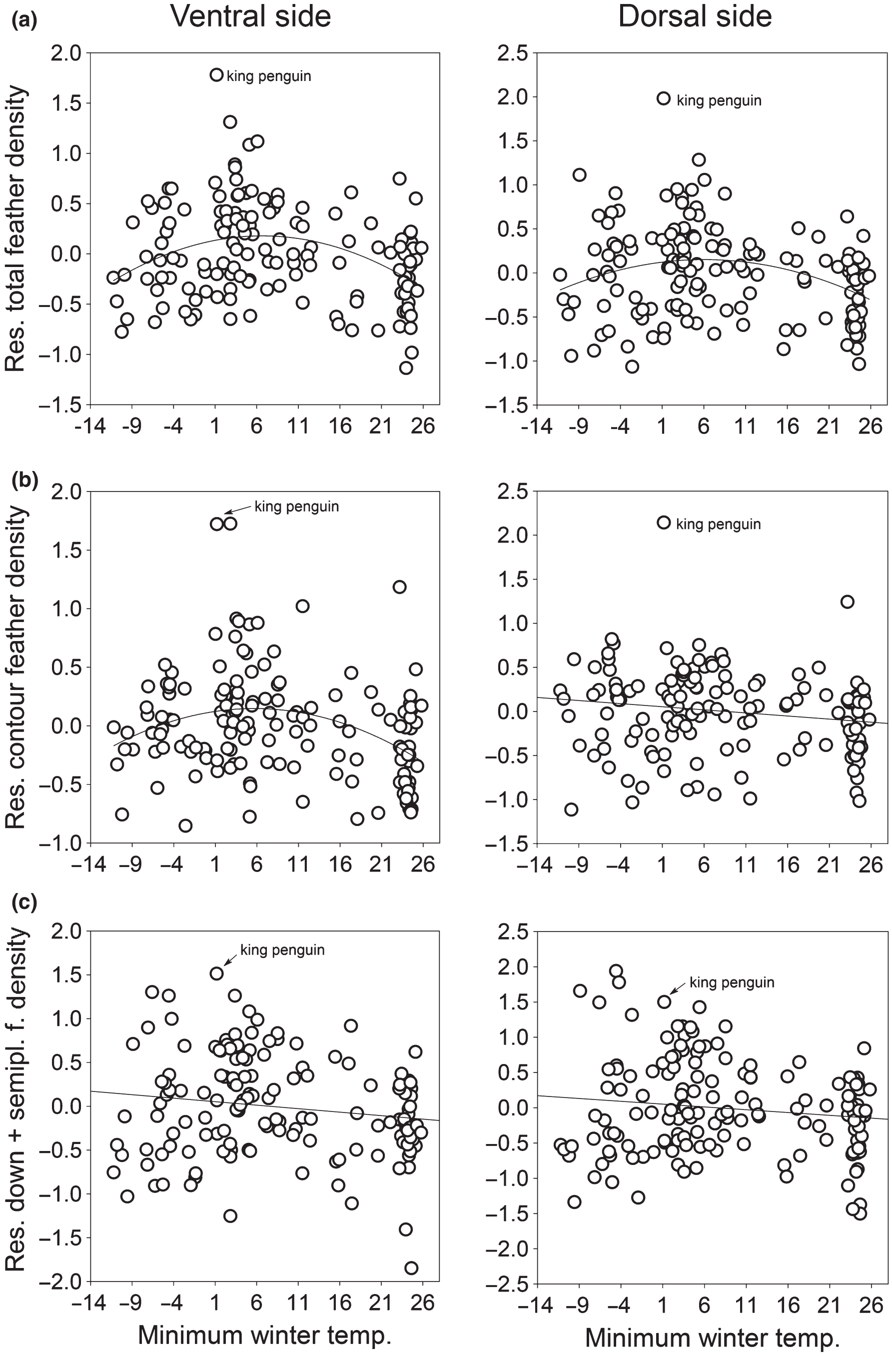

FIGURE 2 The relationship between: total (a), contour (b), and downy + semiplume (c) feather density on ventral and dorsal sides of the body and minimum winter temperature. Lines are quadratic regressions with the equations $y = 0.1174 + 0.0185x - 0.0014x^2$ (a) and $y = 0.1119 + 0.0126x - 0.0011x^2$ (b), and linear regression with the equation $y = 0.0604 - 0.008x$ (c) for the ventral side of the body, and quadratic regression with the equation $y = 0.1094 + 0.014x - 0.0012x^2$ (a), and linear regressions with the equations $y = 0.0611 - 0.007x$ (b) and $y = 0.0604 - 0.008$ (c) for the dorsal side of the body. Regression lines are without the king penguin's data

best of our knowledge, this study presents the first multi-species evidence for the thermoinsulatory function of this trait. We found, however, that the total density of feathers measured on both sides of the body and the density of contour feathers on the ventral side are quadratically related with minimum winter temperature, showing higher feather densities in species wintering under intermediate climates compared to species wintering in colder or hotter climates. Our results suggest that heat retention and heat transfer can be ensured at the same time by low total feather density on the ventral and dorsal sides and by the contour feather density on the ventral side of the body. This relationship between feather density and minimum winter temperature can be related to the variation in the number of air vacuoles in function of these feather traits, whereas the amount of air trapped in the plumage may remain unchanged. This finding indicates that species living in warm or cold environment may show the same adaptation in plumage traits, however, further measurements of feather traits and heat transfer are important to verify this assumption.

We found weak support for the prediction that birds living in warmer environments can reduce heat absorption at the surface of their plumage to prevent it from reaching the skin, or increase heat transfer from the body to the environment, at least through structural

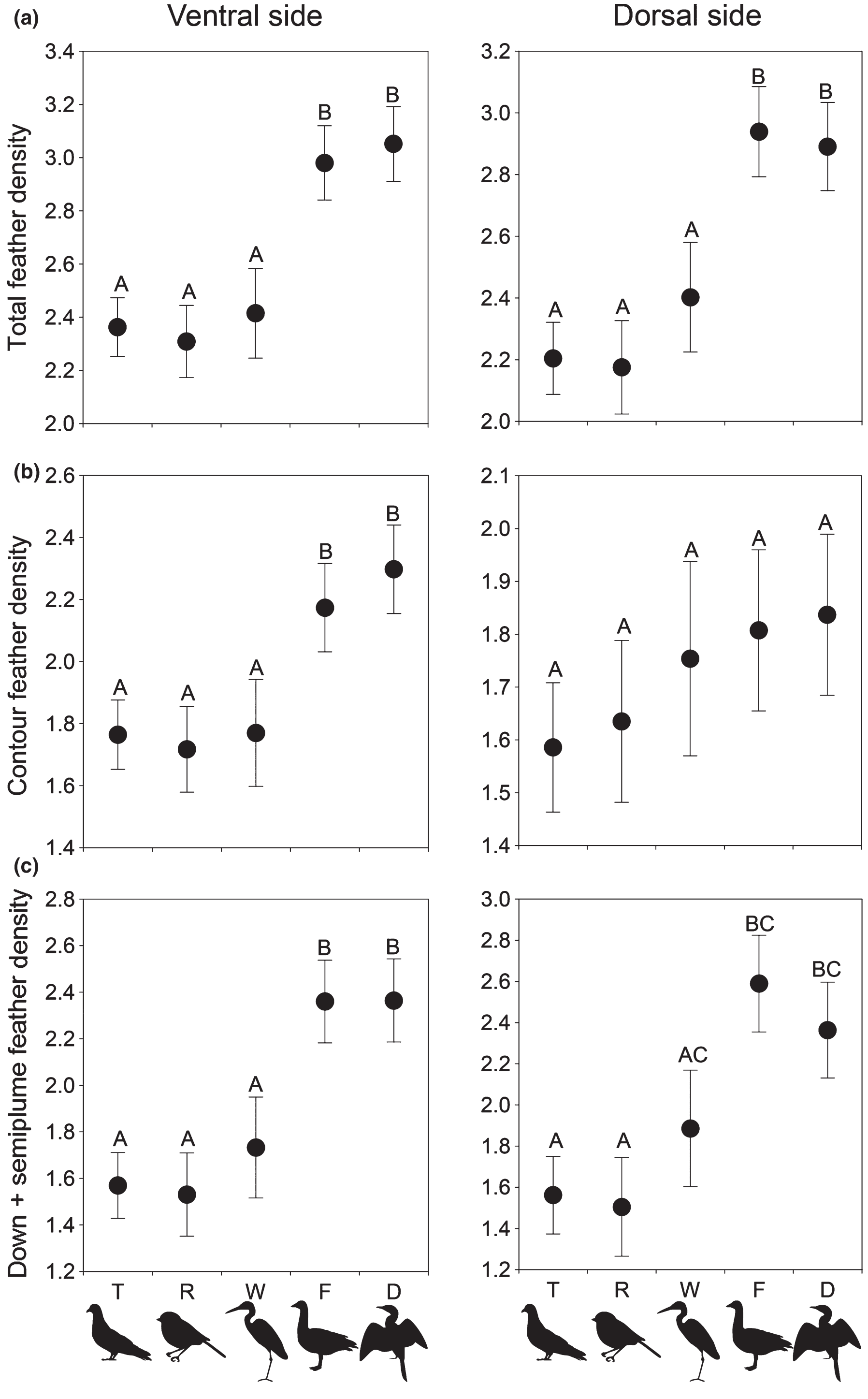

FIGURE 3 The relationship between residual total (a), contour (b) and downy + semiplume (c) feather density on ventral and dorsal sides of the body corrected for body mass (extracted from a single-predictor model controlled for phylogeny) and habitat type (T, terrestrial; R, riparian; W, wading; F, floating; D, diving). Different letters denote significant differences at $p < .05$, as indicated by PGLS models. Values are predicted *M* ± *SE* derived from the models presented in Table 1b without the king penguin's data. Silhouettes are from PhyloPic (http://phylopic.org), contributed by various authors under Public domain licenses

changes of the plumage (Walsberg & King, 1978). Our results weakly support the idea that birds living in warmer areas, as characterized by maximum breeding temperature, have less dense plumage, consistent with an earlier multi-species study that showed that the morphology of individual body feathers is unrelated to the maximum breeding temperature (Pap et al., 2017). A reduced, or completely absent, role for plumage in heat dissipation might be related to the fact that avian skin has a limited capacity for evaporative heat transfer, something that is probably compensated for by other heavily vascularized appendices, including the bill (Tattersall, Arnaout, & Symonds, 2016), and other behavioural or physiological thermoregulatory adaptations seen in birds living in hot climes (Nilsson, Molokwu, & Olsson, 2016; Nord & Williams, 2015). However, it is important to note that our species pool encompass mostly cold adapted temperate breeding birds, which limit our interpretation about the function of bird plumage to reduce heat absorption under warm conditions. Further investigation including warm adapted species will definitely clarify this issue.

TABLE 2 Minimum adequate PGLS models used to investigate the relationships between differences in feather mass on ventral and dorsal sides of the body (Δ; a), and total feather density and density of different body feather types (b) on the ventral and dorsal sides of the body in relation to 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 (see text for details)

		Models including king penguin		Models without king penguin	
	df	*F*	*p*	*F*	*p*
(a)					
Feather mass		Pagel's $\lambda = 0.56$, $N = 147$		Pagel's $\lambda = 0.54$, $N = 146$	
Body mass	1	16.09	<.0001	20.15	<.0001
Habitat	4	3.84	.0054	4.24	.0029
Food	1	8.98	.0032	10.31	.0016
(b)					
Total feather density		Pagel's $\lambda = 0.18$, $N = 147$		Pagel's $\lambda = 0.18$, $N = 146$	
Body mass	1	6.21	.0138	6.42	.0124
Contour feather density		Pagel's $\lambda = 0.42$, $N = 147$		Pagel's $\lambda = 0.42$, $N = 146$	
Body mass	1	0.21	.6503	0.26	.6111
Downy + semiplume feather density		Pagel's $\lambda = 1.01$, $N = 147$		Pagel's $\lambda = 0.51$, $N = 146$	
Body mass	1	5.17	.0245	1.66	.2001
Food	1	4.50	.0356	–	

4.3 | Feather traits and diet

We found that birds that feed mainly on plants have lower feather density than those that feed on insects and vertebrates, corroborating former studies that have suggested that protein limits feather synthesis (Butler et al., 2008; Danner et al., 2015; Dawson et al., 2000; Pap et al., 2008). Intraspecific studies have also demonstrated the presence of a protein limitation on feather synthesis during growth, which shows that given the range of food compositions naturally present in avian diets, feather quality largely depends on ingested food (Dawson et al., 2000). The results of this study suggest that the density of body feathers per unit area is limited across species by food type, possibly suggesting the presence of species-level constraints on food protein content which links with keratin synthesis during feather growth. Earlier comparative studies on feather morphology (Pap et al., 2015, 2017) suggested that type of food has no effect on feather structural traits. Therefore, it appear that across multiple species food quality is associated with the amount and density of feathers but not their structural properties.

Our comprehensive study is the first to explore how natural variation in the amount and density of body feathers in birds relates to lifestyle and the environment. We present the result of a series of tests to determine how habitat and thermal condition affect feather mass, a proxy of the amount of keratin invested per unit area, as well as the density of body feathers measured on the ventral and dorsal sides of the body, using a phylogenetic comparative analysis of 152 bird species. The results of this study demonstrate, for the first time, that body feathers mass, as well as the density of downy + semiplume feathers in birds is higher when environmental conditions are colder. Remarkably, we also show that the density of contour and downy feathers, measured on both sides of the body, is higher in aquatic than in terrestrial birds, whereas diving behaviour does not select for further increases in body feather mass and density.

This comparative study provides a basis for understanding the evolution of variation in body feather characteristics. Although our initial results are tantalizing, more work will be required if we are to understand the factors underlying the evolution of the diverse range of feathers seen in living birds. Future work will extend this study to directly measure the thermal capacity and conductivity of the plumage under various conditions, as well as in air and in water, to correlate these values with plumage traits.

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CONFLICT OF INTEREST

The authors declare they have no conflicts of interests.

AUTHORS' CONTRIBUTIONS

P.L.P. and G.O. designed the research; G.O., T.D., T.F., A.N., J.P., D.V. and C.I.V. collected the data; O.V., P.L.P. and G.O. analysed the data.

G.O., P.L.P. and G.D. wrote the paper with significant contribution from all co-authors. All authors discussed the results and gave final approval for publication.

DATA ACCESSIBILITY

Data associated with this study are available from the Dryad Digital Repository https://doi.org/10.5061/dryad.40r6p (Osváth et al., 2017).

ETHICAL STATEMENT

All feather data were collected from birds that died accidentally or due to natural causes.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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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

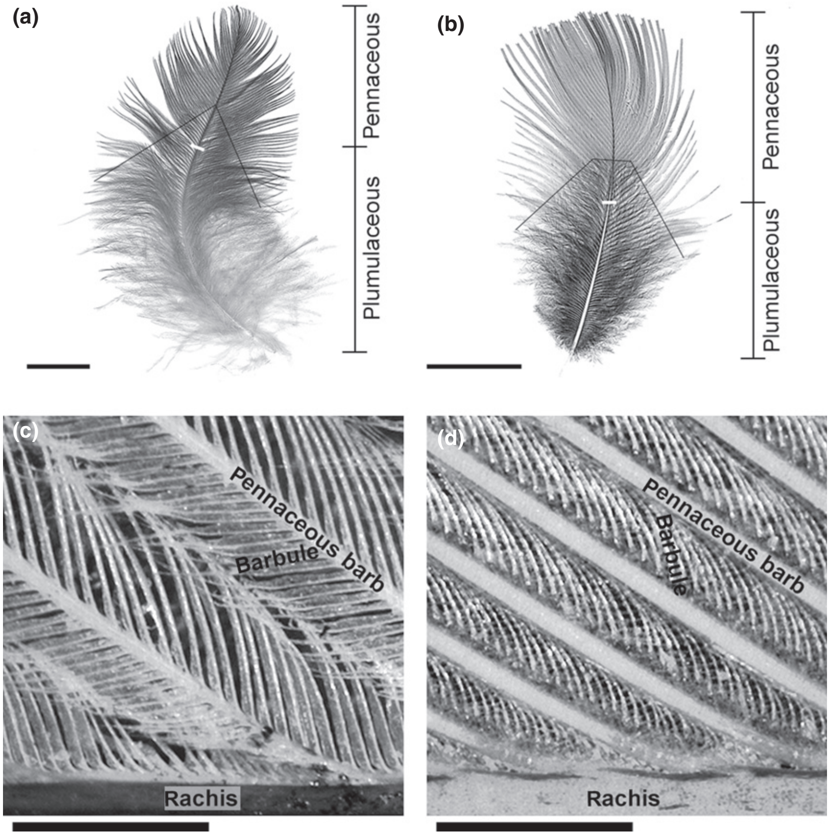

Fig. 1. Different parts of a body feather with distal pennaceous and proximal plumulaceous sections delimited by a white stripe across the rachis at the base of the barb in which the length was at least 33% plumulaceous. The black lines define the boundary of the pennaceous and plumulaceous sections 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 Babeș-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; plumulacous barb density: ICC = 0·79, 0·62–0·86; pennaceous barbule density: ICC = 0·94, 0·85–0·97; all $F > 5{\cdot}83$, $N = 16$, all $P < 0{\cdot}001$].

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 (Lindenfors, Revell & Nunn 2010) show that feather parameters are very similar between the two age classes (in all cases $t < 0{\cdot}01$, $N_{\mathrm{barb}} = 88$, $N_{\mathrm{barbule}} = 87$, $\lambda > 0{\cdot}92$, $P > 0{\cdot}9952$). 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: $Z = 1{\cdot}06$, $N = 24$, $P = 0{\cdot}2905$; pennaceous length: $Z = 0{\cdot}60$, $N = 24$, $P = 0{\cdot}5485$; plumulaceous length: $Z = 1{\cdot}34$, $N = 24$, $P = 0{\cdot}1739$; total barb density: $Z = 1{\cdot}20$, $N = 24$, $P = 0{\cdot}2301$; pennaceous barb density: $Z = 1{\cdot}74$, $N = 24$, $P = 0{\cdot}0814$; plumulacous barb density: $Z = 0{\cdot}26$, $N = 24$, $P = 0{\cdot}7971$; pennaceous barbule density: $Z = 0{\cdot}77$, $N = 24$, $P = 0{\cdot}4445$). Therefore, our results are not biased by degradation of feathers collected from either recently dead or museum specimens. Means ± 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: $t = 0{\cdot}70$, $N = 194$, $P = 0{\cdot}4824$), but pennaceous barb density was lower than plumulaceous barb density ($t = 6{\cdot}92$, $N = 194$, $P < 0{\cdot}0001$).

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: $\lambda = 0{\cdot}91$, $P < 0{\cdot}0001$; pennaceous length: $\lambda = 0{\cdot}76$, $P < 0{\cdot}0001$; plumulaceous length: $\lambda = 0{\cdot}75$, $P < 0{\cdot}0001$; total barb density: $\lambda = 0{\cdot}66$, $P < 0{\cdot}0001$; pennaceous barb density: $\lambda = 0{\cdot}70$, $P < 0{\cdot}0001$; plumulaceous barb density: $\lambda = 0{\cdot}67$, $P < 0{\cdot}0001$; pennaceous barbule density: $\lambda = 0{\cdot}91$, $P < 0{\cdot}0001$).

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.	*F*	*P*		d.f.	*F*	*P*
Body feather total length (Pagel's λ = 0·77, *N* = 194)				Total barb density (whole feather) (Pagel's λ = 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 λ = 0·82, *N* = 194)				Pennaceous barb density (Pagel's λ = 0·78, *N* = 194)			
Body mass	1	241·21	<0·0001	Body mass	1	5·4	0·0211
Plumulaceous length (Pagel's λ = 0·66, *N* = 194)				Plumulaceous barb density (Pagel's λ = 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 λ = 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

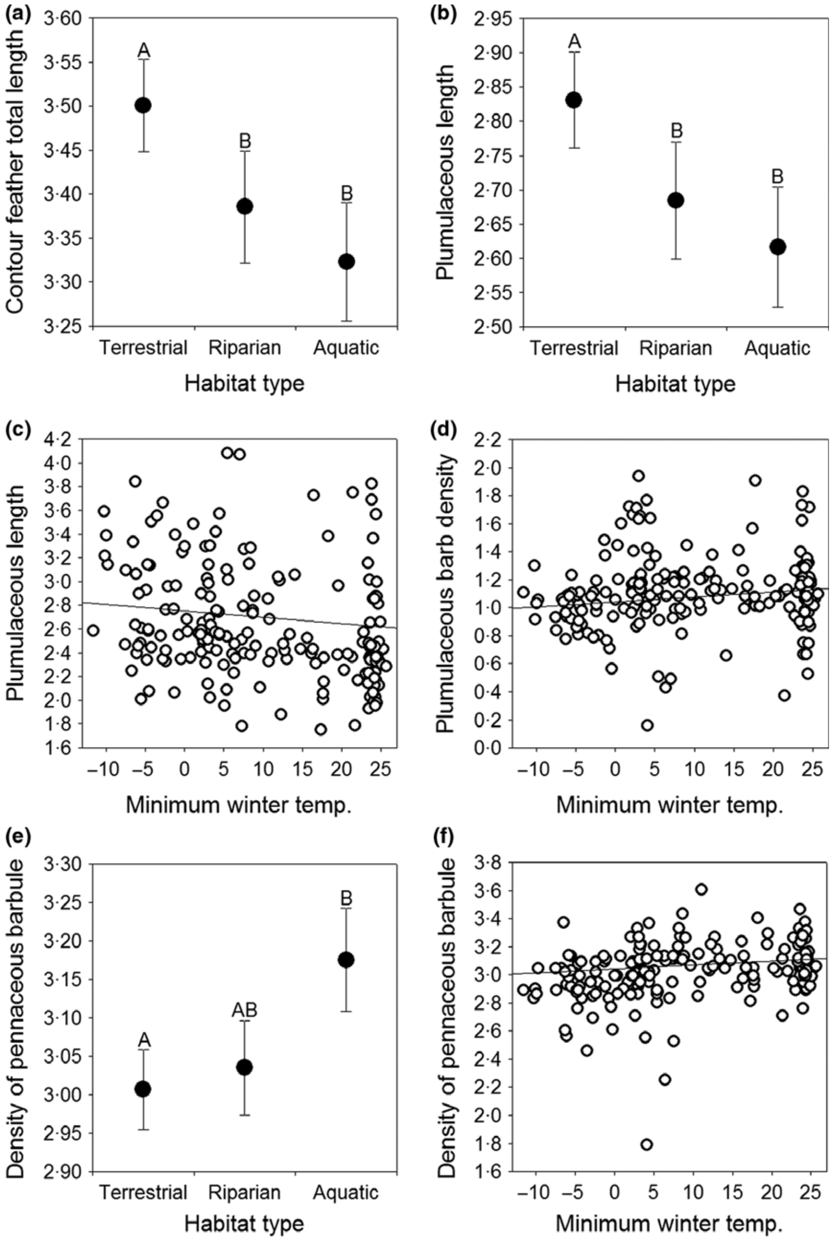

Fig. 2. The relationship between habitat type, minimum wintering temperature, and feather morphological variables. Different letters denote significant differences at $P < 0{\cdot}05$, as indicated by the phylogenetic generalized least squares models. The sample size for the terrestrial, riparian, and aquatic groups are 116, 22 and 56, respectively. The values from figures a and b are shown as mean ± 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.	*F*	*P*	*F*	*P*
		Body feather total length (Pagel's λ = 1·00, *N* = 56)		Total barb density (whole feather) (Pagel's λ = 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 λ = 0·99, *N* = 56)		Pennaceous barb density (Pagel's λ = 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 λ = 0·88, *N* = 56)		Density of pennaceous barbules (Pagel's λ = 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 λ = 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.

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Down feather morphology reflects adaptation to habitat and thermal conditions across the avian phylogeny

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Down feathers are the first feather types that appear in both the phylogenetic and the ontogenetic history of birds. Although it is widely acknowledged that the primary function of downy elements is insulation, little is known about the interspecific variability in the structural morphology of these feathers, and the environmental factors that have influenced their evolution. Here, we collected samples of down and afterfeathers from 156 bird species and measured key morphological characters that define the insulatory properties of the downy layer. We then tested if habitat and climatic conditions could explain the observed between-species variation in down feather structure. We show that habitat has a very strong and clearly defined effect on down feather morphology. Feather size, barbule length and nodus density all decreased from terrestrial toward aquatic birds, with riparian species exhibiting intermediate characters. Wintering climate, expressed as windchill (a combined measure of the ambient temperature and wind speed) had limited effects on down morphology, colder climate only being associated with higher nodus density in dorsal down feathers. Overall, an aquatic lifestyle selects for a denser plumulaceous layer, while the effect of harsh wintering conditions on downy structures appear limited. These results provide key evidence of adaptations to habitat at the level of the downy layer, both on the scale of macro- and micro-elements of the plumage. Moreover, they reveal characters of convergent evolution in the avian plumage and mammalian fur, that match the varying needs of insulation in terrestrial and aquatic modes of life.

KEY WORDS: Afterfeather, down, functional morphology, insulation, water resistance, wind chill.

Bird feathers are one of the most remarkable biological structures. They are extremely lightweight, durable, and flexible and, besides their key roles in flight, sexual display, and camouflage, also serve as outstanding thermal insulators (McCafferty et al. 1997, McCafferty et al. 2013; Grémillet et al. 1998; Dawson et al. 1999; Prum and Brush 2002; Fuller 2015). The plumage, when combined with appropriate thermophysiological responses (Blix 2016), enables birds to survive in some of the harshest environments on Earth, and to sustain a constant high core body temperature even when ambient temperatures drop below –40°C, when exposed to strong winds, and when diving in icy polar waters (e.g., Fahlman et al. 2005; McCafferty et al. 2013; Blix 2016). Most of the insulation is contributed by down feathers (Lucas and Stettenheim 1972; Wolf and Walsberg 2000; Prum and Brush 2002). These provide the lowest warmth-to-weight ratio of all natural fibers known to science, and are able to provide similar insulation to mammalian hair with only half the mass of the latter (Fuller 2015). Besides these advantages, down feathers

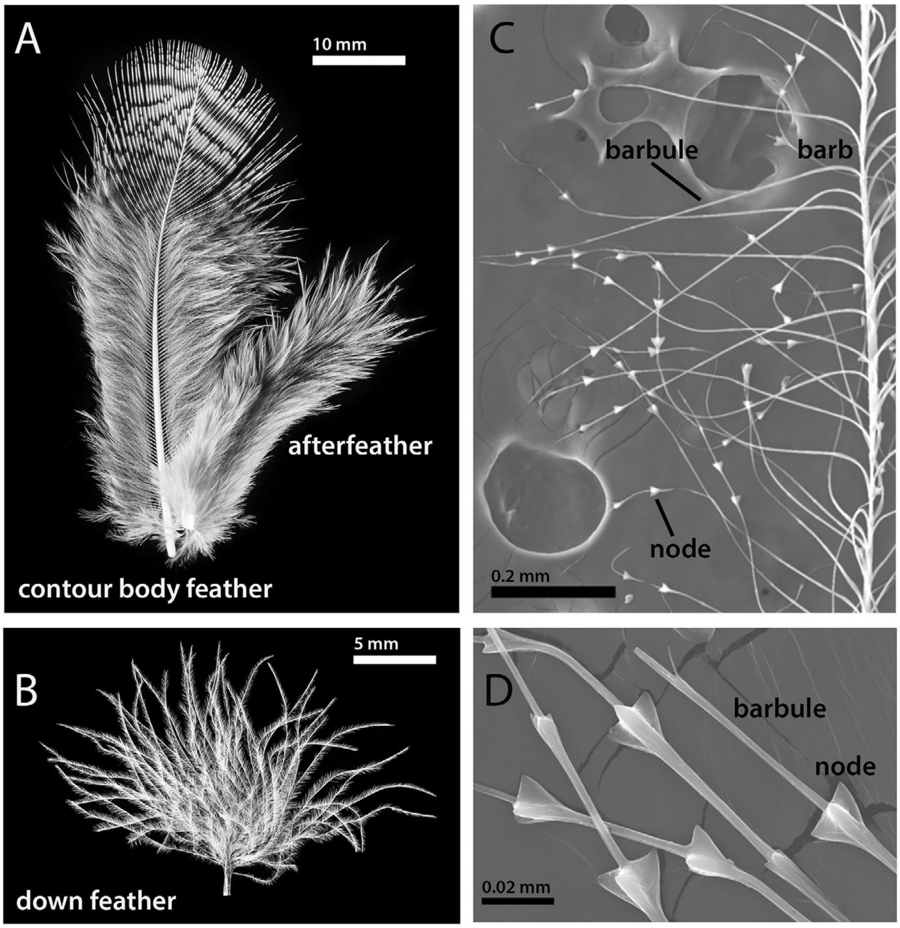

Figure 1. A typical afterfeather attached to a contour feather (A), and a typical down feather (B), from the back of a grey partridge (*Perdix perdix*) and a great white pelican (*Pelecanus onocrotalus*), respectively. Figures C and D illustrate a section of down from the belly of a tufted duck (*Aythya fuligula*) with the rachis and barb to which the barbules attach, and the nodes on the barbules.

also have impressive structural resilience, enabling them to regain their original configuration after compression, such as when birds are diving to great depths (Fuller 2015). Being known from both pterosaurs and non-avian dinosaurs, down feathers potentially appeared as far back as 250 million years ago (Benton et al. 2019; Yang et al. 2019), coinciding with the evolution of higher metabolic rates and endothermy in feathered dinosaurs and early birds. This suggests that the emergence of downy feathers might be key to the evolutionary success of the avian clade, by contributing to the maintenance of homeothermy with low metabolic costs.

The external layer of the bird plumage, that is, the distal ends of the contour feathers, has the primary function of defining coloration and protecting the inner, insulating layer of the plumage against solar radiation, wind, and water (Prum and Brush 2002). The inner insulating layer is composed of proper down feathers, semiplumes, and the proximal downy parts of the contour feathers. In addition, many species have an auxiliary downy or semiplume-like shaft on the contour feathers, which are referred to as "afterfeathers" (Prum and Brush 2002; Osváth et al. 2018). The role of the afterfeather is not clear, but because it has structural properties reminiscent of true down, it has been proposed to function primarily in insulation (Williams et al. 2015). Unlike contour feathers, down feathers have detached barbs that branch into long, soft, and filamentous barbules that lack interlocking hooklets (Figure 1; Lucas and Stettenheim 1972; Prum and Brush 2002). Their barbules are composed of a series of filiform cells, which are interlocked by nodes that vary both in shape and size (Dove 2000; Figure 1). These microstructures ensure that the downy layer remains a loosely packed, lofty matrix that retains warm insulating air close to the body, even when under pressure from wind or water (Walsberg 1988; Wilson et al. 1992). This allows warm air produced by the body to be retained within the plumage, thus aiding thermoregulation (Fuller 2015; D'Alba et al. 2017).

Earlier studies suggest that the insulatory properties of the plumage in aquatic and terrestrial birds might have evolved differently (Dove and Agreda 2007; Pap et al. 2017; Osváth et al.

2018). For instance, it has been shown that aquatic life selects for shorter feathers with short downy elements but longer and denser barbules (Dove and Agreda 2007; D'Alba et al. 2017; Pap et al. 2017). This increased density of keratinous elements is probably essential to maintain insulation of the downy layer when large volume of air cannot be kept within the plumage due to its positive effect on buoyancy. Down feathers of some aquatic birds also have higher nodus density, which might increase cohesion ("stickiness") of the down, which makes it more resistant to pressure from water (Fuller 2015; D'Alba et al. 2017). Nonetheless, it was argued that for better insulation a lofty, less dense plumage (higher fill power; lower barbule density and shorter barbule length) is needed, since feathers with higher fill power trap more air (Fuller 2015; D'Alba et al. 2017). On the other hand, down with lower cohesion (i.e., reduced contact points at barbule nodes) might facilitate diving at the cost of lowered insulation, by increasing expulsion of air from the plumage while underwater (Dove and Agreda 2007; Fuller 2015). The effect of habitat on downy feather microstructure thus remains ambiguous (Dove and Agreda 2007; Fuller 2015; D'Alba et al. 2017; Pap et al. 2017). Ambient environment also affect the plumage depth and the density of feathers per unit skin area. For example, harsh temperatures during winter are associated with higher downy feather densities per unit skin area (Osváth et al. 2018). Moreover, the contour feather length and the plumulaceous section of contour feathers is longer in species wintering under cold environtmental conditions (Pap et al. 2017). It is however little knwon how the structural morphology of down feathers changes along environmental gradients such as habitat or ambient thermal conditions, but diverse taxa need to be samples to assess such patterns. Nonetheless, previous studies investigating properties of down have typically used duck, goose or eider down, paying little or no attention to the great diversity of bird orders, or to the specific ambient environment these species have adapted to (Fuller 2015).

To further our understanding of the evolution of insulation in birds, we now need to undertake studies on the comparative morphology of down feathers across the avian phylogeny, particularly in relation to the ambient conditions in which different species have evolved. This could both generate new knowledge and have wide applicability, since it could inform on the design of superior biomimetic insulating materials (Du et al. 2007; Eadie and Ghosh 2011; Fuller 2015). Here, we bring new evidence to clarify the function and evolution of down feather insulation, by analyzing data from a diverse set of birds occupying different habitats and environmental conditions. Specifically, we test how habitat and thermal conditions, expressed as windchill (a combined measure of the cooling power of the environment as function of ambient temperature and wind speed) affects feather morphology. Our results provide key insights into the evolution of avian thermoregulation, and highlight the convergent evolution of the structural morphology of the pelage in birds and mammals, the two homeothermic vertebrate lineages.

Methods

FIELD DATA COLLECTION AND FEATHER MEASUREMENTS

We collected feather samples from 370 birds encompassing 156 species. Age of each individual was assessed based on plumage characteristics and only individuals in adult plumage, or full-grown birds with no signs of juvenile plumage, were sampled. Feathers were collected all year round and most sampled individuals were of unknown exact ages. Note, however, that our former studies indicated that neither sampling period nor age affects any of the measured feathers traits (Osváth et al. 2018). Moreover, interspecific repeatability of each feather traits measured here was moderate to high ($0.28 \leq r \leq 0.90$ in all cases; see below), indicating limited within-species, and large cross-species variation in the measured traits. Samples were only collected from non-molting individuals and from birds in apparently good body condition, with no apparent signs of sickness or starvation, usually from fresh carcasses found between 2012 and 2016 at several locations across Romania, Hungary, Norway, Sweden, Scotland, or on the sub-Antarctic Crozet Islands archipelago (king penguin, *Aptenodytes patagonicus*). Most individuals died due to natural causes (e.g., road kill, building collision, electrocution, starvation) or were procured from authorized hunters (Osváth et al. 2018). All birds were preserved at −20°C until feather collection in 2016. These samples were complemented with samples from museum specimens belonging to the collection of the Zoological Museum of Babeș-Bolyai University, Cluj-Napoca, Romania. From each handled individual, down feathers, semiplumes, and afterfeathers of contour feathers were collected, separately from the ventral and the dorsal sides of the body. We use the data of down and afterfeathers only because they are similar in structure and are most abundant, while semiplumes are less common and therefore probably contribute less to insulation (Osváth et al. 2018). Ventral feathers were selected from a randomly selected area delimited by the breastbone, the contact point of the wing and leg to the body. Dorsal feathers were collected from a randomly selected area delimited by the shoulders and the tail end of the synsacrum and the sides (see Osváth et al. 2018). Feather samples were stored dry in small zip-lock plastic bags at room temperature until measurements took place in 2018. None of the feathers showed any sign of degradation at the time when photographs and measurements were taken.

To quantify structure, feathers were placed on a metric grid background, or against a stage micrometer and were photographed using a Nikon D80 camera. Photos were imported into

ImageJ (version 1.37, http://rsb.info.nih.gov/ij/) and the following parameters were measured: feather area and length, barb and barbule length, barbule density as well as nodus density on barbules. For each individual, the contour of one randomly selected down and one afterfeather was used to measure the area of the feathers. Length was measured from two randomly selected and unbroken barbs and barbules. These values were averaged per individual for analyses. Barbule density was measured along a 1 cm barb length, and the density of nodes was measured along a 1 mm barbule length section. Because the magnification used did not allow us to differentiate between the two node types (D'Alba et al. 2017), we quantified node density irrespective of node type. We calculated the mean species values of each feather trait and used this value in all analyses. All measurements were performed by T.D.

BODY MASS, ECOLOGICAL TRAITS, AND HABITAT

To control for size, we obtained body mass data for each species from del Hoyo et al. (1992) and Dunning (2007), preferentially from European populations and subspecies. We classified species into one of five habitat groups: terrestrial (i.e., rarely encountering water), riparian (i.e., living in wetland habitats like marshes and sedges), waders (i.e., species that move above, and around the water surface), floaters (i.e., aquatic species that float on the water but do not dive), and divers according to Osváth et al. (2018). 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 and Jones 2005), a global dataset containing interpolated monthly average land temperatures (°C) in a grid of spatial coordinates ($0.5 \times 0.5°$). We used 10 years of temperature data (encompassing the period between 2006 and 2015, downloaded on November 3, 2016), and calculated average monthly mean temperature over this period for each spatial grid cell. Similarly to temperature data, we obtained data on wind speed from the above mentioned online source (CRU CL version 2.0; New et al. 2002). The latter database contains interpolated monthly mean wind speeds between 1961 and 1990 in high spatial resolution (i.e., 10' grid cells). We averaged mean wind speeds over the observation years separately for each month and spatial grid cell. We then created two 12-layer shape files where each layer contained temperature or wind data for a given month. By intersecting these shape files with species distributions, we calculated monthly temperatures and wind speeds for each species on their wintering grounds (Vincze 2016; Pap et al. 2017). Distribution maps (shape files) were retrieved from http://www.birdlife.org/datazone/info/spcdownload (BirdLife International and NatureServe 2015, accessed on October 27, 2016). We extracted the lowest monthly mean temperature ("minimum winter temperature") and the highest monthly wind speed ("maximum winter wind") for the period between December and February (except for the king penguin, for which the calculation was based on the whole year) as a proxy for environmental conditions during winter. Low ambient temperature exacerbates radiative, convective, and conductive heat loss rate, and increased wind speed enhances heat lost by forced convection (Monteith and Unsworth 2013). We used data on winter minimum temperatures (T;°C) and winter maximum wind speeds (V; km/h), to calculate windchill temperature for each species according to Osczevski and Bluestein (2005): wind chill temperature $= 13.12 + 0.6215T - 11.37V^{0.16} + 0.3965TV^{0.6}$. This represents the thermal sensation under a given temperature and wind condition Gómez-Acebo et al. (2010). Although originally developed for humans it is now a widely used environmental indicator in studies of various organisms (Born et al. 1999; Bouchard and Anderson 2011; Krause et al. 2016; Chaise et al. 2018). Environmental conditions were only considered during the wintering period, because our prior investigations indicated no plumage adaptations to extreme heat during breeding, neither in body feather structures nor in plumage density (Pap et al. 2017; Osváth et al. 2018). When calculating climate variables, distribution ranges for most species were only considered between longitudes 20°W and 60°E in order to have the most appropriate range representation of populations sampled for feathers within the framework of this study. In some species, restrictions had to be employed in order to exclude African resident populations or non-European wintering grounds (see list of specific restrictions in Table S3, and Osváth et al. 2018 for the same approach). All data used here are reported in Tables S1–S3.

STATISTICAL ANALYSES

First, in order to determine whether feather parameters were species-specific and suitable for multitaxon comparisons, we tested intraspecific repeatability by assessing the importance of "between-species" compared to "within-species" variance in feather traits using the "icc" function in the R package "irr" (Wolak et al. 2012). Repeatability was tested using raw, non-transformed values. All feather traits proved to be species-specific in the case of both down feathers and afterfeathers, and for both the ventral and the dorsal sides of the body (Table S4). We therefore concluded that species-specific feather traits are accurately characterized even when sample sizes were low for some species. This was further supported by the fact that weighting multivariate models (described below) by within-species sample sizes did not increase model fits, as indicated by likelihood ratio statistics. To test weather structural parameters were affected by museum storage, we conducted phylogenetic paired t-test comparing feather trait between samples of wild and museum origin. Most feather traits did not differ between wild and museum birds (Table S5), except for ventral down barb length and dorsal down and afterfeather barbule density. Note, however, that although sample size in these comparisons was very limited ($6 < n < 15$), we only detected three significant differences out of the 24 tested. On this ground we concluded that there are no general differences between museum and wild collected feathers. We used phylogenetic paired *t*-tests to test if there were directional differences in feather parameters on the two sides of the body. We then calculated the differences in traits values between ventral and dorsal feathers ($\Delta =$ ventral subtracted by dorsal), and fitted multivariate models to these data to test if these differences were influenced by ecology or climatic conditions (see below). Our a priori hypothesis was that aquatic life should have a stronger effect on ventral than dorsal feathers, because when floaters or divers are in the water, their ventral feathers are always in contact with water.

Three out of the six measured feather traits were highly correlated for both down feathers and afterfeathers (feather area, feather length, barb length), on both sides of the body (Figure S1). Therefore, we conducted a phylogenetically controlled principal component analysis (PCA) to reduce the number of variables in the data set (all three traits log-transformed) using the "phyl.pca" function of the R package "phytools" (Revell 2012). The first principal component (PC1) explained 95.50% and 96.53% of variation in ventral and dorsal down, and 91.97% and 90.96% of variation in ventral and dorsal afterfeather structure, respectively (Table S6). PC1 is negatively loaded for all feather size traits and is interpreted as the axis of feather size with lower values marking larger feather size. In order to make the interpretation of the results more straightforward, we multiplied PCs with –1, so that larger PC values correspond to larger feathers. Separate analysis of size parameters incorporated in the PCs are presented in Table S7 and Figure S2.

To investigate the relationship between feather structural parameters and body mass, habitat, and wind chill, we built phylogenetic generalized least squares (PGLS) models using ventral, dorsal, and Δ feather parameters as response variables. Body mass was included to control for size-effects on feather traits. However, because contour feather length might be more directly linked to down and afterfeather size we reran models on ventral down- and afterfeathers using ventral contour feather length as a size-correcting covariate, using a subset of data where such data were available (Pap et al. 2017). These models provided qualitatively similar results. Hence, we only present models using body mass here, as this variable was available for every species sampled. Weighting models by within-species sampling effort or the inverse of within-species variance or sample size did not increase models fits (see also Garamszegi and Møller 2010, 2011). Therefore, we present the results of unweighted models. We accounted for phylogenetic non-independence using trees from http://birdtree.org. We downloaded 100 random trees using the Hackett backbone tree (Hackett et al. 2008), and calculated a rooted, ultrametric consensus tree using the SumTrees software (Sukumaran and Holder 2010). We used this consensus tree in the PGLS model (Pagel 1997, 1999) and set the degree of phylogenetic dependence (Pagel's λ) to the most appropriate value estimated by maximum likelihood in each model separately (Freckleton et al. 2002). Habitat was entered into models as a fixed factor, while body mass (log-transformed) and wind chill were included as covariates.

All statistical analyses were conducted using R version 3.5.2 (R Development Core Team 2018), and all PGLS models were built using the "nlme" package (Pinheiro et al. 2017). Sample sizes vary across models depending on the body side (i.e., ventral or dorsal), as feathers could not be sampled on both sides of the body for some individuals. Barbule density was $\log(x)$-transformed in all statistical models to ensure model residual normality. All other traits were untransformed. Raw data means ± SE are reported in the text and tables. Data in all figures are estimated marginal means ± SE ("emmeans" package, Lenth 2019). Models of the two family of hypothesis were jointly adjusted for multiple comparison (models in Table 1, S7, S8 and in Table 2, respectively) using the Benjamini-Hochberg false discovery rate method (Benjamini and Hochberg 1995).

Results

FEATHER MORPHOLOGY, HABITAT, AND WINDCHILL

Habitat type was a strong predictor of the size (i.e., PC1 score), barbule length and nodus density of down feathers and barbule length of afterfeathers measured on the ventral side of the body (Table 1). Specifically, feather size, barbule length, and nodus density decreased from terrestrial to aquatic birds, with intermediate values seen in riparian and wading taxa (Fig. 2). Although the effect of habitat on size and nodus density of ventral afterfeathers was non-significant (Table 1), there was a gradual transition between habitat groups similar to that seen in down feathers. Barbule density of both down- and afterfeathers were unrelated to habitat type. The results were highly consistent between ventral and dorsal feathers, though the size of the afterfeather was significantly associated with habitat type on the dorsal side (Table S8, Figures S3). Note, however, that the nodus density of the dorsal afterfeather did not show a clear transition from terrestrial to diving species, as did the other feather traits (Figure S3).

Wind chill temperature was significantly associated with only one feather parameter, and the effect was rather weak (Table 1; Table S8). Dorsal nodus density of down feathers decreased with increasing wind chill ($t = \beta$ (SE) $= -0.11$ (0.04), $t = -2.54$, $P = 0.029$). This indicates that species living in cold and windy climates tended to have down feathers with higher node densities on the dorsal side of their body.

Table 1. Phylogenetic generalized least squares models of the relationships between ventral feather traits and body mass, habitat, and wind chill temperature. *P* denotes adjusted value for multiple comparison using the method of Benjamini and Hochberg (1995) false discovery rate.

		Down feather		Afterfeather	
	d.f.	*F*	*P*	*F*	*P*
$PC_{\text{Feather size}}$		Pagel's $\lambda = 0.57$, $n = 150$		Pagel's $\lambda = 0.83$, $n = 117$	
Body mass	1	**89.29**	**<0.001**	**37.87**	**<0.001**
Habitat	4	**4.89**	**0.003**	**2.60**	0.077
Wind chill temperature	1	2.51	0.190	0.53	0.541
Nodus density		Pagel's $\lambda = 0.45$, $n = 151$		Pagel's $\lambda = 0.78$, $n = 117$	
Body mass	1	**28.90**	**<0.001**	**5.74**	**0.039**
Habitat	4	**6.04**	**0.001**	1.67	0.235
Wind chill temperature	1	**4.94**	0.055	2.28	0.204
Barbule length		Pagel's $\lambda = 0.85$, $n = 151$		Pagel's $\lambda = 0.92$, $n = 117$	
Body mass	1	**3.95**	0.087	0.96	0.408
Habitat	4	**10.75**	**< 0.001**	**5.48**	**0.002**
Wind chill temperature	1	0.26	0.658	0.00	0.983
Barbule density		Pagel's $\lambda = 0.26$, $n = 151$		Pagel's $\lambda = 0.70$, $n = 117$	
Body mass	1	**7.68**	**0.016**	0.01	0.944
Habitat	4	0.40	0.836	0.63	0.685
Wind chill temperature	1	1.59	0.280	0.59	0.518

Table 2. Phylogenetic generalized least squares models used to investigate the relationships between differences in ventral and dorsal feather parameters (Δ) in relation to body mass, habitat, and wind chill temperature. *P* denotes adjusted value for multiple comparison using the method of Benjamini and Hochberg (1995) false discovery rate.

		Down feather		Afterfeather	
	d.f.	*F*	*P*	*F*	*P*
$PC_{\text{Feather size}}$		Pagel's $\lambda = 0.30$, $n = 146$		Pagel's $\lambda = 0.04$, $n = 112$	
Body mass	1	0.03	0.942	2.26	0.414
Habitat	4	1.14	0.508	1.21	0.497
Wind chill temperature	1	0.00	1.000	0.04	0.942
Nodus density		Pagel's $\lambda = 0.03$, $n = 147$		Pagel's $\lambda = 0.03$, $n = 112$	
Body mass	1	**9.58**	**0.019**	**4.17**	0.262
Habitat	4	2.16	0.371	1.40	0.442
Wind chill temperature	1	1.06	0.497	0.75	0.550
Barbule length		Pagel's $\lambda = 0.16$, $n = 147$		Pagel's $\lambda = 0.03$, $n = 112$	
Body mass	1	1.41	0.442	**11.88**	**0.010**
Habitat	4	1.59	0.442	1.51	0.442
Wind chill temperature	1	0.39	0.708	0.00	1.000
Barbule density		Pagel's $\lambda = 0.14$, $n = 147$		Pagel's $\lambda = 0.03$, $n = 112$	
Body mass	1	2.23	0.414	2.23	0.414
Habitat	4	1.52	0.442	**6.25**	**0.009**
Wind chill temperature	1	0.06	0.942	0.13	0.942

EFFECTS OF HABITAT AND WINDCHILL ON $\Delta_{\text{FEATHER MORPHOLOGY}}$

Phylogenetic paired *t*-test indicated no directional differences between ventral and dorsal feather structures of either down feathers or afterfeathers (Table S9). Habitat type significantly influenced $\Delta_{\text{barbule density}}$ of afterfeathers, but not of down feathers (Table 2). The former association suggested that barbule density of afterfeathers was higher on the dorsal than on the ventral sides in

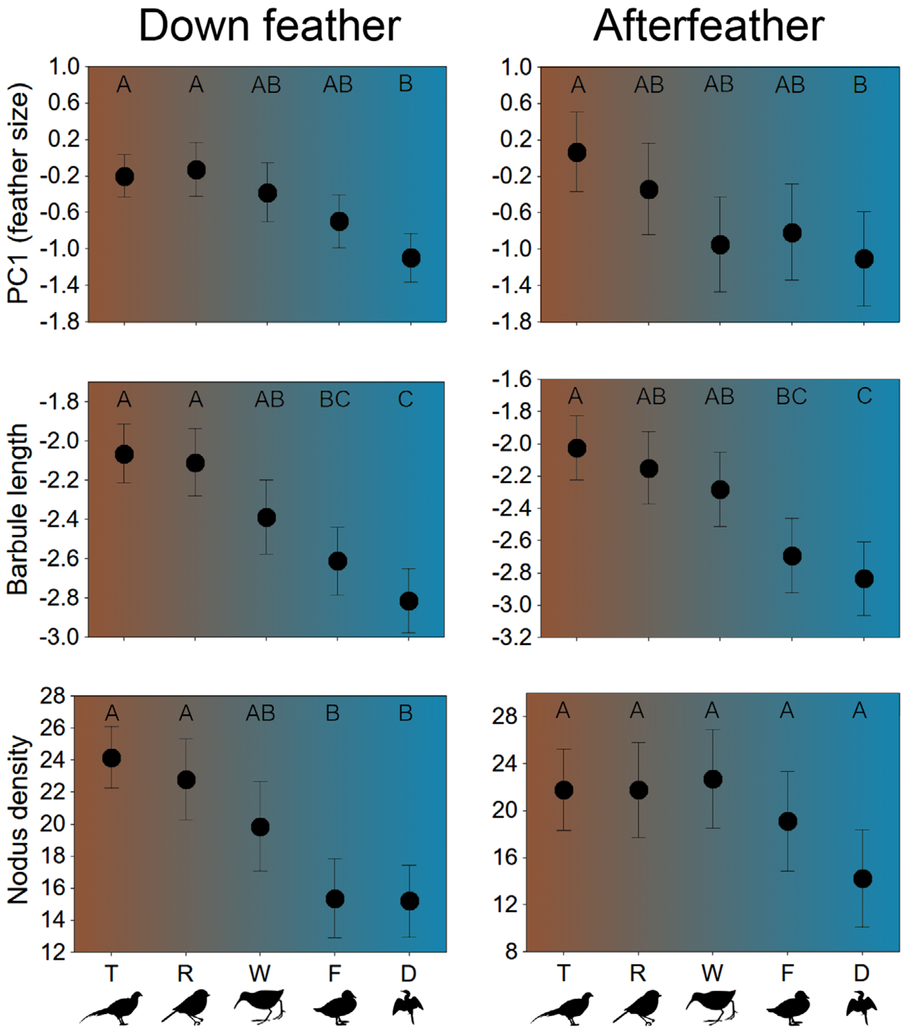

Figure 2. **The relationship between habitat type and ventral feather traits. Different letters denote significant differences ($P < 0.05$), as inferred from the PGLS models. Values are predicted means ± SE from the models presented in Table 1. Species are classified into five habitat groups: T, terrestrial; R, riparian; W, waders; F, floaters; D, divers. Background color reflects the transition from terrestrial (brown) to aquatic (blue) habitat.**

terrestrial, riparian and wading species, but that the opposite applied in floaters and divers. Moreover, the difference in barbule density between ventral and dorsal afterfeathers was markedly higher in diving species compared to all other groups (all $P \leq 0.049$). Wind chill had no effect on $\Delta_{\text{feather morphology}}$ of either down feather or afterfeather structural characteristics (Table 2).

Discussion

FEATHER MORPHOLOGY AND HABITAT

Our study shows that the plumage structure of birds (down feathers and afterfeathers) has evolved to match habitat requirements. Specifically, down feathers in aquatic birds were smaller, had shorter barbules and lower nodus density than in terrestrial species. All these characters exhibited a gradual change from terrestrial toward aquatic lifestyles. Even within the latter, analogous changes could be detected when transitioning from wading to diving species. The similarity in responses between down feathers and afterfeathers confirms former studies suggesting that both feather types are important insulators and serve similar functions (Williams et al. 2015). This study, as well as our previous work, showed that there is a reduction in the depth of the downy plumage layer (i.e., shorter feathers) in aquatic birds (see also Pap et al. 2017). This may appear counter-intuitive at first, because increased insulation provided should be beneficial to counter the higher heat loss rate in water compared to in air (Fuller 2015). However, unlike terrestrial species, aquatic birds (and divers in particular) also face the challenge of keeping buoyancy low. An important parallel can be drawn here with mammals, where the fur represent a structure highly similar with avian plumage in terms of layered structure and function (Liwanag et al. 2012a; Blix 2016). Coarse, long hairs cover an inner, denser, and softer underfur that provide thermal insulation. While terrestrial and many semiaquatic mammals rely on the fur extensively for insulation, aquatic species have evolved very short fur (e.g., fur seals) or have lost their fur entirely (e.g., cetaceans, sirenians; Liwanag et al. 2012b). This is a key step in the evolution of aquatic mammals, as reducing fur depth improves their hydrodynamic and subaquatic movements. Hence, aquatic mammals either possess structural modification to the short fur to efficiently trap air while submerged, and also complement/replace the reduced or missing insulation from the pelage with blubber, a dense and highly vascularized layer of subcutaneous fat (Liwanag et al. 2012b). As in aquatic mammals, hydrodynamic subaquatic movement in birds might be improved by reducing the depth of the plumage and reducing the amount of air trapped within the downy layer (Grémillet et al. 2005), for example, by shortening barbules and reducing nodus density (D'Alba et al. 2017). These adaptations ensure that air can easily escape from the down when birds dive, such that the plumage can be more easiliy compressed to reduce both buoyancy and drag ("air lubrication" hypothesis). This special adaptation is well exemplified by emperor penguins, where the plumage can contain as much as 5 L of air (Davenport et al. 2011; Williams et al. 2015). When underwater, increasing pressure and the contraction of muscles associated with individual feathers allows the bird to expel nearly all air from the plumage. However, the muscles controlling feather motion aid the retention of a small quantity of air near the skin surface by forming a tightly shut feather layer above the thin downy coat. The high–down density in penguins (Williams et al. 2015; Osváth et al. 2018) may facilitate the interlocking of barbules and thus the trapping of air when the animal is submerged, similarly to what is observed in mammals (Liwanag et al. 2012b). This might offset at least parts of the reduction in insulation from compression of the plumage.

Upon ascent, air bubbles in the plumage have another function. With decreasing depth, the air bubbles expand, diffuse through the fine plumage meshwork and form a bubbly coat around the body that drastically reduces drag and helps the penguins to accelerate to twice their normal swimming speed and jump on land. A similar idea of injection of air into boundary layers has been used by engineers to speed the movement of vehicles (ships, torpedoes) through sea water (Davenport et al. 2011). This ingenious "solution" seems to solve the conflict between the need of a thick insulating down layer and the negative effect of positive buoyancy. We might expect similar plumage adaptations of aquatic birds to have features that both confer low buoyancy and thermo-isolation (see Williams et al. 2015), although our knowledge of special solutions for this trade-off is very limited and anecdotal (Grémillet et al. 2005).

Some studies on semi-aquatic mammals that use the pelage as the primary insulator have structural modifications of the fur that increase air-traipping capacity, much like how the bird plumage works. For instance, fur seals and river otters have underhairs with elongated scales, reminiscent of the expanded noduses on down feathers in birds (Liwanag et al. 2012a). These structures are thought to enable the underhairs to interlock more efficiently to maintain air near the skin surface despite the shallow fur (Liwanag et al. 2012a). In line with this, species that use blubber as the primary insulator (e.g., sea lions) have lost similar structural adaptations to the underhairs (Liwanag et al. 2012a). In contrast to this, our results show that aquatic birds have down with lower nodus densities than terrestrial birds, suggesting that aquatic birds release air under hydrodynamic pressure. Hence, alternative insulatory mechanisms, such as local heterothermy or subcutaneous fat stores, might be at play here, similarly to mammals that use subcutaneous fat to keep warm. For example, some seabirds also use local heterothermy in the appendages or the body trunk when diving (Bevan et al. 1997; Handrich et al. 1997; Ponganis et al. 2003, but see Enstipp et al. 2005), which greatly reduces heat loss and contributes to extended dive duration (Scholander 1940). Birds also use large fat deposits as energy stores during migration, or to overcome food shortage in winter (Mortensen et al. 1983; Vincze et al. 2019). However, except in penguins, the role of these fat stores in insulation has not been formally studied (Pond and Mattacks 1985; Blix 2016; Lewden et al. 2017), but the cost of transportation clearly sets an upper limit to fat insulation in volant species.

Our results contradict conclusions in previous studies (Fuller 2015; D'Alba et al. 2017), which found that longer barbules and higher nodus density is more likely to occur in diving than in floating aquatic birds. These studies hypothesized that the compression and bending resistance of down feathers confer thermal benefits since bulkier materials trap more air. By analogy, if the material cannot resist, or recover from compression, then it will be a poor insulator. However, these conclusions are based on studies of only a few aquatic species (greylag goose *Anser anser*, common eider *Somateria mollissima*, domestic goose, and duck). Our study, which was based on 156 species from five habitat categories, support Dove and Agreda's (2007) conclusion of a negative relationship between barbule nodus density and diving behavior. Accordingly, it appears that diving species have fewer expanded nodes and a less buoyant plumage that makes them more efficient at foraging in deep waters compared to floating birds. These microstructural adaptations suggest that the coat of diving birds, especially of those hunting underwater, might have higher thermal conductance in air than that of non-diving species, due to the limited amount of air their plumage can trap (see de Vries and van Eerden 1995). This could explain why in some aquatic birds, such as penguins, much of the thermal insulation is provided by a thick subcutaneous fat layer that compensates for reduced plumage insulation (Wilson et al. 1992). The inverse relationship between a shallow external insulating coat (i.e., fur/plumage) and a deeper subcutaneous fat layer might, thus, represent another example of convergent evolution of mammals and birds during the transition to aquatic life.

With the exception of $\Delta_{\text{barbule density}}$ of afterfeathers, the difference between ventral and dorsal downy feather structures was not influenced by habitat or windchill. Hence, dorsal feathers, despite coming into contact with water less frequently, show similar adaptations in size, barbule length, and nodus density to habitat as do ventral feathers. Finally, the similarity of down and afterfeather microstructure was very consistent across habitats, which strengthen former presumptions about the function of afterfeathers in insulation (Williams et al. 2015). Afterfeathers were not present on one or both sides of the body in several species, reducing the sample size in the analysis of this feather type considerably, possibly also effecting the results of the models. However, association between feather structure in down and afterfeathers with habitat were highly consistent, suggesting that the lower sample size for afterfeathers did not bias the results.

FEATHER MORPHOLOGY AND WIND CHILL TEMPERATURE

Species wintering under cold and windy climatic conditions were found to have dorsal down feathers that harbored slightly higher nodus densities. Higher nodus density in harsher ambient conditions can be explained by the need for increased cohesion of the down (Fuller 2015; D'Alba et al. 2017). This is a key characteristic for ensuring better air trapping capacity (Fuller 2015; D'Alba et al. 2017) and, hence, insulation in strong winds. In line with this, previous work shows that harsher wintering temperatures is associated with longer plumulaceous sections of contour feathers both across (Pap et al. 2017) and within bird species (Cheek et al. 2018). Similar adaptations have been found in mammals, at least in the sense that the insulating values of the fur in Arctic mammals was found to be proportional to the length of the hairs (Scholander et al. 1950).

The effect of windchill on feather traits was overall rather weak. It appears that cold ambient temperatures reinforce selection on plumage mass and density (cf. Osváth et al. 2018), rather than on the length and structure of individual down feathers. Indeed, change in density of plumulaceous feathers probably has a more significant effect on insulation than change in the structure of individual feathers (Pap et al. 2017; Osváth et al. 2018). Another possible explanation for the weak effect of thermal conditions on feather morphology is that these traits are flexible characters which vary geographically in relation to the local environment (see Broggi et al. 2011). Here, we calculated wind chill temperatures for the wide wintering range of each species, but the sampled birds usually originated from one particular geographic location whose thermal conditions would have been different from those of the mean winter range.

FINAL CONCLUSIONS

The results of the current study, together with two extensive former comparative studies on contour feather morphology and plumage density (Pap et al. 2017; Osváth et al. 2018), provides a glimpse into the key elements influencing the evolution of feather and plumage structural morphology in birds. These studies suggest that contact with water, especially the forces encountered during diving, have triggered a series of structural adaptations in body feathers, ultimately leading to a shallow, heavy and densely packed plumage in aquatic species. Moreover, individual feathers are designed to be compressible and resilient in aquatic species, probably as a result of frequent underwater compression, which is ensured by short barbules with fewer nodes. It appears that increased keratin content of the downy structure is also important for insulation in aquatic birds, while terrestrial birds rely more on a fluffy and air-filled downy layers for this purpose. The advantage of high keratin content in the downy coat of aquatic birds serve the function of reducing the amount of air trapped in the plumage and therefore reducing the undesirable buoyancy. At the same time, a reduction of the volume of air trapped in the coat also infers costs, such as lower insulation (see de Vries and van Eerden 1995) and a corresponding need for metabolically expensive heat production, and probably higher net plumage weight. Analogous changes to aquatic life are apparent in mammals, including shallower depth of the coat and structural modification that reduces trapping air, especially in diving taxa. Ambient thermal conditions only weakly influenced the structure of down and afterfeathers. This suggests that selection for cold tolerance acts mostly on the structure of whole plumage and not on individual downy feathers (cf. Osváth et al. 2018). Experimental work is now needed to reinforce and validate the correlative findings reported here (see McGowan et al. 2018; Ivlev 2019). In particular, we suggest that future studies should manipulate specific feather and plumage traits, such as density and morphology of individual feathers, and measure the effects on thermal resistance, buoyancy and water resistance. Moreover, a wider geographic coverage, especially sampling of species inhabiting tropical regions, could help broadening the scope of the results presented here.

AUTHOR CONTRIBUTIONS

P.L.P. and O.V. designed the research; P.L.P, G.O., T.D., A.N., and O.V. performed the research; O.V. and P.L.P. performed all statistical analyses; P.L.P. and O.V. wrote the article, and A.N. and G.O. contributed substantially to revisions.

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DATA ARCHIVING

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Raw data of down feathers.
Table S2. Raw data of afterfeathers.
Table S3. Summary of data on species-specific body mass, habitat (T – terrestrial, R – riparian, W – wading, F – floating, D – diving), and maximum and minimum monthly temperature of breeding and wintering areas, respectively.
Table S4 Repeatability of feather traits of the down and afterfeather measured on the ventral and dorsal sides of the body.
Table S5. Phylogenetic paired *t*-test of the difference in dorsal and ventral down- and afterfeather traits between wild-collected and museum-sourced samples.
Table S6. Results from a phylogenetic PCA on feather size traits with loadings of the first three principal components (PC1, PC2 and PC3), and the percent of variance in feather traits explained by these components.
Table S7. Full phylogenetic generalized least squares models of the effect of body mass, habitat, and wind chill on feather size traits (feather area, total length, barb length) of ventral (A) and dorsal (B) down and afterfeathers.
Table S8. Full phylogenetic generalized least squares models of the effect of body mass, habitat, and wind chill on dorsal feather traits.
Table S9. Phylogenetic paired *t*-test of the difference in feather traits of down and afterfeathers between ventral and dorsal sides of the body.
Figure S1. Correlation plots showing direction and strength of the relationship between six feather traits of dorsal and ventral down and afterfeathers.
Figure S2. The relationship between habitat type and feather size traits (feather area, total length, barb length) of ventral and dorsal down- and afterfeathers.
Figure S3. The relationship between habitat type and feather traits of dorsal down- and afterfeathers measured.

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Morphological characterization of flight feather shafts in four bird species with different flight styles

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Variation in rachis (central shaft) morphology in individual remiges (flight feathers) within and among species reflects adaptations to requirements imposed by aerodynamic forces, but the fine-scale variation of feather morphology across remiges is not well known. Here we describe how the shape of the rachis, expressed by the height/width ratio, changes along the longitudinal and lateral axis of the wing in four bird species with different flight styles: flapping-soaring (white storks), flapping-gliding (common buzzards), passerine-type (house sparrows) and continuous flapping (pygmy cormorants). Overall, in each wing feather, irrespective of species identity, rachis shape changed from circular to rectangular, from the base towards the feather tip. The ratio between the height and width of the calamus was similar across remiges in all species, whereas the ratio at the base, middle and tip of the rachis changed among flight feathers and species. In distal primaries of white storks and common buzzards, the ratio decreased along the feather shaft, indicating a depressed (wider than high) rachis cross section towards the feather tip, whereas the inner primaries and secondaries became compressed (higher than wide). In house sparrows, the rachis was compressed in each of the measurement points, except at the distal segment of the two outermost primary feathers. Finally, in pygmy cormorants, the width exceeds the height at each measurement point, except at the calamus. Our results may reflect the resistance of the rachis to in-plane and out-of-plane aerodynamic forces that vary across remiges and across study species. A link between rachis shape and resistance to bending from aerodynamic forces is further indicated by the change of the second moment of areas along the wing axes.

ADDITIONAL KEYWORDS: *Buteo buteo* – *Ciconia ciconia* – flight style – *Microcarbo pygmaeus* – *Passer domesticus* – rachis morphology – rachis shape – rachis width – second moment of area – wing feather.

INTRODUCTION

To become airborne and move forward, birds need to generate lift and propulsive thrust to overcome their weight and drag forces, respectively (Pennycuick, 2015). Flight feathers provide the largest part of the lifting surface of bird wings, which have to withstand aerodynamic forces during flight (Lindhe Norberg, 2002; Wang *et al.*, 2012; Altshuler *et al.*, 2015). The distribution of forces acting upon the wing changes

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across the wing, and the pattern of this change is influenced by flight style, i.e. whether a bird flies with continuous wing flapping, or soars/glides without flapping its wings. Thus, it can be hypothesized that the structural parameters of the feather shaft varies with the flight style of birds (Ennos *et al.*, 1995; Pap *et al.*, 2015).

Resistance to bending and torsion under cyclic aerodynamic loads is provided by the rachis, the central shaft of a feather. Overall diameter and the cross-sectional shape are the main determinants of the bending pattern of the rachis (Corning & Biewener, 1998; Tubaro, 2003; De la Hera *et al.*, 2010; Sullivan *et al.*, 2017; Wang & Meyers, 2017a). Furthermore, the differential flexural stiffness in the two planes of bending (i.e. dorso-ventral and lateral) is achieved mainly by changes in the cross-sectional morphology of the rachis (Purslow & Vincent, 1978). Therefore, variation in rachis shape between species and among flight feathers that are differently positioned along the wing should reflect adaptations to the requirements imposed by localized differential aerodynamic forces. Consequently, we predict that rachis morphology varies: (1) along the longitudinal axis of individual flight feathers; (2) among flight feathers across the wing span, and (3) between species characterized with different flight styles.

According to Bruderer *et al.* (2010), four main flight styles can be distinguished in birds: flapping and soaring, flapping and gliding, continuous flapping, and passerine-type flight. Flapping-soaring flight is used by relatively large species (e.g. vultures, storks) and is energetically the most efficient flight style, characterized by reduced flapping frequency. Flapping-gliding flight involves relatively long flapping periods and comparably long gliding phases with outstretched wings (e.g. gulls and falcons). Smaller passerine birds have a specific flight style which is characterized by short flapping phases followed by a short parabolic flight path with closed wings and gliding with outstretched wings. Continuous flapping birds flap their wings with constant wing-beat frequency over relatively long distances (e.g. waders, ducks and cormorants). Each of these flight styles is associated with different aerodynamic and inertial properties, which in turn influence the structural properties of the rachis (Pap *et al.*, 2015; Pennycuick, 2015; Lees *et al.*, 2016).

A comprehensive comparative study using 137 species reported that the dorso-ventral rachis diameter (reflecting the bending resistance in dorso-ventral plane) of the innermost primary feathers is greater in soaring and gliding species than in species of continuous flappers and passerine-type flyers (Pap *et al.*, 2015). Furthermore, the study of Pap *et al.* (2015) found a marked increase in rachis width from inner to outer primary feathers in flapping flyers, while in soarers and gliders the change along the wing is less pronounced. The latter result suggests that the aerodynamic forces are distributed more evenly across the primary feathers of soarers and gliders, whereas loading is more biased towards the distal primaries in flapping flyers. However, it remains undocumented how rachis shape varies across the entire wing. This variation might reflect differential adaptation of flight feathers to in-plane (load acting perpendicular to the wing plane) and out-of-plane aerodynamic forces (loads that are applied parallel to the wing plane), see Fig. 1 (Purslow & Vincent, 1978; Ennos *et al.*, 1995).

Distal (primary) and proximal (secondary) feathers have distinct functions in flight, and so have to withstand forces acting from different directions (Norberg, 1985; Videler, 2005; Usherwood, 2010; Muijres *et al.*, 2012). Primary feathers are responsible for generating not only thrust but also lift (Norberg, 1985; Videler, 2005), whereas proximal wing feathers (secondaries) mostly generate lift (Müller & Patone, 1998). These differences correlate with the structural variation in the vanes between these two wing feather groups (Ennos *et al.*, 1995; Bachmann *et al.*, 2007; Heers & Dial, 2011; Feo *et al.*, 2015), but little is known about the variation of their rachis shape.

The aerodynamic load also varies along the axis of individual feathers (Müller & Patone, 1998; Sullivan *et al.*, 2017), which is reflected in the change in the structural and mechanical properties along the rachis (Purslow & Vincent, 1978; Macleod, 1980; Bonser & Purslow, 1995; Cameron *et al.*, 2003; Bachmann *et al.*, 2012). The bending stiffness of the rachis primarily depends on how the material is distributed in the cross-section (i.e. second moment of area) and changing the cross-sectional shape is an effective solution for adjusting the bending stiffness of a rachis to accommodate differential aerodynamic forces (Purslow & Vincent, 1978; Bonser & Purslow, 1995; Weber *et al.*, 2010). It was shown that the shape of the flight feather rachis changes from round to square/rectangular from feather base to feather tip (Wang & Meyers, 2017b). This rectangular form offers greater bending stiffness per unit area and allows flight feathers to twist and resist flexure (Wang *et al.*, 2016; Sullivan *et al.*, 2017; Wang & Meyers, 2017a, b). To date, however, morphological investigations have principally focused on primary feathers, or often on a single feather of the wing. It is still unclear how the shape varies along the rachis within individual feathers, among all of the feathers across the wing, and how these traits differ among species.

Here, we investigated the variation of the rachis shape (1) along the longitudinal axis of individual flight feathers, (2) across remiges and (3) among four bird species with remarkably different flight styles:

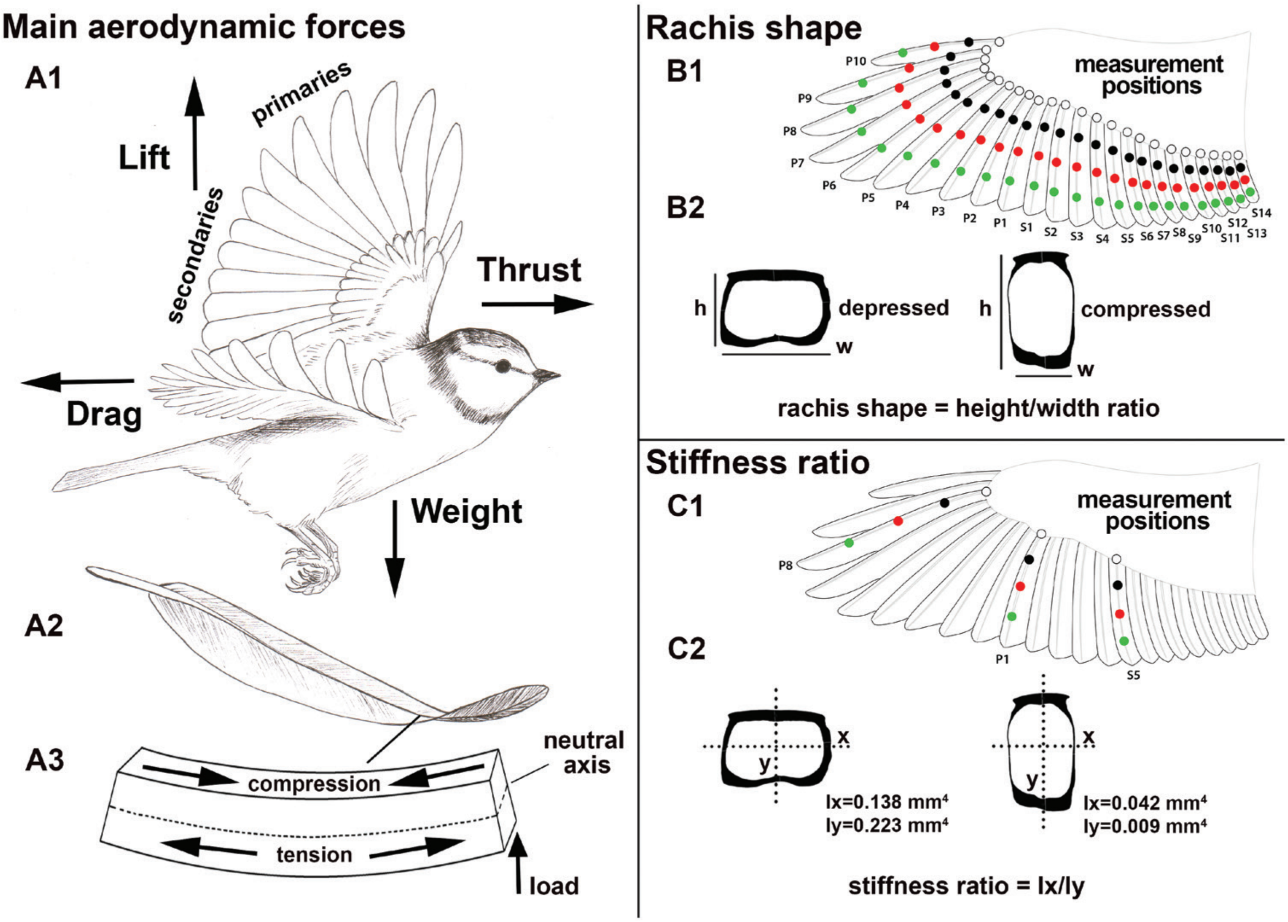

Figure 1. A1, main aerodynamic forces acting on wing feathers. A2, the bending of a feather under aerodynamic load. A3, schematic lateral view of a bent rachis segment. B1, the dots show the measurement positions of rachis shape along the wing (open—calamus, black—base, red—middle, green—tip). B2, the method for calculating rachis shape. C1, positions along the wing from where rachis cross-sections were taken to measure the second moment of area. C2, cross-section of common buzzard rachises at the tip of P1 (right) and P8 (left) after image processing. x is the neutral axis for bending in dorsoventral direction (span-wise bending), y is the neutral axis for bending in lateral direction (chord-wise bending). Ix is the second moment area with respect of x axis (stiffness in dorso-ventral direction), Ix is the second moment area with respect of y axis (stiffness in lateral direction).

flapping-soaring [white stork (*Ciconia ciconia*)], flapping-gliding [common buzzard (*Buteo buteo*)], passerine-type [house sparrow (*Passer domesticus*)] and continuous flapping [pygmy cormorant (*Microcarbo pygmaeus*)]. In order to describe the variation in rachis' shape, we calculated the dorso-ventral/lateral width ratio (i.e. height/width ratio) at four measurement positions (calamus, base, middle and tip defined as the 0%, 25%, 50% and 75% points of the total vane length, respectively) along the rachis, where a value higher than one indicates higher than wider rachis section ('compressed' shape), and values smaller than one indicate wider than higher rachis section ('depressed' shape) (Fig. 1).

We expect that the greatest cross-sectional diameters of remiges are in those planes where the bending forces are largest. Since, in general, inner primaries and secondaries cope with stronger lift forces, we predict that the rachis of secondaries tends to be higher than wide (i.e. compressed shape) in each examined species and at each measurement position of these feathers. Because the outermost primary constitutes the leading edge of the wing, these are likely to experience significant lateral aerodynamic forces from drag, we expect a smaller height to width ratio (i.e. more depressed shape) of the rachis of outermost primary than in primaries proximal to it, which in turn should have a more compressed shape, similar to inner primaries and secondaries. However, we predict in soaring and gliding white storks and common buzzards, a depressed rachis shape in all distal primaries which constitutes the slotted wingtip (the six, respectively five distal primaries in white storks and common buzzards). The rationale behind this expectation is that in a slotted wingtip each feather acts as an individual aerofoil, being exposed to large lateral forces, while moving through the air. In the flapping and passerine-type flyer house sparrows and pygmy cormorants, we predict a compressed shape in all primaries other than the outermost, since the more active flapping flight is associated with stronger bending and twisting forces.

MATERIAL AND METHODS

Data collection and feather measurements

We collected wing feathers from carcasses of white storks (N = 11), common buzzards (N = 10), house sparrows ($N = 10$) and pygmy cormorants ($N = 10$). Only individuals with fully grown remiges were considered. We plucked all remiges (primaries and secondaries) from one of the two wings from each individual bird, totalling 33 feathers from white storks (11 primaries and 22 secondaries), 24 from common buzzards (10 and 14), 25 from pygmy cormorants (10 and 15) and 18 from house sparrows (9 and 9). The innermost secondary (S22) in white storks was missing in eight birds, while the outermost primary (P10) in the house sparrows is vestigial, therefore these feathers were excluded from the analysis. White storks were either juveniles, sub-adults or adults; common buzzards and pygmy cormorants were juveniles or adults, while all house sparrows were in adult plumage.

For each feather collected, we measured the dorso-ventral (= height) and lateral width (= width) of the rachis with a digital caliper (to the nearest 0.01 mm) at four positions along the rachis. Then we calculated the height/width ratio as a proxy of rachis shape, by dividing these two values for each measurement point. These positions are at the base of the vane (calamus), and along the rachis at one-quarter (base), half (middle) and three-quarters of the distance from the base to the tip (Fig. 1). The rachis widths of white storks' feathers were measured by Dragomir-Cosmin David, the common buzzards and house sparrows were measured by Gergely Osváth, and the pygmy cormorants by László Jácint Nagy. Repeatability between observers (i.e. measurements of the same feathers by different observers) was high for all parameters [rachis height: Intra-class Correlation Coefficients (ICC) = 0.99; 95% CI 0.988–0.999; rachis width: ICC = 0.99; 95% CI 0.991–0.999]. Measurements generally showed very high intraspecific repeatability (ICC > 0.78) in all tested feathers and positions. All data are reported in the Supporting Information (Table S1).

Determination of the second moment of area

To illustrate the variation in cross-sectional shape of the rachis, we used the third outermost primary (P7, P8 or P9 depending on species; see Fig. 1 and Fig. 2), the innermost primary (P1), and the fifth secondary feather (S5) from one individual of each species. Feathers were prepared by removing the vanes and by creating cross sections at four positions along the vane; at the calamus, base, middle and tip. Feather pieces were embedded in epoxy and cross-sections were polished using graded sand papers, then each cross-section was photographed. These images were also used to calculate the second moment of area of the different rachis segments in order to confirm the pattern of variation in the dorso-ventral and lateral bending resistance suggested by dorso-ventral/lateral rachis width ratio (i.e. height/width ratio) (Fig. 1). The second moment of area (I, mm^4) is an important parameter in structural engineering, which quantifies the bending resistance of a beam about a given axis of a cross-section: assuming homogeneous material properties, structures with larger values of I are stiffer than those with smaller values of I (Young, 1989). The feather shaft is structurally a tapered cantilever beam in which case the magnitude of I is a reflection of the amount of material (keratin) in cross-section and its distribution perpendicular to a given neutral axis (the square area of keratin resisting compression or tension in the plane of bending) (Pennycuick, 2008). In our study Ix and Iy are measures of the second moment of area about the x and y axis (Fig. 1). Therefore, Ix indicates the stiffness in a dorso-ventral plane, whereas Iy reflects the stiffness of a given cross-section in lateral flexion. The second moment area values were calculated using BoneJ, a plugin of Fiji-ImageJ (Doube *et al.*, 2010; Schindelin *et al.*, 2012). We calculated the stiffness ratio (Ix/Iy), and a value greater than one indicates greater dorso-ventral bending stiffness, whereas values smaller than one indicate greater lateral bending stiffness (Blanco *et al.*, 2009).

Statistical analysis

The morphology of feathers can vary depending on the age of a bird (Butler *et al.*, 2008) and because our dataset includes white storks, common buzzards and pygmy cormorants with both juvenile and adult wing feathers, we tested for differences between different age classes. Rachis shape was similar in adult and juveniles birds (paired t-test): common buzzards: t = -0.23, df = 95, P = 0.81; pygmy cormorants: t = -0.97, df = 91, P-value = 0.33; white storks: t = -1.2811, df = 127, P-value = 0.20). Therefore, it is unlikely that our results are affected by age-specific changes in feather traits.

Non-linear relationships were found between the dependent variable and the explanatory variables.

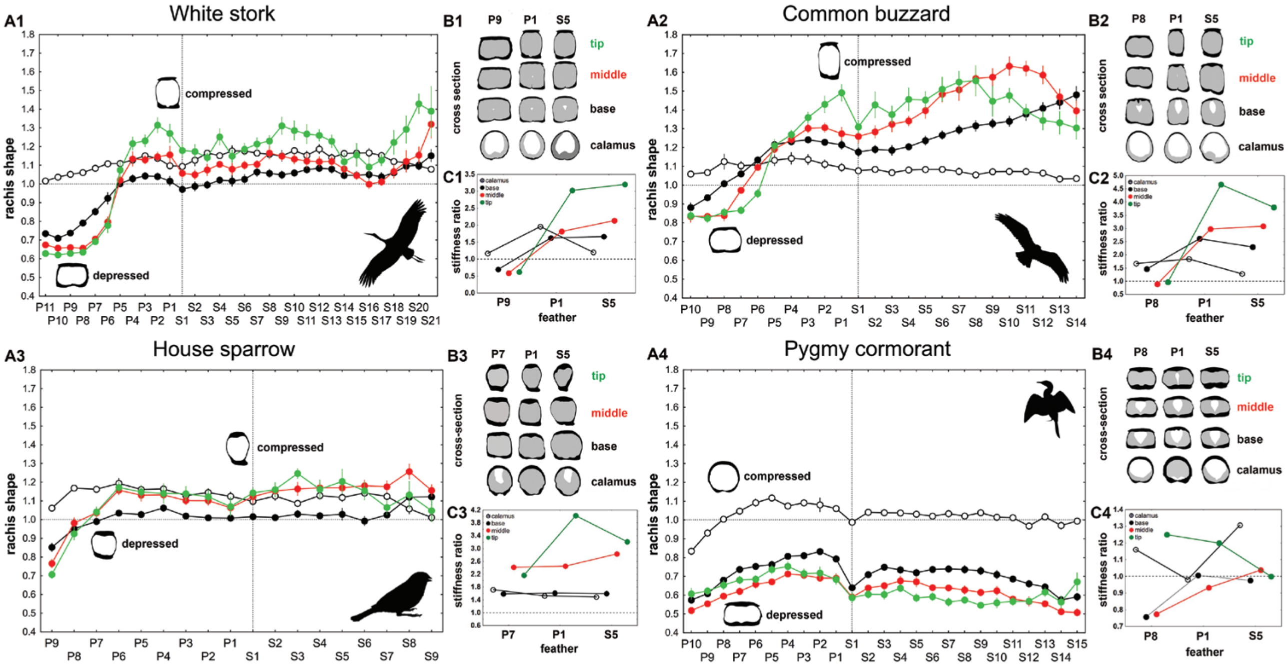

Figure 2. A1-A4, variation in rachis shape of wing feathers of the four examined species in relation to position along the rachis and position along the wing (P = primary; S = secondary). The symbols and colours indicate different position along the feather axis (open—calamus, black—base, red—middle, green—tip, as in Fig. 1). The vertical hatched line marks the border between the primary and secondary feathers. Mean ± SE are presented ($N = 11$ for A1 and $N = 10$ for A2-A4). B1-B4, cross-section of the rachis of the third outermost and the innermost primary along with the medial secondary feather. C1-C4, variation in Ix/Iy ratio among the second to outermost, the innermost primary and the medial secondary feather wing feathers. A higher value than 1 indicates greater dorso-ventral bending resistance, while values smaller than 1 indicate greater lateral bending resistance. Bird silhouettes taken from PhyloPic (http://phylopic.org)

Therefore, to investigate the variation of rachis shape across remiges, among the four measurement positions along the vane and among the four bird species, we performed General Additive Models (GAMs). Rachis shape values were used as dependent variables in the model that included species, feather identity (the individual number of each flight feather), measurement position (calamus, base, middle, tip) and the interaction between explanatory variables: rachis shape ~ species + measurement position + s(feather id) + s(feather id, measurement position) + s(feather id, species). GAMs were carried out using the 'gam' function with a Gaussian error distribution and smoothing function with default settings from the R package 'mgcv' (Wood, 2003, 2004, 2011, 2017; Wood *et al.*, 2016). All statistical analyses were conducted using the R statistical computing environment, v.3.4.2 (R Development Core Team, 2017).

RESULTS

VARIATION IN RACHIS SHAPE ALONG THE VANE, AMONG FLIGHT FEATHERS AND SPECIES

In the four study species, for each examined wing feather, the rachis shape changed from circular/oval to square/rectangular in shape, from the calamus towards the feather tip (Fig. 2). The height/width ratio of the rachis differed significantly among the four feather segments and wing feathers in each species (Table 1). Furthermore, the variation of rachis shape along the longitudinal axis of the rachis and across remiges showed different patterns among species (Fig. 2). All species showed a slightly compressed rachis at the calamus except pygmy cormorants (P10, P9 and S1). In the distal primaries of white storks and common buzzards, the height/width ratio decreased along the feather shaft, indicating that the rachis shape was becoming depressed towards the feather tip, while the inner primaries and secondaries became compressed (Fig. 2A1-A2). In house sparrows, the variation of the height/width ratio among flight feathers was similar, and the rachis was generally compressed at each of the measurement points, except at the distal segments of the two outermost primary feathers (Fig. 2A3). For all species the outermost primaries generally were depressed towards the feather tip (Fig. 2). In pygmy cormorants the height/width ratio at the calamus was close to one (except in the distal primaries) resulting in an almost circular profile, while towards the tip, the feathers showed more accentuated

Table 1. Results of the general additive models explaining the variation in rachis shape as a function of feather identity (i.e. the individual number of each flight feather), measurement position (i.e. position along the shaft: calamus, base, middle, tip) and species. Estimated degrees of freedom (edf); reference degrees of freedom (Ref.df)

Terms	Rachis shape			
A. Parametric coefficients	Estimate	SE	*t*-value	*P*-value
Intercept	0.19	0.0011	163.87	<0.0001
Measurement position	0.22	0.0015	146.94	<0.0001
Species	0.12	0.0015	82.19	<0.0001
B. Smooth terms:	edf	Ref.df	*F*-value	*P*-value
Feather id	4.99	6.09	2.68	0.0129
Feather id: measurement position	24.63	25.84	104.07	<0.0001
Feather id: species	25.16	26.15	163.85	<0.0001

lateral rachis width associated with a depressed rachis shape. The height/width ratio of these segments varied between 0.5 and 0.8, indicating a width twice as large as the height in some feathers of pygmy cormorants (Fig. 2A4). For the other three species, the flattening of the feathers distally in the dorso-ventral plane is much less extreme than that of cormorants.

VARIATION IN STIFFNESS RATIO OF THE RACHIS

In white storks and common buzzards, the value of stiffness ratio (Ix/Iy) was below one at the distal part of the third outermost primary, but greater than one at each measurement point of the innermost primary and medial secondary feather (Table 2; Fig. 2C1-C2). This indicates greater bending stiffness in the lateral direction at the distal part of the outer primary, but relatively greater bending resistance in the dorso-ventral direction in the inner primary and secondary feathers. In house sparrows, the stiffness ratio values indicate greater bending stiffness in the dorso-ventral direction for each feather, and at each measurement point (Table 2; Fig. 2C3). In pygmy cormorants, the stiffness ratio was less than one at the base and middle of the third outermost primary, at the calamus and middle segment of the innermost primary and the base of S5, indicating greater bending resistance in the lateral direction. In contrast, the stiffness ratio indicates superior bending resistance in the dorso-ventral direction at the calamus of the third outermost, tip of the innermost and middle and base of S5. The base and middle segment of P1 and the tip of S5 show similar bending resistance in both directions (Table 2; Fig. 2C4).

DISCUSSION

CHANGE IN RACHIS SHAPE ALONG FEATHER AXIS

We found that the cross-sectional shape of the rachis differs not only along the feather length, but also between wing feathers and species. In general, at the calamus, the rachis was oval in shape or circular in all four species, resulting in similar height and width. A compressed rachis shape was expected in all investigated birds, because of the large dorso-ventral forces acting on the feathers during downstroke, which are redirected towards the bones and tendons of the wing through the calamus (Bachmann *et al.*, 2012; Sullivan *et al.*, 2017). The relatively uniform cross-sectional shape of the calamus across the wing and among species suggests that the force exerted at the base of each feather is of similar orientation relative to the cross-sectional axes irrespective of feather size and position along the wing. The similar orientation of forces selects for the one optimum cross-section (i.e. the shape of the calamus is optimized to resist bending loads in a dorso-ventral direction). This is a realistic assumption, because flight feathers function like tapered cantilevered beams (Lees *et al.*, 2017), so the total force on the feather will be focused at the calamus (the point at which the feather leaves the skin) and the feathers are fixed against rotation at this point (Raikow, 1985). In contrast, the observed variation across the flight feathers and along the rachis suggests that the distribution and orientation of the aerodynamic forces relative to the cross-sectional axes differs both between feathers within a species as well as between species. Again, this is a reasonable assumption, because feather rachises are curved, meaning that forces applied to them, particularly distally, will twist as well as bend the feather (Corning & Biewener, 1998). The degree of twisting and bending depends on feather position with the distal primaries of many species splitting and parting to create wing-tip slots and the amount of this feather deflection differs between species (Withers, 1981; Norberg, 1985; Tucker, 1993, 1995; Lindhe Norberg, 2002). Flattened rachises ensuring resistance to torsional forces during twisting at the distal position of outer primaries found in the species studied here supports this notion.

Table 2. Measurements of the second moment of area in three feathers of each of the four studied species. See *Material and Methods* for sources

Species	Measurement position	Third to outermost primary			Innermost primary			Medial secondary		
		I_x (mm^4)	I_y (mm^4)	I_x/I_y ratio	I_x (mm^4)	I_y (mm^4)	I_x/I_y ratio	I_x (mm^4)	I_y (mm^4)	I_x/I_y ratio
White stork	Calamus	26.0470	22.3000	1.1700	21.6780	11.0440	1.9600	7.0540	5.8990	1.2000
	Base	7.0140	10.0670	0.7000	6.1820	3.8090	1.6200	2.7850	1.6650	1.6700
	Middle	1.2350	2.1060	0.5900	2.3200	1.2770	1.8200	0.8870	0.4140	2.1400
	Tip	0.1380	0.2230	0.6200	0.2270	0.0750	3.0300	0.1090	0.0340	3.2100
Common buzzard	Calamus	5.7880	3.4650	1.6704	2.5860	1.4020	1.8445	1.7770	1.3950	1.2738
	Base	1.6190	1.1100	1.4586	1.3960	0.5350	2.6093	0.8120	0.3530	2.3003
	Middle	0.3530	0.3950	0.8937	0.3690	0.1240	2.9758	0.1910	0.0620	3.0806
	Tip	0.0290	0.0300	0.9667	0.0420	0.0090	4.6667	0.0190	0.0050	3.8000
House sparrow	Calamus	0.0426	0.0248	1.7187	0.0157	0.0103	1.5302	0.0104	0.0070	1.4892
	Base	0.0099	0.0062	1.5896	0.0061	0.0038	1.6148	0.0068	0.0042	1.5933
	Middle	0.0025	0.0010	2.4135	0.0015	0.0006	2.4495	0.0015	0.0005	2.8287
	Tip	0.0004	0.0002	2.1625	0.0004	0.0001	4.0283	0.0003	0.0001	3.2171
Pygmy cormorant	Calamus	0.5200	0.4480	1.1607	0.5470	0.5580	0.9803	0.2550	0.1950	1.3077
	Base	0.1060	0.1400	0.7571	0.1770	0.1760	1.0057	0.0800	0.0820	0.9756
	Middle	0.0410	0.0530	0.7736	0.0550	0.0590	0.9322	0.0270	0.0260	1.0385
	Tip	0.0050	0.0040	1.2500	0.0060	0.0050	1.2000	0.0030	0.0030	1.0000

PATTERNS OF RACHIS SHAPE VARIATION ACROSS REMIGES AND BETWEEN STUDY SPECIES

The height to width ratio of the outer primaries of white storks, common buzzards and house sparrows decreased (i.e. depressed shape) towards the feather tip, whereas the height generally exceeded the width (i.e. compressed shape) in the inner primaries and the secondaries. Furthermore, in white storks and common buzzards, not only the rachis of the outermost primary, but also the rachises of all emarginated distal primaries were wider than they were high. Additionally, the ratio of second moments of area showed greater resistance to lateral forces (i.e. force that acts in the direction parallel to wing) in the outermost primary (third to outermost) and superior resistance to dorso-ventral bending in the innermost primary (P1) and medial secondary (S5). Hence, the rachis of the outer primaries appears to be relatively more resistant to lateral forces, while the rest of the wing feathers (i.e. inner primaries and secondaries) are stiffer in dorso-ventral than in lateral flexion. A generally compressed shape, indicative of greater bending resistance dorso-ventrally in wing flight feathers, is expected because the resultant relative forces on the wing are oriented dorso-ventrally, particularly during the wing's downstroke (Norberg, 1985). However, as aforementioned, the distal primaries split and spread (Withers, 1981) and they are oriented perpendicular to the direction of travel, so it is likely they experience significant lateral aerodynamic forces from drag even when not spread (Purslow & Vincent, 1978). Rachis morphology is also likely influenced by the need to resist torsional forces during twisting and because torsion reduces the bending moment required to cause buckling failure (Young, 1989), perhaps to increase resistance to lateral buckling. It should be noted, however, that although feather diameters and second moment of area (at the umbilicus) provide a broad indication of a flight feather's material properties within a species, across species comparisons are limited, because microstructure (keratin fibre matrix) likely differs markedly between species (Lees *et al.*, 2017). Therefore, a broader rachis in one direction or greater second moment of area does not necessarily mean increased stiffness or strength when comparing across species.

Among the outer primaries, the height/width ratio gradually increased from the outermost towards the inner primaries, and from the tip to the base of individual feathers. Hence, the cross section of the distal segment of the rachis progressively changes from depressed to compressed from the outermost towards the inner primary feathers. This pattern of variation was most pronounced among the emarginated remiges that form the wingtip in white storks and common buzzards. When separated to create wing-tip slots, each primary feather acts like an individual aerofoil (Tucker, 1995). Pennycuick (2008) suggested that the flexibility of feather shafts in outer primaries is graduated, which allows them to form the slotted wingtip passively by variations in the bending resistance of the shafts: the first feather bends until its tip points almost straight upwards, and subsequent feathers bend less, resulting in a cascade of up to six feathers around the wing tip. Hence, a depressed rachis shape may equate to an increase in flexibility, which makes our rachis cross-section analyses congruent with Pennycuick's (2008) hypothesis. A depressed rachis shape of distal primaries may also result in decreased profile drag allowing more efficient flight in laminar air flow (Bonser & Purslow, 1995; Cameron *et al.*, 2003).

In pygmy cormorants, width exceeds the height at each measurement point except at the calamus, representing a surprising pattern of a species characterized by high wing-beat frequencies, and therefore high aerodynamic loading on the wing's feathers from the dorso-ventral direction (Usher wood, 2003; Pap *et al.*, 2015, 2019). Pygmy cormorants dive and swim underwater using foot-propulsion, while the wings are held close to the body (Kato *et al.*, 2006). Therefore, swimming is unlikely to significantly influence flight feather morphology in terms of hydrodynamic forces, particularly as aerodynamic forces during flight will be far greater than forces experienced when submerged. It is possible that morphological adaptations of feather vanes to aquatic life [e.g. increased barb and barbule density (Pap *et al.*, 2015, 2017)], that aid water repellence or reduce buoyancy (i.e. reduce the amount of air trapped) during diving, also influence rachis morphology; however, further work is needed before this can be unequivocally concluded.

CONCLUSION

We found that the cross-sectional shape of the rachis varies along the longitudinal axis of the feathers and along the wing of the four species studied. The morphology of the rachis appears to be driven by the orientation of *in situ* aerodynamic forces and the need to provide sufficient rigidity to resist them. The calamus was oval and slightly compressed in all birds and all remiges, indicating a shape optimized to resist bending loads in a dorsoventral direction. In white storks, common buzzards and house sparrows the distal part of the outermost primaries, which forms the leading edge of the wing, had a depressed rachis shape and were relatively more resistant to lateral forces, while the rachis of the rest of wing feathers was compressed and stiffer in dorso-ventral than in lateral flexion. In addition, the gradual increase in the height to width ratio among the emarginated primaries of white storks and common buzzards suggest adaptation to bending that allows the slotted wingtip to be formed passively. Contrary to our predictions, in pygmy cormorants we found a depressed rachis shape at each measurement position, except at the calamus, which is perhaps an adaptation to aquatic locomotion. However, further research is needed in order to corroborate this hypothesis.

In conclusion this study adds new details to our understanding of the functional morphology of the rachis of flight feathers and overall, highlights the striking variation in feather structural morphology within individual feathers; across the wing and between examined species of birds. Hopefully, our study will be a catalyst for future work investigating the driving forces behind this diverse structural morphology, which will ultimately contribute to our understanding of avian wings and aerodynamics.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Raw data: morphological traits of the rachis.

Biological Journal of the Linnean Society, 2019, **126**, 256–267. With 3 figures.

Vane macrostructure of primary feathers and its adaptations to flight in birds

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The selection pressures that drive flight feather morphology are poorly understood. Using a phylogenetic comparative approach and data from 178 species of birds, we investigated whether both position along the wing length and flight feather length affected vane structure. We found that barb density was lower on distal primaries than on proximal primaries of the leading feather vane. In contrast, on the trailing vane only mid-point barb density differed and, here, it showed denser barbs on the distal primaries. This difference was greater at the feather base than at the tip. Barb angle was higher along the full length of the leading edge vane on the proximal primaries than on the distal primaries. Overall, barb density decreased from base to tip on both trailing and leading vanes on both the proximal and the distal primaries. In general, barb angle was less acute at the feather base than at the tip. Barbs were in general denser in continuous flapping fliers than in soarers and the angle of barbs on both the proximal and the distal primaries was affected by flight type. However, we did not identify consistent differences in the pattern of barb angle change among flight style groups. These findings add new perspectives to our understanding of the functional morphology of the flight feather vane, although we still have limited knowledge on how aerodynamic forces, particularly during take-off and landing, affect the morphology of the feather vane.

ADDITIONAL KEYWORDS: barb angle – barb density – feather vane – flight – flight feathers – functional morphology.

INTRODUCTION

Primary feathers are long, stiff and asymmetrically shaped feathers on the distal part of the wings of birds, and whose main function is to generate lift and thrust during flight. These primary feathers make up a large part of the wing area, constituting approximately 50% of the total wing span depending upon the species (Nudds, 2007; Nudds *et al.*, 2011). Hence, the primaries determine the wings' ability to withstand forces during flight, which are equal to the bird's body weight during gliding flight, but are much higher when the bird is changing direction or during the flapping phases of active flight (Lees *et al.*, 2017).

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The macrostructure of the primary feather vane is a mesh formed by barbs and barbules. The barbs branch off from the shaft, are parallel to each other and are connected via hook- and bow barbules that branch off. This mesh structure ensures that feather vanes are both light and flexible and are highly resistant to aerodynamic pressure (Prum & Brush, 2002). The response of the vane to aerodynamic forces is determined by at least two traits of this mesh structure. Firstly, the density of the barbs and barbules determines the air transmissivity of the vane, which may affect flight performance (Müller & Patone, 1998; Heersi*et al.*, 2011; Dial *et al.*, 2012). Secondly, the branching angle of the barbs determine the vane's response to in-plane and out-of-plane forces during both the down- and upstroke of the wing (Ennos *et al.*, 1995). Therefore, we expect these feather macrostructure traits to show differences at multiple levels: (1) among species that differ in flight style (wing flappers vs. soarers/gliders), (2) among primary feathers that differ in their position on the wing (proximal vs. distal) and (3) between parts of the same primary feather (basal vs. apical part or leading vs. trailing vane). The rationale behind these expectations is that the wing of birds that differ in flight type, the different feathers on the wing and the different parts of the same feather should be subject to different aerodynamic forces (see below). However, these expectations have rarely been tested in a comparison across a large variety of bird species.

Flight type (e.g. flapping, soaring/gliding or hovering flight) may be a key contributer to the diversification seen in the functional morphology of flight feathers, because different flight behaviours subject the wing feathers to different aerodynamic loads (Pennycuick, 1989). Hence, it is conceivable that the morphology and the mechanical response of the primary feathers to airflow varies with predominant flight styles among species (e.g. Nudds & Dyke, 2010; Wang *et al.*, 2011, 2012; Feo *et al.*, 2015; Pap *et al.*, 2015). Common to all species, however, is that the aerodynamic forces exerted on individual feathers vary across the wing span, with distal primary feathers needing to withstand considerably higher aerodynamic forces than more proximal primaries or secondary feathers (Ennos *et al.*, 1995; Bachmann *et al.*, 2007; Heers *et al.*, 2011; Dial *et al.*, 2012; Feo *et al.*, 2015; Pap *et al.*, 2015), particularly during downstrokes in flapping flight (Corning & Biewener, 1998). Therefore, the vane of distal feathers has to resist larger out-of-plane forces than the proximal primaries, which might ultimately result in differentiation in barb density and barb angle. Also, profile (friction) drag will exerting in-plane force, and the direction of that force relative to the vanes will change as the presentation of the feathers differs along the span of the wing (and through the wingbeat cycle). For similar force distribution reasons, feather morphology will also differ along the shaft. This is exemplified by shaftwise changes in vane geometry and in the vane's resistance to bending (Bachmann *et al.*, 2007; Wang *et al.*, 2012; Feo *et al.*, 2015; Pap *et al.*, 2015). Vane emarginations (i.e. marked narrowing of the vane) observed in primary feathers, particularly the distal primaries, result in separation of the feather tips when the wing is spread (Norberg, 1990). These non-overlapping tips act as independent aerofoils during flight, which requires even greater resistance to aerodynamic forces, particularly at the tips of these feather sections (Tucker, 1995). Except for the most distal primary feathers, the largest pressure gradient on a fixed (non- or gentle-flapping) bird wing is toward the leading edge (Norberg, 1990) and, therefore, the base of individual feathers will experience higher pressure gradients than the tips (Müller & Patone, 1998). This probably explains why vane barb density is higher at the base than at the tip of flight feathers in the two species studied to date, the barn owl (*Tyto alba*) and the feral pigeon (*Columba livia*) (Bachmann *et al.*, 2007). Transmissivity was also found to be greater on the leading vane than on the trailing vane of each feather, perhaps indicating a greater barb density on the trailing vane (Müller & Patone, 1998). This difference in transmissivity was suggested to result in a differential pressure gradient upon the vanes, resulting in a tilting moment pushing the trailing edge of the feather against the leading edge of the adjacent more proximal feather, thereby forming a continuous smooth lifting surface (Müller & Patone, 1998). The branching angle of the barbs decreases from the base to the tip of pigeon flight feathers (Ennos *et al.*, 1995; Bachmann *et al.*, 2007). Ennos *et al.* (1995) showed that resistence to out-of-plane moments (stiffness) correlates broadly with barb angle, with the more acute angle found at the tip being associated with more resistance. It is likely that the tips of the feathers are structured to resist greater out-of-plane and hence aerodynamic forces than the bases, which are overlayed and supported by covert feathers (Ennos *et al.*, 1995). Therefore, there is evidence supporting differentiation in barb angle and barb density across the wingspan, along the feather length, and between the leading and trailing feather vanes, all probably driven by variation in the aerodynamic forces experienced at those points. To the best of our knowledge, however, no study has formally addressed how the macrostructure and geometry of primaries vary between proximal and distal feathers along the wing, along the shaft of individual feathers, and between trailing and leading vanes among species with different flight styles. Understanding how vane morphology varies among birds with different flight styles is key to unravelling the question of how feather vanes have evolved to meet the aerodynamic requirements of flight. Such knowledge could also aid our understanding of selection pressures that drove the evolution of flight feather morphology and its variation among birds (Feo *et al.*, 2015; Pap *et al.*, 2015; Wang *et al.*, 2017). Using a phylogenetic comparative approach and data on 178 species of volant birds, we test how feather vane macrostrucure varies across the wing (between proximal and distal primaries), along the axis of individual feathers, and between leading and trailing vanes in species characterized as having different flight types. We measured barb density and barb angle in distal and proximal primary feathers at three positions along the feather vanes (base, middle and tip) on both the trailing and the leading vane of the feathers (see Fig. 1). We analysed how the observed among-species variation relates to flight styles, which was assigned to one of the four categories [flapping and soaring (FS), flapping and gliding (FG), continuous flapping (CF) and passerine type (PT); following Pap *et al.* (2015)] according to the proportion of flapping flight (Pennycuick, 2008; Bruderer *et al.*, 2010).

We predicted higher barb density of the feather vanes of species using flapping (CF and PT) rather than soaring flight (FS and FG), and we expected it to be more pronounced on distal than on proximal primaries, due to greater aerodynamic forces experienced when flapping the wing. Furthermore, we predicted a decrease in barb density from the base towards the tip of the feathers because lift generation and, hence, the need for low transmissivity to air is highest at the base. In accordance with Müller & Patone (1998), we also predicted a greater density of barbs on the trailing vane than on the leading vane.

Barb angle was predicted to decrease from the base toward the tip because of the association between a lower branching angle and resistence to out-of-plane loading, which is highest at the tip. We also predicted a more acute branching angle of the barbs on the leading than on the trailing vane, with a more marked difference on the distal primary, because the outer primaries function as individual aerofoils (acting as leading edge slats) during demanding flight manoeuvres (Ennos *et al.*, 1995). We predicted a more acute branching angle for flapping flyers, and we expected it to be more pronounced on distal than on proximal primaries. However, barb angle on the leading vane is probably reduced in the serrated wings of some soaring birds, where the distal parts of distal primaries are separated and thus act as individual airfoils.

MATERIAL AND METHODS

DATA COLLECTION AND FEATHER MEASUREMENTS

We collected feather samples from 399 adult birds of 178 species at several locations across Romania, Hungary, Norway, Sweden and Scotland between 2003 and 2017, and at the Pointe Géologie Archipelago, Terre Adélie (66°40′S, 140°01′E), Antarctica, between 2012 and 2017. All sampled individuals were dead at the time of sampling and died due to natural causes (e.g. road kill, building collision, electrocution, starvation) or were procured from authorized hunters. None of the birds were killed for the purpose of the present study. We collected a proximal primary (innermost primary, hereafter P1) and a distal primary feather (P8) from each individual (Fig. 1A). We chose to sample P8 to characterize the distal part of the wing, because it is the longest flight feather in most birds and is thus more exposed to aerodynamic forces than the more proximal P1. On each feather, we measured barb density and angle (determined by the barb axis and the distal part of the rachis) on the trailing and the leading vane sides of the rachis at three different positions along the rachis (base, middle and tip, defined as 25, 50 and 75% of the total vane length, respectively). These measurements were made on digital photographs of the feathers lying on a metric grid background, or a stage micrometer, using ImageJ v.1.37 (http://rsb.info.nih.gov/ij/). The density of barbs was calculated as the number of barb embranchments along a 1-cm rachis section. Barb density was measured by B.B. and barb angle by Z.S.

EXPLANATORY VARIABLES

We obtained body mass data from Dunning (2007) for both sexes. In species for which the data of several subspecies or populations were reported, we only used European populations and subspecies with European distributions (except for species originating from Antarctica). Species-specific body mass used in the analyses was calculated as the mean of male and female body masses. Flight type was assigned to one of four categories according to the proportion of flapping flight following Bruderer *et al.* (2010) and Pennycuick (2008), with slight modification after Pap *et al.* (2015): FS, FG, CF and PT. The four flight type categories differ in wing beat frequency, with steady increase from FS to PT; the aerodynamic pressure on primaries and its effect on feather morphology was assumed to be higher in soarers than in flappers. All data are reported in Supporting Information Tables A1–A3.

STATISTICAL ANALYSES

Large within-species variation of the studied traits can cause significant biases in comparative

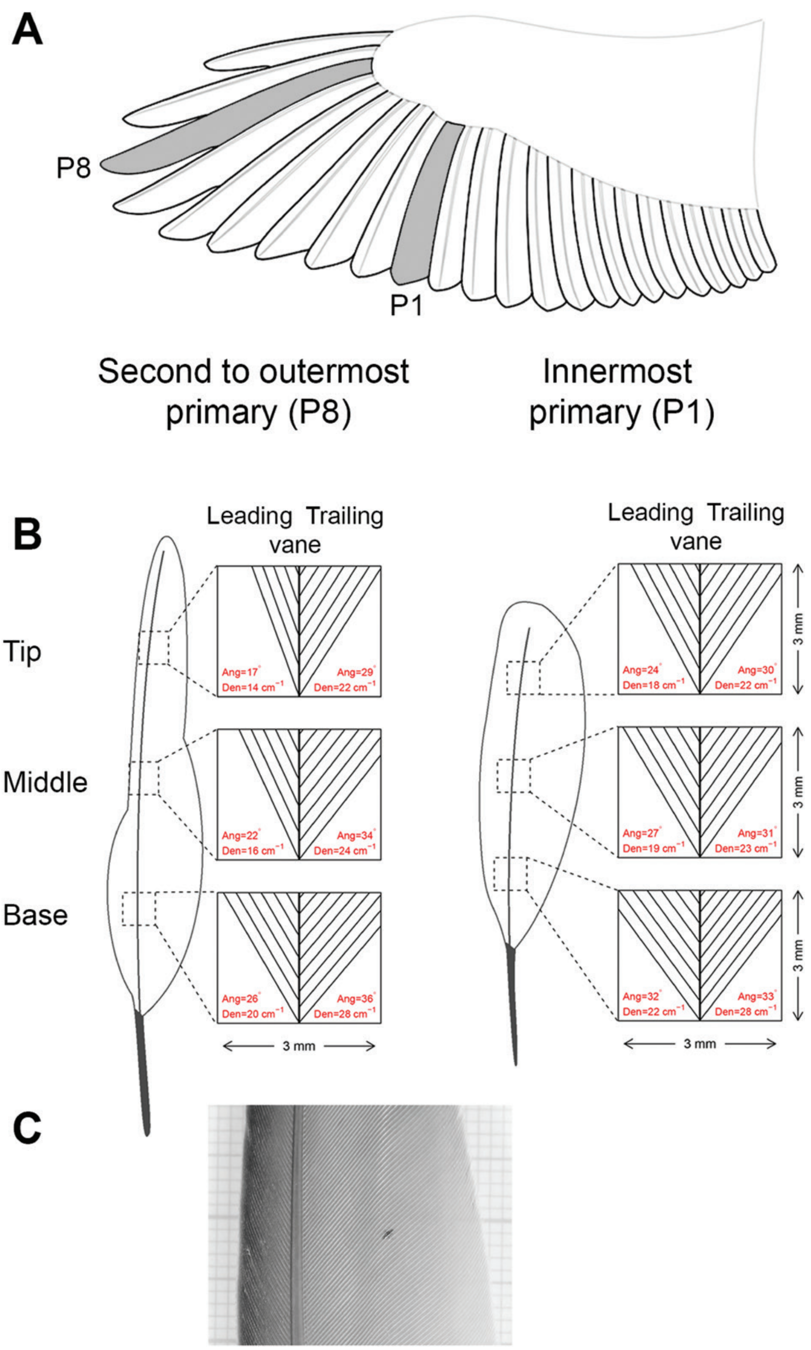

Figure 1. Schematic drawing of the innermost (P1) and distal (P8) primaries (A), the measurement positions (B) and photograph of the mesh structure of the vane of a common buzzard (*Buteo buteo*) with parallel barbs that branch off from the shaft (C). Measurements were taken on the trailing and the leading vane sides of the rachis at three different positions along the rachis (base, middle and tip, defined as 25, 50 and 75% of the total vane length, respectively). Values in red indicate barb angle (Ang) and density (Den) for each measurement position. Wing drawing courtesy of Erzsébet Ferencz.

phylogenetic analyses. Therefore, as a first step, we tested whether feather traits were species-specific and thus suitable for multispecies comparisons. We tested the species-specificity of the measured feather morphological traits by assessing the importance of 'between-species' compared to 'within-species' variance using the function 'ICCest' from R package 'ICC' (Wolak *et al.*, 2012). This was computed using raw, non-transformed data. The species-specificity of feather traits varied from moderate to high with generally narrow confidence intervals, indicating that the measured feather morphological traits are species-specific (Supporting Information, Table A4) and that they adequately characterized feather traits of the studied species.

In a previous study (Pap *et al.*, 2015) we found differences in proximal and distal primary feather morphology that were related to flight style. Accordingly, here, we used a series of statistical tests to test whether feather traits differed between proximal and distal feathers (i.e. P1 vs. P8), among different sections along the shaft (i.e. base vs. middle vs. tip) and between the two sides of the shaft (i.e. leading vs. trailing vane). Firstly, using phylogenetic paired *t*-tests we investigated whether feather morphological parameters differed between P1 and P8, separately at each position along the shaft (base, middle and tip), for both the trailing and leading vanes. Secondly, we used phylogenetic paired *t*-tests (Lindenfors *et al.*, 2010) to test whether feather morphology differed between the trailing and leading vanes and between different positions (base, middle and tip) along the shaft of both P1 and P8. Thirdly, using phylogenetic generalized least squares (PGLS) models (Pagel, 1997, 1999), we investigated how flight type influenced feather morphology of P1 and P8, while controlling for the confounding effect of body mass. We chose to control for the effect of body mass instead of feather mass, because these two traits are strongly correlated, our dataset for body mass was more comprehensive than for feather mass, and we know from our previous work that body mass may affect the relationship between flight style and feather morphology (Pap *et al.*, 2015). Finally, using PGLS models, we tested how flight type influenced morphological differences between proximal and distal primaries (hereafter ΔP), which was computed by subtracting the value of P1 from P8 for each morphological trait.

To account for phylogeny, we used trees provided by http://birdtree.org (Jetz *et al.*, 2012). We generated 100 trees using the higher order phylogeny of Hackett *et al.* (2008) and derived a rooted ultrametric consensus phylogenetic tree from these using the SumTrees software (Sukumaran & Holder, 2010). Feather morphological characters were used as response variables in the PGLS models, while body mass and flight type were introduced as explanatory variables. In all analyses, we set the degree of phylogenetic dependence (Pagel's λ) to the most appropriate degree evaluated for each model based on likelihood ratio statistics.

All statistical analyses were conducted using the R statistical computing environment v.3.4.3 (R Development Core Team, 2017). PGLS models were built as implemented in the package 'nlme' (Pinheiro *et al.*, 2017). All phylogenetic paired *t*-tests were two tailed and were performed using the 'phyl.pairedttest' function of the 'phytools' package in R (Lindenfors *et al.*, 2010). We report data as means ± standard errors (SE). Graphical presentation of paired *t*-tests is based on raw data. Graphical presentation of PGLS models is based on predicted marginal means and their contrasts, obtained using the 'lsmeans' package in R (Lenth, 2016). Barb density, barb angle and body mass were $\log_e$-transformed in all statistical models to ensure normality of residuals.

RESULTS

DIFFERENCES BETWEEN PROXIMAL AND DISTAL PRIMARIES

Barb density of the trailing vane was only greater on P8 than on P1 at the middle of the vane, with no differences detected between the two feathers at the base or at the tip (Table 1; Fig. 2A). In contrast, barb density of the leading vane was higher at all measurement positions along the shaft on P1 compared with P8. Barb angle was similar between P8 and P1 at all three measurement points along the shaft on the trailing vane, but higher on P1 than on P8 at all three positions on the leading vane (Table 1; Fig. 2B).

DIFFERENCES ALONG THE SHAFT

In general, barb density decreased from the base to the tip of the feathers on both the trailing and the leading vanes of both P1 and P8. However, the degree of change along the shaft differed between P1 and P8, and also between the two sides of the vane (Table 2; Fig. 2A). For the trailing vane of P1, barb density

Table 1. Results of phylogenetic paired *t*-tests investigating differences in feather morphological parameters between the innermost (P1) and distal (P8) primaries measured at different positions (base, 25%; middle, 50%; tip, 75%) along the shaft on the trailing and leading vanes of the primaries (*N* = 178 species)

	Barb density		Barb angle	
	t	*P*	*t*	*P*
Trailing vane				
Base	–0.26	0.7915	1.33	0.1857
Middle	2.45	0.0152	1.00	0.3194
Tip	–0.52	0.6048	–1.58	0.1160
Leading vane				
Base	–2.98	0.0033	–3.15	0.0019
Middle	–2.75	0.0065	–2.24	0.0263
Tip	–2.92	0.0039	–3.47	0.0006

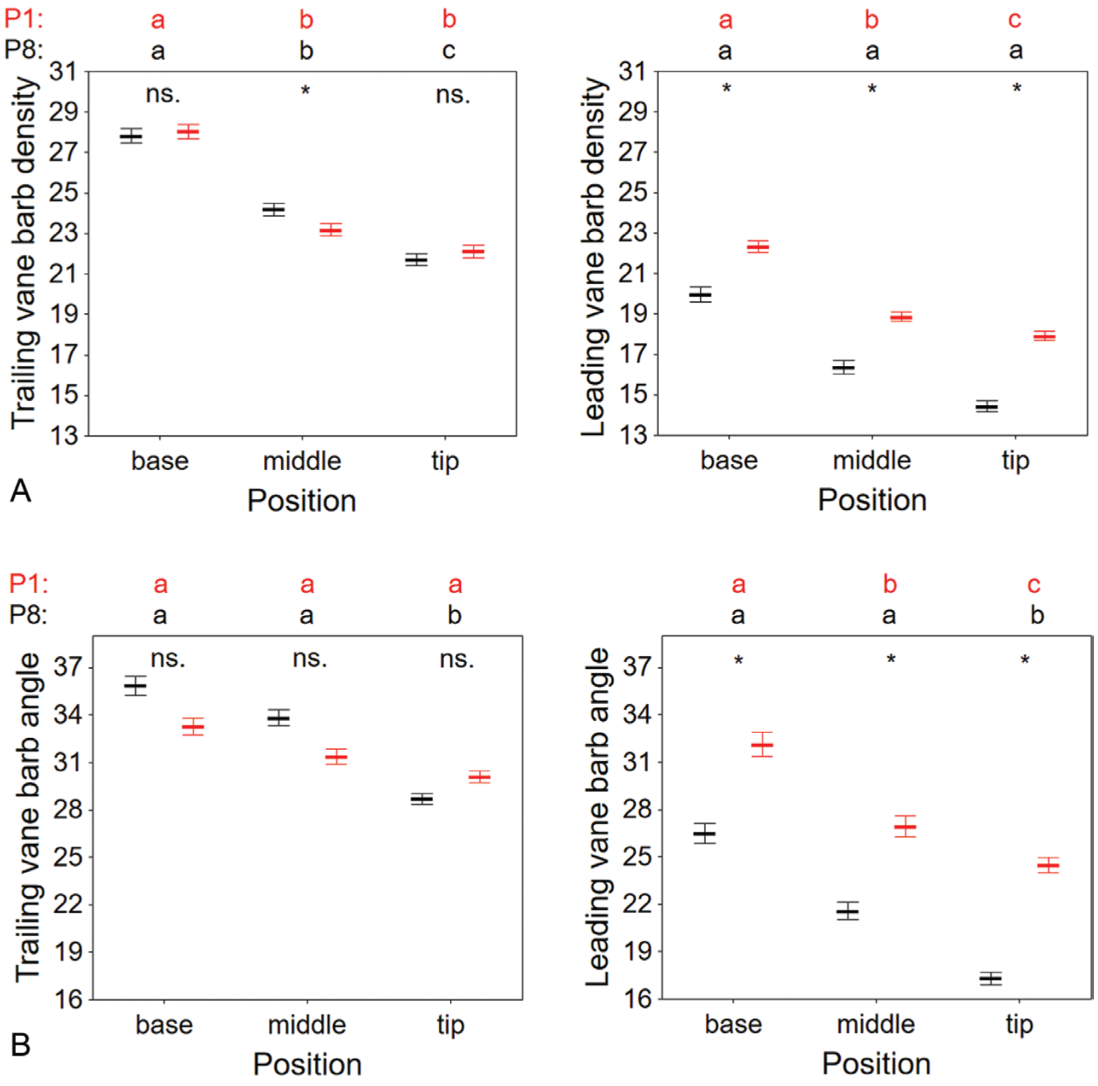

Figure 2. Differences in barb density (A) and barb angle (B) between the innermost (P1) and distal (P8) primaries, measured at three positions along the feathers. Values are means ± SE calculated from the raw data, and group-wise contrasts are extracted from the models. Different letters (a, b, c) denote significant ($P < 0.05$) differences in feather traits measured at different vane positions, and asterisks denote significant differences ($P < 0.05$) between P1 and P8.

decreased significantly between the base and middle positions, while for P8 the decrease was continuous and significant among all three positions along the shaft. For the leading vane of P1, barb density decreased continuously and significantly among all three positions along the shaft, while the change for P8 was non-significant. Barb angle decreased from the base to the tip on both P1 and P8, and on both sides of the vane. However, the degree of change along the shaft differed between P1 and P8, and between the trailing and leading sides of the vane (Table 2; Fig. 2B). For the trailing vane of P1, the change in barb angle was non-significant, while for P8 the decrease was sharp and significant between the middle and tip positions. For the leading vane of P1, barb angle decreased continuously and significantly among all three positions along the shaft, while the decrease for P8 was significant only between the middle and tip (Table 2; Fig. 2B).

Difference between leading and trailing vanes

Barb density of the trailing vane was significantly higher than that of the leading vane at each point along the vane for both P1 and P8 (phylogenetic paired *t*-test, P1, base: $t = 2.15$, $P = 0.0328$; middle: $t = 2.47$, $P = 0.0145$; tip: $t = 3.46$, $P = 0.0007$; P8, base: $t = 4.69$, $P < 0.0001$; middle: $t = 4.91$, $P < 0.0001$; tip: $t = 5.70$,

Table 2. Results of phylogenetic paired *t*-tests investigating differences in feather morphological parameters between different positions (base, 25%; middle, 50%; tip, 75%) along the shaft on the trailing and leading vanes of the innermost (P1) and distal (P8) primaries ($N = 178$ species)

	Barb density				Barb angle			
	P1		P8		P1		P8	
	t	*P*	*t*	*P*	*t*	*P*	*t*	*P*
Trailing vane								
Base–middle	5.49	<0.0001	6.08	<0.0001	1.39	0.1672	0.96	0.3387
Middle–tip	1.23	0.2197	2.36	0.0195	1.47	0.1439	3.33	0.0011
Base–tip	4.87	<0.0001	4.26	<0.0001	1.89	0.0608	3.33	0.0011
Leading vane								
Base–middle	3.22	<0.0015	1.26	0.2082	3.93	0.0001	1.35	0.1783
Middle–tip	2.95	<0.0036	0.93	0.3535	2.89	0.0043	2.18	0.0304
Base–tip	3.53	<0.0005	1.85	0.0653	3.32	0.0011	2.59	0.0103

$P < 0.0001$). The barb angle of the leading vane was significantly lower than that of the trailing vane along the entire shaft of both P1 and P8, with the exception of the base of P1 (P1, base: $t = -0.26$, $P = 0.7918$; middle: $t = 2.37$, $P = 0.0188$; tip: $t = 3.95$, $P = 0.0001$; P8, base: $t = 4.91$, $P < 0.0001$; middle: $t = 5.83$, $P < 0.0001$; tip: $t = 8.38$, $P < 0.0001$).

Flight type and feather morphology

Flight style had a significant effect on barb density on both the trailing and the leading vane of P1 and P8, with the exception of the base of the leading vane of P1 and the tip of the leading vane of both P1 and P8 (Table 3; Fig. 3A). In general, barb density was higher in flapping (CF and PT) than in gliding flyers (FS and FG), with the largest difference observed between flight types FS and CF (Fig. 3A). However, the effect of flight type differed between P1 and P8, between the two sides of the vanes and along the shaft. The effect of flight type on barb density was most pronounced on the distal vane of P8 and less marked on the proximal vane of P1, and on the trailing side vane of both feathers.

Flight type was significantly related to barb angle on the trailing vane of P8 at all three positions along the shaft, although we did not find a clear difference between gliders (FS and FG) and flappers (CF and PT) (Table 3; Fig. 3B). In contrast, flight type had no effect on barb angle on the trailing vane of P1 (Table 3; Fig. 3B). The effect of flight type on the barb angle of the leading vane differed between P1 and P8, and along the shaft; differences were most pronounced on the tip of both P1 and P8, weaker on the middle-vane measurement of P8 and non-significant on the other measurement points. Nonetheless, the difference between gliders and flappers was unclear.

Effect of flight type on variation in feather morphology along the feather axis and on the difference between P8 and P1 (ΔP)

In general, the change in barb density along the shaft on both sides of the vane was weakly associated with flight type (Table 4) and was significant in two cases only: the difference between base and tip of the trailing vane on P1, and the difference between middle and tip of the leading vane on P8 (Table 4). In both cases, the increase in barb density toward the tip of the vane was significantly higher ($P < 0.05$) for CF than for FS flight styles. The change in barb angle in P8 was significantly related to flight type in three cases, but the differences did not seem to follow differences in wing beat frequency (Table 4; P8, trailing vane, base vs. middle: CF < FS; middle vs. tip: CF > FS, CF > PT; P8, leading vane, middle vs. tip: CF > FS; all $P < 0.05$). Variation in barb angle along the shaft of P1 was not related to flight type.

ΔP of barb density was not related to flight type, except for the leading vane at mid-shaft (Table 5). In this case, the difference between P8 and P1 was significantly higher for CF than for FS ($P < 0.05$). ΔP of barb angle varied little with flight type, except for the trailing vane measured at the base and middle positions along the shaft (Table 5). However, group differences did not seem to reflect differences in flight type (trailing vane, base: CF < FG, PT < FG; middle: CF > FS; all $P < 0.05$).

DISCUSSION

Vane morphology and variation within and between primaries

The sample of 178 species used in the current study shows that the variation in vane macrostructure (barb

Table 3. Results of PGLS models investigating whether the feather morphological parameters measured at different positions (base, 25%; middle, 50%; tip, 75%) along the shaft on the trailing and leading vanes of the innermost (P1) and distal (P8) primaries differed between species characterized by different flight types (N = 178 species)

		Barb density				Barb angle			
		P1		P8		P1		P8	
	d.f.	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Trailing vane									
Base									
Body mass	1	26.67	<0.0001	12.32	0.0006	30.82	<0.0001	23.35	<0.0001
Flight type	3	8.71	<0.0001	7.72	0.0001	0.60	0.6181	3.07	0.0292
Middle									
Body mass	1	30.45	<0.0001	20.01	<0.0001	28.47	<0.0001	6.29	0.0131
Flight type	3	4.75	0.0033	7.23	0.0001	1.51	0.2055	4.78	0.0032
Tip									
Body mass	1	26.27	<0.0001	17.33	<0.0001	13.48	0.0003	9.09	0.0030
Flight type	3	3.08	0.0290	5.49	0.0013	2.38	0.0715	5.52	0.0012
Leading vane									
Base									
Body mass	1	0.38	0.5410	0.02	0.8779	22.04	<0.0001	7.71	0.0061
Flight type	3	2.37	0.0721	3.14	0.0267	0.72	0.5409	1.05	0.3713
Middle									
Body mass	1	2.45	0.1194	2.84	0.0936	30.71	<0.0001	1.88	0.1721
Flight type	3	3.19	0.0250	6.18	0.0005	1.40	0.2456	3.32	0.0211
Tip									
Body mass	1	3.79	0.0533	5.18	0.0241	40.46	<0.0001	1.48	0.2248
Flight type	3	2.55	0.0572	1.17	0.3244	5.77	0.0009	4.37	0.0054

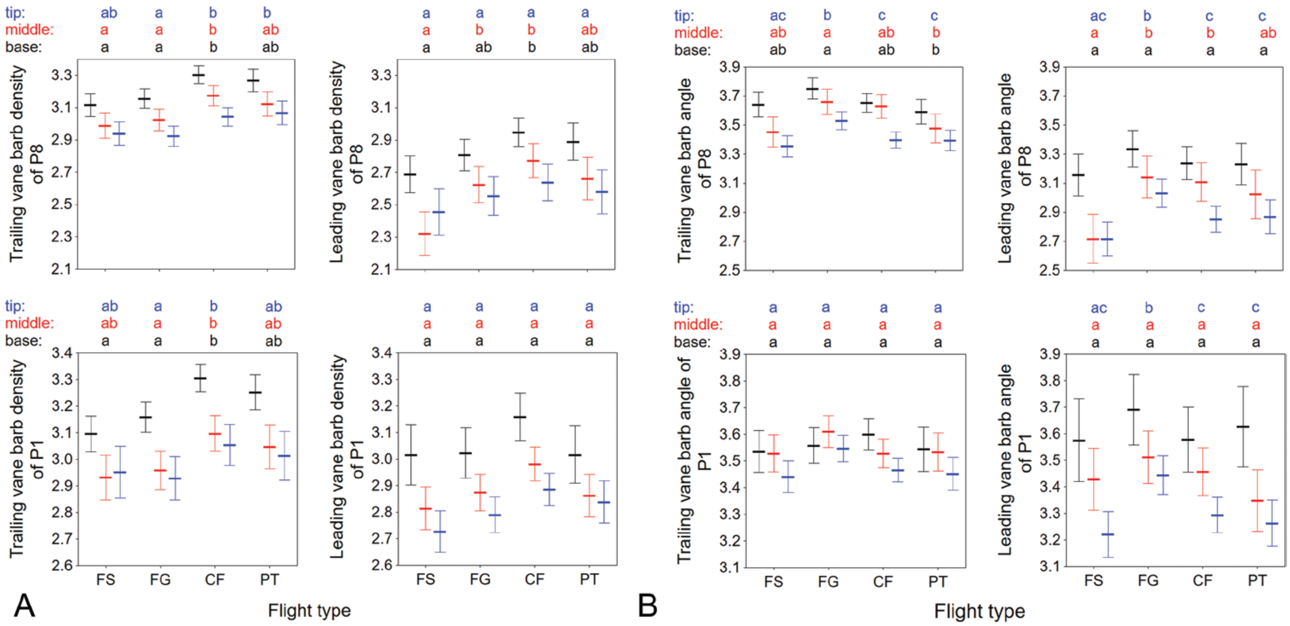

Figure 3. The relationship between flight type and barb density (A) and barb angles (B), measured at three positions along the innermost (P1) and distal (P8) primaries. Values are mean ± SE predicted from the models, and different letters (a, b) denote significant differences ($P < 0.05$), as inferred from the PGLS models. Flight types: FS, flapping and soaring; FG, flapping and gliding; CF, continuous flapping; PT, passerine type. Predicted values are on log scale (natural logarithm).

Table 4. Results of PGLS models investigating whether the change in feather morphological parameters along the shaft on the trailing and leading vanes of the innermost (P1) and distal (P8) primaries differed between species characterized by different flight types (N = 178 species)

		Barb density				Barb angle			
		P1		P8		P1		P8	
	d.f.	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Trailing vane									
Base–middle									
Body mass	1	0.33	0.5645	0.06	0.8101	0.36	0.5518	10.01	0.0018
Flight type	3	1.48	0.2223	0.15	0.9265	2.65	0.0506	4.41	0.0051
Middle–tip									
Body mass	1	0.37	0.5437	0.00	0.9909	5.46	0.0206	0.54	0.4619
Flight type	3	1.47	0.2239	2.64	0.0513	0.23	0.8754	4.32	0.0058
Base–tip									
Body mass	1	0.29	0.5923	0.02	0.8809	4.74	0.0309	11.54	0.0008
Flight type	3	3.22	0.0242	2.28	0.0814	2.67	0.0494	0.37	0.7744
Leading vane									
Base–middle									
Body mass	1	0.40	0.5292	2.72	0.1010	6.69	0.0105	4.72	0.0312
Flight type	3	1.07	0.3640	0.44	0.7231	1.54	0.2065	1.08	0.3590
Middle–tip									
Body mass	1	0.01	0.9137	0.20	0.6518	13.96	0.0003	0.94	0.3336
Flight type	3	0.69	0.5564	3.82	0.0111	1.16	0.3271	3.23	0.0237
Base–tip									
Body mass	1	0.15	0.7020	2.30	0.1315	10.91	0.0012	5.47	0.0205
Flight type	3	0.89	0.4499	1.14	0.3356	0.31	0.8164	0.37	0.7779

density and barb angle) between P1 and P8, along the feather shaft, and between the trailing and leading vanes largely reflects the variation in aerodynamic forces along the feather and at different positions on the wing (Bachmann *et al.*, 2007; Dial *et al.*, 2012; Pap *et al.*, 2015). Specifically, the vanes of the distal primary feathers (e.g. P8), which are subject to higher aerodynamic forces during the flapping flight wingstroke, can resist larger out-of-plane forces than proximal primaries (e.g. P1; Ennos *et al.*, 1995). The greater barb density of the trailing vane at mid-shaft of P8 compared with P1 supports this statement and also corroborates our previous findings, which showed that barb density of the trailing vane is higher in distal compared than in proximal primaries (Pap *et al.*, 2015). However, the difference in barb density between the base and the tip of the trailing vane was similar on P1 and P8, indicating that the relative distribution of forces along the feather is similar across the distal wingspan. Therefore, morphological adaptation concerning vane barb density of primary feathers is influenced by feather position, by the position along the shaft, and by the side of the vane (i.e. leading or trailing). The greater barb density of the leading vane of P1 compared with that of P8 is probably related to the fact that peak aerodynamic forces are higher for the distal part of the wing. Barb angle of the leading vane was greater for P1 than for P8, reinforcing the role of barb angle in modulating vane width and vane asymmetry (Ennos *et al.*, 1995): P8, particularly when emarginated, generally has a narrower leading vane and greater vane asymmetry than P1.

In agreement with a study on barn owls and feral pigeons (Bachmann *et al.*, 2007), and in support of the idea of higher air pressure gradients at the base of the feather (Müller & Patone, 1998), barb density on both vanes (i.e. leading and trailing) decreased from the base to the tip of the feathers. Barb density of the leading vane, however, decreased from the base toward the tip on P1, but not on P8. Perhaps because the more distal primary (P8) orientates in the wingspan plane, whereas P1 orientates more in the chordwise plane, means that the leading edge of P8 experiences similar aerodynamic forces over its entire length. However, if this were the explanation, we might expect to see a similar result for the trailing vane of P8 too. Our results, and those of others, suggest that a major evolutionary driver of feather morphology diversity was different transmissivity needs (Müller & Patone, 1998; Heers *et al.*, 2011; Dial *et al.*, 2012; Pap *et al.*, 2015).

Table 5. Results of PGLS models investigating whether the difference in feather morphological parameters between the distal (P8) and innermost (P1) primaries (ΔP), measured at different positions (base, 25%; middle, 50%; tip, 75%) along the shaft on the trailing and leading vanes, differed between species characterized by different flight types (*N* = 178 species)

		Barb density		Barb angle	
	d.f.	*F*	*P*	*F*	*P*
Trailing vane					
Base					
Body mass	1	6.50	0.0116	0.10	0.7496
Flight type	3	0.27	0.8472	5.21	0.0018
Middle					
Body mass	1	4.52	0.0349	3.53	0.0619
Flight type	3	0.56	0.6453	3.43	0.0183
Tip					
Body mass	1	4.16	0.0428	1.96	0.1635
Flight type	3	1.13	0.3390	0.95	0.4191
Leading vane					
Base					
Body mass	1	1.53	0.2178	1.65	0.2013
Flight type	3	0.86	0.4606	0.43	0.7314
Middle					
Body mass	1	0.42	0.5167	7.85	0.0057
Flight type	3	3.07	0.0294	2.50	0.0610
Tip					
Body mass	1	0.00	0.9982	15.50	0.0001
Flight type	3	0.07	0.9751	0.16	0.9240

In general, the branching angle of the barbs decreased from the base to the tip on both the leading and the trailing vanes, although the change was significant only on P8. A smaller branching angle of the barbs and hence greater resistance to out-of-plane forces (Ennos *et al.*, 1995) towards the tip of the distal primary makes sense because this is the area of the feather where aerodynamic forces are likely to be highest. For example, the distal primaries can be twisted up to almost 180° to produce lift on the upstroke during take-off (Tobalske *et al.*, 2003; Crandell & Tobalske, 2011). In the case of the innermost primary, P1, the non-significant change in barb angle along the shaft on the trailing vane may reflect a more even distribution of forces resulting from the more chordwise plane of the feather (Ennos *et al.*, 1995) in comparison to that of P8.

Barb density of the trailing vane was significantly higher than that of the leading vane at all three positions along the shaft and on both primaries, suggesting that flight feathers show morphological adaptations that ensure low transmissivity to air and, therefore, high resistance to aerodynamic forces. Additionally, in accordance with Müller & Patone (1998), the difference in transmissivities of the trailing and leading vanes may induce a pressure gradient, which pushes the less transmissive trailing vane towards the overlying, more transmissive leading vane to help maintain a continuous flight surface. Moreover, the lower branching angle of the leading edge barbs may help to produce the great degree of planform asymmetry, which allows feathers to function as independent aerofoils during flight, take-off and landing (Norberg, 1990). Lower branching angles of leading vane barbs may also increase the stiffness of the leading vane, which helps prevent barb detachment when the feather is pulled through the air (Ennos *et al.*, 1995). Currently, however, it is not possible to determine whether barb angle is driven by a need for a narrow vane, whilst maintaining structural integrity, or whether barb angle is linked directly to aerodynamic loading. Finally, it should be noted that transmissivity to air and the dynamic response of the feather vane to aerodynamic forces are determined not only by barb density and geometry, but also by the diameter and shape of the barbs, and barbules, measures which were not quantified in the present study.

FEATHER MORPHOLOGY AND FLIGHT TYPE

After controlling for body mass, flight type significantly affected barb density on the vanes of both proximal (P1) and distal (P8) primaries at all three positions along the axis of the feathers, except for the leading vane of P1 and the tip of the leading vane of P8. This corroborates one of our previous comparative studies, in which we found that the barb density of the trailing vane was lowest in birds with the FS flight type (Pap *et al.*, 2015). In general, barb density was higher in flappers (CF and PT) than in soarers (FS and FG), but the differences between the flight types were somewhat obscured by intermediate values for the PT group. The lack of a clear difference in barb density may indicate that feather morphology is affected by more than just steady-state flight type: e.g. the high forces generated during take-off and landing. Contrary to our prediction, flight type was not related to feather morphology differences along the shaft, and the effect of flight type was similar on both vanes of P1 and P8. This indicates that, in general, flight (regardless of flight type) affects morphological adaptation of vane barb density similarly along the shaft despite the fact that the forces acting on the feather tips during flapping flight are probably greater than during soaring flight (Müller & Patone, 1998; Pap *et al.*, 2015). This idea is further supported by the lack of a clear difference in the barb density of P1 and P8 between flappers (CF and PT) and soarers (FS and FG) (Table 5; Fig. 3A). The lack of an effect of flight type on feather vane macrostructure contrasts with our previous comparative study on a smaller number of species (Pap *et al.*, 2015). This highlights the importance of further studies on more species with flight types not included in these studies. Additionally, there is a need for more data on the wing kinematics of different species of birds, because it is likely that the broad qualitative categorizations of flight styles currently available lack sufficient resolution to accurately define the aerodynamic forces to which any given species are subjected. In addition, further measures of the macro- and microstructure of the vane (i.e. barbule morphology and geometry) is needed to better understand the mechanic response of the feathers to flight.

Flight type affected the angle of barbs on both P1 and P8 (Fig. 3B). However, we could not find any consistent differences in the direction of change between flight type groups. Hence, adaptation of this important component of vane geometry does not seem to be driven by variation in flight style. Instead, barb angle might be more strongly affected by selective forces other than the steady-state flight type, such as the high forces generated during take-off and landing.

CONCLUSIONS

The vanes of no two feathers are alike: the geometry of one proximal and one distal primary feather differs from each other and these fine-scale differences appear to be adaptations to the particular aerodynamic conditions to which each feather is exposed. Hence, this study adds new perspectives to our understanding of the functional morphology of flight feather vanes. To test the suggestions put forward in our paper, it would be desirable to measure other traits of the vane, such as barbule morphology and geometry, and its mechanical response to air pressure. There is also a need to investigate a wider range of birds, ranging from gliding specialists, such as albatrosses and petrels (groups which are under-represented in the present study) whose wings are mainly subjected to aerodynamic forces from below, to hovering specialists, such as hummingbirds, whose wings must withstand forces from both below and above (because of an active upstroke). Ideally, a categorization of flight styles that provides a higher resolution, more accurate description of flight behaviour would also be desirable. Furthermore, we have limited knowledge on how aerodynamic forces generated during take-off and landing affect the morphology of the feather vane and how such forces might differ between flight types, a topic that clearly deserves further investigation.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table A1. Barb density for the innermost (P1) and second to outermost (P8) primaries. *N* is the sample size for each parameter. See Material and methods for sources.

Table A2. Barb angle to rachis for the innermost (P1) and second to outermost (P8) primaries. *N* is the sample size for each parameter. See Material and methods for sources.

Table A3. Body masses and flight types (FS, flapping and soaring; FG, flapping and gliding; CF, continuous flapping; PT, passerine-type) for the species included in the study. *N* is the sample size for each species. See Material and methods for an explanation of flight types.

Table A4. Repeatability of feather traits for the innermost (P1) and second to outermost (P8) primaries. ICC is the intraclass correlation and the values in parentheses denote the 95% confidence interval. Only species with at least two individuals measured are included in the analyses (*N* = 123). See Material and methods for sources.

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Subject:	PhD Publication List

Candidate: Gergely Osváth
Doctoral School: Pál Juhász-Nagy Doctoral School of Biology and Environmental Sciences
MTMT ID: 10062057

List of publications related to the dissertation

Foreign language scientific articles in international journals (5)

1. Pap, P. L., **Osváth, G.**, Daubner, T., Nord, A., Vincze, O.: Down feather morphology reflects adaptation to habitat and thermal conditions across the avian phylogeny.
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Foreign language scientific articles in international journals (11)

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