Supporting Information

The evolution of avian wing shape and previously unrecognized trends in covert feathering Wang and Clarke

Included:

1.Supplementary methods.

2. Supplementary results: results from ancestral state optimization (Figure S1) and depiction of the wing phylomorphospace. (Figure S2)

3. Supplementary Results from comparison of PC1 with aerodynamic variables and previous descriptors of wing shape. (Figure S3)

4. Supplementary results: assessment of the wing of *Archaeopteryx*. (Figure S4)

5. Supplementary results from Phylogenetic generalized least squares (Table S1)

6. Supplementary results: PhyloANOVA (Table S2)

7.Supplementary data (Tables S3,S4)

1. Supplementary methods.

Description of sampling, landmark placement, phylogenetic tree and assessment of phylogenetic signal

Taxon sampling: In total 498 specimens, from 105 species and 30 orders (out of a total of 36; SI Table1), representing all major clades proposed by Hackett et al. [1]. We included multiple images of species where possible to give an indication of intraspecific variation. The spread wings were prepared in Burke Museum. They were pinned and dried with the primary feather slots open. The images were taken using a standard background, including a scale, preening and straightening feathers, and cleaning wings and they were carefully taken directly from above to minimize variation caused by perspective. The dataset for analysis of PC values and aerodynamic parameters (i.e., flight speed and wing beat frequency) is smaller than that used in the flight style comparisons. These data have been assessed for fewer avian taxa. Specifically, flight speed data were available for 30 species representing 27 genera and 13 traditional orders [2]; wing beat frequency data [3] were available for 32 species, representing 30 genera and 17 traditional orders.

Landmark placement: Our sampled avian wings differ significantly in flight feather (18-30) and covert feather number (16-30) (all specimens must possess the same number of landmarks in geometric morphologic analysis), which make it impossible to use feather tips as landmarks. A compromise was necessary between resolution of wing geometry and consistency of landmarks across taxa. Consequently, we used 60 equally spaced points (the first point at the point where should start, the last point located at the last secondary feather or tertiary feather if it is about the same length of secondaries) to define the wing shape outline, similar to Brewer and Hertel [4], where 64 points were used. Given that the maximal number of major coverts is 30, the same number equally spaced points (the first point located at the first major primary covert and the last point located at the last major secondary covert) along the curvature defined by the distribution of covert feathers were also applied. This way, the overall geometry of the variety of wing morphologies can be captured.

The wing tip slots that developed in most soaring birds (e.g. vultures, storks) and small agile birds (e.g. wood warblers) were not digitized in this study. Again, as the same numbers of landmarks in geometric morphologic analysis are required and the points we used are equally spaced, fixed number (around 30) of points are located along the wing tip. However, to delineate those wings with slots (number and size varies) by using equally spaced points, much more points will automatically locate around wing tip, which will cause great difference in the number of points that delineate wing tip shape. Consequently, to make the position of each point as consistent as possible for all the species, wing tip slots were not considered in this study.

Estimation of the consensus wing: We calculated a consensus wing for each species and then pooled these to estimate of the consensus wing for all species following the methods of Brewer and Hertel (2007). However, we also investigated the potential implications of this approach compared to a second approach also applied by Brewer and Hertel (2007); we combined all specimens directly to estimate the consensus wing and then calculated species means. Consistent with the findings of Brewer and Hertel (2007), the relative contributions to PC1 and PC2 and % variance explained are nearly identical regardless of approach taken to estimation of the consensus wing; greater primary covert extent and secondary feather extent were again the most heavily weighted variables in PC1 which explain 59% of the variance (vs. 64% with the same relative weights in our primary analysis) and PC2 explains 16% variance (vs. 13% and wing tip shape and greater secondary covert extent are again most heavily weighted. Thus, our conclusions are not affected by the approach used in estimating the consensus wing for all taxa.

Phylogenetic tree and branch lengths

Dated phylogenies of the majority of extant bird species have been recently built [5] and sets of 100000 pseudo-posterior samples of these phylogenies are available for download at [http://birdtree.org/.](http://birdtree.org/) 5000 trees for the possible phylogenetic affinities of these 105 birds were retrieved. These trees take the Hackett et al. (2008) topology as a backbone. A strict consensus tree was then built in Mesquite 2.75 [6]. However, this consensus tree is not fully resolved and thus could not be used in all of our phylogenetic analyses. We then resolved this tree based on the fully resolved phylogenetic hypothesis from Hackett et al. [1] . The branch lengths from the ultrametric time tree of the Fig. 3 topology of [1, 7, 8] were also used for all available nodes. For those unavailable nodes, the method used in [9] was used to obtain branch length. To prevent under/overestimation of evolutionary change on unequal terminal branches, we used the ultrametricize function in Mesquite to adjust branch lengths of terminal taxa in the consensus tree. The branch lengths and phylogenetic relationship for well-nested species taxa within traditional families should thus be considered a best approximation but they are not estimated directly; a molecular phylogeny of all extant taxa assessed was outside the scope of our analysis. The tree based on the backbone of Hackett et al. [1], is referred to as the "H" tree. This tree was adjusted based on recently published genome-scale avian phylogeny [10], and the adjusted tree was named "J" tree. Branch lengths in substitution units were used for all available nodes from Jarvis et al. [10]. For those unavailable nodes, the corresponding branch length from "H" tree divided by 1000 was used to get comparable branch length as in substitution units. These two trees were used in the following analyses.

Assessment of phylogenetic signal

A strong phylogenetic signal means that closely-related species tend to fall out closer in morphospace than more distantly related species [11]. First, we performed a permutation test in MorphoJ, where the phylogeny is held constant, and the PC scores for each taxon are randomly swapped across the tree 10 000 times [11]. If less than 5% of permutations result in a tree length (calculated using squared-change parsimony) that is shorter than or equal to the value obtained from the original data, the null hypothesis of no phylogenetic signal is rejected. Second, we assessed Pagel's lambda values for PC 1 and 2 [12]. The influence of the phylogeny increases with lambda from 0 (no phylogenetic signal) and 1 (strong phylogenetic signal). To determine whether lambda is significantly different from zero, we used a likelihood ratio test in R [13]. Finally, we estimated Blomberg's K [14]. Blomberg's K [14] is used to test whether the observed distribution of traits exhibits more or less divergence than expected for traits evolving under Brownian motion. Values of K close to 1 indicate trait similarity is proportional to divergence and a Brownian motion model of evolution fits the data. K > 1 indicates that close relatives are more similar than expected, and K < 1 indicates more divergence between taxa than expected under a Brownian model.

2. Supplementary results: Ancestral state optimization and phylomorphospace for the Hackett et al. (2008) phylogenetic tree.

Figure. S1. Ancestral state estimation for wing shape based on ventral PC1 scores, PC1vs *(a)* and dorsal PC1 scores, PC1ds *(b)* utilizing weighted squared-change parsimony for the Hackett et al. (2008) phylogenetic tree. Vector-based output was used to visualize how shape differed across the phylogeny. Each landmark of the consensus wing represents the vector base, and arrows show the direction and magnitude of variation at any point along the wing. Ducks, Grebes, Charadriiformes and Core Waterbirds show a trend toward more elongate primary covert feathers and shorter secondary feathers regardless of flight style or body size. Core Landbirds show a trend toward shorter primary coverts.

phylomorphospace described by principle components 1 and 2 for the 105-taxon sample. Internal nodes are placed according to a squared-change parsimony optimization. Major changes in wing shape along the two PC axes are depicted on deformation grids from the consensus wing (insets). Each landmark on the consensus wing represents the vector base, and arrows show the direction and magnitude of variation at any point along the wing. In the wing insets, changes (in blue) along PC1 reflect a shortening of the greater primary coverts and greater secondary feather length. Changes (in blue) along PC2 reflect

a narrowing of the wing tip. Dot color for individual taxa reference major avian subclades recovered in each phylogenetic analysis.

3. Supplementary Results: Comparison of aerodynamic variables with PC1 values.

Figure. S3. Phylogenetic generalized least squares (PGLS) plots of PC1 on aspect ratio (AR), wing loading (Q), wing beat frequency (Hz), flight speed (V), body mass (BM) of dorsal wing (PC1d, right column) and ventral wing (PC1v; left column) for the Jarvis et al. (2014) topology.

4. Supplementary Results: Assessment of *Archaeopteryx*

Figure. S4. Digitization and location of the reconstructed ventral wing (A) of *Archaeopteryx* (Rietschel,1985) in the phylomorphospace of living birds. In Rietschel (1985), ventral surface of the right wing was reconstructed while in Wellnhofer (2008), both dorsal and ventral surfaces were reconstructed with the greater secondary covert length on the ventral side shown as much longer than those on the dorsal side. Given that only ventral coverts were exposed in the Berlin specimen [15, 16], the basis for

the short dorsal secondary coverts reconstructed in Wellnhofer (2008) is unclear. *Archaeopteryx* lies in a space not occupied by any other living taxa but shows a primary covert length similar to aquatic taxa. Both the Jarvis et al. 2014 (top) and Hackett et al. 2008 (bottom) phylogenetic trees are projected in this morphospace.

5. Supplementary results from Phylogenetic generalized least squares.

Table S1. Phylogenetic generalized least squares (PGLS) of PC1 on aspect ratio (AR), wing loading (Q), wing beat frequency (Hz), flight speed (V), body mass (BM) of dorsal wing (PC1d) and ventral wing (PC1v) for the Hackett et al. (2008), "H"; and Jarvis et al. (2014), "J" topologies.

6. Supplementary results: PhyloANOVA.

Table S2. PhyloANOVA using the Jarvis et al. 2014 (top) and Hackett et al. 2008 (bottom) topologies with post-hoc tests for means of PC1 scores between any two flight styles. CF, 'continuous flapping', occurs in birds with high wing-loading, comprising mainly waders and waterfowl; FG, 'flapping and gliding', comprises relatively long flapping phases and comparably long gliding phases with fully stretched wings; FS, 'flapping and soaring', relatively big birds with large wing-area for soaring in thermals and gliding between thermals or in extended updrafts and also include dynamic soaring; PT, 'passerine type flight', is a combination group of flap-gliding, partially flexed wings and completely flexed wings during flight.; PC1v, ventral side; PC1d, dorsal side.

*significant level at 0.05.

7. Supplementary Data

Table S3. List of included species and specimen numbers per species. UWBM, Burke Museum University of Washington. Ecological category: 1, terrestrial; 2, aquatic/semi-aquatic. Two sets of ecology category data were used. In the second sample, more littoral/terrestrial taxa (indicated with with an *) in Charadriifromes, Anatidae and Ciconiformes were exclude from aquatic ecology group.

Table S4. PC scores and other data used. Body mass (BM) data are from Dunning [17]. Wing aspect ratio (AR, aspect ratio, wing span²/wing area) and wing loading (Q, wing loading, body mass/ wing area) measurements are from a dataset used in [18] and [19]. Wingspan and wing area include the area of the body equal to the proximal wing chord. Cruising speed (V) and wing beat frequency (Hz) data are from the literature [19, 20, 21] . It should be noted that flight speed data are from distinct populations and seasons and were not collected under identical conditions [19]. Four flight styles for living birds were defined most recently by Bruderer et al. [19]. These categories, 'continuous flapping' (CF); 'flapping and soaring' (FS); 'flapping and gliding' (FG) and 'passerine type' (PT) flight, and the assignment of taxa to these categories are based on this recent review by Bruderer et al. [19].

References

- 1. Hackett SJ*, et al.* 2008 A phylogenomic study of birds reveals their evolutionary history. *Science* **320**,1763-1768.
- 2. Alerstam T, Rosén M, Bäckman J, Ericson PG, Hellgren O 2007 Flight speeds among bird species: allometric and phylogenetic effects. *PLoS Biol.* **5**,e197.
- **3.** Bruderer B, Peter D, Boldt A, Liechti F 2010 Wingbeat characteristics of birds recorded with tracking radar and cine camera. *Ibis* **152**,272-291.
- 4. Brewer ML, Hertel F 2007 Wing morphology and flight behavior of pelecaniform seabirds. *J. Morphol*.**268**,866-877.
- 5. Jetz W, Thomas G, Joy J, Hartmann K, Mooers A 2012 The global diversity of birds in space and time. *Nature* **491**,444-448.
- 6. Maddison W, Maddison D 2011 Mesquite 2.75: a modular system for evolutionary analysis.
- 7. Brown JW, Rest JS, García-Moreno J, Sorenson MD, Mindell DP (2008) Strong mitochondrial DNA support for a Cretaceous origin of modern avian lineages. *BMC Biol.* **6**,6.
- 8. Han K-L, Braun EL, Kimball RT, Reddy S, Bowie RC, Braun MJ, Chojnowski JL, Hackett SJ,Harshman J, Huddleston CJ 2011 Are transposable element insertions homoplasy free?: an examination using the avian tree of life. *Syst. Biol*.**60**,375-386.
- 9. Wang X , Clarke JA 2014 Phylogeny and forelimb disparity in waterbirds. *Evolution* **68**,2847-2860.
- 10. Jarvis ED*, et al.* 2014 Whole-genome analyses resolve early branches in the tree of life of modern birds. *Science* **346**,1320-1331.
- 11. Klingenberg CP, Gidaszewski NA 2010 Testing and quantifying phylogenetic signals and homoplasy in morphometric data. *Syst. Biol.* **59**,245-261.
- 12. Pagel M 1999 Inferring the historical patterns of biological evolution. *Nature* **401**(6756):877-884.
- 13. Harmon LJ, Weir JT, Brock CD, Glor RE, Challenger W 2008 GEIGER: investigating evolutionary radiations. *Bioinformatics* **24**,129-131.
- 14. Blomberg SP, Garland T, Ives AR 2003 Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* **57**,717-745.
- 15. Rietschel S 1985 Feathers and wings of *Archaeopteryx*, and the question of her flight ability. In *The beginnings of birds,* pp.251-260.
- 16. Wellnhofer P 2008 *Archaeopteryx. Spektrum der Wissenschaft* **8**.100.
- 17. Dunning JB 1993 Handbook of avian body masses. *CRC, London*.
- 18. Nudds RL, Dyke GJ, Rayner JMV 2007 Avian brachial index and wing kinematics: putting movement back into bones. *J. Zool.* **272**, 218-226.
- 19. Bruderer B, Peter D, Boldt A, Liechti F 2010 Wingbeat characteristics of birds recorded with tracking radar and cine camera. *Ibis* **152**,272-291.
- 20. Pennycuick C 1990 Predicting wingbeat frequency and wavelength of birds. *J. Exp. Biol*. **150**,171-185.
- 21. Alerstam T, Gudmundsson GA, Larsson B 1993 Flight tracks and speeds of Antarctic and Atlantic seabirds: radar and optical measurements. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **340**,55-67