Article

Evolution of avian foot morphology through anatomical network analysis

Julieta Carril [1,2] ✉, Ricardo S. De Mendoza [1,2], Federico J. Degrange[3], Claudio G. Barbeito[1,2] & Claudia P. Tambussi[3]

Avian evolution led to morphological adaptive variations in feet. Diverse foot types are accompanied by a diverse muscle system, allowing birds to adopt different primary lifestyles, and to display various locomotor and manipulative skills. We provide insights of evolutionary and functional significance on the avian foot architecture through Anatomical Network Analysis, a methodology focused on connectivity patterns of anatomical parts. Here, we show that: (1) anatomical parts largely conserved in living birds and already present in ancestral dinosaurs exhibit the highest connectivity degree, (2) there is no link between the more complex foot networks and the ability to perform more specialized skills or a higher number of different tasks, (3) there is a trend towards the simplification of foot networks on a macroevolutionary scale within birds, and (4) foot networks are phylogenetically constrained and conserved in all birds despite their foot type diversity, probably due to stabilizing selection.

Over ~100 million years of evolutionary diversification, but mainly the explosive phyletic radiation following the K–Pg extinction event[1], resulted in about 11,000 living bird species (Neornithes) within 254 families[2]. The avian evolutionary journey led to a body plan (bauplan) that unequivocally characterizes birds in high taxonomic levels, in addition to morphological adaptive variations in their feet at lower taxonomic levels (e.g., orders, families).

In birds, hind limbs are highly modified for bipedal locomotion, as well as for leaping from and landing on the substrate[3,4]. The most important evolutionary changes at their hind limbs are the fusion of independent bones forming the tibiotarsus (tibia + proximal tarsal bones) and the tarsometatarsus (metatarsals 2–4 + distal tarsal bone), in addition to the retroversion of the digit I (hallux)[5]. Avian feet are traditionally classified in several categories based on the number of digits, and their positional arrangement and mobility. These include anisodactyl, didactyl, tridactyl, heterodactyl, zygodactyl, ectropodactyl, semizygodactyl and pamprodactyl[3] (Fig. 1a-c). Furthermore, they are also classified based on the presence and extent of skin, lobes, or web between digits into syndactyl, lobate, semipalmate, palmate

and totipalmate[3] (Fig. 1d). These greatly diverse types of foot are accompanied by an extremely complex and also diverse system of muscles, enabling birds to perform a great variety of movements such as coordinated or individual flexion, extension, abduction and adduction. This versatility in motion allows birds to display various locomotor and manipulative skills (e.g., walking, running, hopping, wading, perching, climbing, swimming, diving, hanging upside down, grasping), and thus, to explore and conquer several niches and display several behaviours[3,4]. Moreover, tradeoffs between the forelimbs and hind limbs have influenced the evolution of several locomotor strategies[6], leading birds to adopt different primary lifestyles; including terrestrial, arboreal, aquatic and hyperaerial.

Hypothesizing about the mechanisms leading to evolutionary changes and functional skills based on morphological variation is a classical conceptual tool. The morphology of the feet of birds has been widely studied using standard methodological tools such as those analysing bone shape, phalanx size proportions, toe and metatarsals trochleae orientations, claw curvature, and digital muscle and tendon configurations[3,7–15]. We aim to provide insights of evolutionary and

[1]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), La Plata, Argentina. [2]Laboratorio de Histología y Embriología Descriptiva, Experimental y Comparada (LHYEDEC), Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, Av. 60 y 118, Buenos Aires 1900, Argentina. [3]Centro de Investigaciones en Ciencias de la Tierra (CICTERRA), Universidad Nacional de Córdoba-CONICET, Ing. Ismael Bordabehere y Av. Haya de la Torre, Córdoba 5016, Argentina. ✉e-mail: julyetacarril@gmail.com
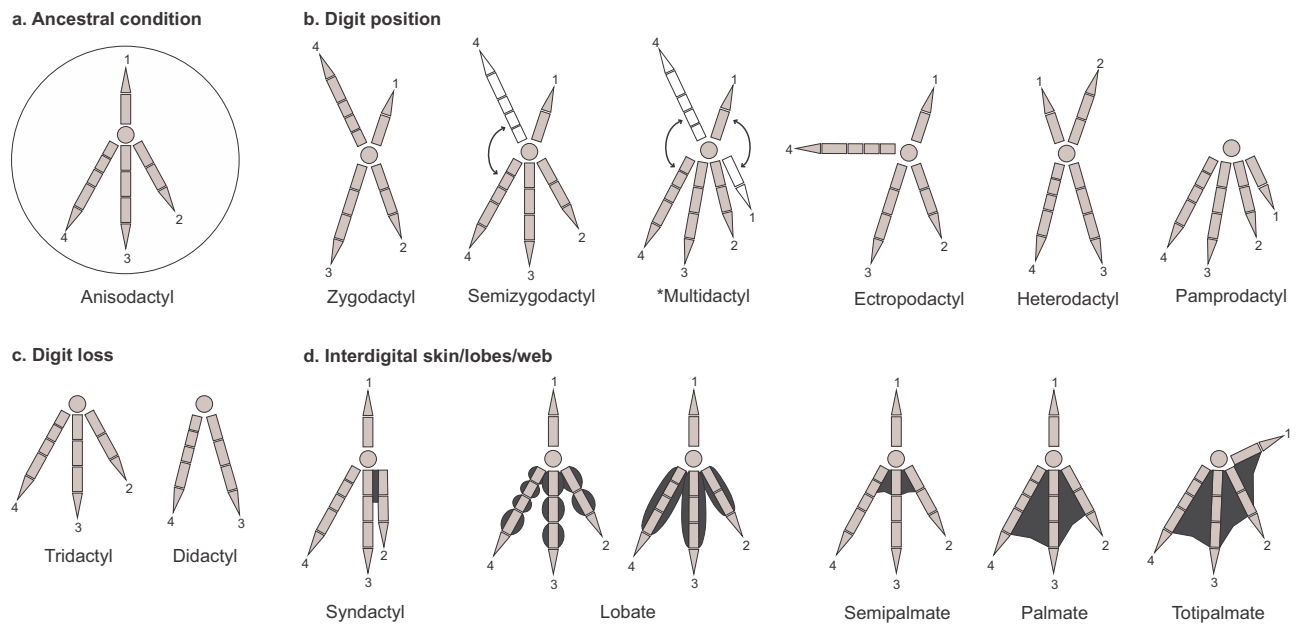
Fig. 1 | Foot types of birds. a the ancestral foot type, from which the other foot types evolved[3,7]. Classification based on: (**b**) the positional arrangement of digits and ability to rotate and change the position of some of them, (**c**) the secondary loss of digits and the remaining number of digits, and (**d**) the presence and extent of skin, lobes or web between digits. *We proposed the term multidactyl for birds able to rotate both the first and fourth digits to either a cranial or a caudal position (e.g., *Colius striatus*).

functional significance on the avian foot architecture through Anatomical Network Analysis (AnNA)[16]. By using connectivity data, AnNA complements the information on the comprehensive concept of form provided by the traditional linear and geometric morphometrics, thus allowing the comparison of disparate anatomies like 2-toed, 3-toed, and 4-toed birds. AnNA has been widely and successfully adopted in the scientific community over the past 10 years to address functional, evolutionary and developmental questions related to the musculoskeletal system of limbs in various taxa[17–24], including birds as studied by our research group[25,26].

In this work, we analyse the connectivity patterns and the mutual influence of the anatomical parts of the musculoskeletal system of the foot of 62 species representatives of most major avian lineages (Supplementary Data 1). In doing so, we address the following subject matters: (1) the use of the connectivity degree (ki), a parameter related to the co-dependence of an anatomical part with others within the network, as a proxy for evolutionary constraints of the anatomical parts and their role in the avian body plan; (2) the potential link between the structural and functional complexity of the foot with the complexity of their anatomical networks; (3) the birds' foot networks disparity and its potential link with the primary lifestyles, foot skills, and nest attendance type, (4) the correspondence between the diversity of foot types and the connectivity patterns of their networks; (5) the role played by the phylogenetic history in shaping the foot networks; and (6) the trace of the evolutionary history of network parameters across Neornithes.

Results

Network anatomical parts and body plan

There is a set of features that unequivocally distinguishes birds from other lineages of tetrapods. These are the result of a progressive assembly process that unfolded over 160 million years, leading to the acquisition of the avian body plan[27]. Most features characterizing living birds (Neornithes) are restricted to the skeleton, including a reorganization of limbs due to loss or fusion of bones, or both. Of all the evolutionary events describing the assembly of the avian body plan, 43% belong to the hind limbs[28].

The avian foot networks revealed some patterns that can be associated with the avian body plan at its crown group level. Bony elements are conserved in all species, except in the few species that secondarily lost the digit 1 (tridactyls like *Dromaius* and *Eudromia*) and digit 2 (the sole didactyl *Struthio*). The avian foot is characterized by the fusion of metatarsals II-IV forming the tarsometatarsus. All digits contact the tarsometatarsus and all the intrinsic muscles originate from it (Fig. 2a). Not surprisingly, this is the bone with the highest connectivity degree (ki) in almost all species (Fig. 2d; Supplementary Data 2), a network parameter that measures the co-dependency of an anatomical part with others and is related to its constraints for evolutionary change[16].

Extrinsic muscles of the foot (i.e., those arising from the femur, tibiotarsus and/or fibula, and inserting on the digits) are largely conserved, with few exceptions (e.g., the absence of mm. flexor hallucis longus in *Apteryx* and *Pterocles*, flexor perforatus digiti 2 in *Chaetura* and *Podiceps*, flexor perforans et perforatus digiti 2 in *Struthio*). Specifically, the extrinsic muscles flexor digitorum longus and extensor digitorum longus, which are responsible for the coordinated and simultaneous flexion and extension of the three forward toes[3], are the muscles with the highest ki (Fig. 2d; Supplementary Data 2). The digital flexor group evolved from two muscles into seven or more among the lineage leading to crown-group birds, but how or when this subdivision proceeded is still unknown[28]. In contrast, the digital extensor group, which includes the mm. extensor digitorum longus and extensor hallucis longus and their insertions on the ungual phalanges, was already present in ancestral dinosaurs[28]. Undoubtedly, both the antagonistic flexor digitorum longus and extensor digitorum longus, along with an opposable incumbent hallux, are the basic precursors needed for grasping[15] and for an arboreal lifestyle; an adaptation acquired ~185–145 Mya ago[27].

Intrinsic muscles of the foot (i.e., those arising from the tarsometatarsus and inserting on the digits) provide independent action of the toes[3]. Their presence/absence varies between taxa: some intrinsic muscles are present in a few species, while others are exclusive to certain species (i.e., mm. extensor propius digiti 3 accesorius in *Amazona* and extensor propius digiti 4 in *Strix* and *Colius*). Also, the
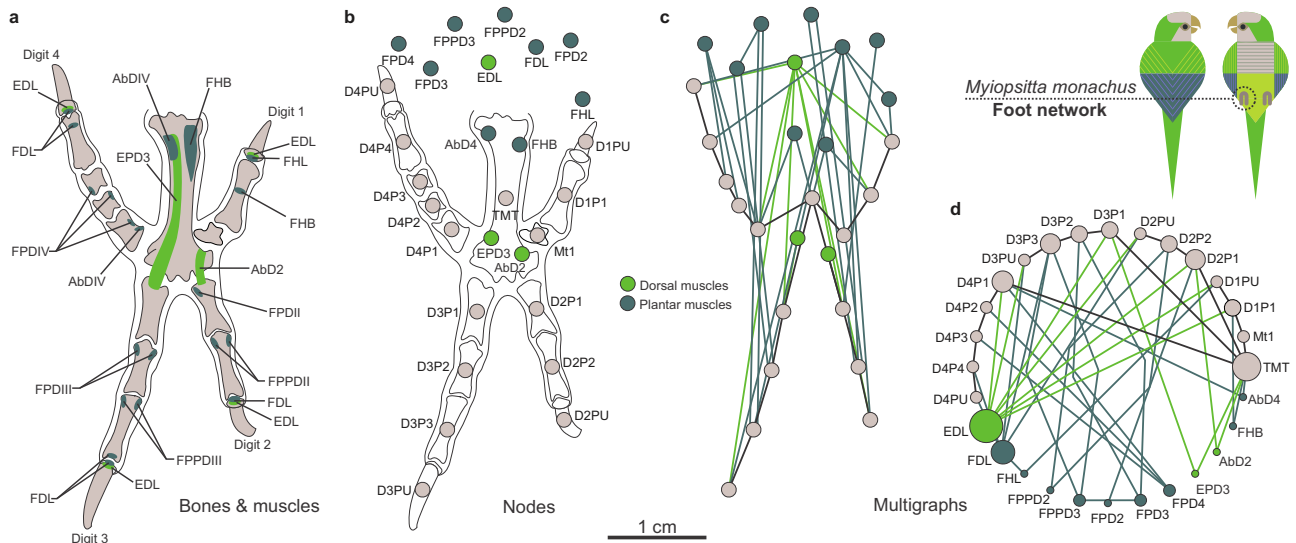
Fig. 2 | Foot network of the monk parakeet (*Myiopsitta monachus*, Psittaciformes). a Tarsometatarsus and phalanges, showing the muscles' attachments. **b** Detail of bone and muscle nodes. Multi-network models following: **c** foot topology, and **d** circular layout showing the proportional size of nodes according to their connectivity degree (k_i). For abbreviations of bones and muscles, see Supplementary Data 12. Illustration of the monk parakeet attributed to Scott Partridge.

intrinsic muscles' k_i values are low (Fig. 2d; Supplementary Data 2). Individual digit movements might be useful in climbing and/or in manipulating objects or food items, specialized skills probably acquired later in the evolutionary history of birds. Most intrinsic muscles are absent in the Passeriformes, a pattern that may be related with the evolutionary trend in phalanx length proportions (i.e., the penultimate phalanxes of digits II-IV are longer than the more proximal phalanxes)[11]. However, their secondary loss cannot be explained through connectivity, because they share similar k_i values with other anatomical elements. A plausible explanation may rely on the changes of the muscles' early developmental mechanisms throughout the course of evolution. For example, regulatory changes in the expression of genes controlling hind limb musculoskeletal development and patterning (e.g., Tbx4)[29], or in the signalling pathways regulating cleavage of muscle mass precursors into individual intrinsic muscles (e.g., retinoic acid mediating apoptosis in myogenic cells, vessels and platelet derived growth factor-B PDGFB involvement in muscle splitting)[30,31].

Exploring foot network complexity

Complexity in morphology is usually related to the high number of anatomical parts[32]. However, when studying the morphology with AnNA, complexity is defined as the high number of structural and functional interactions among different anatomical parts[32]. In AnNA, the focus of the study shifts from the anatomical parts to the connections between those anatomical parts. Within this framework, complexity can be quantified by the following network parameters: density of connections (D), average clustering coefficient (ACC), and average shortest path length (APL)[16,32]. These parameters measure the abundance of connections (D), the interdependence or integration (ACC), and the proximity between nodes (APL). Therefore, complex systems are expected to have higher D and ACC, and lower APL. In contrast, simple systems are expected to have lower D and ACC, and higher APL. By intuition, it is expected that complex anatomical systems are capable of performing complex behaviours. For example, in musculoskeletal systems, more connections (high D) could be linked to the achievement of greater ranges of motion and greater potentiality of action, or both[32]. Additionally, the proximity of anatomical parts (low APL) could be linked to a greater efficiency for spreading biomechanical forces[33]. However, the observed pattern for the foot networks of birds does not align with this expectation, as detailed below.

The specialized skilled foot is mainly restricted to a few bird species (for details, see Supplementary Data 3 and 4). Brinkworth et al.[34] found that birds with more complex appendicular skeletons tend to occupy specialized dietary and habitat niches, establishing a link between complex morphology and ecological specialization. We do not find this association when studying the musculoskeletal networks of birds' feet. Our results (Fig. 3, Supplementary Data 5) show that almost 13% of the analysed species have complex systems, and almost 30% of the analysed species are able to perform one or more specialized skills with their feet such as climbing, powerful grasping, digital dexterity, and hanging upside down. Unexpectedly, most of those species have systems that are neither complex nor simple. The only species capable of performing a specialized skill as climbing which also has a complex network is the hoatzin *Opisthocomus*. This species is characterized for having the highest D value, and the lowest -and notably different from the rest of the analysed species- APL value. However, hoatzins climb with the help of their beaks (similar to psittacids), and also with the use of their wing claws when they are young[2]. Therefore, it would be interesting to explore the complexity parameters of the foot network of a true scansorial (climber) foot, such as the ectropodactyl foot of woodpeckers[7], for which detailed musculoskeletal information is still not available. In addition, taxa with complex foot networks do not perform any of the specialized skills (i.e., *Dromaius*, Galliformes and *Uria*); and many of the species able to perform specialized skills have simple foot networks (i.e., 16% of the analysed species; *Chordeiles*, *Chaetura*, *Pterocles*, Strigiformes, *Colius*, *Merops*, *Galbula*, *Caracara*, and *Tyrannus*). This means that more complex foot networks do not necessarily perform more specialized skills, and that the simplicity of a foot network does not limit its potential for specialization.

Some birds can perform more functions than others. The functional complexity[35] refers to the number of different tasks that an organism can perform. Within our sample, psittacids are the species having the greatest variety of foot tasks. They can walk, run, hop on land and trees, and are capable of climbing and hanging upside down on branches. Besides, they are distinguished for their digital dexterity while manipulating food and other objects. Despite the ability of psittacids to perform a high number of tasks, their foot networks are neither complex nor simple. The same is true for other birds capable of performing a wide number of tasks (e.g., *Coccyzus*, *Tapera*, *Nannopterum*, *Anhinga*, *Ixobrychus*, *Accipiter*, *Fringilla*, and *Turdus*). Moreover, the mousebird
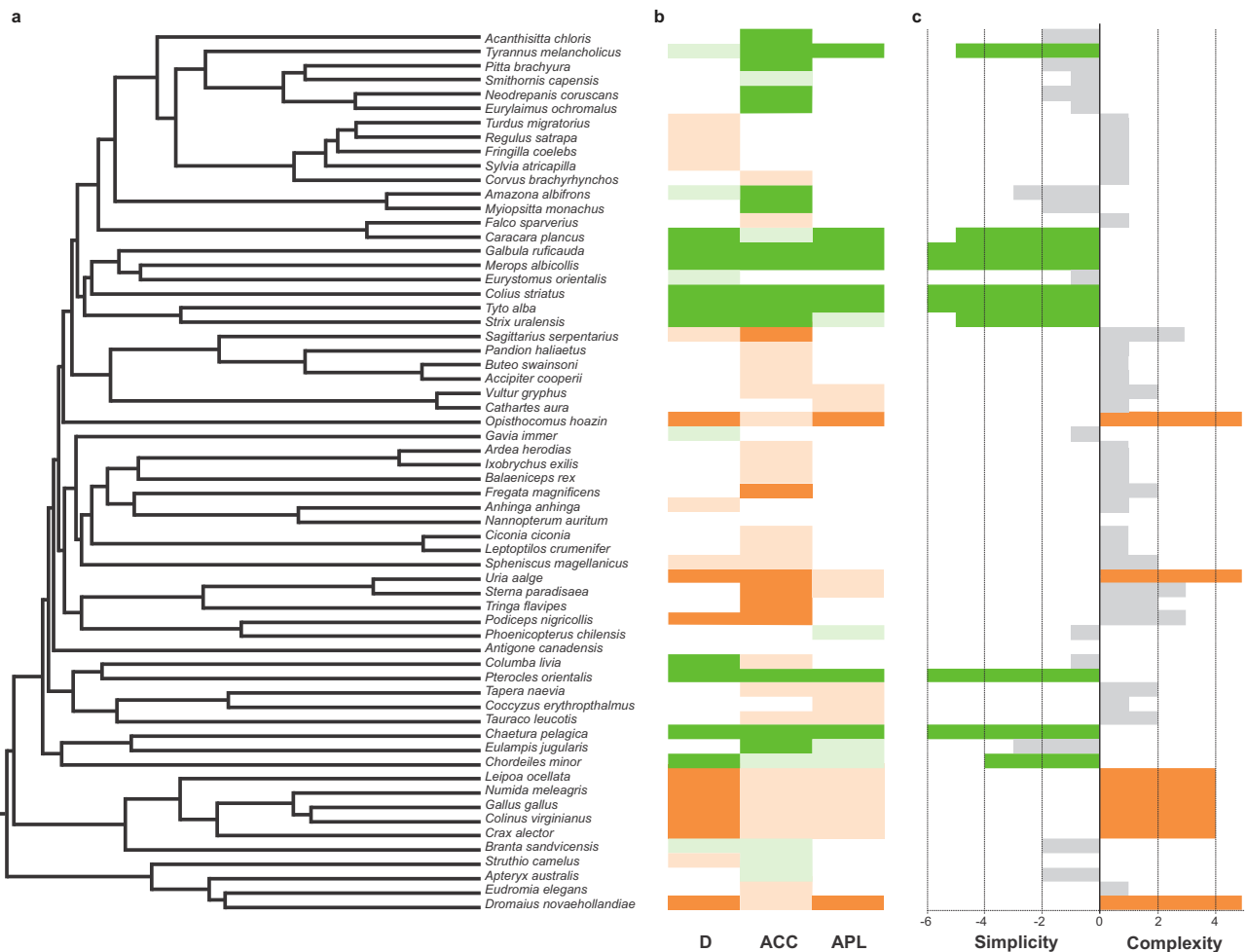
Fig. 3 | Complexity. a Time-calibrated phylogeny of birds (Prum et al.[40]). **b** Heatmap of the network parameters that capture the morphological complexity (in orange) or simplicity (in green) of an entire network: density of connections (D), average cluster coefficient (ACC), average shortest path length (APL). **c** Scores of the parameters (D, ACC, and APL) determining the complexity (score of 4 or higher) or simplicity (score of −4 or less) of the systems. For details, see Supplementary Data 5.

Colius and Strigiformes can perform a wide range of tasks despite having simple foot networks. On the contrary, birds capable of performing fewer tasks, such as cursorials (e.g., *Struthio* and *Dromaius*), hyperaerials (e.g., *Eulampis* and *Fregata*), and the terrestrial *Pterocles*, have foot networks that are complex, simple, or neither of them. This suggests that more complex foot networks do not necessarily perform more tasks; and that although form and function are closely linked, morphological complexity within AnNA and functional complexity are not. This is not exclusive to birds. For example, in humans, the hind limb network complexity is the same as in chimpanzees, although they have different hind limb functional complexity[20].

In general, complex foot networks belong to species with more plesiomorphic morphologies, whereas simple foot networks are associated with species with more derived morphologies. This suggests a potential evolutionary trend towards the simplification of foot networks. Simpler networks may provide benefits in terms of energetic saving[36] without compromising foot functionality. The extent of morphological changes in relation to the complexity of the avian body plan on a macroevolutionary scale, and its comparison with the morphological divergence of birds remains to be elucidated.

Phylomorphospace and mapping

The foot networks of birds do not form distinct clusters in the phylomorphospace (Fig. 4a, Supplementary Data 6 and 7). Most networks are distributed around the centre of the phylomorphospace, except for four species located at opposite limits, clearly separated from the rest of the species. At the negative limit of principal component (PC) 2 is located the ostrich *Struthio*. This is the sole living dydactyl bird and, in consequence, it is characterized by having the lowest number of nodes (N) and connections (L) in its network (Supplementary Data 2). Dydactyl foot type is considered a high-speed running adaptation[3], making the ostrich the fastest of all birds, reaching speeds of 70 km/h[37]. At the opposite and positive limit of PC2 is the mousebird *Colius*, the sole multidactyl bird of our analysis. It is characterized for having a low value of number of connections (L) and the lowest value of density of connections (D) (Supplementary Data 2). Mousebirds have the ability to rotate both the first and fourth digits of the foot to either a cranial or a caudal position, and thus, being able to adopt an anisodactyl, zygodactyl or pamprodactyl feet configuration[38]. Multidactyly provides mousebirds with a great variety of movements, postures and manipulative skills useful in locomotion, feeding, and even during aggressive encounters with other birds[38]. Finally, at the positive limit of PC1 is the hoatzin *Opisthocomus*, while at the negative limit of PC1 is the sandgrouse *Pterocles*. Functional explanation for the distribution of these two anisodactyl species is hard to find. However, considering the hoatzin is a monotypic taxon, its position in the phylomorphospace could be due to its long independent evolutionary history rather due functional constraints.
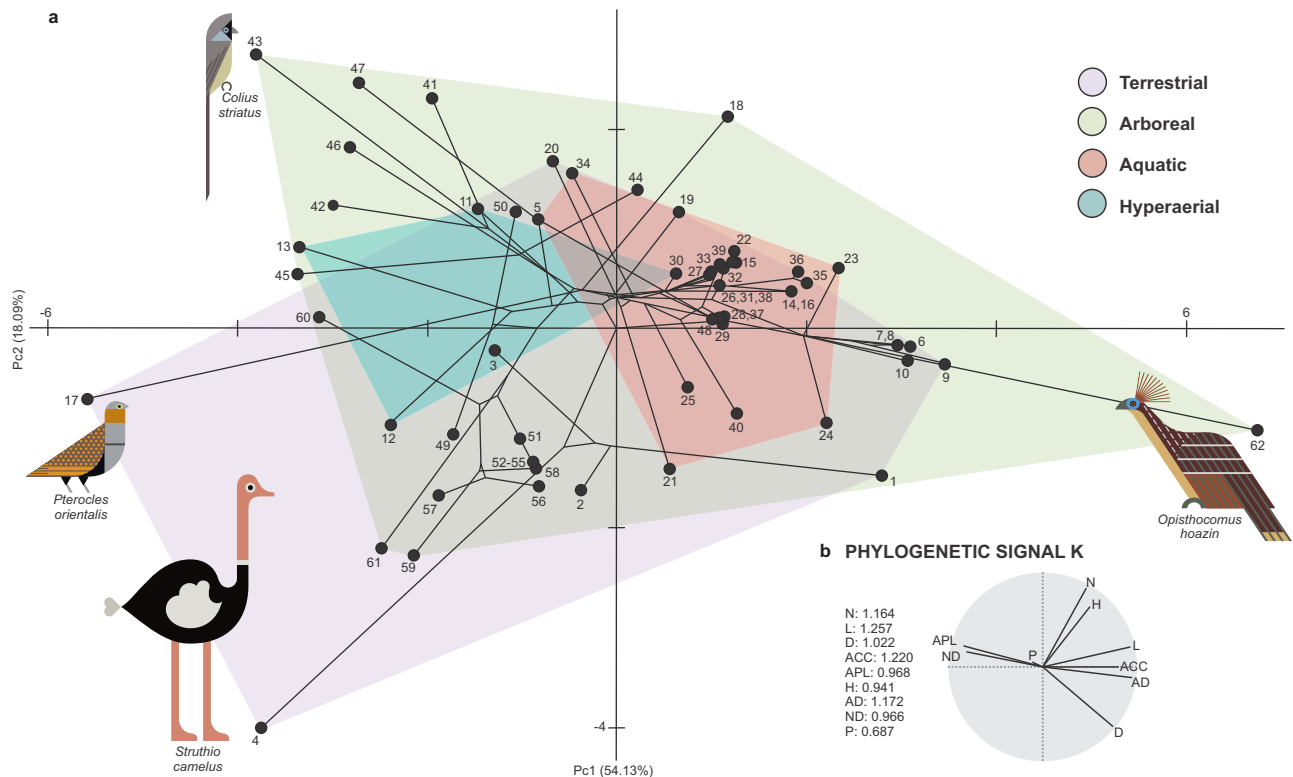
Fig. 4 | Phylomorphospace of PCA of network parameters. a The 62 bird species from the time-calibrated phylogeny of Prum et al.[40] are grouped by colours according to their primary lifestyle. Number labels represent the species (for references see the birds' species list in the Supplementary Data 1). **b** Contributions to each parameter and their phylogenetic signal (K). Network parameters: average cluster coefficient (ACC), average degree (AD), average shortest path length (APL), density of connections (D), heterogeneity (H), number of connections (L), number of nodes (N), network diameter (ND), and parcellation (PA) (for details see Supplementary Data 2). Illustration of birds attributed to Scott Partridge.

When grouping foot networks according to their primary lifestyles, all groups overlap around the centre; although they clearly differ in the amount of morphospace occupied by each lifestyle: arboreal and terrestrial birds are more dispersed than aquatic and hyperaerial birds (Fig. 4a, Supplementary Data 7a). This distribution pattern in the phylomorphospace finds support in the results obtained from the OU/BM evolutionary tests. The best model for fitting the network parameters into the evolution of the different primary lifestyles was a Brownian motion model with multiple possible optima (BMM) (Akaike Information Criterion, AIC = 443.7492, Supplementary Data 8), revealing thus a change of rates between the different primary lifestyles. The rates of evolution along PC1 of arboreal birds was 4.9 times faster than aquatic birds, and 1.1 times faster than hyperaerial birds; while the rate of terrestrial birds was 2.5 times faster than aquatic birds, and 1.6 times slower than hyperaerial birds (Supplementary Data 8). Slower rates in the more plesiomorphic terrestrial birds and faster rates in the more derived arboreal birds could have led to a greater dispersion in the phylomorphospace for birds of both lifestyles. Meanwhile, a possible evolutionary scenario for the lesser dispersion in the phylomorphospace of aquatic and hyperaerial birds could result from reaching the morphological limits for those lifestyles, at slower or faster rates, respectively. The rate of evolution along PC2 was very similar across all primary lifestyles (Supplementary Data 8). Finally, the best model for fitting the network parameters into the evolution of the different nest attendance types and foot types was a Brownian motion model, but with a single possible optimum (BM1) (AIC = 447.4479, Supplementary Data 8), indicating the same rate of evolution across all nest attendance types and foot types.

When grouping foot networks according to their skills, flightless birds and species capable of terrestrial hopping, wadding, swimming, foot propelled diving, and grasping are more clustered in the phylomorphospace in relation to the rest of the species (Supplementary Data 6 and 7), but only the difference between flying and flightless birds resulted to be statistically significant ($F = 55.445$, $p = 0.02$). Finally, when analysing the nest attendance, precocial birds are the most dispersed (Supplementary Data 6q and 7q). All this suggests that the distribution of foot networks in the phylomorphospace is not largely influenced by the primary lifestyles, skills, or nest attendance type.

Regarding foot types (Supplementary Data 6p and 7p), anisodactyl birds show the greatest dispersion, which translates into a great morphological diversity. The rest of the foot types converge in the centre of the phylomorphospace, except for the sole foot types aforementioned: the didactyl *Struthio* and the multidactyl *Colius*. These results go against our expectations, as we hypothesized a correspondence between the diversity of foot types and the connectivity patterns of their networks. On the contrary, our results demonstrate that, although the adaptive radiation of birds led to several foot types, the connectivity of their anatomical parts remained conserved.

PERMANOVA tests validate there is no significant distinction between groups in the phylomorphospace occupation that could be explained by primary lifestyles ($F = -6.7147$, $p = 0.8143$), nest attendance type ($F = 25.258$, $p = 0.3206$), or foot types (F = 0.4989, $p = 0.6813$).

The character mapping and the ancestral reconstruction of the network parameters onto a molecular phylogeny of birds reveal that the ancestor of all Neornithes likely occupied a portion of the phylomorphospace close to the centre (Fig. 4a, Supplementary Data 9). The ancestor reconstructed position, located near and from where most species are distributed, allows for uncovering foot evolutionary pathways. A low variation at the connectivity level could offer high resistance to evolutionary changes, as it carries less potential on which selection can act[39]. In fact, of the nine parameters analysed, only parcellation (PA) showed low phylogenetic signal, indicating that most

network parameters carry more phylogenetic signal than expected under Brownian motion (Fig. 4b). Moreover, PC1/PC2 vs PCoA1/PCoA2 showed grouping of foot networks of the main clades along all the ecological variables (Supplementary Data 10), and the correlations between PC1/PC2 and PCoA axes turned to be not significant (slope for the intercept = -0.1082808, slope for Axis 1 = -0.1415643, slope for Axis 2 = -3.4641495; all p-values > 0.05, pseudo $R^2 = 0.1455967$). Therefore, the distribution of birds in the phylomorphospace is largely constrained by their evolutionary history. This high phylogenetic signal could, in turn, result in entrenchment into the connectivity of anatomical parts and could have acted as a limit in the production of further diversity.

Discussion

The study of avian foot networks revealed some patterns that can be associated with the avian body plan. Anatomical parts largely conserved in Neornithes and already present in ancestral dinosaurs, like the tarsometatarsus and the digital flexor/extensor system, are the ones with the highest connectivity degree (ki), a parameter related to the co-dependence of an anatomical part with others and its constraints for evolutionary change.

Most birds have foot networks that are neither complex nor simple. There is no evident link between more complex foot networks and the ability to perform more specialized skills (climbing, powerful grasping, digital dexterity, and hanging upside down) or more functions (number of different tasks). The simplicity of the foot network does not limit its potential functions and, on a macroevolutionary scale within the entire clade Aves, there is a trend toward the simplification of foot networks.

The network connectivity pattern of the diverse foot musculoskeletal system in birds is constrained by their lineage-specific phylogenetic history. Moreover, foot networks do not align with the highly diverse foot types of birds, which are classified based on the number, positional arrangement, mobility, and the presence and extent of skin/lobes/or web between digits. This scenario could be the result of stabilizing selection acting specifically on foot network connectivity rather than on foot type variation.

Methods

Data acquisition

A total of 62 species representatives of most major avian lineages from the phylogenetic proposal of Prum et al.[40] were selected (Supplementary Data 1 and 11). Birds were classified based on their primary lifestyles[2]. Most birds are capable of several locomotor and manipulative different skills, namely, the behaviours that are known to be capable of being performed. Therefore, in order to capture the plasticity of bird foot usage, we scored as either absent or present all the possible skills for each species[2]. We also classified the birds according to the nest attendance[2,41], and foot type[2,3] (for details on the characters, see Supplementary Data 3). Gross anatomical data of the musculoskeletal system of the foot (i.e., the absence/presence of bones and their articulations, and the absence/presence of muscles and their origins/insertions) was acquired by reviewing the descriptions in the bibliography (for details of the sources of the anatomical descriptions for each species see Supplementary Data 1). Osteological nomenclature follows Baumel & Witmer[5], and myological nomenclature follows Vander Berge & Zweers[42]. Bones and muscles abbreviations and synonyms between the muscle names used by the different authors cited are presented in Supplementary Data 12.

Network modelling

We constructed musculoskeletal anatomical multi-network models of the foot (Fig. 2c, d) for all the species in our data set, considering each bone and each muscle as nodes. We included the tarsometatarsus, metatarsal 1, phalanges, and the muscles responsible for the movements

of the toes. Bone-bone, bone-muscle, and muscle-muscle connections were represented as unweighted and undirected connections between pairs of nodes. For bones, the connections represent their articulations; while for muscles the connections represent their origins and insertions, and the tendinous connections between muscles. The topological information on node relationships was coded in adjacency matrices (i.e., symmetric not binary matrices of size NxN, where 0 indicates absence and 1 or more indicates presence of connection)[43].

Network analysis

Networks and statistical analysis and visualizations were performed in R 4.3.0[44]. Different parameters were obtained by using the R package Igraph[45]. These include: (1) connectivity degree (ki), which is the sum of connections that a specific node has with other nodes in the network; (2) number of nodes (N), which is the simple count of nodes of each network; (3) number of connections (L), which is the total number of connections among nodes of each network; (4) density of connections (D), which is the number of actual connections of each network with respect to the maximum possible; (5) average cluster coefficient (ACC), which is the average of the number of interconnections between the neighbours of all nodes in the networks; (6) average shortest path length (APL), which is the average length of all shortest paths (i.e., the minimal number of connections every two nodes) in a network; (7) heterogeneity (H), which is a measure of how even are the nodes according to their number of connections (specifically, the ratio between the standard deviation of the connections along the network and the average number of connections); (8) average degree (AD), which is the average of the connectivity degree (ki) of the network; (9) network diameter (ND), which is the length of the longest path; and (10) parcellation (PA), which is related to the subdivision of the network in modules. An introduction to anatomical network analysis can be found in Rasskin-Gutman & Esteve-Altava[16]. Phenograms[46] were constructed for the different network parameters using the function phenogram of the phytools package[47] (Supplementary Data 13). In order to detect nodes with high (and low) burden rank, we search for those nodes with a connectivity degree (ki) value consisting of two standard deviations (SD) above (and below) the mean (Supplementary Data 2).

Complexity

High values of average cluster coefficient (ACC) and density (D), and low values of average shortest path length (APL) capture the morphological complexity of an entire network[16,27]. In order to determine the complexity (or simplicity) of the systems, we have assigned a scoring system for each of the three parameters. If D and ACC values are below/above one SD, the assigned value is -2/2; and if it is below/above 1/2 SD, the assigned value is -1/1. And if APL values are below/above one SD, the assigned value is 2/-2, and if it is below/above 1/2 SD, the assigned value is 1/-1. If the sum of the scores results in a score of −4 or less, we consider the system as simple; if the sum results in a score of 4 or higher, we consider the system as complex; and if the sum of the scores results in a score between -4 and 4, we consider the system neither complex nor simple.

Phylomorphospace occupation, PCA, PCoA, and regressions

A principal component analysis (PCA) of the network parameters using the function prcomp of the base package of R was performed. A phylomorphospace was generated with the phylomorphospace function of the phytools package[47] also in R using the comprehensive and time-calibrated phylogeny[40] (tree available in Supplementary Data 11). Bird distribution in the phylomorphospace was grouped according to the variables primary lifestyle, foot skills, nest attendance and foot type, as explained above. We performed a non-phylogenetic PERMANOVA with 10,000 iterations on the resulting first five PC's to test whether network parameters discriminate between the different variables. To compare the correlation between the network parameter

values and the variables of primary lifestyle, nest attendance and foot type, the character matrix with the variables (Supplementary Data 4) was transformed into a Gower distance matrix and then converted into a Principal Coordinates Analysis (PCoA) using the function pcoa implemented in the R ape package[48]. The first two PC's were compared with the first two coordinate axes using a phylogenetic Generalised Least Squares regression[49] using the gls function implemented in the R package nlme[50].

Phylogenetic signal

The amount of phylogenetic signal was assessed for the network parameters by calculating the kappa statistic (K)[51] under a Brownian motion model of evolution, using the phylosig function of the geiger package[52] of the programming language R.

Character mappings

The network parameters were mapped as characters in the time-calibrated phylogenetic tree[40] using the function FastAnc of the R phytools package[46] for estimation of ancestral states using Maximum Likelihood. As the parameter of ND only assumes integer values, we mapped the transitions between states considering an equal rates (i.e., equal probability) model, using the function fitER of the R phytools package[47].

We tested the possible shift of means and/or rates for different evolutionary regimes regarding the different primary lifestyles, nest attendance types and foot types, by fitting of the network parameters according to an Ornstein-Uhlenbeck model with multiple possible optima for a shift of means or with a single possible optimum for a single mean, and according to a Brownian motion model with multiple possible optima for a shift of rates or with a single possible optimum for a single rate. These OU/BM tests, that account for phylogeny, were performed with the functions mvOU (model = OUM and OU1, for multiple possible optima and a single possible optimum, respectively) and mvBM (model = BMM and BM1, for multiple possible optima and a single possible optimum, respectively) using the R mvMORPH package[53]. We compared the results of the different models (Supplementary Data 8) by selecting the model with the lowest Akaike information criterion (AIC) value following[54,55].

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The authors declare that all data supporting the findings of this study are available within the paper and its supplementary files. Adjacency matrices are in an external Zenodo repository (https://doi.org/10.5281/zenodo.13924386)[43]. Source data for Fig. 3 is in Supplementary Data 5. Source data for Fig. 4 is in Supplementary Data 2 (sheet 'parameters') and 11.

Code availability

All R scripts used in this study are fully available in an external Zenodo repository (https://doi.org/10.5281/zenodo.13924420)[56]. The code for making Fig. 3 is in the file 'R Script Fig. 3.R'. The code for making Fig. 4 is in the file 'R Script Phylomorphospace.R'.

References

1. Feduccia, A. Big bang' for tertiary birds? *Trends Ecol. Evol.* **18**, 172–176 (2003).
2. CornellLab. *Birds of the World.* https://birdsoftheworld.org/bow/home (2024).
3. Raikow, R. J. Locomotor System. In, *Form and Function in Birds*, Vol. 3 (eds. King, A. S. & Mc Lelland, J.) 57–147 (Academic Press, 1985).
4. Abourachid, A. & Höfling, E. The legs: a key to bird evolutionary success. *J. Ornithol.* **53**, 193–198 (2012).
5. Baumel, J. & Witmer, L. Osteologia. in *Handbook of Avian Anatomy: Nomina Anatomica Avium* (eds. Baumel, J., King, A. S. & Breazile, J. E.) 45–132 (Cambridge University Press, 1993).
6. Heers, A. M. & Dial, K. P. Wings versus legs in the avian bauplan: development and evolution of alternative locomotor strategies. *Evolution* **69**, 305–320 (2015).
7. Bock, W. J. & Waldron DeWitt Miller. The scansorial foot of the woodpeckers, with comments on the evolution of perching and climbing feet in birds. *Am. Mus. Novit.* **1931**, 1–45 (1959).
8. Middleton, K. M. The morphological basis of hallucal orientation in extant birds. *J. Morphol.* **250**, 51–60 (2001).
9. Hopson, J. A. Ecomorphology of avian and nonavian theropod phalangeal proportions: implications for the arboreal versus terrestrial origin of bird flight. In, *New Perspectives on the Origin and Early Evolution of Birds: Proceedings of the International Symposium in Honor of John H. Ostrom* (eds. Gauthier, J. & Gall, L. F.) 211–227 (Peabody Museum of Natural History, Yale University, 2001).
10. Sustaita, D. et al. Getting a grip on tetrapod grasping: form, function, and evolution. *Biol. Rev.* **88**, 380–405 (2013).
11. Backus, S. B., Sustaita, D., Odhner, L. U & Dollar, A. M. Mechanical analysis of avian feet: multiarticular muscles in grasping and perching. *R. Soc. Open Sci.* **2**, 140350 (2015).
12. Abourachid, A., Fabre, A.-C., Cornette, R. & Höfling, E. Foot shape in arboreal birds: two morphological patterns for the same pincer-like tool. *J. Anat.* **231**, 1–11 (2017).
13. De Mendoza, R. S. & Gómez, R. O. Ecomorphology of the tarsometatarsus of waterfowl (Anseriformes) based on geometric morphometrics and its application to fossils. *Anat. Rec.-Adv. Integr. Anat. Evolut. Biol.* **305**, 3243–3253 (2022).
14. Leblanc, K., Pintore, Romain, Galvão, A., Heitz, E. & Provini, P. Foot adaptation to climbing in ovenbirds and woodcreepers (Furnariida). *J. Anat.* **242**, 607–626 (2022).
15. Pouydebat, E., Boulinguez-Ambroise, G., Manzano, A., Abdala, V. & Sustaita, D. Convergent evolution of manual and pedal grasping capabilities in tetrapods. In, *Convergent Evolution. Fascinating Life Sciences* (eds. Bels, V. L. & Russell, A. P.) 323–389 (Springer, Cham, 2023).
16. Rasskin-Gutman, D. & Esteve-Altava, B. Connecting the dots: anatomical network analysis in morphological EvoDevo. *Biol. Theory* **9**, 178–193 (2014).
17. Diogo, R., Esteve-Altava, B., Smith, C., Boughner, J. C. & Rasskin-Gutman, D. Anatomical network comparison of human upper and lower, newborn and adult, and normal and abnormal limbs, with notes on development, pathology and limb serial homology vs. homoplasy. *PLOS One* **10**, e0140030 (2015).
18. Dos Santos, D. A., Fratani, J., Ponssa, M. L. & Abdala, V. Network architecture associated with the highly specialized hindlimb of frogs. *PLOS ONE* **12**, e0177819 (2017).
19. Molnar, J., Esteve-Altava, B., Rolian, C. & Diogo, R. Comparison of musculoskeletal networks of the primate forelimb. *Sci. Rep.* **7**, 10520 (2017).
20. Diogo, R., Molnar, J. L., Rolian, C. & Esteve-Altava, B. First anatomical network analysis of fore- and hindlimb musculoskeletal modularity in bonobos, common chimpanzees, and humans. *Sci. Rep.* **8**, 6885 (2018).
21. Esteve-Altava, B. et al. Evolutionary parallelisms of pectoral and pelvic network-anatomy from fins to limbs. *Sci. Adv.* **5**, eaau7459 (2019).
22. Fernández, M. S. et al. Fingers zipped up or baby mittens? two main tetrapod strategies to return to the sea. *Biol. Lett.* **16**, 20200281 (2020).
23. Fontanarrosa, G., Fratani, J. & Vera, M. C. Delimiting the boundaries of sesamoid identities under the network theory framework. *PeerJ* **8**, e9691 (2020).

24. Krahl, A. & Werneburg, I. Deep-time invention and hydrodynamic convergences through amniote flipper evolution. *Anat. Rec. Adv. Integr. Anat. Evol. Biol.* **306**, 1323–1355 (2022).

25. Carril, J., Tambussi, C. P. & Rasskin-Gutman, D. The network onto-geny of the parrot: altriciality, dynamic skeletal assemblages, and the avian body plan. *Evolut. Biol.* **48**, 41–53 (2020).

26. De Mendoza, R. S., Carril, J., Degrange, F. J. & Tambussi, C. P. Connectivity patterns of the hindlimb musculoskeletal system in living and fossil diving birds. *Evolut. Biol.* **49**, 292–302 (2022).

27. Cau, A. The assembly of the avian body plan: a 160 million year long process. *Boll. Della Soc. Paleontol. Ital.* **57**, 1–25 (2018).

28. Hutchinson, J. R. The evolution of hindlimb tendons and muscles on the line to crown-group birds. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **133**, 1051–1086 (2002).

29. Hasson, P. et al. Tbx4 and Tbx5 Acting in connective tissue are required for limb muscle and tendon patterning. *Dev. Cell* **18**, 148–156 (2010).

30. Rodriguez-Guzman, M. et al. Tendon-muscle crosstalk controls muscle bellies morphogenesis, which is mediated by cell death and retinoic acid signaling. *Dev. Biol.* **302**, 267–280 (2007).

31. Tozer, S. et al. Involvement of vessels and PDGFB in muscle splitting during chick limb development. *Development* **134**, 2579–2591 (2007).

32. Esteve-Altava, B. & Rasskin-Gutman, D. Anatomical network analysis in Evo-Devo. In, *Evolutionary Developmental Biology* (eds. Nuño de la Rosa, L. & Müller, G. B.) 977–995 (Springer, Cham, 2021).

33. Esteve-Altava, B., Marugán-Lobón, J., Botella, H. & Rasskin-Gutman, D. Structural constraints in the evolution of the tetrapod skull complexity: Williston's law revisited using network models. *Evolut. Biol.* **40**, 209–219 (2013).

34. Brinkworth, A. et al. Bird clades with less complex appendicular skeletons tend to have higher species richness. *Nat. Commun.* **14**, 5817 (2023).

35. McShea, D. W. Functional complexity in organisms: parts as proxies. *Biol. Philos.* **15**, 641–668 (2000).

36. Simon, H. A. The architecture of complexity. *Proc. Am. Philos. Soc.* **106**, 467–482 (1962).

37. Abourachid, A. & Renous, S. Bipedal locomotion in ratites (Paleog-natiform): examples of cursorial birds. *Ibis* **142**, 538–549 (2008).

38. Berman, S. L. & Raikow, R. J. The hindlimb musculature of the mousebirds (Coliiformes). *Auk* **99**, 41–57 (1982).

39. Guillerme, T. et al. Innovation and elaboration on the avian tree of life. *Sci. Adv.* **9**, eadg1641 (2023).

40. Prum, R. O. et al. Convergent evolution of manual and pedal grasping capabilities in tetrapods. *Nature* **526**, 569–573 (2015).

41. Starck, J. M. & Ricklefs, R. E. Patterns of development: the altricial-precocial spectrum. In *Avian Growth and Development. Evolution Within The Altricial Precocial Spectrum* (eds. Starck, J. M. & Ricklefs, R. E.) 1–30 (Oxford University Press, 1998).

42. Vanden Berge, J. C. & Zweers, G. A. Myologia. In, *Handbook of Avian Anatomy: Nomina Anatomica Avium* (eds. Baumel, J., King, A. S. & Breazile, J. E.) 189–247 (Cambridge University Press, 1993).

43. Carril, J., De Mendoza, R. S., Degrange, F. J., Barbeito, C. G. & Tambussi, C. P. Adjacency matrices for Carril et al. Evolution of avian foot morphology through anatomical network analysis. *Zenodo* https://doi.org/10.5281/zenodo.13924386 (2024).

44. R Core Team. R: A language and environment for statistical computing. *R Foundation for Statistical Computing* https://r-project.org (Vienna, Austria, 2021).

45. Csardi, G. & Nepusz, T. The Igraph software package for complex network research. *Int. J. Complex Syst. Sci.* **5**, 1–9 (2006).

46. Evans, M. E. K., Smith, S. A., Flynn, R. S. & Donoghue, M. J. Climate, niche evolution, and diversification of the 'bird-cage' evening primroses (*Oenothera*, Sections *Anogra* and *Kleinia*). *Am. Nat.* **173**, 225–240 (2009).

47. Revell, L. J. Phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* **3**, 217–223 (2012).

48. Paradis, E., Claude, J. & Strimmer, K. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**, 289–290 (2004).

49. Adams, D. C. A method for assessing phylogenetic least squares models for shape and other high-dimensional multivariate data. *Evolution* **68**, 2675–2688 (2014).

50. Pinheiro, J, Bates, D & R Core Team. *nlme: Linear And Nonlinear Mixed Effects Models. R Package Version 3.1–165*. https://CRAN.R-project.org/package=nlme. (2024).

51. Blomberg, S. P., Garland, T. & Ives, A. R. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* **57**, 717 (2003).

52. Harmon, L. J., Weir, J. T., Brock, C. D., Glor, R. E. & Challenger, W. GEIGER: investigating evolutionary radiations. *Bioinformatics* **24**, 129–131 (2007).

53. Clavel, J., Escarguel, G. & Merceron, G. mvMORPH: an R package for fitting multivariate evolutionary models to morphometric data. *Methods Ecol. Evol.* **6**, 1311–1319 (2015).

54. Burnham, K. P. & Anderson, D. R. *Model Selection and Multi-Model Inference: A Practical Information-Theoric Approach* 2nd edn, Vol. 488 (New York: Springer-Verlag, 2002).

55. Jhwueng, D., Huzurbazar, S., O'Meara, B. C. & Liu, L. Investigating the performance of AIC inselecting phylogenetic models. *Stat. Appl. Genet. Mol. Biol.* **13**, 459–475 (2014).

56. Carril, J., De Mendoza, R. S., Degrange, F. J., Barbeito, C. G. & Tambussi, C. P. R scripts for Carril et al. Evolution of avian foot morphology through anatomical network analysis. *Zenodo* https://doi.org/10.5281/zenodo.13924420 (2024).

Acknowledgements

Author contributions

J.C., R.S.D.M., F.J.D., C.G.B. and C.P.T. conceived the study and super-vised the project equally. J.C. constructed the matrix data, with con-tributions from R.S.D.M.. R.S.D.M. performed the formal analysis, with contributions from J.C. and F.J.D.. J.C. and C.P.T. wrote the manuscript. R.S.D.M., F.J.D. and C.G.B. reviewed the manuscript. J.C. and R.S.D.M. edited the final manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41467-024-54297-9.

Correspondence and requests for materials should be addressed to Julieta Carril.

Peer review information *Nature Communications* thanks the anon-ymous reviewers for their contribution to the peer review of this work. A peer review file is available.

Reprints and permissions information is available at http://www.nature.com/reprints

Publisher's note Springer Nature remains neutral with regard to jur-isdictional claims in published maps and institutional affiliations.