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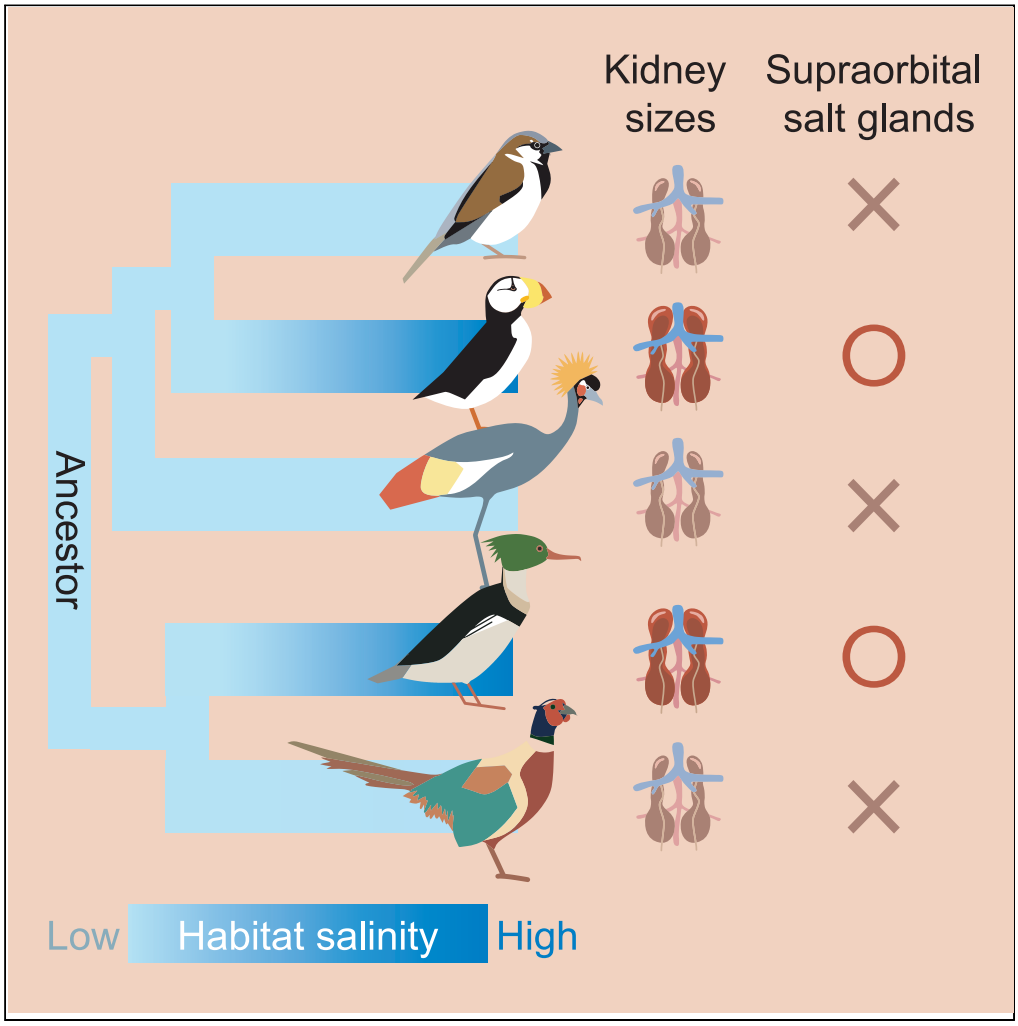

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Highlights

Birds have independently evolved larger kidneys and salt glands multiple times

Kidney sizes and salt glands in birds correlate positively with habitat salinity

Habitat salinity constrains the evolution of bird kidney sizes and salt glands

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Convergent evolution of kidney sizes and supraorbital salt glands for birds living in saline habitats

Chi-Cheng Chiu,[1] Cheng-Te Yao,[2] Ben-Yang Liao,[3,*] and Shou-Hsien Li[1,4,*]

SUMMARY

Only a small number of avian species inhabit salty environments. To understand how they adapted, we examined the evolution of kidney sizes, supraorbital salt glands (SSGs), and the utilization of salty habitats across 230 species spanning 25 avian orders. Phylogenetic analysis indicates that SSGs, large kidneys, and thriving in salty habitats emerged convergently in birds. Transition rate analysis reveals that species possessing SSGs and large kidneys tended to move from low-to high-salinity environments, while others moved in the opposite direction. However, habitat salinity also influenced kidney evolution; lineages residing in high-salinity environments tended to develop larger kidneys than those in low-salinity environments. Our findings suggest that SSGs and large kidneys may have evolved through adaptation to high salinity. Overall, habitat conditions and physiological traits influenced avian adaptation to salty environments in a reciprocal manner. These results shed the new light on the evolutionary mechanisms underlying functional diversity in birds.

INTRODUCTION

Oceans provide vast space and rich resources for organisms. However, organisms must overcome the osmotic challenge of saltwater intake to live in such environments. Therefore, less than 3% of avian species (i.e., seabirds) live entirely or partially in high-osmolarity environments.[1]

Supraorbital salt glands (SSGs, salt glands) in birds, also known as glandulae nasalis,[2,3] produce concentrated solutions primarily consisting of NaCl or KCl, which help maintain proper internal solute levels while minimizing water loss.[4–6] Glands with a similar function might have evolved, independently and convergently in reptiles such as turtles, sea snakes, lizards, and crocodiles.[7,8] These glands are widespread and found in various parts of the skull across different evolutionary lineages, although their homology remains to be examined. Moreover, the unique positioning of the salt glands above the eye is a distinctive feature of birds.[3,4] Supraorbital salt glands have been discovered in at least 40 bird families, which cover almost all traditional bird orders except for the Passeriformes.[8] These glands are particularly common in marine birds such as gulls, petrels, albatrosses, auks, and penguins but can also be found in some freshwater species, such as dabbling ducks, mallards, and rails.[9] Desert-dwelling birds such as ostriches and North African partridges[1,8] and some carnivorous birds with high-protein diets such as Tawny Eagles[10] also possess supraorbital salt glands. Avian supraorbital salt glands display significant levels of plasticity in both their structure and function,[1,6,11,12,44] but their size and excretory capacity largely depend on the species' environmental salinity and diet.[13,14]

Birds also use their kidneys to maintain the osmotic balance of their blood.[15] The avian kidney comprises units of lobule with a renal medulla containing loops of Henle able to extract water from urine with higher salt concentrations than in the plasma. It has been shown that the mass of the kidney is positively correlated with the number of medullary cones, the smaller units of a renal medulla, which are associated with its ability to regulate osmotic pressure.[15] How birds physiologically maintain their osmostasis has been studied intensively.[14] However, we know little about how the avian physiological traits associated with osmoregulation, such as the SSGs and large kidneys, have evolved in response to the types of habitats they use.

Non-passerine birds with SSGs tend to have larger kidneys (compared with their body sizes) than others.[1] Hughes, therefore, proposed that birds with high-salinity tolerance have larger kidneys for better osmoregulation. However, shared ancestry among species can lead to a significant association between two traits evolving independently.[19] Without controlling for such "phylogenetic signals" (as Hughes did not[1]), we do not know whether high-salinity environments could have driven these two physiological traits to evolve in birds. Independent evolution of similar traits, convergent evolution, in the same environmental conditions, has long been considered convincing evidence of adaptation.[56] If a salty environment could drive the evolution of a larger kidney and the presence of an SSG, could it lead to the convergent evolution of the

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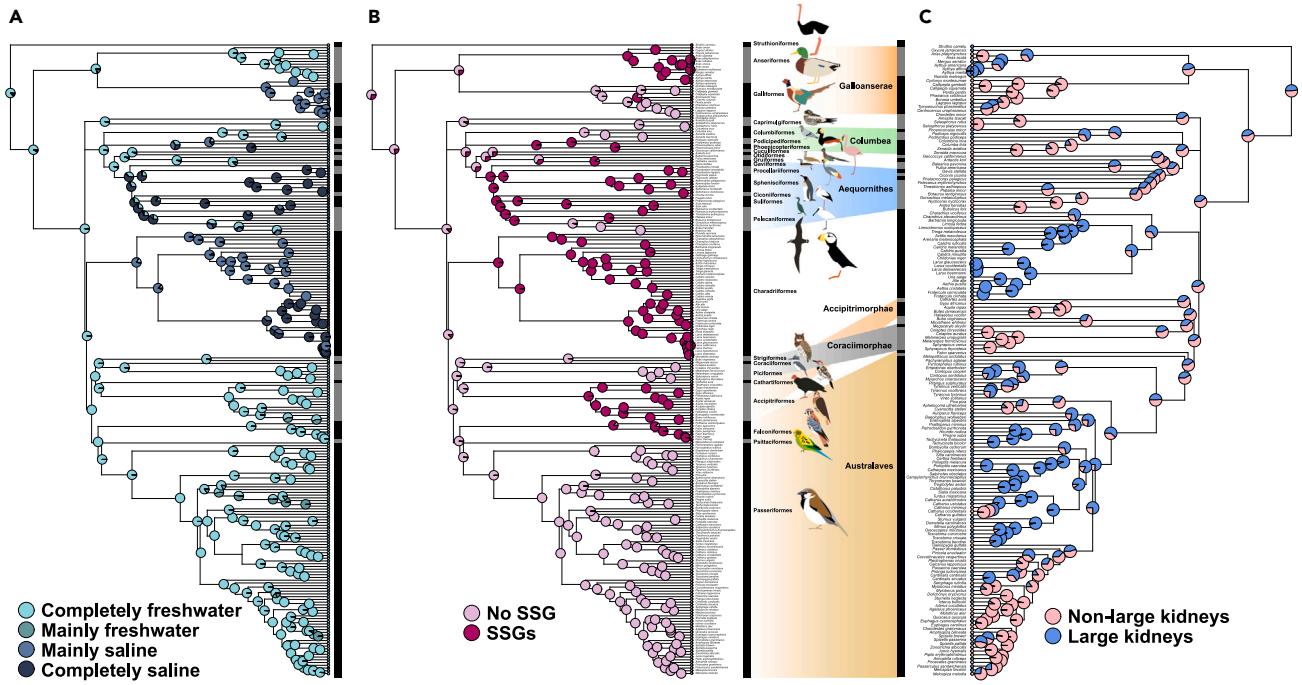

Figure 1. Estimating ancestral states and reconstructing traits in avian species: habitat salinity, presence of SSGs, and kidney sizes

Estimated ancestral states for the level of habitat salinity (A) and the presence of SSGs (B) for the 230 avian species studied and kidney sizes for the 167 avian species studied (C). The characteristics of each species are shown at the tips of the phylogenetic tree. The posterior probability of the reconstructed characteristic state of each trait is shown in the pie on each ancestral node. The gray and black bars next to the phylogenetic tree indicate the order of the species. The color patterns at the rightmost indicate the characteristic state of the extant species.

larger kidneys and SSGs in different avian lineages? It is also unclear whether the evolution of the two physiological traits was correlated or independent in birds.

RESULTS

Convergent evolution of larger kidney sizes, presence of supraorbital salt glands, and the use of high salinity habitat

In our dataset, species in completely or mainly saline habitats usually have large kidneys (residual kidney weight ≧ 0.13, 67%) and SSGs (91%); in contrast, few species in mainly and completely freshwater habitats have "large kidneys" (88% had residual kidney weight <0.13), and most (80%) do not have SSGs. Ancestral characteristics reconstruction suggested that the ancestor of birds probably lived in freshwater environments, had no SSG, and had medium-sized kidneys (the probabilities of having "non-large kidneys" and of having "large-kidneys" are roughly equal) (Figure 1).

Interestingly, our results suggest that the birds' utilization of high-salinity habitats evolved together with the growth of their kidneys and the development of SSGs. The use of high-salinity habitats (sum of posterior probability for completely or mainly saline habitats >0.5) occurred independently at least 14 times in 12 orders of birds (Anseriformes, Charadriiformes, Coraciiformes, Gaviiformes, Gruiformes, Phoenicopteriformes, Podicipediformes, Pelecaniformes, Passeriformes, Suliformes and Sphenisciformes); SSGs evolved independently 8 times in 14 orders of birds (Accipitriformes, Anseriformes, Charadriiformes, Falconiformes, Galliformes, Gaviiformes, Gruiformes, Pelecaniformes, Phoenicopteriformes, Podicipediformes, Procellariiformes, Sphenisciformes, Struthioniformes and Suliformes) (Figure 1). Intriguingly, both the utilization of high-salinity habitats and the presence of SSGs evolved in the same nodes for at least four avian lineages (Columbea, Aequornithes, and part of Galloanserae and Charadriiformes). Furthermore, SSGs are present in the common ostrich (*Struthio camelu*), the 13 Accipitriformes species, and 5 Falconiformes species that do not utilize high-salinity habitats. In contrast, four species in Passeriformes, the ash-throated flycatcher (*Myiarchus cinerascens*), the marsh wren (*Cistothorus palustris*), the snow bunting (*Plectrophenax nivalis*), and the Savannah sparrow (*Passerculus sandwichensis*) and one species in Coraciiformes, the belted kingfisher (*Megaceryle alcyon*), have evolved to utilize high-salinity habitats without having SSGs.

The use of high-salinity habitats and the presence of SSGs are evolutionarily inert in birds. Only five lineages, the pin-tailed duck (*Anas acuta*), the redhead (*Aythya Americana*), the greater scaup (*Aythya marila*), the upland sandpiper (*Bartramia longicauda*), and the long-billed dowitcher (*Limnodromus scolopaceus*), have evolved from ancestors utilizing high salinity habitats to descendants using freshwater habitats. Only four lineages, the white stork (*Ciconia Ciconia*), the African fish eagle (*Haliaeetus vocifer*), the American kestrel (*Falco sparverius*), and the Robert Falco (*Falco jugger*) have lost SSGs possessed by their ancestors.

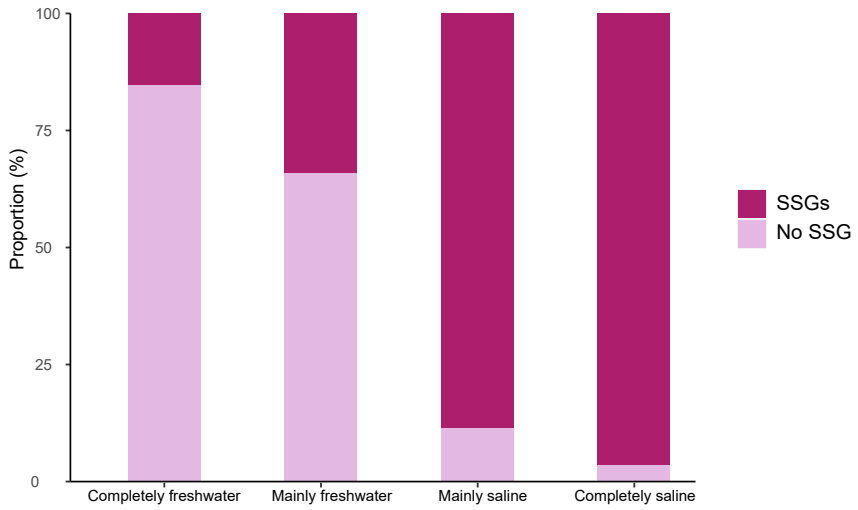

Figure 2. The proportion of avian species living in completely saline, mainly saline, mainly freshwater, and completely freshwater habitats with and without supraorbital salt glands

By contrast, the evolution of birds' kidney size has been relatively labile. Large kidneys evolved at least 25 times in twelve orders of birds (Struthioniformes, Anseriformes, Galliformes, Phoenicopteriformes, Podicipediformes, Gaviiformes, Gruiformes, Pelecaniformes, Suliformes, Charadriiformes, Coraciiformes, and Passeriformes). In addition, lineages derived from those with large kidneys have 16 times reverted to having residual kidney weight <0.13, namely the mallard duck (*Anus platyrhynchos*), the pin-tailed duck (*A. acuta*), the redhead (*Aythya Americana*) in the non-Passeriformes, and the horned lark (*Eremophila alpestris*), the American bushtit (*Psaltriparus minimus*), the cedar waxwing (*Bombycilla cedrorum*), the cactus wren (*Campylorhynchus brunneicapillus*), the northern mockingbird (*Mimus polyglottos*), the Bendire's thrasher (*Toxostoma bendirei*), the zebra finch (*Taeniopygia guttata*), the chipping sparrow (*Spizella passerina*), the grey-cheeked thrush (*Catharus minimus*), the russet nightingale-thrush (*C. occidentalis*), the hermit thrush (*C. guttatus*), the ancestors of corvoidea, and all species of passeroidea in Passeriformes. However, birds in other avian lineages re-acquired large kidneys. These include the Eurasian magpie (*Pica pica*) in corvoidea, the painted redstart (*Myioborus pictus*), Bullock's oriole (*Icterus bullockii*), Brewer's blackbird (*Euphagus cyanocephalus*), the rufous-crowned sparrow (*Aimophila ruficeps*), the Savannah sparrow, and Lincoln's sparrow (*Melospiza lincolnii*) in passeroidea, Carduelinae and a "masked" clade (Piranga, Cardinalis, and Caryothraustes) in Cardinalidae. It is worth noting that birds adapted to high-salinity habitats have all evolved at least one characteristic assumed to be related to osmostasis except the snow bunting (*Plectrophenax nivalis*).

In addition, the results of search.conv clarify that when birds inhabit saline environments, the kidney size and SSGs in different lineages tend to be similar (found highly significant under "state," p = 0.001). Furthermore, birds' habitat diversity within each clade is diverse. Different clades do not exhibit convergent evolution toward the same direction (found no significance under "clade," p = 0.117).

Correlated evolution of large kidneys and functional supraorbital salt glands

Our results show high phylogenetic signals in levels of habitat salinity ($\lambda = 0.78$, $p \ll 0.001$; $K = 0.26$, p = 0.001; null hypothesis λ and $K = 0$), the presence of SSGs ($\lambda = 1$, $p \ll 0.001$; $K = 1.373$, p = 0.001; null hypothesis λ and $K = 0$), and residual kidney weight ($\lambda = 0.7$, $p \ll 0.001$; K = 0.2, p value = 0.012; null hypothesis λ and $K = 0$). After controlling for the phylogenetic signal, the PGLS results show that birds in high-salinity environments tend to have larger kidneys; the residual kidney weight is significantly positively correlated with levels of habitat salinity ($\lambda = 0.54$, K = 0, $\delta = 0.26$, estimate = 0.07, s.e. = 0.01, $t = 7.38$, $p \ll 0.001$, $R^2 = 0.25$). The evolutionary dependency between residual kidney weight and habitat types is also supported by the results of the correlated evolution analysis, except at the 25th percentile cut-off point (logBF for the set of cut-off points: 25th = 0.67; 50th = 10.872; 75th = 23.041).

The results of our PGLM analysis show that the presence of SSGs is significantly positively correlated with levels of habitat salinity ($\lambda = 0.91$, $K = 0.42$, $\delta = 2.09$, estimate = 0.488, s.e. = 0.226, t = 2.16, p = 0.03, $R^2 = 0.099$) (Figure 2). The results of the correlated evolutionary analyses also support such an association; the salt gland characteristic and habitat types are highly evolutionarily dependent on each other (logBF = 16.012). Furthermore, the presence of SSGs is positively correlated with kidney size ($\lambda = 0.46$, $K = 0$, $\delta = 0.3$, estimate = 0.21, s.e. = 0.03, $t = 6.4$, $p \ll 0.001$, $R^2 = 0.19$) (Figure 3). The results of the correlated evolutionary analyses show that the residual kidney weight and salt gland characteristics are strongly dependent on each other (logBF in the set of the cut-offs point $25^{th} = 8.85$; $50^{th} = 16.618$; $75^{th} = 16.305$).

Supraorbital salt glands and larger kidneys drove the evolution of salinity adaptation

Transition rate analysis indicates that the presence of SSGs drove birds to shift from low-to high-salinity habitats, while the lack of SSGs drove the evolution of habitat use in the other direction. For species with SSGs, the transition rate from low to high-salinity habitats was 4.56, while the reverse was 1.73; in contrast, for species without SSGs, the transition rate was biased toward low-salinity habitats (18.38 vs. 1.09 for low-to

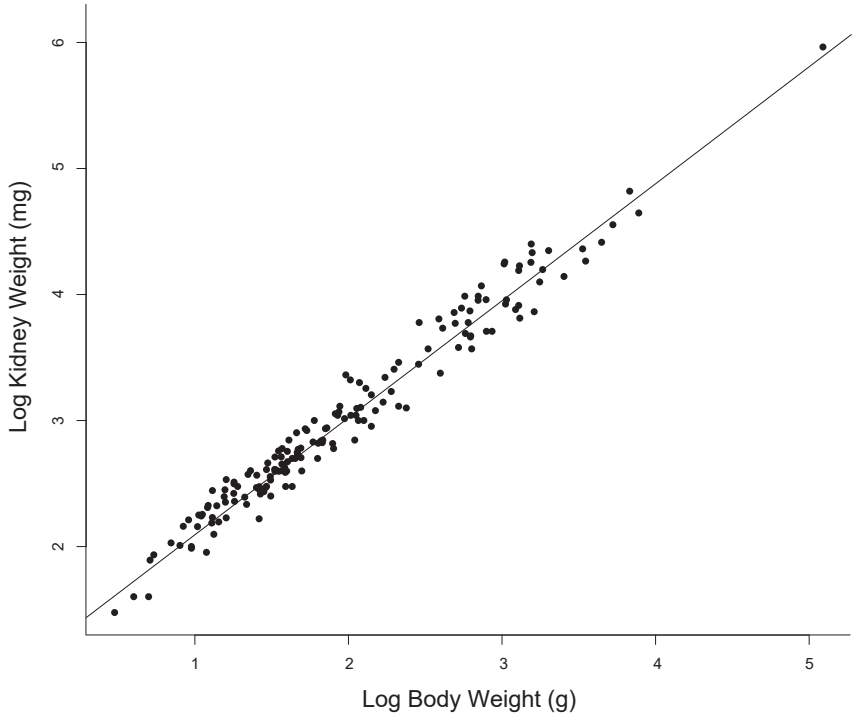

Figure 3. The allometric effect of body weight on kidney weights

Linear regression between the log(body weight) and log(kidney size) is log(kidney size) = 1.164994 ± 0.072970 + (0.928436 ± 0.019287) log(body weight) ($p < 2.2e\text{-}16$; adjusted $r^2 = 0.933$).

high-salinity habitats transition; Figure 4A). Similarly, kidney size probably drove the evolution of birds' habitat use; for species with large kidneys, the transition rate from high-to low-salinity habitats was about 13.4 times higher than that from low-to high-salinity habitats (16.59 vs. 1.24 for low-to high-salinity habitats transition, Figure 4B).

Results of the transition rate analysis also support correlated evolution between the presence of SSGs and kidney sizes. For species without SSGs, the transition rate from large to non-large kidneys was 12.57, and the reverse was 7.68, whereas for species with SSGs, the transition rate from non-large to large kidneys was 17.81 and the reverse was 2.45 (Figure 4C).

Salinity is a force to fine-tune the evolution of avian kidney size

Our results suggest that different levels of environmental salinity might have modulated the evolution of kidney size (Figure 4B). For species living in low-salinity habitats, the transition rate from large to non-large kidneys was 12.09, and the reverse was 7.75, whereas, for species in high-salinity habitats, the transition rate from non-large to large kidneys (1.08) was about five times higher than that from large to non-large kidneys (0.18).

DISCUSSION

Our ancestral state reconstruction suggests that high-salinity habitats evolved independently in different lineages of birds (Figure 1), as it did with sea snakes.[29] Furthermore, we found that this ecological trait was quite evolutionarily inert; among the 230 species we sampled, only five species whose ancestors had used saline habitats reverted to using freshwater habitats. Similarly, we found that the two physiological traits related to salt tolerance, namely the presence of SSGs and large kidneys, also evolved convergently in birds. Of these, the presence of SSGs was also highly evolutionarily conserved (Figure 1). Therefore, our results suggest that the evolutionary potential of organisms might be constrained by the cost of niche shift.[30,31] The "cost" of niche shift may arise because of antagonistic pleiotropy, in which a mutation in a single gene controlling multiple traits increases fitness in one trait while also reducing fitness in another one.[33] Alternatively, such "cost" may be rendered by a reduction in evolvability, the capacity to generate variation useful for adaptive change, after shifting to a new ecological niche.[34,35]

Shifting to high salinity habitats might have allowed these avian lineages to escape from the intensive competition with other species using freshwater habitats and paved the way for further adaptive radiations.[36] It allowed avian lineages (such as Charadriiformes[37]) to radiate by filling various empty niches in such habitats. However, ecological niche shift may constrain changes in other directions and thus limit niche diversity and evolvability[38,39] later. Therefore, birds using high-salinity habitats might become an "evolutionary dead-end"[40,41] after the empty salty niche is filled. The speciation rate for these birds could be reduced or even followed by an increasing extinction rate.

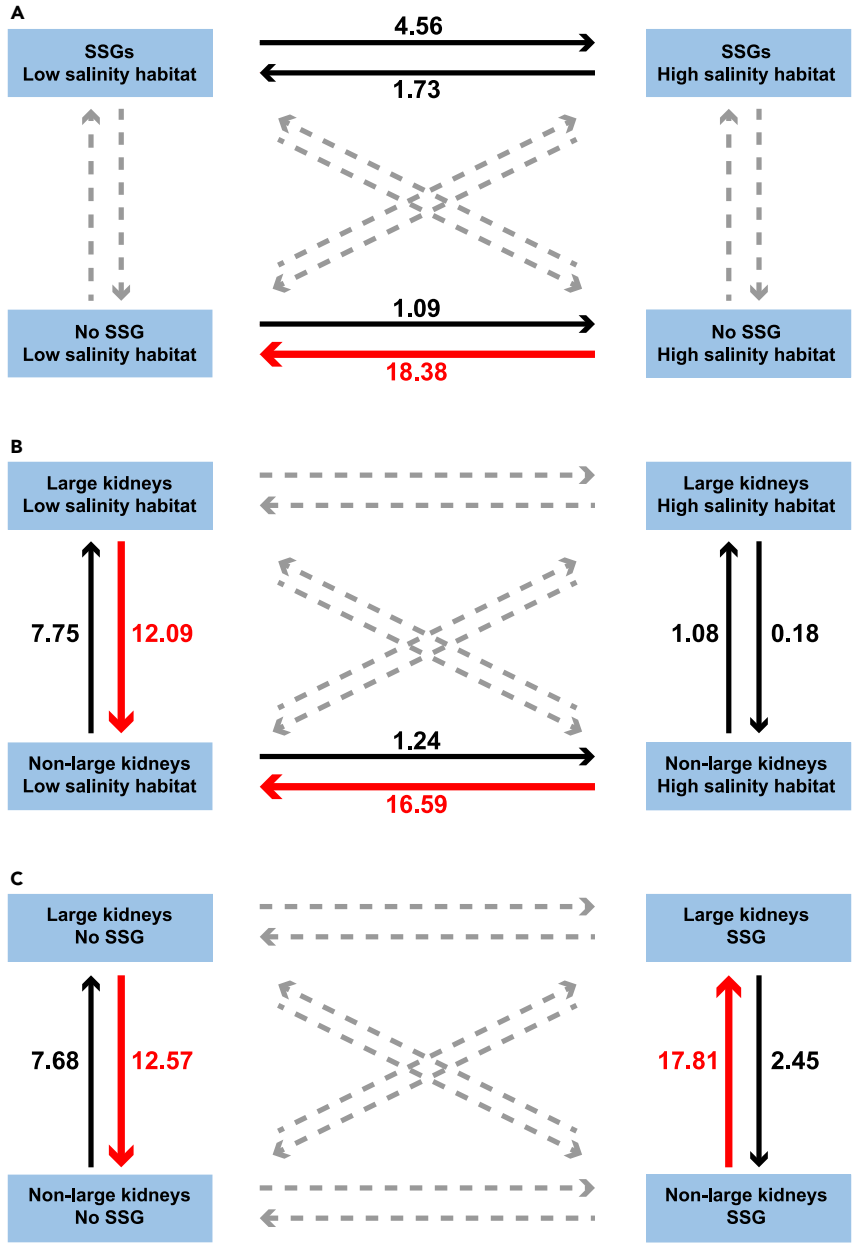

Figure 4. Evolutionary transition rates of salinity adaptation traits in avian species

Evolutionary transition rates between the use of high salinity habitats and (A) the presence of SSGs, (B) larger kidneys, and (C) between the larger kidneys and the presence of SSGs. For each characteristic, the transition rates between the three different combinations of characteristic categories were estimated by the dependent model in BayesTraits[13,14] with the assumption that the two characteristics evolved interdependently. The dashed arrows are the estimated minimum transition rates (≒0). The thin black arrow indicates the estimated lower transition rates (<10). The thick red arrows indicate the estimated higher transition rates (>10).

Convergent evolution (the independent evolution of similar phenotypes[8]) to use high-salinity habitats also implies that different lineages of birds might have adopted different genetic routes to develop complex traits when adapting to high-salinity habitats.[42,43] For instance, the supraorbital salt glands of birds have been considered to be derived from the nasal gland, which can also be found in reptiles and most mammals.[8] The convergent evolution of salt glands in the tetrapod has been suggested to be the result of the cooption of an existing nasal gland in the ancestor of tetrapods.[8] The convergent evolution of SSGs we found in this study implies that the development of this complex phenotype could have been modulated by the same set of developmental toolkits for the same existing gland in different lineages of birds independently. Therefore, the evolution of salt tolerance could have been driven by mutations in different compartments of the same genetic

pathways, or genetic toolkit, that regulates the developmental process of SSGs, kidney size, and other physiological traits related to salt tolerance or osmostasis. Convergent phenotypic evolution with divergent genetic causes has been shown in the cases of four Passerellidae sparrows,[45] the Savannah sparrow (*P. sandwichensis beldingi*), Nelson's sparrow (*Ammospiza nelsoni subvirgatus*), the song sparrow (*Melospiza melodia pusillula*), and the swamp sparrow (*M. georgiana nigrescens*); fewer than 50% of genes that were considered to contribute to adaptation to salty coastal habitats were shared among them. Hence the convergent evolution of salt-tolerance phenotypes suggests that birds should provide a rich opportunity to investigate the diverse genetic mechanisms underpinning their salt-tolerant phenotype.

In 1970, Hughes[1] found that species in avian orders composed mainly of non-saline species have relatively smaller kidneys whereas species associated with saline habitats have larger kidneys. However, he neither tested for the positive associations between kidney size and environmental salinity nor controlled for the effect of phylogenetic constraints on the evolution of kidney size and habitat use. Therefore, our results provide the first modern evolutionary test to demonstrate that avian species living in high-salinity environments have larger kidneys. Such an evolutionary trend can be illustrated by avian orders whose species mainly use freshwater habitats. For example, species specialized to high-salt habitats, such as the black-faced spoonbill of Pelecaniformes, the American coot (*Fulica Americana*) of Gruiformes, and the Savannah sparrow of Passeriformes, all have the largest kidneys recorded in their order (Table 1). The importance of kidney size in osmoregulation is also supported by the result that lineages without large kidneys tend to shift their habitats from high-to low-salinity habitats (Figure 4B).

In addition to kidney size, our results suggest that the presence of SSGs in species also correlates with their habitats' salinity. We found that species with SSGs tend to become high-salinity specialists; in contrast, species without SSGs shift from high-salinity habitats to low-salinity habitats (Figure 4). Therefore, the transition rate between the SSG and environmental salinity (from low-to high-salinity habitats was 16.47, while the reverse was 3.35). This not only supports the important role of the SSG in osmoregulation in high-salinity environments[13,46] but also suggests that the presence of SSGs could be a physiological prerequisite for birds in some lineages to use the harsh high-salinity environment.

Hughes proposed that SSGs should be associated with increased renal mass.[1] According to our PGLS results, the presence of SSGs is positively correlated with kidney size; our evolutionary dependency analysis results also suggest that SSGs tend to be highly correlated with the evolution of kidney sizes. This is also supported by the results of transition rate analysis; kidney size tended to decrease in species without SSGs and increase in species with SSGs (Figure 4). Our results support the intimate interactions between the kidneys and the salt glands for the osmoregulation suggested by Hughes.[1]

The correlated evolution of two traits could be caused either by the linkage between the genes underpinning the two traits (genetic correlation) or because the two traits respond to the same selection pressure.[47] Genetic correlation is the proportion of variance that two traits share due to genetic causes.[48] Such correlations may arise from linkage disequilibrium (i.e., the non-random association of alleles at two or more loci) or pleiotropy, which occurs when a single gene influences two or more characteristics.[48] However, if the correlated evolution between the kidney size and functional salt glands had been caused by genetic linkage, we would have found a perfect (or nearly perfect) correlation between these two traits. Although the result of the correlated evolutionary analysis was highly significant (logBF >8.0) for the kidney size and presence of SSGs, the r^2 between relative kidney size and the functional salt gland was only 0.19. Therefore, our results suggest that the association between the large kidneys and the presence of SSGs was probably driven by the selection force of environmental salinity influencing both of them independently. This result implies that birds may need large kidneys and salt glands working synergistically to regulate the osmotic pressure in high-salinity habitats effectively. Or if multiple genes control each of the two traits, and some of the associated genes are linked, the correlations between the traits could be imperfectly correlated. Or perhaps the evolution of the traits was genetically correlated, and initially led to a perfect or near-perfect correlation between them which has to some extent broken down more recently., but has not they could still drift apart recently.

We also found that the correlation between kidney size and habitat type used (r^2 = 0.25) is much higher than that between the presence of supraorbital salt glands and habitat type used (r^2 = 0.02). The lower correlation between the presence of SSGs and habitat use might be because birds that can produce hypertonic salt secretions from SSGs are not restricted to species living in high-osmotic environments. For instance, the salt glands of raptor species (e.g., the tawny eagle), were found to produce secretions in response to a high protein diet.[10] The common ostrich also produces salt secretions from the salt gland when facing heat stress.[49,50]

Our analysis suggests that larger kidneys and the presence of SSGs are highly associated with the level of habitat salinity in birds. Almost all birds adapted to high-salinity habitats have evolved at least one characteristic that is assumed to be related to osmostasis. There are two lines of evidence to support the conclusion that salty environments drive the evolution of SSG and large kidneys. (1) Transition rate analysis indicates that species living in high salinity habitats tend to develop larger kidneys and SSGs; in contrast, species living in low-salinity habitats tend to develop smaller kidneys and to lose their SSGs (Figures 4B and 4C). (2) The results of our investigation of morphological convergence reveal that birds' kidney size and SSGs tend to undergo convergent evolution when they occupy saline water environments. Therefore, the salty environment might not only play a role in facilitating the emergence of physiological traits that participate in osmoregulation but could also further fine-tune the evolutionary dynamics of these physiological traits.

Our results suggest that environmental salinity could be a powerful driving force for large kidneys' convergent and correlated evolution and the presence of SSGs in birds. The strong association between the presence of SSGs and large kidneys suggests that birds might need both to handle the strong selection pressure posed by high-salinity environments. Because it is highly energetically demanding to maintain osmotic balance through SSGs and large kidneys in high-salinity environments,[51,52] evolution to utilize salty habitats may be constrained in birds. Such physiological constraints might explain why less than 3% of avian species live entirely or partially in such high-osmolarity environments.[53] Our results provide tantalizing evidence of correlated evolution between different physiological traits to handle high osmotic pressure in high-salinity environments and suggest that such osmotic pressure has independently contributed to the expansion of avian physiological and functional diversity.

Table 1. List of avian species with information on the sample size, presence/absence (+/−) of supraorbital salt glands (SSGs), kidney weight in grams, body weight in grams, the residual of kidney weight/body weight (K/W), and habitat type (completely freshwater, mainly freshwater, mainly saline, completely saline)

Species	Sample size	SSG	Kidney (g)	Body (g)	K/W residuals	Habitat type
Accipitriformes						
Accipiter gentilis[10]	1	+	NA	NA	NA	Complete freshwater
Accipiter striatus[10]	1	+	NA	NA	NA	Complete freshwater
Aquila rapax[2]	3	+	13.9	2532	−0.187	Completely freshwater
Aquila verreauxii[10]	1	+	NA	NA	NA	Complete freshwater
Buteo rufofuscus[10]	1	+	NA	NA	NA	Mainly freshwater
Buteo jamaicensis[15]	1	–	7.6	1225	−0.155	Completely freshwater
Gyps africanus[2]	1	–	35.8	5270	−0.073	Completely freshwater
Haliaeetus vocifer[2]	1	–	18.4	3500	−0.197	Completely freshwater
Gyps coprotheres[10]	1	+	NA	NA	NA	Complete freshwater
Polemaetus bellicosus[10]	1	+	NA	NA	NA	Complete freshwater
Terathopius ecaudatus[10]	1	+	NA	NA	NA	Complete freshwater
Torgos tracheliotos[10]	1	+	NA	NA	NA	Complete freshwater
Anseriformes						
Anas platyrhynchos[1]	1	+	6.5	1305	−0.249	Mainly saline
Anas acuta[15]	1	+	5.1	862	−0.186	Mainly freshwater
Anas rubripes[54]	1	+	NA	NA	NA	Mainly freshwater
Anas crecca[55]	1	+	NA	NA	NA	Mainly saline
Anas clypeata[55]	1	+	NA	NA	NA	Mainly saline
Aythya valisineria[55]	1	+	NA	NA	NA	Mainly saline
Anser anser[57]	1	+	NA	NA	NA	Mainly saline
Cygnus atratus[58]	1	+	NA	NA	NA	Mainly saline
Aythya americana[15]	2	+	8.4	1055	−0.051	Mainly freshwater
Aythya marila[2]	1	+	9.1	787	0.102	Mainly freshwater
Aythya affinis[2]	1	+	18.1	1041	0.288	Mainly saline
Oxyura jamaicensis[1]	1	+	5.4	411	0.139	Mainly saline
Mergus serrator[2]	1	+	9.7	700	0.178	Mainly saline
Somateria mollissima[59]	1	+	NA	NA	NA	Completely saline
Caprimulgiformes						
Chordeiles minor[15]	3	–	0.6	80	−0.152	Completely freshwater
Amazilia tzacatl[2]	1	–	0.04	5	−0.204	Completely freshwater
Selasphorus platycercus[15]	2	–	0.03	3	−0.129	Completely freshwater
Selasphorus rufus[15]	1	–	0.04	4	−0.114	Completely freshwater
Cathartiformes						
Cathartes aura[1]	1	–	12.6	1761	−0.083	Completely freshwater
Charadriiformes						
Charadrius alexandrinus[1]	1	+	0.6	37	0.161	Completely saline
Charadrius vociferus[15]	3	+	1.3	88	0.145	Mainly saline
Arenaria melanocephala[1]	1	+	2	118	0.214	Completely saline
Bartramia longicauda[15]	1	+	1.6	141	0.045	Completely freshwater
Actitis macularius[15]	2	+	0.7	41	0.186	Mainly saline
Tringa melanoleuca[15]	2	+	2.9	212	0.137	Mainly saline
Calidris melanotos[15]	2	+	1.1	85	0.087	Mainly saline

(*Continued on next page*)

Table 1. *Continued*

Species	Sample size	SSG	Kidney (g)	Body (g)	K/W residuals	Habitat type
Calidris minutilla[1]	1	+	0.3	19	0.13	Mainly saline
Calidris ruficollis[1]	1	+	0.8	46	0.198	Mainly saline
Limnodromus scolopaceus[15]	2	+	1.8	130	0.129	Mainly freshwater
Calidris pusilla[15]	2	+	0.4	23	0.177	Mainly saline
Limosa fedoa[1]	1	+	6.4	388	0.236	Mainly saline
Larus californicus[60]	1	+	NA	NA	NA	Mainly saline
Larus marinus[59]	1	+	NA	NA	NA	Completely saline
Larus hyperboreus[61]	1	+	NA	NA	NA	Completely saline
Larus delawarensis[15]	4	+	7.2	488	0.194	Mainly saline
Larus heermanni[1]	1	+	7.8	544	0.185	Completely saline
Larus glaucescens[1]	1	+	7.4	618	0.111	Mainly saline
Larus occidentalis[1]	2	+	15.5	1283	0.136	Completely saline
Chlidonias niger[15]	3	+	1	60	0.187	Mainly saline
Aethia cristatella[1]	2	+	6	289	0.328	Completely saline
Aethia pusilla[1]	2	+	2.3	96	0.358	Completely saline
Fratercula cirrhata[1]	1	+	11.7	734	0.24	Completely saline
Uria aalge[1]	1	+	17.5	1031	0.277	Completely saline
Alle alle[2]	2	+	2.1	103	0.29	Completely saline
Cepphus grylle[61]	1	+	NA	NA	NA	Completely saline
Uria lomvia[61]	1	+	NA	NA	NA	Completely saline
Alca torda[61]	1	+	NA	NA	NA	Completely saline
Recurvirostra americana[62]	1	+	NA	NA	NA	Mainly saline
Rynchops niger[59]	1	+	NA	NA	NA	Mainly saline
Fratercula corniculata[1]	2	+	9.7	572	0.26	Completely saline
Fratercula arctica[61]	1	+	NA	NA	NA	Completely saline
Charadrius hiaticula[61]	1	+	NA	NA	NA	Mainly saline
Pluvialis apricaria[61]	1	+	NA	NA	NA	Mainly saline
Rissa tridactyla[61]	1	+	NA	NA	NA	Completely saline
Actitis hypoleucos[61]	1	+	NA	NA	NA	Mainly saline
Calidris alpina[61]	1	+	NA	NA	NA	Mainly saline
Calidris canutus[61]	1	+	NA	NA	NA	Completely saline
Calidris minuta[61]	1	+	NA	NA	NA	Mainly saline
Calidris alba[61]	1	+	NA	NA	NA	Mainly saline
Gallinago gallinago[61]	1	+	NA	NA	NA	Mainly saline
Limosa lapponica[61]	1	+	NA	NA	NA	Mainly saline
Tringa glareola[61]	1	+	NA	NA	NA	Completely freshwater
Tringa ochropus[61]	1	+	NA	NA	NA	Completely freshwater
Ciconiiformes						
Ciconia ciconia[2]	3	–	23	3350	−0.082	Completely freshwater
Columbiformes						
Columba livia[15]	1	–	2.8	286	0.001	Completely freshwater
Zenaida asiatica[15]	3	–	0.9	141	−0.205	Mainly freshwater
Zenaida macroura[1]	1	–	0.7	110	−0.214	Completely freshwater
Columbina inca[15]	2	–	0.3	43	−0.201	Completely freshwater

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Table 1. *Continued*

Species	Sample size	SSG	Kidney (g)	Body (g)	K/W residuals	Habitat type
Coraciiformes						
Megaceryle alcyon[15]	2	–	2.2	173	0.1	Mainly saline
Cuculiformes						
Geococcyx californianus[15]	2	–	1.7	190	−0.05	Completely freshwater
Falconiformes						
Falco sparverius[2]	1	–	1.1	112	−0.025	Completely freshwater
Falco biarmicus[10]	1	+	NA	NA	NA	Complete freshwater
Falco cherrug[10]	1	+	NA	NA	NA	Mainly freshwater
Falco chicquera[10]	1	+	NA	NA	NA	Complete freshwater
Falco jugger[10]	1	–	NA	NA	NA	Complete freshwater
Falco peregrinus[10]	1	+	NA	NA	NA	Mainly saline
Polihierax semitorquatus[10]	1	+	NA	NA	NA	Complete freshwater
Galliformes						
Bonasa umbellus[15]	2	–	4.9	577	−0.041	Completely freshwater
Lagopus lagopus[15]	5	–	6	602	0.03	Completely freshwater
Tympanuchus phasianellus[15]	1	–	5.1	791	−0.151	Completely freshwater
Centrocercus urophasianus[15]	2	–	22.3	2013	0.111	Completely freshwater
Callipepla squamata[15]	2	–	1.4	168	−0.084	Completely freshwater
Callipepla gambelii[15]	3	–	1.2	150	−0.105	Completely freshwater
Cyrtonyx montezumae[15]	2	–	1.3	212	−0.211	Completely freshwater
Phasianus colchicus[15]	4	–	8.2	1283	−0.14	Completely freshwater
Perdix perdix[15]	2	–	3.8	521	−0.11	Completely freshwater
Numida meleagris[2]	1	–	7.3	1620	−0.286	Completely freshwater
Coturnix coturnix[63]	18	+	NA	NA	NA	Mainly freshwater
Ammoperdix heyi[49]	1	+	NA	NA	NA	Completely freshwater
Gaviiformes						
Gavia stellata[2]	3	+	25.2	1549	0.27	Mainly saline
Gruiformes						
Gallirallus owstoni[64]	4	+	NA	NA	NA	Completely freshwater
Balearica pavonina[2]	2	–	26	4448	−0.144	Completely freshwater
Fulica americana[1]	1	+	9	699	0.146	Mainly saline
Otidiformes						
Ardeotis kori[2]	2	–	44.3	7770	−0.138	Completely freshwater
Pelecaniformes						
Pelecanus erythrorhynchos[15]	2	+	66	6777	0.09	Mainly saline
Ardea herodias[15]	1	–	15.8	1840	−0.002	Mainly saline
Nycticorax nycticorax[15]	4	–	4.6	623	−0.099	Mainly freshwater
Botaurus lentiginosus[15]	2	–	4.7	625	−0.091	Mainly saline
Platalea minor[c]	4	+	21.52	1573	0.196	Completely saline
Threskiornis aethiopicus[c]	3	+	9.08	1068	−0.022	Mainly saline
Gorsachius melanolophus[c]	3	–	2.38	397	−0.203	Completely freshwater
Bubulcus ibis[c]	3	–	1.26	238	−0.271	Completely freshwater
Pelecanus occidentalis[59]		+	NA	NA	NA	Completely saline

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Table 1. *Continued*

Species	Sample size	SSG	Kidney (g)	Body (g)	K/W residuals	Habitat type
Phoenicopteriformes						
Phoeniconaias minor[2]	5	+	18	1541	0.126	Completely saline
Piciformes						
Colaptes auratus[15]	1	–	1	126	−0.114	Completely freshwater
Colaptes chrysoides[15]	2	–	1	116	−0.08	Completely freshwater
Melanerpes uropygialis[15]	3	–	0.7	68	−0.019	Completely freshwater
Melanerpes formicivorus[15]	2	–	0.5	63	−0.134	Completely freshwater
Sphyrapicus varius[15]	2	–	0.5	45	0.002	Completely freshwater
Sphyrapicus thyroideus[15]	3	–	0.5	43	0.021	Completely freshwater
Podicipediformes						
Podiceps nigricollis[15]	2	+	3.7	330	0.064	Mainly saline
Podilymbus podiceps[15]	2	+	5.9	496	0.101	Mainly saline
Procellariiformes						
Phoebastria immutabilis[65]	1	+	NA	NA	NA	Completely saline
Phoebastria nigripes[65]	1	+	NA	NA	NA	Completely saline
Phoenicopterus ruber[65]	1	+	NA	NA	NA	Completely saline
Phoebastria irrorata[66]	1	+	NA	NA	NA	Completely saline
Psittaciformes						
Melopsittacus undulatus[15]	6	–	0.3	39	−0.162	Completely freshwater
Sphenisciformes						
Eudyptula minor[65]	1	+	NA	NA	NA	Completely saline
Pygoscelis adeliae[65]	1	+	NA	NA	NA	Completely saline
Pygoscelis papua[65]	1	+	NA	NA	NA	Completely saline
Spheniscus mendiculus[44]	1	+	NA	NA	NA	Completely saline
Spheniscus humboldti[65]	1	+	NA	NA	NA	Completely saline
Strigiformes						
Bubo virginianus[1]	1	–	3.7	635	−0.201	Completely freshwater
Micrathene whitneyi[15]	2	–	0.4	37	−0.015	Completely freshwater
Struthioniformes						
Struthio camelus[2]	1	+	920	123000	0.06	Completely freshwater
Suliformes						
Urile pelagicus[1]	1	+	16.9	1300	0.168	Completely saline
Fregata minor[65]	1	+	NA	NA	NA	Completely saline
Aptenodytes patagonicus[65]	1	+	NA	NA	NA	Completely saline
Sula nebouxii[65]	1	+	NA	NA	NA	Completely saline
Sula sula[65]	1	+	NA	NA	NA	Completely saline
Passeriformes						
Pachyramphus aglaiae[15]	1	–	0.295	29	−0.049	Completely freshwater
Tyrannus tyrannus[15]	3	–	0.604	49	0.05	Completely freshwater
Tyrannus verticalis[15]	1	–	0.388	38.8	−0.048	Completely freshwater
Tyrannus vociferans[15]	2	–	0.557	46.4	0.037	Completely freshwater
Pitangus sulphuratus[15]	1	–	0.657	79	−0.107	Completely freshwater
Contopus sordidulus[15]	3	–	0.157	14.3	−0.036	Completely freshwater

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Table 1. *Continued*

Species	Sample size	SSG	Kidney (g)	Body (g)	K/W residuals	Habitat type
Contopus cooperi[15]	1	–	0.515	36.5	0.1	Completely freshwater
Pyrocephalus rubinus[15]	2	–	0.211	13.9	0.104	Completely freshwater
Empidonax oberholseri[15]	1	–	0.204	12.1	0.145	Completely freshwater
Myiarchus cinerascens[15]	4	–	0.299	26.3	−0.003	Mainly freshwater
Eremophila alpestris[15]	4	–	0.284	27.5	−0.044	Mainly freshwater
Tachycineta thalassina[15]	2	–	0.282	15.7	0.18	Mainly freshwater
Tachycineta bicolor[15]	3	–	0.315	18	0.173	Mainly freshwater
Hirundo rustica[15]	3	–	0.325	18	0.187	Completely freshwater
Petrochelidon pyrrhonota[15]	5	–	0.374	22.2	0.163	Completely freshwater
Progne subis[15]	2	–	0.544	46.6	0.025	Mainly freshwater
Cyanocitta stelleri[15]	2	–	1.101	103.7	0.007	Completely freshwater
Aphelocoma ultramarina[15]	2	–	1.274	120.2	0.01	Completely freshwater
Pica pica[15]	4	–	2.556	198.2	0.11	Completely freshwater
Baeolophus wollweberi[15]	4	–	0.144	10.4	0.055	Completely freshwater
Auriparus flaviceps[15]	4	–	0.107	7	0.087	Mainly freshwater
Psaltriparus minimus[15]	1	–	0.102	8	0.012	Mainly freshwater
Sitta carolinensis[15]	1	–	0.225	15.8	0.08	Completely freshwater
Certhia familiaris[15]	2	–	0.145	8.4	0.145	Mainly freshwater
Troglodytes aedon[15]	3	–	0.175	11	0.117	Completely freshwater
Cistothorus palustris[15]	2	–	0.278	13	0.255	Mainly saline
Thryomanes bewickii[15]	1	–	0.212	12.2	0.159	Completely freshwater
Campylorhynchus brunneicapillus[15]	5	–	0.424	38.4	−0.005	Completely freshwater
Catherpes mexicanus[15]	1	–	0.18	11.2	0.122	Completely freshwater
Salpinctes obsoletus[15]	2	–	0.249	15.5	0.132	Mainly freshwater
Mimus polyglottos[15]	3	–	0.508	49.1	−0.026	Completely freshwater
Dumetella carolinensis[15]	1	–	0.452	36.8	0.04	Completely freshwater
Toxostoma bendirei[15]	1	–	0.66	63.6	−0.017	Completely freshwater
Toxostoma curvirostre[15]	3	–	0.875	72.4	0.053	Completely freshwater
Toxostoma crissale[15]	2	–	0.677	58.8	0.026	Completely freshwater
Oreoscoptes montanus[15]	3	–	0.57	40	0.107	Completely freshwater
Turdus migratorius[15]	3	–	1.172	86.6	0.107	Completely freshwater
Catharus guttatus[15]	1	–	0.359	31	0.009	Completely freshwater
Catharus ustulatus[15]	1	–	0.461	29.8	0.134	Mainly freshwater
Catharus minimus[15]	1	–	0.396	33.2	0.024	Completely freshwater
Catharus occidentalis[15]	1	–	0.252	31.2	−0.147	Completely freshwater
Catharus aurantiirostris[15]	1	–	0.405	33.8	0.027	Completely freshwater
Sialia mexicana[15]	4	–	0.408	29.3	0.088	Mainly freshwater
Polioptila caerulea[15]	2	–	0.078	5.1	0.078	Completely freshwater
Polioptila melanura[15]	3	–	0.086	5.4	0.097	Completely freshwater
Bombycilla cedrorum[15]	4	–	0.471	40.1	0.023	Completely freshwater
Phainopepla nitens[15]	3	–	0.368	25.3	0.103	Completely freshwater
Sturnus vulgaris[15]	4	–	1.133	82.2	0.113	Mainly freshwater
Vireo solitarius[15]	1	–	0.169	16	−0.05	Completely freshwater
Setophaga ruticilla[15]	1	–	0.1	9.5	−0.066	Mainly freshwater
Myioborus pictus[15]	1	–	0.163	9.1	0.163	Completely freshwater

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Table 1. *Continued*

Species	Sample size	SSG	Kidney (g)	Body (g)	K/W residuals	Habitat type
Myioborus miniatus[15]	1	–	0.097	9.5	−0.079	Completely freshwater
Passer domesticus[15]	5	–	0.301	29.3	−0.044	Completely freshwater
Taeniopygia guttata[15]	2	–	0.09	11.9	−0.203	Completely freshwater
Dolichonyx oryzivorus[15]	3	–	0.396	35.2	0.001	Mainly freshwater
Sturnella neglecta[15]	2		1.247	113.4	0.025	Completely freshwater
Agelaius phoeniceus[15]	4	–	0.668	67.5	−0.036	Mainly freshwater
Icterus cucullatus[15]	2	–	0.294	25.2	0.007	Completely freshwater
Icterus bullockii[15]	3	–	0.41	33.1	0.041	Completely freshwater
Euphagus carolinus[15]	1	–	0.687	66.2	−0.016	Mainly freshwater
Euphagus cyanocephalus[15]	3	–	0.863	70.9	0.055	Mainly freshwater
Quiscalus quiscula[15]	3	–	1.037	94.3	0.019	Completely freshwater
Molothrus ater[15]	2	–	0.398	49.7	−0.137	Completely freshwater
Piranga ludoviciana[15]	2	–	0.514	33.2	0.138	Completely freshwater
Cardinalis cardinalis[15]	3	–	0.59	47	0.057	Completely freshwater
Cardinalis sinuatus[15]	1	–	0.574	35.1	0.163	Completely freshwater
Passerina caerulea[15]	1	–	0.273	28.1	−0.07	Completely freshwater
Coccothraustes vespertinus[15]	1	–	0.86	52.3	0.177	Completely freshwater
Pinicola enucleator[15]	1	–	0.833	53.5	0.154	Completely freshwater
Passerculus sandwichensis[15]	3	–	0.261	17.7	0.254	Completely saline
Pipilo erythrophthalmus[15]	1	–	0.397	39.6	−0.046	Mainly freshwater
Pooecetes gramineus[15]	2	–	0.285	26.3	−0.024	Completely freshwater
Chondestes grammacus[15]	1	–	0.166	26.2	−0.257	Completely freshwater
Aimophila ruficeps[15]	1	–	0.264	17.9	0.099	Completely freshwater
Amphispiza bilineata[15]	6	–	0.154	12.9	−0.003	Completely freshwater
Junco hyemalis[15]	2	–	0.216	21.7	−0.067	Completely freshwater
Spizella passerina[15]	1	–	0.125	13.3	−0.106	Completely freshwater
Spizella pallida[15]	1	–	0.17	13	0.037	Completely freshwater
Spizella breweri[15]	4	–	0.178	10.6	0.14	Completely freshwater
Zonotrichia albicollis[15]	3	–	0.279	26.5	−0.036	Completely freshwater
Melospiza lincolnii[15]	5	–	0.228	18.1	0.03	Mainly freshwater
Melospiza melodia[15]	2	–	0.247	21.1	0.003	Completely freshwater
Calcarius lapponicus[15]	2	–	0.261	26.7	−0.068	Mainly freshwater
Plectrophenax nivalis[15]	1	–	0.337	31.1	−0.019	Mainly saline

Data sources are shown in superscript after the species name; a superscript C indicates data collected in the current study.

Limitations of the study

Since kidney weight measurements require fresh samples, they are not easy to obtain. Only about 56% of the orders of birds were included in this study. However, such limitations probably only affect the estimation of the numbers of species having adapted to high-salinity habitats, changed kidney size, or developed or lost SSGs in the evolutionary process, rather than the trend of the results from the phylogenetic analysis, the ancestral state reconstruction, and the correlated evolution analysis.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact

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AUTHOR CONTRIBUTIONS

Shou-Hsien Li, Chi-Cheng Chiu, and Ben-Yang Liao designed the research; Chi-Cheng Chiu, and Cheng-Te Yao performed the research; Chi-Cheng Chiu analyzed data; and Shou-Hsien Li, Chi-Cheng Chiu, Ben-Yang Liao, and Cheng-Te Yao wrote the article. All authors approved the final submission.

DECLARATION OF INTERESTS

None of the authors of this article have any affiliations with or involvement in any organization or entity with any financial or non-financial interest (such as patent or stock ownership, membership of a company board of directors, membership of an advisory board or committee for a company, and consultancy for or receipt of speaker's fees from a company) in the subject matter or materials discussed in this article.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Pelicaniforme samples	Taiwan Biodiversity Research Institute	SPBorgan001 SPBorgan002 SPBorgan003 SPBorgan004 ASibis001 ASibis002 ASibis003 BcNH001 BcNH002 MNH001 MNH002 MNH003 Bubibis001 Bubibis002 Bubibis003 GE001 GE002
Deposited data		
Analyzed data	This paper	https://doi.org/10.17632/bkvjx52nsr.1
Software and algorithms		
R software	Team, R.C.[67]	https://www.r-project.org/
phytools	Revell[19]	https://github.com/liamrevell/phytools
caper	Orme et al.[23]	https://github.com/cran/caper/blob/master/R/brunch.R
phylolm	Ho et al.[24]	https://github.com/lamho86/phylolm
RRphylo	Castiglione et al.[32]	https://github.com/cran/RRphylo
BayesTraits 3.0	Pagel and Meade[26]	http://www.evolution.reading.ac.uk/BayesTraitsV3/BayesTraitsV3.html

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Shou-Hsien Li (e-mail: t43028@ntnu.edu.tw).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Data: All the raw data enrolled in this study have been deposited to the Digital Commons Data and are publicly available as of the date of publication. The accession number is listed in the key resources table.
- Code: This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

METHOD DETAILS

Collection of characteristics

Data on kidney and body weight were collected for 161 avian species and on SSGs for 224 species, both featuring known phylogenetic information from the literature.[1,2,10,15,44,54–66] In 2023, we conducted searches using Google Scholar, utilizing various combinations of search terms such as 'salt gland', 'kidney size', and 'osmotic regulation' to obtain the most pertinent data. Because information on kidney weight was only published for two species of Pelicaniformes, we measured kidney and body weights from six additional Pelicaniformes species, namely the great egret (*Ardea alba*; n= 2), cattle egret (*Bubulcus ibis*; n= 3), black-crowned night heron (*Nycticorax nycticorax*; n= 2), Malayan night heron (*Gorsachius melanolophus*; n= 3), black-faced spoonbill (*Platalea minor*; n= 4), and African sacred ibis (*Threskiornis aethiopicus*; n= 3) which live in habitats with various levels of salinity (Table 1). All Pelicaniforme samples measured in this study were from carcasses of roadkill or of rescued individuals archived in the Taiwan Biodiversity Research Institute (TBRI). Before measurement, carcasses were stored in sealed plastic bags to prevent desiccation, and frozen at -20°C. After thawing, we followed the method of Maryanne (1970) to weigh the birds to the nearest 0.1 g; then, we took out the kidneys, dried them with absorbent paper, and weighed them to the nearest 0.001 g. Our subsequent analyses related to kidney size included 167 species and those relating to SSGs covered 230 species representing 25 avian orders.

We categorized the habitat type used by these birds into different levels of salinity: (1) completely freshwater (completely living in freshwater habitats, such as forests, grasslands, streams, lakes, and reservoirs.), (2) mainly freshwater (almost exclusively living in freshwater habitats; only a few records appear in saline habitats such as saltmarshes, saltpans, mangroves, seacoasts, beaches, and marine environments), (3) mainly saline (including the ocean and habitats associated with high salinity; sometimes appearing in freshwater habitats or just for parts of the year), and (4) completely saline habitats (exclusively living in habitats with high salinity) by assessing the habitat information on these species described in Birds of the World.[16] The completely saline habitat has the highest level of environmental salinity, followed by mainly saline, mainly freshwater, and then completely freshwater.

Standardization of kidney sizes

Since the data obtained from the literature mostly only provides the average values of multiple individuals for each species, this study uses the average values of each species for subsequent analysis. Because both the body and kidney sizes of the avian species included in this study differ by up to five orders of magnitude (3 ~ 123,000 g for body weight; 0.03 ~ 920 g for kidney weight), we log-transformed body and kidney weights to better meet the statistical assumptions of a regression test. We used the residual from linear regressions (Log(kidney size) = 1.165 $\pm$ 0.073 + (0.928 $\pm$ 0.0193) log(body weight) ($p < 2.2e\text{-}16$; adjusted r^2: 0.933; Figure 3).) of kidney weight on body weight as a proxy for relative kidney sizes to control the allometric effect.

Ancestral state reconstruction

To infer the macroevolutionary history of the ecological and physiological traits associated with salt tolerance, we extracted 10,000 avian phylogenetic trees of all 230 species based on the complete Bayesian maximum clade credibility (MCC) species-level avian phylogeny from http://birdtree.org [17] with the Hackett constraint.[18] Using the R package, phytools,[19] we constructed a consensus tree based on the majority rule of the 10,000 trees we extracted. We estimated the branch length with a least square method. In the consensus tree of the 167 avian species the values were: Minimum: 0.188, 1st Quartile: 3.565, Median: 7.831, 3rd Quartile: 17.514, Maximum: 108.376. And in the consensus tree of the 230 avian species the values were: Minimum: 0.12, 1st Quartile: 3.252, Median: 7.337, 3rd Quartile: 15.979, Maximum: 108.376. Then, we used a Bayesian inference method (stochastic characteristic mapping with the "make.simmap" function) implemented in the phytools package for R[26] to reconstruct the ancestral state of the kidney size of the 167 avian species and SSG and habitat type of the 230 avian species. To compare the continuous data of residual kidney weight with the discrete data of SSGs and habitat type, we recoded the residual kidney weight data as a binary variable using the fourth quartile (the 75th percentile) of residual kidney weights as a reference. Species with residual kidney weights equal to or greater than 0.13 (the 75th percentile) were considered to have 'large kidneys', while those with residual kidney weights less than 0.13 were considered to have 'non-large' kidneys. The parameters were set with 1,000 MCMC generations and the ARD ("All Rates Different") model, which assumes that rates of trait evolution were not the same among all branch. The transition rates between different characteristic states were calculated by simulations, and posterior probabilities were mapped to the phylogeny using the "densityMap" function in phytools.[19] The simulation involves two steps: first, simulating ancestral states at each internal node by sampling the posterior distribution of states; second, generating a substitution (mutational) history by sampling the posterior distribution conditioned on the reconstructions and observed states at the tips of the topology. Posterior probability values $\geq$ 50% indicated a characteristic state being ancestral to a clade.

Phylogenetic signals in kidney sizes, possession of SSGs, and habitat salinity

After using the Shapiro-Wilk test to check the normality of the data, we used the function 'phylosig' in the package 'phytools[19]' to calculate the Pagel's λ[20] and Blomberg et al.'s K[21] for the phylogenetic signal in the variation of relative kidney sizes, the presence of SSGs, and the category of habitat. Assuming a Brownian motion model of trait evolution, a λ of 0 indicates that trait correlation between species is independent of their shared evolutionary history; by contrast, a λ of 1 suggests that trait correlation between species is constrained by their shared evolutionary history.[20] Also, assuming a Brownian motion model of trait evolution, K values greater than 1 indicate a higher trait variance among

clades than expected, whereas *K* values smaller than 1 imply a lower trait variance within clades than expected.[22] Therefore, low *K* values suggest that their shared evolutionary history constrains the trait variance. We used phylogenetic generalized least-squares (PGLS) in the R package 'caper'[23] to control the phylogenetic effect and infer the association between variations in relative kidney size and habitat types with different levels of salinity and between variations in relative kidney size and the presence of SSGs. We used the phylogenetic generalized linear model (PGLM) in R package 'phylolm'[24] to infer the linear association between the possession of SSGs and habitat salinity.

Evolutionary transitions in kidney size, SSGs and levels of habitat salinity

We used the Discrete module of BayesTraits 3.0[25,26] to estimate the transition rates of correlated evolution between habitat salinity, relative kidney sizes, and the possession of SSGs on a phylogeny. We took the dependent model approach, which assumes that the rate of change in one trait depends on the state of the other trait. We estimated the log Bayes factor (logBF) for the dependent model (allowing correlation between variables) against the independent model (null model, which fixes all correlations to be zero) as twice the difference between the estimated log marginal likelihoods using the formula logBF = 2*(log marginal likelihood dependent model – log marginal likelihood, independent model). Then, we interpreted comparisons where logBF > 2 as having weak support, logBF > 5 as having moderate support, and logBF > 10 as having strong support to reject the null model.[27]

Because the transition rate of correlated evolutionary analyses can only be calculated from binary data, residual kidney weight was transformed into a binary variable by reference to the fourth quartile of residual kidney weights; species with residual kidney weights equal to or larger than 0.13 (the 75th percentile) were considered to have 'large kidneys,' and those with residual kidney weights less than 0.13 were supposed to have 'non-large kidneys.' To test the sensibility of this cutoff value for our analysis, we repeated these analyses setting the cut-off points to the 25th and 50th percentiles of residual kidney weight, as suggested by Fristoe et al.[28] We similarly re-grouped the habitat categories into two by combining the entirely freshwater habitat and the mainly freshwater habitat to be 0, and the mainly saline habitat and the completely saline habitat to be 1. We ran an MCMC chain with 5.05 million iterations and a burn-in of 50,000 iterations and sampled every 1000 iterations. We scaled the branch length of the phylogenetic trees by 0.001 and used an exponential prior with a mean of 10 for all parameters.

Searching for morphological convergence among species in diverse saline habitat types

We used the RRphylo[32] method to perform phylogenetic ridge regression on trees and data, yielding branch-wise evolutionary rates and ancestral character estimates (ACEs) at each node. This process is applied independently to each phenotype component for multivariate data, using a normalization factor to prevent extreme rate values and multicollinearity while assuming minimized rate variation within clades. We also employed search.conv,[32] a fast and effective method for identifying phenotypic convergence among clades or species groups within specific categories. With search.conv, the phenotypic distance between species is quantified as the angle between their phenotypic vectors (i.e., multivariate phenotypes for each species). Under a Brownian motion model of evolution, this angle should increase proportionally to the patristic distance, the sum of the lengths of the branches that link two nodes in a tree, between species. However, when morphological convergence is present, the angle (per unit time) becomes smaller than the expected.

When comparing clades, the function calculates the mean angle over all possible combinations of species pairs, taking one species per clade, and divides this value by the patristic distance between the nodes subtending the clades (i.e., the phylogenetic distance between the most recent common ancestors, MRCAs, of the clades). This value is contrasted with a random distribution of 1,000 angle-by-time values to assess significance. When comparing species, the function randomly samples two species within the tree, computes the angle between them, and divides it by the patristic distance between their immediate ancestors.

Given two clades presumed to evolve under convergence, search.conv derives the ACEs for the MRCAs for both clades from the RRphylo results and calculates the angle between them. This angle is added to the mean angle between species and divided by the patristic distance between the MRCAs. If the "summed" angle is smaller than expected by chance, it indicates that the clades converged since their origin and subsequently followed parallel phenotypic evolutionary trajectories. The significance level is assessed as described above by randomizing phenotypes across the tree tips. Finally, if no specific hypothesis about converging clades is available, the function automatically scans the phylogeny to identify instances of convergence.

Under the "state case", search.conv computes the mean angle over all possible combinations of species pairs using one species per state. Each angle is divided by the patristic distance between the species. Significance is assessed by contrasting this value with a family of 1,000 random angles obtained by shuffling the state across the species.

In this study, we employed search.conv for both "clade" and "state" scenarios, by categorizing species based on their usage of saline and non-saline habitats. We further conducted the analyses by functional variable values (kidney sizes and presence of SSGs) for each species, utilizing these vectors as input for search.conv.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical Analysis Methods and Software: Used R v 4.2.3 language and related packages (phytools, caper, phylolm, RRphylo) and BayesTraits V3.0, to perform various statistical analyses, including ancestral state reconstruction, correlated evolutionary transition rate estimation, phylogenetic signal testing, phylogenetically adjusted least squares regression, phylogenetically adjusted generalized linear models, phylogenetic ridge regression, and detection of morphological convergence.

Location of Statistical Details: All statistical details of this research, such as the types of statistical tests, the exact value and meaning of sample size n, the definition and measurement of central tendency and dispersion, the setting of significance level, etc., were found in Table 1 and Method details.

Premises and Strategies of Statistical Analysis: Before performing statistical analysis, we tested the normality of the data and log-transformed or binarized the data to meet the assumptions of the statistical model. This study obtained all species with available characteristic values and phylogenetic information, stratified them based on their habitat types and physiological characteristics, and did not exclude any data or species during the analysis process.