A comparative test of the correlated evolution of flightlessness and relative brain size in birds

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Abstract

Secondary flightlessness has evolved independently many times in birds. Morphological changes in the pectoral girdle and flight feathers and changes in body size have been associated with the evolution of flightlessness, and it has also been suggested that flightless birds have relatively small brains. We therefore tested whether flightlessness is related to changes in relative brain size. Relative brain size was compared between volant and flightless species using both conventional statistics and modern comparative methods within nine taxonomic groups. No significant difference was found between flightless and volant species in six of these groups, regardless of whether body mass or tibiotarsal measurements were used as estimates of body size. Species with relatively smaller brains compared with their volant relatives were the great auk *Pinguinus impennis*, the kakapo *Strigops habroptilus* and some species of penguin. Thus, we found no evidence of a general correlation between the evolution of secondary flightlessness and the evolution of relatively small brains in birds. This suggests that neural requirements are not significantly different between flightless and volant species, although our methods may have overlooked subtle neurological changes that do not result in markedly different endocranial volumes.

Key words: brain size, flightless, comparative method, correlated evolution, birds

INTRODUCTION

Although flightlessness would seem to be the antithesis of the evolutionary trajectory of the class Aves, many different lineages of birds have independently lost the ability to fly. Numerous skeleto-muscular and even physiological changes have been correlated with the evolution of flightlessness, so it was natural to ask whether changes also may have taken place in other organ systems. To this end, Bennett & Harvey (1985) investigated relative brain size and concluded that flightless birds have proportionately smaller brains than volant species. Their taxonomic sample was small, however, suggesting that a more exhaustive study would be needed to confirm their original prediction. Therefore, a survey was undertaken of brain size in a much greater diversity of flightless species and their closest volant relatives.

The evolutionary loss of the ability to fly has occurred repeatedly and is taxonomically widespread in birds (Feduccia, 1999). Modern flightless species are known in at least 26 avian families in 17 orders (Livezey, 1995). The number of flightless species within each of these taxonomic groups varies from all species (e.g. penguins, Sphenisciformes) to single species (e.g. parrots, Psittaciformes). In some families, flightlessness has evolved independently many times (e.g. rails, Rallidae and waterfowl, Anseriformes). Flightless birds include deep-diving piscivores such as penguins and the great auk *Pinguinus impennis*, various semi-aquatic and terrestrial waterfowl, a folivorous parrot *Strigops habroptilus* and the nocturnal invertebrate-feeding kiwis *Apteryx* sp.

In typical flightless birds, the keel of the sternum, the long bones of the wing, and the pectoral muscles are much reduced in size, whereas the bones and musculature of the hindlimb are often larger than in related volant species. In most cases, shortening of the wing progresses from distal to proximal bony elements such that the manus and antebrachium are relatively shorter than the humerus in flightless species (Livezey, 1995).

In addition to skeleto-muscular changes the integument may be affected as well. The vanes of flight feathers usually lose their asymmetry (Feduccia & Tordoff, 1979), and barbules may lose their ability to interlock (Feduccia, 1995), which in the most extreme form results in the hair-like feathers of kiwis. Such extreme morphological changes are not, however, present in all flightless birds. For example, some flightless species, such as grebes (Podicipedidae) show little morphological divergence

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from volant relatives (Livezey, 1989). Similarly, because wing-propelled diving birds, such as the penguins and flightless auks, 'fly' through a medium many times denser than air, they have the pectoral girdle hypertrophied rather than reduced. Thus, the wing-propelled divers may be considered flightless only because they cannot fly in air.

The ability to fly clearly constrains the body size of birds. All of the world's largest birds, including ratites such as ostriches, elephant birds and moas (Worthy & Holdaway, 2002); the Australian dromornithids (Murray & Megirian, 1998), and the Holarctic *Diatryma* (Andors, 1992), are flightless. In fact, most flightless birds are heavier and more robust than their closest volant relatives (Livezey, 1995; Feduccia, 1999).

The morphological correlates of secondary flightlessness in birds mentioned above are well known, but the implications of flightlessness for tissues that are not directly related to flight have received less attention. One such tissue that may exhibit changes in size and structure that are correlated with flightlessness is the brain. This was, in fact, tested in Bennett & Harvey's (1985) comparison of relative brain size and ecology in birds. Although Bennett & Harvey (1985) found that flightless species have relatively smaller brains, they were diffident about this conclusion because their analysis included only ratites and a penguin but no species from orders that include both volant and flightless species. Because many features of avian life history and morphology, including relative brain size (Nealen & Ricklefs, 2001), are significantly affected by phylogenetic relationships (Bennett & Owens, 2002), it is important to test for such differences within clades that possess both volant and flightless species. Therefore relative brain size was investigated in nine different clades representing at least 15 independent instances of evolution of secondary flightlessness that could be used to test for significant differences in relative brain size using both conventional statistics and modern comparative methods (Harvey & Pagel, 1991).

MATERIALS AND METHODS

Data

The adult brain sizes of 2577 specimens representing 417 species across 10 taxonomic groups were measured using fixed brain masses and endocranial volumes (see Iwaniuk, 2003 for the complete dataset). The methods used to measure endocranial volume and brain masses are described in full elsewhere (Iwaniuk & Nelson, 2001, 2002; Iwaniuk, 2003). Briefly, endocranial volumes were measured by filling the skulls of skeletal specimens with lead shot and decanting the shot into modified syringes and graduated cylinders to estimate brain volume. Brain masses, on the other hand, were taken from formalin fixed specimens collected from zoos and veterinary clinics. Each brain was dissected out of the skull, the meninges removed and the brain weighed to the nearest milligram. Body masses could not be obtained for all specimens

owing to missing data on museum specimen tags or from the source of their collection. Thus, for some species, body masses were obtained from the literature (a complete list of references is provided in Iwaniuk, 2003) as well as estimated from tibiotarsal measurements (see below).

Flightless representatives of 9 taxa were measured: paleognaths (Tinamiformes and Struthioniformes), 10 spp. (6 volant/4 flightless); grebes (Podicipediformes), 10 spp. (9/1); cormorants (Phalacrocoracidae), 9 spp. (8/1); parrots (Psittaciformes), 189 spp. (188/1); auks (Alcinae), 14 spp. (13/1); waterfowl (Anseriformes), 92 spp. (85/7); rails (Rallidae), 35 spp. (29/6); ibises (Threskiornithidae), 15 spp. (13/2); penguins, 6 spp. The anseriforms included 3 subspecies of the Canada goose (*Branta canadensis minima*, *B. c. moffitti* and *B. c. taverneri*) because recent evidence suggests that some of the subspecies are more closely related to the Hawaiian geese examined (the nene *Branta sandvicensis*, and the flightless giant Hawaii goose *Branta* sp.) than others (Paxinos *et al.*, 2002). The Procellariiformes (37 spp.) were used for comparison with penguins because there are no extant volant penguins and these 2 orders probably represent sister clades (Olson, 1985; Kennedy & Page, 2002).

Several extinct and fossil taxa were included to maximize the number of flightless species analysed within each group. One of these was the great auk. The endocranial volumes of 10 great auk skulls excavated from Funk Island (Newfoundland, Canada) were cleaned of debris and measured. Several extinct species were also examined within the Anseriformes: Auckland Islands merganser *Mergus australis*, giant Hawaii goose *Branta* sp. (H. F. James & S. L. Olson, pers. obs.), nene-nui *Branta hylobadistes*, *Ptaiochen pau* and *Thambetochen chauliodous*. The merganser became extinct in the early 1900s (Marchant & Higgins, 1990), whereas the other 4 species are known only from fossil material collected in the Hawaiian islands (Olson & James, 1991). The giant Hawaii goose and nene-nui were both large geese resembling other species of *Branta* (Olson & James, 1991). The former species seems to have been flightless whereas the latter species has been described as a 'weak flier' (Olson & James, 1991). Both *Ptaiochen* and *Thambetochen* were large goose-like members of the dabbling duck clade (Sorenson *et al.*, 1999) that were both flightless and herbivorous (James & Burney, 1997). Within the rallidae, the extinct species included: Dieffenbach's rail *Gallirallus dieffenbachii*, Laysan crake *Porzana palmeri*, and Wake Island rail *Rallus wakensis*. Like the Auckland Islands' merganser, all of these species became extinct following European contact (Taylor, 1998). Lastly, 2 flightless ibises, *Apteribis brevis* and *Apteribis* sp., from the Hawaiian islands were also measured. Both of these are known from fossils collected on Maui. An additional species, *Apteribis glenos*, is known from Molokai (Olson & Wetmore, 1976), but intact crania with an associated tibiotarsus (see below) were wanting. The undescribed species of *Apteribis* was larger than *Apteribis brevis* and its species status remains uncertain (Olson & James, 1991).

Body mass estimation

To include extinct flightless species in the study it was necessary to estimate their body masses from bone measurements. The method of Campbell & Marcus (1992), who provide regression equations for the relationships of the least shaft circumference of the femur and tibiotarsus against body mass for several groups of birds, was used. In choosing these bone measurements, Campbell & Marcus (1992) reason that these 2 bones are designed to bear the full weight of bipedal animals and their weakest part is their narrowest transverse plane, which therefore should provide a reasonable estimate of maximum body size.

To estimate body masses for the present study, tibiotarsi were measured for 461 specimens representing 121 species. The minimum circumference of the tibiotarsus was estimated by wrapping a cotton thread around the tibiotarsus and measuring the minimum circumference with a pair of digital callipers calibrated to the nearest 0.01 mm. The only extinct species for which body mass was not estimated was the great auk because the crania available were disassociated from the tibiotarsi. However, Livezey (1988) estimated a mean body mass of 5000 g for the great auk based on a series of morphometric measurements, so this estimate was used for scaling purposes. Note, however, that using body masses as low as 4000 g and any value over 5000 g for the great auk yielded similar results. Body masses from the specimens examined and from the literature were then log-transformed and regressed against the log-transformed tibiotarsal measurements.

Using species as independent data points, regressions were calculated for the tibiotarsal circumference–body mass relationship in the Anseriformes, Rallidae, and Threskiornithidae (Table 1). These formulae differed considerably from those provided in Campbell & Marcus (1992). In their study, Campbell & Marcus (1992) calculated 3 regression lines describing the allometric relationship between tibiotarsal circumference and body mass using reduced major axis (RMA), major axis (MA) and generalized least-squares (GRS) regressions, for anseriforms and a 'long legged' bird assemblage that included rails and ibises as well as herons and other taxa. We found that paired tests between these estimates and actual values from specimens were significantly different from one another for both the RMA ($t = 3.11$, d.f. $= 65$, $P < 0.01$) and MA formulae ($t = 7.32$, d.f. $= 65$, $P < 0.01$),

Table 1. Slopes ($\pm 95\%$ confidence interval) and intercepts ($\pm 95\%$ confidence interval), and correlation coefficients (r^2) for least-squares linear regressions of log-transformed minimum tibiotarsal circumference (mm) against log-transformed body masses (g)

Taxon	n	Slope	Intercept	r^2
Anseriformes	50	2.004 ± 0.208	0.781 ± 0.236	0.88
Rallidae	14	2.999 ± 0.556	-0.758 ± 0.558	0.91
Threskiornithidae	4	2.831 ± 0.598	-0.529 ± 0.754	0.99

Table 2. Paired t-test values are given for the paired comparisons of body masses from measured specimens (Actual) and literature values (Literature) and body mass estimates derived from the reduced major axis (RMA), major axis (MA) and generalized least-squares (GSR) regression models from Campbell & Marcus (1992). Significant differences are bold

Taxon	Body mass source	d.f.	RMA	MA	GSR
Anseriformes	Actual	49	**2.01**	**6.09**	1.11
	Literature	77	**2.63**	**6.92**	0.61
Rallidae	Actual	13	**3.11**	**4.27**	**3.24**
	Literature	33	**3.82**	**5.42**	**4.00**
Threskiornithidae	Actual	4	1.85	**6.60**	2.36
	Literature	11	2.10	**6.17**	**2.57**

but did not differ for the GRS formula ($t = 0.64$, d.f. $= 65$, $P = 0.52$). Similar results were found when the estimates were compared with literature values (RMA: $t = 4.34$, d.f. $= 120$, $P < 0.01$; MA: $t = 9.07$, d.f. $= 120$, $P < 0.01$; GRS: $t = 1.83$, d.f. $= 120$, $P = 0.07$). When broken down into taxonomic groups, the degree of difference between estimates and actual masses varied between the formulae and between groups (Table 2).

The body masses for species for which we lacked specimen-specific body masses were then estimated using the equations in Table 1 and compared with literature values. No significant differences were present between these estimates and literature values for the anseriforms ($t = 0.99$, d.f. $= 27$, $P = 0.33$), rails ($t = 0.90$, d.f. $= 19$, $P = 0.38$) or ibises ($t = 0.67$, d.f. $= 7$, $P = 0.52$), nor were there any significant differences when all species were combined ($t = 1.32$, d.f. $= 56$, $P = 0.19$). Given the variations in body masses estimated using the various formulae, the equations calculated in Table 1 as well as the RMA (Rallidae) and GRS (Anseriformes) formulae in Campbell & Marcus (1992) were used to estimate body masses (Table 3). It should be noted, however, that one of the specimens of *Thambetochen* possessed a much larger endocranial volume and tibiotarsal circumference (and larger body mass; Table 3) than the other 2 specimens. Because this specimen also originated from a different locality (Lanai) from the other 2 specimens (Maui), it was therefore treated as a separate species for statistical purposes. Whether the Lanai population of *Thambetochen* was morphologically distinct from *T. chauliodous* remains to be investigated. For all specimens, the mean of the body mass estimates was used in the statistical analyses outlined below.

Statistical analyses

Two scaling methods were used to examine relative brain size variation between flightless and volant species. The first of these used the conventional scaling measure of body mass. For the extinct species, with the exception of the great auk (see above), the average body mass estimate was used. Body masses were not available for all specimens of extant species, so in many instances, literature values were used. The second scaling measure

Table 3. Body mass estimates (± SD) derived from equations 1 in Table 1 and from the generalized least-squares (Anseriformes) and reduced major axis (Threskiornithidae) equations from Campbell Marcus (1992)

Taxon	Species	n	Present study	Campbell & Marcus (1992)
Anseriformes	*Branta hylobadistes*	3	3544.3g ± 263.7	3216.9g ± 228.0
	Branta sp.	4	7545.6g ± 425.2	6606.3g ± 354.5
	Ptaiochen pau	4	5237.6g ± 520.1	4665.7g ± 441.1
	Thambetochen chauliodous	3	6227.5g ± 220.0	5502.6g ± 186.8
	Thambetochen sp.[a]	1	8425.1g	7338.2g
Rallidae	*Gallirallus dieffenbachii*	1	274.6g	–
	Porzana palmeri	1	46.3g	–
	Rallus wakensis	1	99.2g	–
Threskiornithidae	*Apteribis brevis*	5	1248.0g ± 119.9	1298.4g ± 121.5
	Apteribis sp.	1	1947.4g	2003.1g
	Nipponia nippon	1	1831.2g	1886.5g

[a] Note that this specimen of *Thambetochen* originated from a different locality from the other three specimens and possessed a much larger tibiotarsal circumference and hence body mass estimate.

used was tibiotarsal circumference in the anseriforms, rails and ibises. As with the body mass analyses, log-transformed brain volume was regressed against log-transformed tibiotarsus circumference.

Significant differences between flightless and volant species were first tested with Mahalanobis distances (Weisberg, 1980) in JMPIN v3.2.1 (SAS Institute, Cary, NC). This method tests whether any of the values are significant outliers relative to the other data points. The second test performed was a paired test (paired *t*-tests and Wilcoxon signed-rank tests) of predicted brain volumes for the flightless species and the observed values. This was performed both within families/orders and across all species. As these are relatively crude methods, an attempt was also made to use multiple regression techniques (i.e. ANCOVA) to test for differences in relative brain size between flightless and volant species. The body size measure (body mass or tibiotarsus circumference) and volant/flightless were used as covariates of brain volume. A significant interaction factor indicates a difference in slope (i.e. brain size scales at a different rate) whereas a significant volant/flightless factor indicates a difference in intercept (i.e. grade shift). This is dependent upon a sufficient sample size of flightless species and was therefore only possible within the waterfowl, seabirds and rails.

The above analyses treat species as independent data points, an approach that can inflate the probability of type I error in comparative analyses by ignoring the influence of shared phylogenetic history (Harvey & Pagel, 1991). Phylogenies for each of the groups were assembled from published (Siegel-Causey, 1988; Sibley & Ahlquist, 1990; Friesen *et al.*, 1996; Johnson & Sorenson, 1999; McCracken *et al.*, 1999; Sorenson *et al.*, 1999; Kennedy *et al.*, 2000; Fleischer & McIntosh, 2001; Kennedy & Page, 2002; Paxinos *et al.*, 2002; Slikas, Olson & Fleischer, 2002; Ribas & Miyaki, in press; Russello & Amato, in press) as well as unpublished sources (Rallidae: B. Slikas pers. comm.; Anseriformes: M. D. Sorenson, pers. comm.). The phylogenetic relationships within the

parrots (Psittaciformes) and grebes (Podicipediformes) are largely uncertain and the relationships of the flightless species to the volant species are unknown. To perform the comparative analyses, the kakapo was considered to be a sister-species to the kaka *Nestor meridionalis* and kea *Nestor notabilis*, and the Attitlan grebe *Podilymbus gigas* a sister-species to the pied-billed grebe *Podilymbus podiceps*. Although not reported herein, alternative sister-species for the kakapo (e.g. *Pezoporus wallicus*, *Cyanoramphus* spp.) did not significantly alter the results reported below.

Using these phylogenies, differences in relative brain size were tested using independent contrasts and phylogenetically corrected analyses of covariance. Independent contrasts (Felsenstein, 1985; Harvey & Pagel, 1991) were calculated using PDTREE, a program within the PDAP software package (available from T. Garland Jr on request) (Garland, Harvey & Ives, 1992). Since most of the phylogenies were compiled from disparate sources, arbitrary equal and unequal branch lengths were used to calculate the contrasts. Adequate standardization of the contrasts was then assessed by performing regression analyses on the absolute value of the contrasts against their standard deviation following the procedures in Garland *et al.* (1992).

Phylogenetically corrected analyses of covariance were also performed using the PDAP package. Briefly, this method uses Monte Carlo simulations of continuous traits along a phylogeny to create a phylogenetically correct and empirically scaled null distribution of *F* statistics (Garland *et al.*, 1993). A conventional ANCOVA can then be performed on the data with the critical value derived from the 95th percentile of the simulated *F* distribution. Using the PDSIMUL program, 1000 simulations were performed under both a gradual and a speciational model of evolutionary change. Values for body size were restricted to biologically realistic values for both body size and brain size within each group tested. That is, the limits of body mass, brain volume and tibiotarsal circumference were set just below and above that of the smallest and
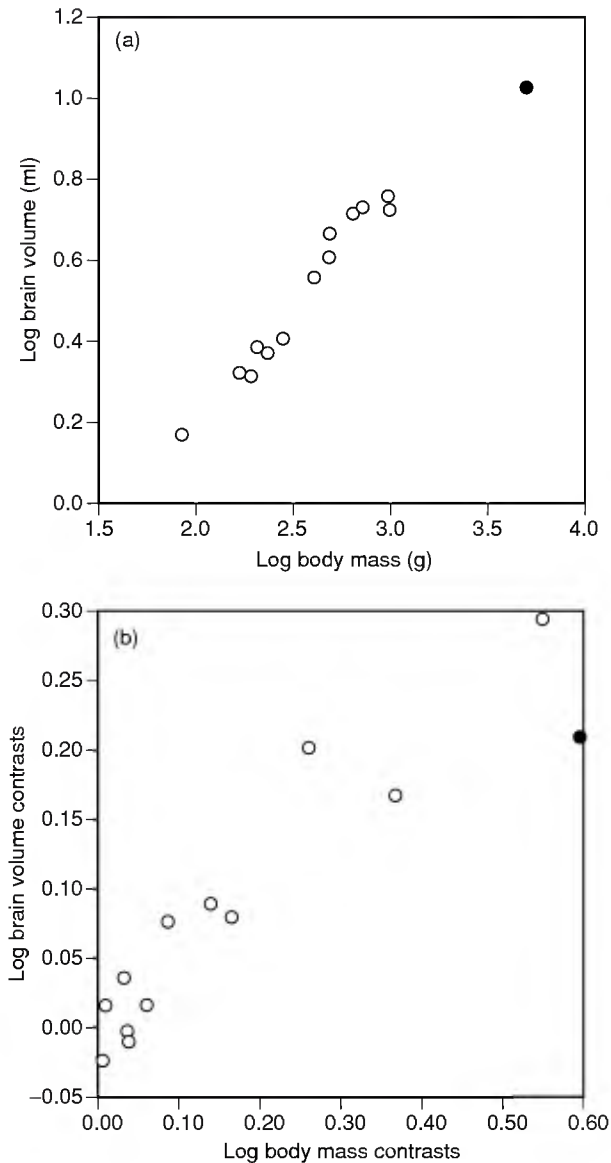
Fig. 1. Log-transformed brain volumes against log-transformed body masses for the Alcinae (14 species) examined using species as independent data points (a) and independent contrasts (b). Open circles, all volant species and the great auk (*Pinguinus impennis*); closed circles, contrast between great auk and razorbill *Alca torda*.
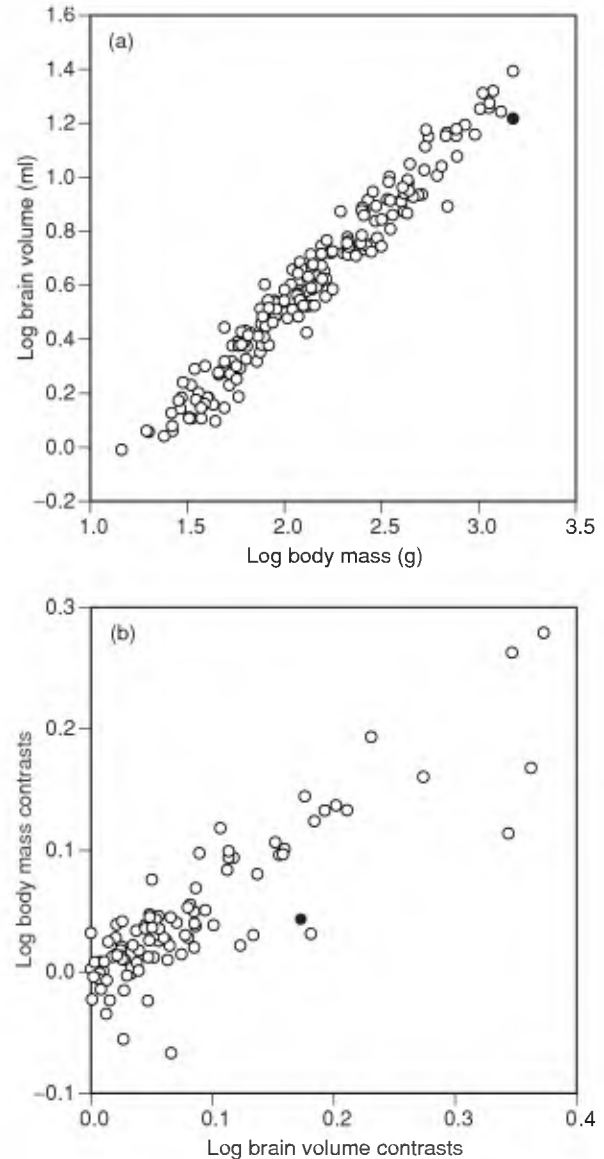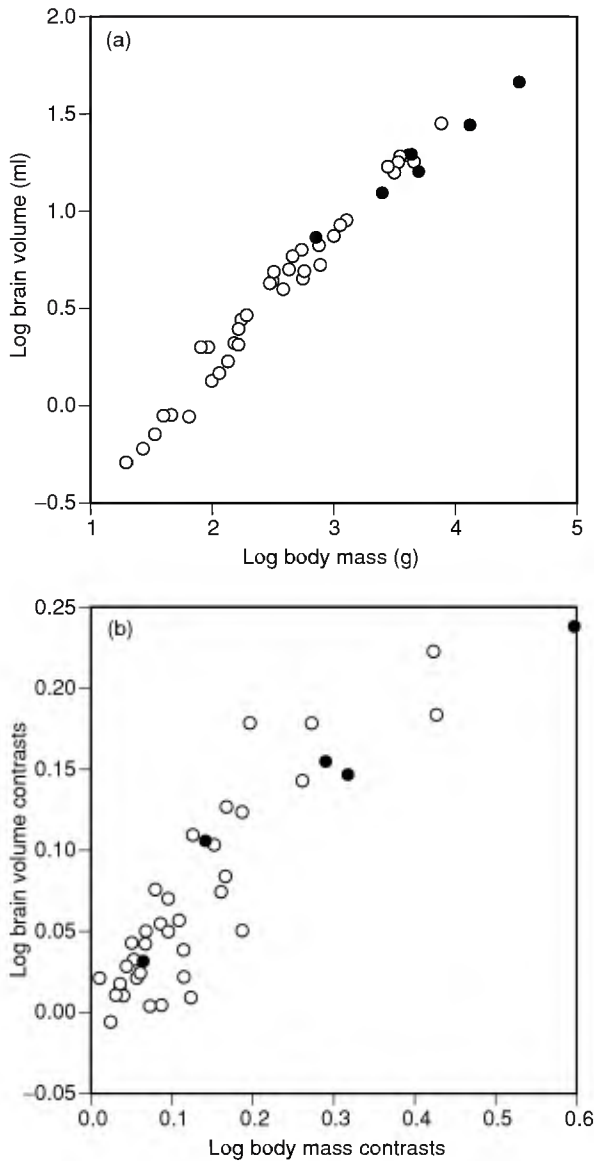
Fig. 2. Log-transformed brain volumes against log-transformed body masses for the Psittaciformes (189 species) examined using species as independent data points (a) and independent contrasts (b). Open circles, all volant species; closed circle, kakapo *Strigops habroptilus*.

largest species within each group. This prevents the generation of values that may be biologically unrealistic for the taxa being examined. Both brain and body size measures were log₁₀ transformed before simulation. The null distributions of the phylogenetically corrected F statistics were then created with PDANOVA and the 95th percentile calculated.

RESULTS

Outlier analyses indicated only four significant outliers that were flightless: the great auk within the Alcinae (Fig. 1); the kakapo within the Psittaciformes (Fig. 2);
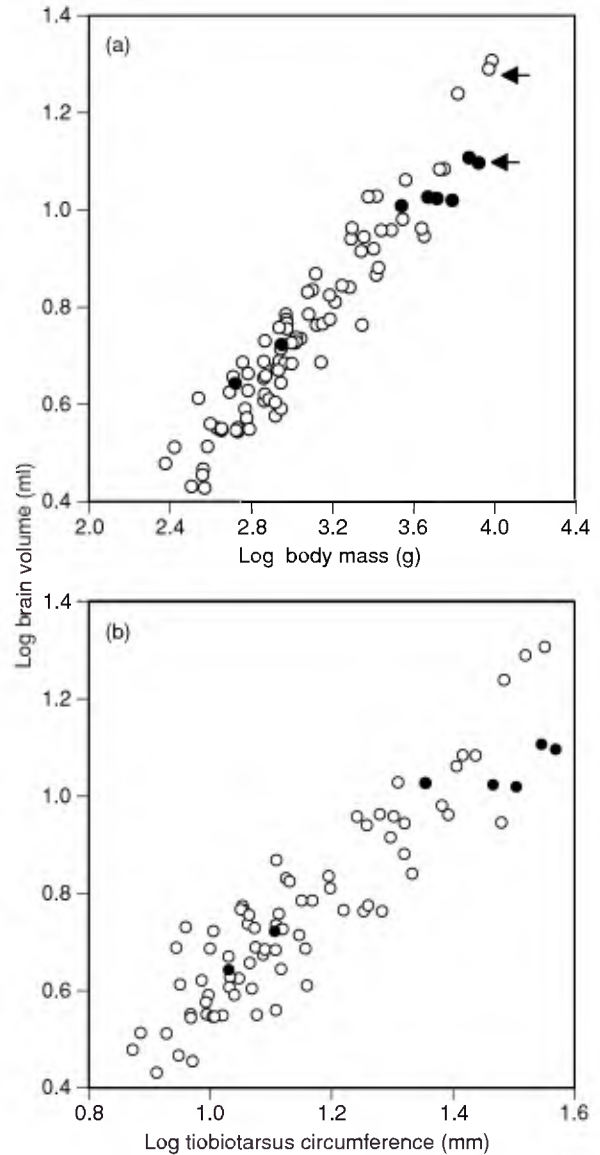
the emperor penguin (*Aptenodytes forsteri*) in the Procellariiformes/Sphenisciformes (Fig. 3); and the lanai *Thambetochen* (Fig. 4). The first two of these were supported by both independent contrasts analyses (Figs 1b & 2b), but the latter two were not. Thus, the flightless ratites, flightless cormorant *Phalacrocorax harrisi*, flightless ibises *Apteribis*, Attitlan grebe, flightless rallids and the other penguins were not significant outliers in any of the analyses. Similar results were also found with the use of tibiotarsus circumference as an estimate of body size in the Anseriformes, Rallidae and Threskiornithidae. The only flightless species identified as a significant outlier in these three groups was the lanai *Thambetochen*, but

Fig. 3. Log-transformed brain volumes against log-transformed body masses for the Procellariiformes (37 species) and Sphenisciformes (6 species) examined using species as independent data points (a) and independent contrasts (b). Open circles, all volant species (i.e. procellariiforms); closed circles, flightless species (i.e. penguins).

Fig. 4. Log-transformed brain volumes against log-transformed body masses (a) and log-transformed tibiotarsus circumference (b) for the Anseriformes (92 species). Open circles, all volant species; closed circles, flightless species and contrast between flightless and volant species; arrows, significant outliers.

again, this was not corroborated by independent contrasts analyses.

Comparisons of predicted vs observed brain volumes yielded similar results (Table 4). Paired comparisons performed across all taxonomic groups revealed no significant differences between the observed and predicted brain volumes of flightless species ($t = -0.32$, d.f. $= 29$, $P = 0.75$). Although not shown, this was also true for those taxa for which tibiotarsus circumference was used as an estimate of body mass ($t = 1.05$, d.f. $= 12$, $P = 0.31$). Paired tests within the Anseriformes ($Z = -1.54$, $n = 8$, $P = 0.12$), Sphenisciformes ($Z = -0.24$, $n = 6$, $P = 0.81$), Paleognatha ($Z = -1.83$, $n = 4$, $P = 0.07$) also

yielded no significant differences between the predicted and observed brain volumes for the flightless species. A comparison within the Rallidae did yield a significant difference ($Z = -2.03$, $n = 7$, $P = 0.04$), but contrary to the predictions, the observed values were higher than the predicted values. Thus, flightless rallids possess larger brains than predicted by body mass.

ANCOVAs were performed within the anseriforms, procellariiforms/spheniscifoms and rallids (Table 5). The anseriformes analysis yielded a significant difference in both slopes and intercepts when body mass was used as an estimate of body size. An examination of the scatterplot suggests that contrary to the predictions, the flightless species tend to have relatively larger brains than

Table 4. The predicted and observed brain volumes (ml) for each of the flightless species examined in this study. The predicted brain volumes were derived from least-squares linear regressions of brain volume against body mass for volant species of each taxonomic group

Taxon	Species	Observed brain volume (ml)	Predicted brain volume (ml)
Alcinae	*Pinguinus impennis*	10.63	16.00
Anseriformes	*Anas aucklandica*	4.40	3.88
	Branta sp.	12.81	13.97
	Ptaiochen pao	10.56	11.72
	Tachyeres leucocephalus	10.20	9.65
	Tachyeres pteneres	10.63	11.15
	Thambetochen chauliodons	10.47	12.74
	Thambetochen sp.	12.50	14.73
Paleognatha	*Apteryx owenii*	7.55	3.75
	Casuarius casuarius	36.35	23.71
	Dromaius novaehollandiae	28.88	19.84
	Rhea americana	18.88	16.95
Phalacrocoracidae	*Phalacrocorax harrisii*	13.58	12.81
Podicepediformes	*Podilymbus gigas*	3.73	3.12
Psittaciformes	*Strigops habroptilus*	16.50	22.05
Rallidae	*Habroptila wallacii*	5.19	4.51
	Gallirallus australis	5.11	4.14
	Gallirallus dieffenbachii	3.50	2.77
	Porphyrio mantelli	8.40	7.03
	Porzana atra	1.27	1.32
	Porzana palmeri	0.93	0.90
	Rallus wakensis	1.45	1.26
Sphenisciformes	*Aptenodytes forsteri*	46.19	74.99
	Aptenodytes patagonicus	27.90	40.37
	Eudyptes chrysocome	12.42	13.55
	Eudyptula minor	7.36	5.96
	Pygoscelis adeliae	19.66	19.55
	Spheniscus humboldti	15.98	21.34
Threskiornithidae	*Apteribis brevis*	7.87	7.74
	Apteribis sp.	8.27	9.89

Table 5. The F values for the ANCOVAs of body size (body mass (g) or tibiotarsus circumference (mm)) and flight ability (volant/flightless) as covariates of brain volume (ml) are presented. Calculated, calculated F value for each comparison; critical, critical F value derived from tables in Rohlf & Sokal (1990); G, F value calculated from simulations assuming a gradual model of evolutionary change; S, F value calculated from simulations assuming a speciational model of evolutionary change

Taxon	Body size	d.f.	Factor	F-values			
				Calculated	Critical	G	S
Anseriformes	Body mass	1, 89	Slope	5.79	3.96	4.33	4.07
			Intercept	4.90	3.96	5.63	5.85
	Tibiotarsus	1, 80	Slope	2.28	3.97	3.91	4.09
			Intercept	1.66	3.97	4.91	4.85
Procellariiformes/ Sphenisciformes	Body mass	1, 40	Slope	13.55	4.08	5.17	4.90
			Intercept	10.09	4.08	35.12	19.24
Rallidae	Body mass	1, 31	Slope	0.58	4.17	4.05	4.70
			Intercept	2.76	4.17	4.21	4.69
	Tibiotarsus	1, 31	Slope	0.90	4.17	4.66	4.44
			Intercept	0.46	4.17	4.84	4.63

the volant species (Fig. 4). This appeared to be due to presence of three significant outliers (*Cygnus buccinator*, *C. columbianus* and *C. cygnus*) (Fig. 4). The exclusion of these three species yielded no significant difference in either slopes ($F = 3.62$, d.f. = 1, 86, $P = 0.06$) or intercepts ($F = 0.37$, d.f. = 1, 87, $P = 0.55$) and this was also upheld with reference to phylogeny-corrected critical F values. The ANCOVAs using tibiotarsus circumference

as an estimate of body size did not, however, yield any significant results, regardless of whether conventional or phylogeny-corrected critical *F*s were used (Table 5).

A similar scenario was discovered in the comparison of procellariiforms and sphenisciforms. A significant difference in slopes was detected using both conventional and phylogeny-corrected critical *F* values (Table 5). A difference in intercepts was also detected using a conventional critical *F*, but not in comparison to the two phylogeny-corrected *F* values. An inspection of the scatterplot (Fig. 3) revealed that owing to the difference in slopes, smaller penguins (e.g. *Eudyptula minor*) have relatively large brains whereas larger penguins (e.g. both species of *Aptenodytes*) have relatively small brains compared with procellariiforms (see also Table 4). Thus, only some penguin species possess significantly smaller brains than similarly sized procellariiforms.

ANCOVAs performed within the Rallidae did not yield a significant difference between slopes or intercepts, regardless of whether conventional or phylogeny-corrected critical *F*s were used or whether body mass or tibiotarsus circumference were used as an estimate of body mass (Table 5). This was corroborated by an inspection of the scatterplots (Fig. 5). Therefore, flightless rallids do not have significantly smaller relative brain volumes than volant rallids.

DISCUSSION

In general, flightless species do not have significantly different brain volumes, relative to their body mass, than species capable of flight. The exceptions to this pattern seem to be the great auk and the kakapo, both of which have much smaller relative brain volumes. The differences in results between the present study and Bennett & Harvey (1985) probably reflect the levels of comparison and the species sampled. In their study, Bennett & Harvey (1985) compared the ratites and penguins with a limited dataset of other species whereas we examined instances of flightlessness within the order or family in which it evolved. Class-wide comparisons of relative brain size variation in birds are inherently problematic because of the strong association between developmental differences and relative brain size (see reviews in Nealen & Ricklefs, 2001; Iwaniuk & Nelson, 2004) and because the statistical power of such a test would be compromised by a difference in sample size of several orders of magnitude between volant and flightless species.

The significantly smaller relative brain volumes of both the kakapo and the great auk suggest that a compromise between neural and somatic growth has occurred in both species. In the case of the kakapo, energy seems to have been re-allocated to the development of a large gastrointestinal (GI) tract (Livezey, 1992; Kirk, Powlesland & Cork, 1993). This has been accompanied by an increase in the amount of fat deposition in the kakapo as well, resulting in a heavier bird without a proportional increase in brain size. A similar compromise
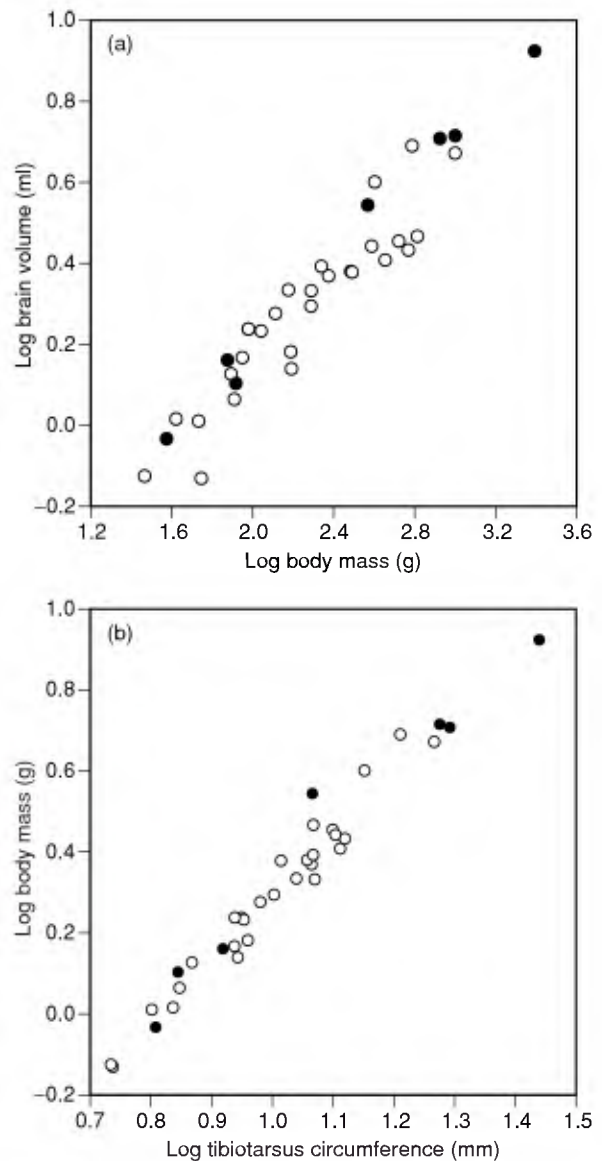
Fig. 5. Log-transformed brain volumes against log-transformed body masses (a) and log-transformed tibiotarsus circumference (b) for the Rallidae (35 species). Open circles, all volant species; closed circles, flightless species and contrast between flightless and volant species.

between the GI tract and the brain is also found in other folivorous species, such as the koala *Phascolarctos cinereus* (Haight & Nelson, 1987), folivorous primates (Clutton-Brock & Harvey, 1980), sloths (Eisenberg, 1981) and the hoatzin *Opisthocomus hoazin* (Iwaniuk, 2003). This enlargement of the GI tract is associated with the microbial fermentation of plant matter. Folivory is not, however, always associated with relatively small brains. For example, the folivorous and flightless takahe does not possess a relatively small brain or an enlarged GI tract (Suttie & Fennessy, 1992), despite its greater reliance on plant matter than the closely related pukeko *Porphyrio porphyrio melanotus* (Trewick, 1996). Similarly, there is no difference in relative brain size between folivorous and

other dietary types of anseriforms (Iwaniuk & Nelson, 2001) or between the folivorous white-tipped plantcutter *Phytotoma rutila* and the related cotingas and manakins (A. N. Iwaniuk, pers. obs.). The adoption of folivory by anseriforms, plantcutters and the takahe may not represent a marked change in diet compared to their relatives or perhaps they are less extremely specialized for feeding on fibrous plant material than the kakapo.

The great auk, on the other hand, was piscivorous (Olson *et al*., 1979; Hobson & Montevecchi, 1991) and since predatory species typically possess short GI tracts (Ziswiler & Farner, 1972), it is unlikely that energy was re-allocated to the digestive system. Instead, the great auk increased its overall body size, possibly to aid in thermoregulation (Livezey & Humphrey, 1986; Livezey, 1988; Montevecchi & Kirk, 1997) or to enable deeper dives during foraging bouts (Livezey, 1988). The latter could have also been achieved by increasing bone density such that the body became denser and heavier overall. This could also explain why the larger and deeper diving *Aptenodytes* penguins have relatively small brains compared with procellariiforms and smaller, shallower diving penguins, such as the fairy penguin *Eudyptula minor*. Regardless of the reason, it would seem that a larger body size was selected for in the great auk, without a proportional increase in brain volume.

While the kakapo and great auk provide some evidence of a compromise between neural and somatic resource allocation, the lack of a significant difference in relative brain size between flightless and volant species of other taxa indicates that this is not systematic. One possible explanation is that although flightlessness can evolve quickly, it is not so fast that increases in brain volume lag behind that of increases in body mass. This may also explain the lack of evidence for an evolutionary time lag in primates (Deaner & Nunn, 1999), but its presence in several captive-bred and/or domesticated strains of mammals (Kruska, 1987). In addition, there are several species that have changed little in body mass or have decreased in mass with the evolution of flightlessness. For example, flightless rails exhibit a range of body sizes on islands where they coexist (Olson, 1973; Trewick, 1997) and the Auckland Islands teal is smaller than its closest relative the chestnut teal *Anas castanea* (Marchant & Higgins, 1990). Thus, body mass does not necessarily increase in flightless species and, as a result, relative brain size remains similar to that of volant species. There are several other reasons that there is no association between flightlessness and relative brain size, such as the diversity of evolutionary scenarios that lead to a flightless condition (Feduccia, 1999) and competing neural demands of behaviours that are not directly related to flight (e.g. foraging, terrestrial locomotion). Assessing the relative effects of these myriad factors and flightlessness and relative brain volume is, however, a significant task that is unlikely to be resolved with currently available data.

Our finding that relative brain volume does not systematically decrease with the loss of flight in birds also goes counter to Jerison's (1973) hypothesis that the evolution of flight was correlated with an increase in relative brain size. He based this hypothesis on his observation that the relative brain size of *Archaeopteryx* is intermediate between that of birds and non-avian 'reptiles'. The relatively large brains of birds were attributed to the invasion of the 'aerial niche' (Jerison, 1973). Bats, however, do not have relatively larger brains than other mammals (Jerison, 1973; Eisenberg, 1981; Baron, Stephan & Frahm, 1996), nor do the gliding forms of other mammalian taxa compared with their non-gliding relatives (Mace, Harvey & Clutton-Brock, 1981; Haight & Nelson, 1987). Thus, neither the gain of flight/gliding ability (mammals) nor the secondary loss of this ability (birds) is correlated with a change in relative brain volume. It seems more likely that endothermy and social learning, as well as the occupation of a diverse array of dietary niches (Wyles, Kunkel & Wilson, 1983), are responsible for the evolution of relatively large brains in birds and not simply the ability to fly.

It is important to acknowledge, however, that our results do not negate the possibility that changes in brain regions may be occurring with the evolution of flightlessness that do not affect overall brain size. The brain is a heterogeneous organ and individual regions can and do change in size independently of one another (Barton & Harvey, 2000; Iwaniuk, Dean & Nelson, 2004) without affecting overall brain size. In an analogous situation to flightlessness in birds, changes in the sizes of three brain regions were correlated with the degree of limb reduction in lizards without a demonstrable correlation with overall brain size (Black, 1983). It is therefore possible that similar changes in brain structure occur with the evolution of flightlessness. Most flightless birds are either extinct or endangered, so the availability of specimens for comparisons of internal structures is limited. There are, however, several predictions that can be made. For example, because the cerebellum is a major area of motor coordination in the central nervous system, cerebellar volume may be relatively smaller in flightless species. Similarly, there may also be changes in aspects of the visual system that are important in movement, such as the accessory optic nuclei (Wylie, Bischof & Frost, 1998) and the nucleus rotundus (Rt) (Wang & Frost, 1992; Wang, Jiang & Frost, 1993). There is the possibility that peripheral nervous system changes are coincident with the evolution of flightlessness as well. One expectation is that the size of the spinal ganglia innervating the forelimbs will be reduced in flightless species that do not use their forelimbs for other forms of locomotion (Giffin, 1995). These would include the flightless rails, kakapo and ratites. Any neuroanatomical differences between volant and flightless species could, however, also be related to other evolutionary changes in ecology and behaviour such as folivory (see above), nocturnal foraging (kakapo, kiwis) and shifts in morphological and behavioural development. Therefore, it will be necessary for future investigations into the neural ramifications of a flightless existence to adopt a holistic approach to tease apart these multiple selection pressures on the central and peripheral nervous systems.

Acknowledgements

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