Morphometrics of the Avian Small Intestine Compared with That of Nonflying Mammals: A Phylogenetic Approach

Shana R. Lavin[1]
William H. Karasov[1,*]
Anthony R. Ives[2]
Kevin M. Middleton[3,†]
Theodore Garland Jr.[3]
[1]Department of Wildlife Ecology, University of Wisconsin, Madison, Wisconsin 53706; [2]Department of Zoology, University of Wisconsin, Madison, Wisconsin 53706; [3]Department of Biology, University of California, Riverside, California 92521

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ABSTRACT

Flying animals may experience a selective constraint on gut volume because the energetic cost of flight increases and maneuverability decreases with greater digesta load. The small intestine is the primary site of absorption of most nutrients (e.g., carbohydrates, proteins, fat) in both birds and mammals. Therefore, we used a phylogenetically informed approach to compare small intestine morphometric measurements of birds with those of nonflying mammals and to test for effects of diet within each clade. We also compared the fit of nonphylogenetic and phylogenetic models to test for phylogenetic signal after accounting for effects of body mass, clade, and/or diet. We provide a new MATLAB program (Regressionv2.m) that facilitates a flexible model-fitting approach in comparative studies. As compared with nonflying mammals, birds had 51% less nominal small intestine surface area (area of a smooth bore tube) and 32% less volume. For animals <365 g in body mass, birds also had significantly shorter small intestines (20%–33% shorter, depending on body mass). Diet was also a significant factor explaining variation in small intestine nominal surface area of both birds and nonflying mammals, small intestine mass of mammals, and small intestine volume of both birds and nonflying mammals. On the basis of the phylogenetic trees used in our analyses, small intestine length and nominal surface area exhibited statistically significant phylogenetic signal in birds but not in mammals. Thus, for birds, related species tended to be similar in small intestine length and nominal surface area, even after accounting for relations with body mass and diet. A reduced small intestine in birds may decrease the capacity for breakdown and active absorption of nutrients. Birds do not seem to compensate for reduced digestive and absorptive capacity via a longer gut retention time of food, but we found some evidence that birds have an increased mucosal surface area via a greater villus area, although not enough to compensate for reduced nominal surface area. We predict that without increased rate of enzyme hydrolysis and/or mediated transport and without increased passive absorption of water-soluble nutrients, birds may operate with a reduced digestive capacity, compared with that of nonflying mammals, to meet an increase in metabolic needs (i.e., a reduced spare capacity).

Introduction

In birds and mammals, the small intestine is the primary site of enzymatic breakdown and absorption of carbohydrates, amino acids, and fatty acids (excluding short-chain fatty acids). Small intestine brush border enzymes (e.g., disaccharidases, peptidases) that break down nutrient polymers and nutrient transporters (e.g., the Na^+-D-glucose transporter) that absorb subsequent monomers are in the greatest quantity in the small intestine (Southgate 1995). Most of the free mono- and disaccharides and amino acids are completely absorbed in the small intestine (Riesenfeld et al. 1980; Southgate 1995; Klasing 1998), with the exception of those species that lack specific enzymes (e.g., some passerine species lack sucrase [Martinez del Rio 1990], and pinnipeds lack lactase [Klurfeld 1999]). Furthermore, the small intestine is the major site of absorption of such minerals and electrolytes as calcium, phosphate, and potassium, as well as such vitamins as B_6 (Levin 1984; Heard and Annison 1986; Van Der Klis et al. 1990).

A larger small intestine volume or nominal surface area (the area of a smooth bore tube) presumably allows more area over which these nutrients can be digested and absorbed. In birds and mammals, digestive adjustments to higher feeding rates almost always include an increase in gut size and, thus, an increase in digestive enzymes and nutrient transporters and associated breakdown and absorption of nutrients (Karasov and McWilliams 2005). For birds that fly, however, the size of the digestive tract and, consequently, the digesta it carries may be minimized because the cost of flight increases with load carried

* Corresponding author; e-mail: wkarasov@wisc.edu.
† Present address: Department of Biology, California State University, San Bernardino, California 92407.

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and takeoff and maneuverability can be impaired at heavier masses (Guillemette 1994; Norberg 1995; Nudds and Bryant 2002). Thus, nominal surface area, a function of circumference that also corresponds to gut volume, may be reduced in volant species because of selection pressure. Ideally, this hypothesis would be tested by comparing flying birds with flightless birds. However, there are relatively few extant flightless birds, those that do exist are mainly large and/or endangered, and very little information detailing their gastrointestinal tracts is available. Therefore, we ask whether flying birds have reduced small intestines compared with those of comparably sized nonflying mammals. A complementary comparison of flying and nonflying mammals is considered elsewhere (Caviedes-Vidal et al. 2007), and we address emerging patterns in volant mammals in relation to our study in "Discussion."

Anecdotal evidence suggests that birds tend to have intestines relatively shorter than those of mammals (McClelland 1979), and shorter intestines would be associated with relatively less nominal surface area and volume. Studies have compared gastrointestinal morphology within primates; domesticated and small mammals; and gallinaceous, passerine, North American, and Mediterranean birds (Leopold 1953; Chivers and Hladik 1980; Herrera 1984; Ricklefs 1996; Snipes 1997; DeGolier et al. 1999). A broad comparison of small intestine length, volume, and area between birds and nonflying mammals has never been made.

We surveyed the literature for gastrointestinal morphometric measurements (e.g., small intestine length, nominal surface area, and volume; supplemental data are available as an Excel file or a tab-delimited ASCII file; sources for the supplemental data are listed in App. B). We used body mass as a covariate in statistical analyses because such morphometric traits as surface area are strongly positively correlated with body mass when a broad range of body sizes are considered (McMahon and Bonner 1983). We also tested for effects of diet because previous studies have shown that the overall size of the gut varies in relation to diet (Leopold 1953; Ziswiler and Farner 1972; Walsberg 1975; Ankney 1977; Pulliainen et al. 1981; Herrera 1984; Barnes and Thomas 1987; Moss 1989; Karasov 1990; Richardson and Wooller 1990; Ricklefs 1996). We applied both conventional and phylogenetically based models because not accounting for hierarchical evolutionary relationships among species potentially violates statistical assumptions of conventional statistical methods, such as residuals from a regression model being independent and identically distributed (Felsenstein 1985; Garland and Adolph 1994; Garland et al. 2005). Violation of assumptions can lead to inflated Type I error rates (claiming statistical significance too often) and estimates of parameters (e.g., allometric slopes) that are not minimum variance. In addition to testing for differences between the bird lineages and the mammal lineages, we tested for generalized phylogenetic signal (Blomberg et al. 2003) by the use of maximum likelihood techniques that compare the fit of a continuum of models ranging between one that assumes a star phylogeny (i.e., a conventional, nonphylogenetic analysis) and one that assumes a specified hierarchical phylogenetic tree with a particular set of branch lengths (Grafen 1989; Freckleton et al. 2002; Chown et al. 2007; Duncan et al. 2007). This approach allows for the possibility that for a given analysis, a star phylogeny may provide a better fit to the data. To accomplish these analyses, we developed a new MATLAB program (Regressionv2.m) that is available on request.

Material and Methods

We searched the literature for gastrointestinal morphometric data on birds and nonflying mammals, including searches within Biological Abstracts and Web of Knowledge databases (all years) for keywords such as "intestine," "morphology," "gastrointestinal," and "surface area." We incorporated only measurements in our database for which there was a corresponding body mass included in the publication (Excel file; ASCII file; App. B; $n = 493$ species). Species were classified by diet (carnivore [1], omnivore [2], herbivore [3], nectarivore [4], or frugivore [5]). Whenever possible, species were classified into diet categories based on the publication from which intestinal measurements were acquired. A species was considered a carnivore if it was noted to consume arthropods and was considered an omnivore if it was noted to consume both arthropods and plant matter. Categorizing species into distinct diet guilds is difficult; while our method allowed the most consistent and unbiased means of diet categorization, it did not include anecdotal records of occasional foods that may have been consumed by particular individuals within species. For example, the waxwing *Bombycilla cedrorum* is classified as a frugivore because it consumed mixed fruits according to the source from which most of the morphometric measurements were taken. *Bombycilla garrulous*, on the other hand, is categorized as an omnivore because the source of morphometric measurements notes that this species feeds on berries, insects, and seeds.

Our dependent variables included small intestine length (from stomach to cecum; cm), nominal surface area (the area of an equivalent smooth bore tube; cm^2), small intestine volume (cm^3), wet mass (g), and villus amplification ratio (ratio of villus area to nominal area). We also recorded measurements of hindgut dimensions when available. Nominal surface area, along with length, defines the intestine volume and partly defines the intestinal surface area for breakdown and absorption. The surface area is further increased by villi and microvilli (the latter not accounted for in this study because of lack of data in the literature), but most measures of intestinal breakdown and absorption in the literature are expressed per unit nominal area and reflect increases in activity imparted by surface magnification from villi and microvilli.

Studies have used various techniques to quantify the villus amplification ratio (the ratio of villus area to nominal area, also known as the mucosal-to-serosal amplification ratio; Harris et al. 1988), the histological surface magnification ratio (Kisielinski et al. 2002), or the surface enlargement factor (Snipes

Table 1: Allometric equations for ln small intestine morphometric traits in relation to ln body mass

		OLS					RegOU					
Trait	N	Y-Intercept (SE)	Slope (SE)	r^2	ln Likelihood	AIC	Y-Intercept (SE)	Slope (SE)	r^2	ln Likelihood	AIC	d
Length (cm):												
Bird	220	1.117 (.0654)	.520 (.0126)	.886	−67.8	141.7	1.397 (.1018)	.468 (.0169)	.779	−25.2	58.4	.621
Mammal	64	2.047 (.0924)	.390 (.0144)	.922	−11.3	28.6	2.010 (.1085)	.395 (.0160)	.908	−9.9	27.9	.191
Nominal surface area (cm^2):												
Bird	86	−.029 (.0833)	.757 (.0225)	.931	−20.0	46.0	.070 (.1042)	.733 (.0255)	.908	−17.0	42.1	.243
Mammal	114	.773 (.1173)	.704 (.0155)	.948	−68.3	142.7	.785 (.1305)	.703 (.0172)	.937	−66.9	141.8	.125
Wet mass (g):												
Bird	34	−2.705 (.2816)	.838 (.0451)	.915	−20.9	47.7	−2.928 (.3690)	.844 (.0608)	.858	−18.3	44.7	.747
Mammal	60	−.345 (.270)	.926 (.0319)	.935	−41.0	88.1	−3.452 (.2983)	.926 (.0351)	.923	−37.2	82.3	.386
Volume (cm^3):												
Bird	65	−3.062 (.1795)	.887 (.0416)	.878	−52.8	111.6	−3.100 (.2042)	.891 (.0455)	.859	−51.3	110.6	.212
Mammal	91	−2.744 (.2011)	.968 (.0265)	.938	−82.9	171.9	−2.714 (.2102)	.964 (.0276)	.932	−82.8	173.6	.375

Note. OLS = ordinary least squares; RegOU = phylogenetic regression with Ornstein-Uhlenbeck process; AIC = Akaike Information Criterion. Values for r^2 from OLS and RegOU models are not comparable; d is the restricted maximum likelihood estimate of the Ornstein-Uhlenbeck transformation parameter.

1997). Results have yielded different estimates for the same species, depending on the method used. For example, there were significantly different estimates for villus magnification in laboratory rats, depending on whether the Fisher-Parsons (1950) method or the Harris method (Harris et al. 1988) was used (Kisielinski et al. 2002). Consequently, in order to determine whether the allometric slopes for the villus area and the nominal surface area versus body mass were parallel and whether the villus amplification scaled with body mass, we used data from Ricklefs (1996; for birds) and Snipes (1997; for mammals) so that consistent methodology was used within taxa. Despite differences in methodology, we used the villus amplification from these two studies as well as from the whole data set to test whether there was a significant difference in villus amplification between birds and mammals.

We constructed a composite phylogeny including all 493 species of birds and nonflying mammals using Mesquite (ver. 1.12; http://mesquiteproject.org). Tree topology was based on existing published phylogenetic hypotheses (for complete list of sources, see App. C). Although we strove for a fully bifurcating arrangement, 68 polytomies remained in the final tree because of either incomplete phylogenetic information or unresolved polytomies in published trees. Branch lengths were specified by Pagel's (1992) arbitrary method.

The Mesquite tree was then exported to a PDI file. This file was read into the DOS PDDIST program (Garland and Ives 2000; http://biology.ucr.edu/people/faculty/Garland/PDAP.html), and a phylogenetic variance-covariance matrix was output.

For all statistical analyses, both the response variable and body mass were natural-log transformed, and the raw data were graphically represented with $\log_{10}$ axes to portray the data clearly. The new MATLAB program Regressionv2.m (see App. A) was then used to implement linear statistical models via both ordinary (i.e., nonphylogenetic) least squares (OLS) and phylogenetic generalized least squares (PGLS) regressions (Garland and Ives 2000; Garland et al. 2005). For the OLS analyses, results were confirmed with SPSS, version 11.5. OLS regression assumes that the unexplained residual variation is independent among species, whereas PGLS assumes that residual variation among species is correlated, with the correlation given by a

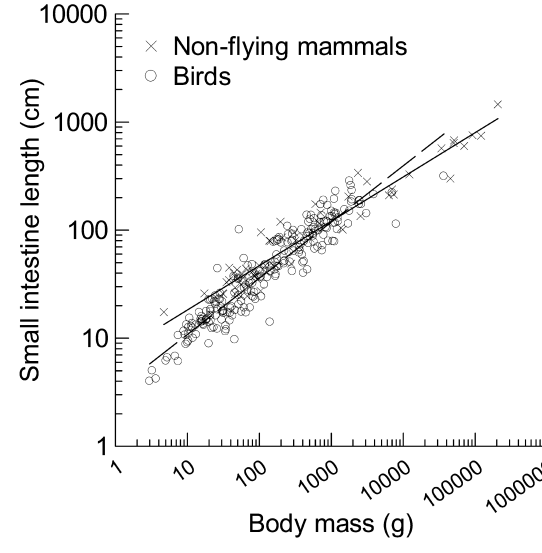

Figure 1. Small intestine length (cm) versus body mass (g) in birds and nonflying mammals on a double-logarithmic scale. Nonflying mammal species are depicted by crosses and solid line (regression statistics in Table 1), and birds are depicted by circles and dashed line. Birds <365 g had small intestines significantly shorter than those of mammals (bird slope, 0.468 ± 0.0169; mammal slope, 0.395 ± 0.0160). See text for statistical comparisons.

Table 2: Statistical tests of the effect of clade (bird vs. mammal) on the allometric relation between small intestine length and body mass ($N = 284$)

Simple Allometry			
Model	d	ln Maximum Likelihood	AIC
OLS		−104.8	215.7
PGLS		−67.9[a]	141.8
RegOU	.613	−44.9[a]	97.8

ANCOVA with Same Slope but Different Intercepts (Clade)							
Model	d	ln Maximum Likelihood	AIC	χ^2 for LRT vs. Simple Allometry	P for LRT (df = 1)	Partial F for Clade	P for Partial F (df = 1, 281)
OLS		−100.0	207.9	9.75	.0018	9.81	.0019
PGLS		−67.8[a]	143.6	.25	.6179	.25	.6201
RegOU	.599	−42.9[a]	95.8	4.06	.0439	3.91	.0488

ANCOVA with Different Slopes (Clade × Mass) and Intercepts (Clade)							
Model	d	ln Maximum Likelihood	AIC	χ^2 for LRT vs. Different Intercepts	P for LRT (df = 1)	Partial F for Clade and Clade × Mass Interaction	P for Partial F (df = 2, 280)
OLS		−79.9	169.9	40.02	.0001[b]	26.81	2^{-11}[c]
PGLS		−66.3[a]	142.6	2.98	.0843[b]	1.60	.2068[c]
RegOU	.551	−38.5[a]	89.1[d]	8.68	.0032[b]	6.78	.0013[c]

Note. Akaike Information Criterion (AIC; smaller is better) is computed as (−2 × ln maximum likelihood) + (2 × no. parameters). d is the restricted maximum likelihood estimate of the Ornstein-Uhlenbeck (OU) transformation parameter. For maximum likelihood and AIC, maximum likelihood estimates of d were used but are not reported. Within each subtable, the following three linear regression models are compared: ordinary (nonphylogenetic) least squares (OLS), phylogenetic generalized least squares (PGLS), and regression in which the residual variation is modeled as an OU process (RegOU) along the specified phylogenetic tree.

[a] On the basis of likelihood ratio tests (LRTs), the PGLS model is statistically significantly better than the OLS model, and the RegOU model is statistically significantly better than the PGLS model (and the OLS model).

[b] LRT comparing model with different slopes and intercepts with model that has parallel slopes.

[c] Partial F-test comparing model with different slopes and intercepts with simple allometry model.

[d] Best model by criterion of lowest AIC. See Table 1 for separate allometric equations for birds and mammals.

process that acts like Brownian motion evolution along the nominal, or starter, phylogenetic tree. When the PGLS model fits the data better than the OLS model (as judged by the Akaike Information Criterion [AIC]), then the residual variation in the dependent variable is said to exhibit phylogenetic signal (Freckleton et al. 2002; Blomberg et al. 2003), after accounting for effects of body mass and any other factors in the model (e.g., clade, diet).

Because the foregoing analyses assume either no (OLS) or relatively strong (PGLS) phylogenetic signal, we also performed an analysis in which the strength of phylogenetic signal in the residual variation was estimated simultaneously with the regression coefficients (e.g., see Grafen 1989; Freckleton et al. 2002; Chown et al. 2007; Duncan et al. 2007). For this, we assumed that the correlation in residual variation was given by an Ornstein-Uhlenbeck (OU) evolutionary process along the phylogenetic tree; this is often used to model the effects of stabilizing selection around an optimum (see App. A). We refer to this model as regression under an OU process (RegOU). The program Regressionv2.m estimates the optimal OU transformation parameter, d, using restricted maximum likelihood (REML). A d value of 1 indicates that the statistical model with the original starter branch lengths (i.e., the PGLS model) best fits the data (residuals from the multiple regression equation), a d value of 0 indicates that a star phylogeny (i.e., the OLS model) best fits the data, and a d value between 0 and 1, which is most typically found, indicates that branch lengths that are intermediate between the starter and a star phylogeny provide the best fit. Compared with PGLS or OLS models, the RegOU model contains one more estimated parameter. When the RegOU model fits the data significantly better than the OLS model (as judged by a likelihood ratio test), in which case d is estimated to be significantly greater than 0, then the residual variation in the dependent variable exhibits statistically significant phylogenetic signal.

For each trait, we considered increasingly complex models, in the following order. For pooled analyses of birds and mammals, we compared models that specified simple allometry, ANCOVA with different intercepts but the same pooled slope, and ANCOVA with different intercepts and different slopes. For separate analyses of birds and mammals, we tested for diet effects by comparing models that specified simple allometry, ANCOVA with different intercepts but the same pooled slope, and ANCOVA with different intercepts and different slopes.

Table 3: Statistical tests of the effect of diet (carnivore, omnivore, herbivore, nectarivore, or frugivore) on the allometric relation between small intestine length and body mass in birds ($N = 220$)

Simple Allometry			
Model	d	ln Maximum Likelihood	AIC
OLS		−67.8	141.7
PGLS		−41.5[a]	88.9
RegOU	.621	−25.2[a]	58.4[b]

ANCOVA with Same Slope but Different Intercepts (Diet)							
Model	d	ln Maximum Likelihood	AIC	χ^2 for LRT vs. Simple Allometry	P for LRT (df = 4)	Partial F for Diet	P for Partial F (df = 4, 214)
OLS		−63.0	140.1	9.61	.0476	2.39	.0521
PGLS		−38.4[a]	90.9	6.04	.1965	1.49	.2069
RegOU	.627	−22.8[a]	61.7	4.76	.3133	1.18	.3198

ANCOVA with Different Slopes (Diet × Mass) and Intercepts (Diet)							
Model	d	ln Maximum Likelihood	AIC	χ^2 for LRT vs. Different Intercepts	P for LRT (df = 4)	Partial F for Diet and Diet × Mass Interaction	P for Partial F (df = 8, 210)
OLS		−56.8	135.7	12.40	.0146[c]	2.76	.0064[d]
PGLS		−37.7[a]	97.3	1.55	.8177[c]	.92	.5000[d]
RegOU	.619	−20.8[a]	65.5	4.13	.3893[c]	1.08	.3808[d]

Note. See Table 2 note for additional information and definitions of variables and abbreviations.

[a] On the basis of LRTs, the PGLS model is statistically significantly better than the OLS model, and the RegOU model is statistically significantly better than the PGLS model (and the OLS model).

[b] Best model by criterion of lowest AIC.

[c] LRT comparing model with different slopes and intercepts with model that has parallel slopes.

[d] Partial F-test comparing model with different slopes and intercepts with simple allometry model.

Models were compared in several ways in an attempt both to determine what model best fit the data and to test particular hypotheses (bird vs. mammal, effect of diet; Ludden et al. 1994; Johnson and Omland 2004). For all four primary traits analyzed (small intestine length, nominal surface area, mass, and volume), body mass explained a large proportion of the total variance. Therefore, the simple-allometry model can be considered "good" in a general sense. Thus, the question becomes whether more complex models are warranted. We used maximum likelihood ratio tests (LRTs) when one was a nested subset of the other (i.e., RegOU vs. either PGLS or OLS). Twice the difference in the ln maximum likelihoods of two models will be distributed approximately as a χ^2 with degrees of freedom equal to the difference in the number of parameters estimated in the two models, with this approximation improving as sample sizes increase. We also used LRTs to compare PGLS with OLS models, which have the same number of parameters. In such comparisons with 0 df, a difference in ln likelihoods >3.8414 (which is the ninety-fifth percentile of the distribution of χ^2 with 1 df) is often taken to indicate a significant difference in the fit of two models (e.g., see Felsenstein 2004, p. 309). As an alternative to LRTs for the nested models that should generally yield similar results, we also report partial F-tests to gauge the significance of clade or diet effects. Although the phylogenetic tree we used contained a number of soft polytomies, indicating uncertainty about the true branching relationships, for simplicity we did not subtract any degrees of freedom for these hypothesis tests (Purvis and Garland 1993; Garland and Díaz-Uriarte 1999); thus, because these tests do not explicitly include the uncertainty in the phylogenetic tree, they might tend to be liberal (Rohlf 2006).

As a heuristic indicator of the support of models, we report the AIC using the smaller-is-better formulation (AIC = (−2 × ln maximum likelihood) + (2 × no. parameters)). When comparing a series of models, nested or not, the one with the lowest AIC is considered to be the best. As a rule of thumb, models whose AIC is ≤2 units larger can also be said to have substantial support (Burnham and Anderson 2002; Duncan et al. 2007). Note that maximum likelihoods are used for computing AIC and LRTs, whereas REML is used for estimating coefficients in the model, such as the allometric scaling exponent. REML estimates of d are also reported.

For comparability with previous studies, we present allometric equations from both OLS and RegOU models, separately for birds and for mammals. In most cases, the RegOU models were preferred on the basis of the AIC values. The equations presented can be used for general predictions, but those predictions could be improved by use of the methods of Garland and Ives (2000) and could be implemented in the DOS PDTEE program (http://biology.ucr.edu/people/

Table 4: Statistical tests of the effect of diet (carnivore, omnivore, herbivore, or frugivore) on the allometric relation between small intestine length and body mass in mammals ($N = 64$)

Simple Allometry			
Model	d	ln Maximum Likelihood	AIC
OLS		−11.3	28.6
PGLS		−23.8	53.6
RegOU	.191	−9.9	27.9[a]

ANCOVA with Same Slope but Different Intercepts (Diet)							
Model	d	ln Maximum Likelihood	AIC	χ^2 for LRT vs. Simple Allometry	P for LRT (df = 3)	Partial F for Diet	P for Partial F (df = 3, 59)
OLS		−9.4	30.8	3.78	.2863	1.20	.3190
PGLS		−22.2	56.4	3.16	.3680	.99	.4017
RegOU	.213	−8.6	31.2	2.67	.4452	.79	.5058

ANCOVA with Different Slopes (Diet × Mass) and Intercepts (Diet)							
Model	d	ln Maximum Likelihood	AIC	χ^2 for LRT vs. Different Intercepts	P for LRT (df = 3)	Partial F for Diet and Diet × Mass Interaction	P for Partial F (df = 6, 56)
OLS		−5.3	28.5	8.28	.0405[b]	1.94	.0909[c]
PGLS		−17.3	52.7	9.74	.0209[b]	2.08	.0696[c]
RegOU	.140	−5.1	30.2	7.05	.0702[b]	1.43	.2210[c]

Note. See Table 2 note for additional information and definitions of variables and abbreviations.

[a] Best model by criterion of lowest AIC.

[b] LRT comparing model with different slopes and intercepts with model that has parallel slopes.

[c] Partial F-test comparing model with different slopes and intercepts with simple allometry model.

faculty/Garland/PDAP.html) as well as Mesquite PDAP:PDTree (http://mesquiteproject.org/pdap_mesquite/index.html). The Johnson-Neyman technique, applied nonphylogenetically, was used to determine regions over which the allometric relations did not significantly differ between birds and mammals. This technique has not yet been developed to include phylogeny; however, it is useful when regression lines are not parallel. Intersecting regression lines cannot be statistically compared using a simple ANCOVA because the data violate the assumption of homogeneity of regression slopes. The Johnson-Neyman technique allows one to determine the range of X values for which there is no significant difference between experimental groups (White 2003).

Results

Clade-specific allometric equations for each of the four primary traits are shown in Table 1. For birds, the phylogenetic RegOU models, which contain one additional parameter (d), always provided better fits to the data (had lower AIC values) as compared with the nonphylogenetic OLS models. LRTs indicated that, with the exception of small intestine volume, the RegOU models fit the data significantly ($P < 0.05$) better than the OLS models. Thus, for at least three of the four traits in birds, the residual values can be said to have statistically significant phylogenetic signal. For mammals, the RegOU model fit the data significantly better than the OLS model (LRTs) only for small intestine wet mass ($P = 0.0054$); thus, for the rest of the traits, phylogenetic signal was not apparent ($P > 0.05$).

Small Intestine Length

As suggested by Figure 1 and demonstrated statistically in Table 2, comparison of birds with mammals indicated that a model with different slopes and intercepts provided the best fit (i.e., it had the lowest AIC value). The separate allometric equations for birds and mammals are shown in Table 1. The Johnson-Neyman technique indicated that birds <365 g had small intestines significantly shorter than those of comparably sized mammals. For all models, LRTs indicated that the phylogenetic regression model with the OU transform provided a significantly better fit than the PGLS model, which in turn provided a better fit than the OLS model; thus, small intestine length had significant phylogenetic signal even after accounting for the strong correlation with body mass and the clade difference between birds and mammals.

With diet in the model (Table 3), significant phylogenetic signal in bird small intestine length was again indicated by LRTs that showed significantly better fits for RegOU versus PGLS and for PGLS versus OLS. For the RegOU model, both LRTs and partial F-tests indicated that diet did not significantly affect small intestine length in birds.

In mammals, the best-fitting model was again simple allom-

Table 5: Statistical tests of the effect of clade (bird vs. mammal) on the allometric relation between small intestine nominal surface area and body mass ($N = 200$)

Simple Allometry			
Model	*d*	ln Maximum Likelihood	AIC
OLS		−124.6	255.1
PGLS		−136.3	278.6
RegOU	.495	−110.7[a]	229.5

ANCOVA with Same Slope but Different Intercepts (Clade)							
Model	*d*	ln Maximum Likelihood	AIC	χ^2 for LRT vs. Simple Allometry	*P* for LRT (df = 1)	Partial *F* for Clade	*P* for Partial *F* (df = 1, 197)
OLS		−96.0	200.0	57.12	<.0001	65.13	<.0001
PGLS		−136.0	280.1	.51	.4751	.50	.4803
RegOU	.192	−91.4[a]	192.7[b]	38.74	<.0001	49.75	<.0001

ANCOVA with Different Slopes (Clade × Mass) and Intercepts (Clade)							
Model	*d*	ln Maximum Likelihood	AIC	χ^2 for LRT vs. Different Intercepts	*P* for LRT (df = 1)	Partial *F* for Clade and Clade × Mass Interaction	*P* for Partial *F* (df = 2, 196)
OLS		−94.6	199.2	2.83	.0925[c]	34.26	<.0001[d]
PGLS		−135.9	281.8	.24	.6242[c]	.37	.6912[d]
RegOU	.185	−90.9[a]	193.8	.94	.3323[c]	25.53	<.0001[d]

Note. See Table 2 note for additional information and definitions of variables and abbreviations.

[a] On the basis of LRTs, the RegOU model is statistically significantly better than the PGLS model and the OLS model.

[b] Best model by criterion of lowest AIC. See Table 1 for separate allometric equations for birds and mammals.

[c] LRT comparing model with different slopes and intercepts with model that has parallel slopes.

[d] Partial *F*-test comparing model with different slopes and intercepts with simple allometry model.

etry by RegOU (Table 4), and LRTs and partial *F*-tests indicated that diet did not significantly affect small intestine length. Unlike in birds, the RegOU model was not much better than OLS (ΔAIC = 0.7; LRT, $\chi^2 = 2.8$, df = 1, $0.05 < P < 0.10$), indicating relatively weak evidence for significant phylogenetic signal in body mass–adjusted small intestine length.

Small Intestine Nominal Surface Area

Comparison of birds with mammals indicated that models with the same slope but different intercepts provided the best fit (Table 5); birds had small intestine nominal surface area significantly less than that of mammals (Fig. 2). The RegOU models always provided the best fit (lowest AIC), and they were significantly better than OLS models by LRTs; thus, small intestine nominal surface area had significant phylogenetic signal, even after statistically accounting for the strong correlation with body mass and the clade difference between birds and mammals.

With diet in the model for birds (Table 6), the best fit was provided by the ANCOVA with different slopes and intercepts by OLS; therefore, diet significantly affected small intestine nominal surface area and in a fairly complicated way. In mammals, the best-fitting model was also the ANCOVA with different slopes and intercepts by OLS (Table 7), so again diet

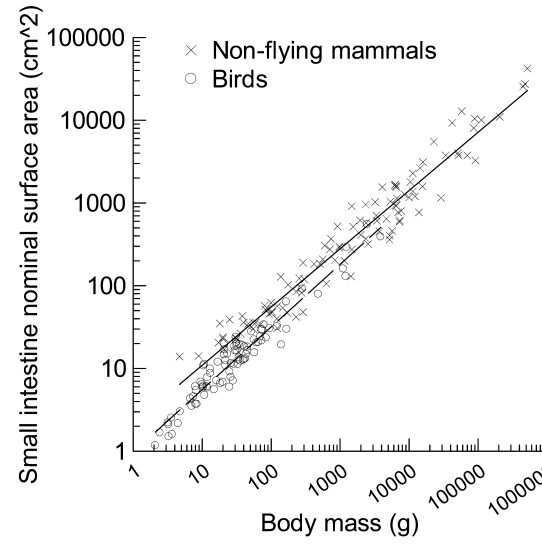

Figure 2. Small intestine nominal surface area (cm^2; area of a smooth bore tube) versus body mass (g) in birds and nonflying mammals on a double-logarithmic scale. Nonflying mammals are depicted by crosses and solid line (regression statistics in Table 1), and birds are depicted by circles and dashed line. Birds had small intestine area significantly smaller than that of mammals (by 51%; bird slope, 0.733 ± 0.0255; mammal slope, 0.703 ± 0.0172). See text for statistical comparisons.

Table 6: Statistical tests of the effect of diet (carnivore, omnivore, herbivore, nectarivore, or frugivore) on the allometric relation between small intestine nominal surface area and body mass in birds ($N = 86$)

Simple Allometry			
Model	d	ln Maximum Likelihood	AIC
OLS		−20.0	46.0
PGLS		−36.4	78.8
RegOU	.244	−17.0[a]	42.1

ANCOVA with Same Slope but Different Intercepts (Diet)							
Model	d	ln Maximum Likelihood	AIC	χ^2 for LRT vs. Simple Allometry	P for LRT (df = 4)	Partial F for Diet	P for Partial F (df = 4, 80)
OLS		−13.6	41.3	12.74	.0126	3.19	.0061
PGLS		−31.5	77.0	9.83	.0434	2.42	.0551
RegOU	.190	−12.6	41.2	8.90	.0636	2.14	.0834

ANCOVA with Different Slopes (Diet × Mass) and Intercepts (Diet)							
Model	d	ln Maximum Likelihood	AIC	χ^2 for LRT vs. Different Intercepts	P for LRT (df = 4)	Partial F for Diet and Diet × Mass Interaction	P for Partial F (df = 8, 76)
OLS		−8.4	38.8[b]	10.41	.0341[c]	2.93	.0066[d]
PGLS		−28.2	78.5	6.53	.1629[c]	1.99	.0590[d]
RegOU	.181	−7.5	39.0	10.18	.0375[c]	2.32	.0277[d]

Note. See Table 2 note for additional information and definitions of variables and abbreviations.

[a] On the basis of LRTs, the RegOU model is statistically significantly better than the PGLS model and the OLS model.

[b] Best model by criterion of lowest AIC.

[c] LRT comparing model with different slopes and intercepts with model that has parallel slopes.

[d] Partial F-test comparing model with different slopes and intercepts with simple allometry model.

significantly affected small intestine nominal surface area, but there was no significant phylogenetic signal.

Small Intestine Mass

There was a noticeable outlier for small intestine mass for the largest mammal (*Tursiops truncates*; Chivers and Hladik 1980). The value for this small intestine mass (430 g) was about an order of magnitude less than what we would expect on the basis of the species' body mass (450 kg). Thus, we removed this data point from our database and our analyses. For the combined bird plus mammal data set (Table 8), LRTs and partial F-tests indicated that the clades did not have significantly different small intestine mass (Fig. 3). The best-fitting model was simple allometry by RegOU, and it was statistically better than the OLS model (LRT, $\chi^2 = 13.0$, df = 1, $P = 0.003$), so phylogenetic signal was significant.

For birds alone, diet did not significantly affect small intestine mass (Table 9). The best-fitting model was simple allometry by RegOU, and it was significantly better than the OLS model (LRT, $\chi^2 = 5.2$, df = 1, $P = 0.0226$), so phylogenetic signal was significant.

In mammals, the best-fitting model based on AIC was OLS (Table 10), so phylogenetic signal was not apparent. However, diet did significantly affect small intestine mass on the basis of LRTs and partial F-tests with the OLS model (Table 10).

Small Intestine Volume

As suggested by Figure 4 and demonstrated statistically in Table 11, comparison of birds with mammals indicated that a model with the same slope and different intercepts provided the best fit; the small intestine volume of birds is significantly less than that of mammals. The RegOU model provided a slightly better fit than the OLS model (difference in AIC = 0.9) but not significantly so (LRT, $\chi^2 = 3.0$, df = 1, $P = 0.0833$), so small intestine volume did not exhibit statistically significant phylogenetic signal.

For birds alone, the ANCOVA model with different intercepts was significantly better than simple allometry (LRTs and partial F-tests), so diet affected small intestine volume (Table 12). The RegOU model was not significantly better than the OLS model (LRT, $\chi^2 = 3.0$, df = 1, $P = 0.0833$). A model with different slopes and intercepts could not be estimated because some diet categories had too few species.

In mammals, diet did not significantly affect small intestine volume, based on LRTs and partial F-tests (Table 13). The phylogenetic models fit the data worse than the OLS models, so phylogenetic signal was not significant.

Small Intestine Villus Amplification Ratio

The villus amplification ratio relates the villus area to the nominal surface area. On the basis of analyses of two previous

Table 7: Statistical tests of the effect of diet (carnivore, omnivore, or herbivore) on the allometric relation between small intestine nominal surface area and body mass in mammals ($N = 114$)

Simple Allometry			
Model	d	ln Maximum Likelihood	AIC
OLS		−68.3	142.7
PGLS		−92.1	190.1
RegOU	.125	−66.9	141.8

ANCOVA with Same Slope but Different Intercepts (Diet)							
Model	d	ln Maximum Likelihood	AIC	χ^2 for LRT vs. Simple Allometry	P for LRT (df = 2)	Partial F for Diet	P for Partial F (df = 2, 110)
OLS		−67.0	144.1	2.62	.2698	1.28	.2821
PGLS		−91.4	192.9	1.26	.5326	.61	.5452
RegOU	.131	−65.6	143.3	2.55	.2794	1.23	.2963

ANCOVA with Different Slopes (Diet × Mass) and Intercepts (Diet)							
Model	d	ln Maximum Likelihood	AIC	χ^2 for LRT vs. Different Intercepts	P for LRT (df = 2)	Partial F for Diet and Diet × Mass Interaction	P for Partial F (df = 4, 108)
OLS		−61.3	136.6[a]	11.45	.0033[b]	3.55	.0092[c]
PGLS		−89.5	193.0	3.85	.1459[b]	1.24	.2983[c]
RegOU	.048	−61.0	137.9	9.37	.0092[b]	3.05	.0200[c]

Note. See Table 2 note for additional information and definitions of variables and abbreviations.

[a] Best model by criterion of lowest AIC.

[b] LRT comparing model with different slopes and intercepts with model that has parallel slopes.

[c] Partial F-test comparing model with different slopes and intercepts with simple allometry model.

studies, we found (Table 14) that the allometric slopes for villus area and nominal surface area were not significantly different from each other within either birds (data from Ricklefs 1996) or mammals (data from Snipes 1997), so we subsequently tested whether the villus amplification ratio (villus area/nominal surface area) scaled with body mass. This ratio did not scale with body mass in either birds ($F = 1.15$, df = 1, 24, $P = 0.29$) or mammals ($F = 0.50$, df = 1, 18, $P = 0.49$).

Using the full data set, we again found that villus amplification ratio did not scale with body mass (top half of Table 15). However, birds had significantly larger small intestine villus amplification ratios compared with those of mammals on the basis of both LRTs and partial F-tests (bottom half of Table 15). In both analyses, OLS models had AIC values lower than those of PGLS or RegOU models, thus indicating no evidence for significant phylogenetic signal beyond the clade difference.

Discussion

We used a statistical approach that incorporates phylogenetic information to estimate allometric scaling relations for small intestine morphometric traits; to determine whether they differ, on average, between birds and nonflying mammals; to test for effects of diet within both clades; and to test for the presence of phylogenetic signal (the tendency for related species to resemble each other). The allometric equations presented in Table 1 can be used as general descriptors for birds or for nonflying mammals (not accounting for diet effects). Whether the OLS or the RegOU version is better can be judged by which has the lower AIC value and also by an LRT to determine whether the more complex model (RegOU) is statistically significantly better than the simpler one. In most cases in Table 1, the phylogenetic model not only had a lower AIC but also fit significantly ($P < 0.05$) better than the OLS model based on the LRT. This indicates that the residuals from the regression model contain significant phylogenetic signal. A separate issue is whether the estimates of the slopes from the two models are different. That can be judged by computing the 95% confidence interval (CI) about the RegOU slope and asking whether it includes the point estimate of the slope from the OLS model. For example, the RegOU estimate of the allometric slope for small intestine length of birds is 0.468 with a 95% CI of 0.435–0.501. This does not include the point estimate of 0.520 from the OLS model. The RegOU model is preferred on the basis of the AIC and is statistically significantly better on the basis of the LRT (Table 1); as a consequence, if the inferior OLS model were used, then the resulting estimate of the slope would fall outside the 95% CI of the best slope estimate. Thus, as has been pointed out before (e.g., Garland et al. 1993; Garland and Ives 2000), phylogenetic models can yield different estimates of slopes in allometric analyses, even when the range of body masses is

Table 8: Statistical tests of the effect of clade (bird vs. mammal) on the allometric relation between small intestine mass and body mass ($N = 94$)

Simple Allometry			
Model	d	ln Maximum Likelihood	AIC
OLS		−64.2	134.4
PGLS		−74.4	154.9
RegOU	.472	−57.7[a]	123.3[b]

ANCOVA with Same Slope but Different Intercepts (Clade)							
Model	d	ln Maximum Likelihood	AIC	χ^2 for LRT vs. Simple Allometry	P for LRT (df = 1)	Partial F for Clade	P for Partial F (df = 1, 91)
OLS		−63.2	134.5	1.94	.1637	1.90	.1715
PGLS		−74.4	156.9	.004	.9496	.003	.9564
RegOU	.482	−57.6[a]	125.3	.09	.7642	.06	.8070

ANCOVA with Different Slopes (Clade × Mass) and Intercepts (Clade)							
Model	d	ln Maximum Likelihood	AIC	χ^2 for LRT vs. Different Intercepts	P for LRT (df = 1)	Partial F for Clade and Clade × Mass Interaction	P for Partial F (df = 2, 90)
OLS		−62.0	134.0	2.49	.1146[c]	2.18	.1190[d]
PGLS		−74.4	158.9	.01	.9203[c]	.007	.9930[d]
RegOU	.467	−56.4[a]	124.8	2.40	.1213[c]	1.15	.3212[d]

Note. See Table 2 note for additional information and definitions of variables and abbreviations.

[a] On the basis of LRTs, the RegOU model is statistically significantly better than the PGLS and the OLS model.

[b] Best model by criterion of lowest AIC. See Table 1 for separate allometric equations for birds and mammals.

[c] LRT comparing model with different slopes and intercepts with model that has parallel slopes.

[d] Partial F-test comparing model with different slopes and intercepts with simple allometry model.

large. Such differences in estimates of allometric slopes are of crucial importance for testing alternative hypotheses about why traits scale with particular allometries (e.g., Chown et al. 2007).

We found that small birds (<365 g in body mass) had significantly shorter small intestines and avian species generally had less small intestine surface area and a smaller volume of small intestine as compared with nonflying mammals (Figs. 1–4). For example, on the basis of our data set, a 20-g bird would have a small intestine that is 33% shorter than that of a comparably sized mammal, and a 200-g bird would have a small intestine that is 20% shorter than that of a comparably sized mammal. Moreover, birds have 51% less nominal surface area and 32% smaller small intestine volumes compared with those of mammals. While small intestine surface area and volume were significantly smaller in birds compared with nonflying mammals, the small intestine wet mass was not significantly different between clades (Fig. 3; Table 8). Assuming a 1 : 1 ratio for gut volume and digesta volume and a density of 1 g $(cm^3)^{-1}$ for tissue and digesta, intestine mass is a small proportion of the sum total of the whole intestine with digesta. Comparable intestine mass between taxa, yet a smaller gut volume in birds compared with that in nonflying mammals, is consistent with the advantage of decreasing digesta volume and the total mass of the gut plus digesta, thus minimizing two kinds of costs associated with flight: the cost of flight increases with load carried, and takeoff and maneuverability can be impaired at

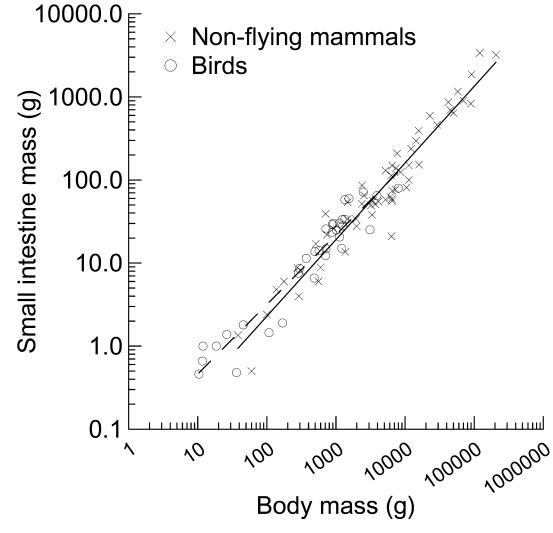

Figure 3. Small intestine wet mass (g) versus body mass (g) in birds and nonflying mammals on a double-logarithmic scale. Nonflying mammals are depicted by crosses and solid line (regression statistics in Table 1), and birds are depicted by circles and dashed line. Small intestine wet mass did not differ significantly between birds and mammals (bird slope, 0.844 ± 0.0608; mammal slope, 0.926 ± 0.0351). See text for statistical comparisons.

Table 9: Statistical tests of the effect of diet (carnivore, omnivore, or herbivore) on the allometric relation between small intestine mass and body mass in birds ($N = 34$)

Simple Allometry			
Model	d	ln Maximum Likelihood	AIC
OLS		−20.9	47.7
PGLS		−21.5	48.9
RegOU	.747	−18.3[a]	44.7[b]

ANCOVA with Same Slope but Different Intercepts (Diet)							
Model	d	ln Maximum Likelihood	AIC	χ^2 for LRT vs. Simple Allometry	P for LRT (df = 2)	Partial F for Diet	P for Partial F (df = 2, 30)
OLS		−19.2	48.4	3.33	.1892	1.54	.2309
PGLS		−19.9	49.8	3.18	.2039	1.47	.2460
RegOU	.728	−16.6[a]	45.1	3.59	.1661	1.64	.2109

ANCOVA with Different Slopes (Diet × Mass) and Intercepts (Diet)							
Model	d	ln Maximum Likelihood	AIC	χ^2 for LRT vs. Different Intercepts	P for LRT (df = 2)	Partial F for Diet and Diet × Mass Interaction	P for Partial F (df = 4, 28)
OLS		−18.5	51.1	1.32	.5169[c]	1.03	.4091[d]
PGLS		−18.3	50.6	3.17	.2049[c]	1.44	.2469[d]
RegOU	.776	−15.7[a]	47.5	1.65	.4382[c]	1.21	.3287[d]

Note. See Table 2 note for additional information and definitions of variables and abbreviations.

[a] On the basis of LRTs, the RegOU model is statistically significantly better than the PGLS model and the OLS model.

[b] Best model by criterion of lowest AIC.

[c] LRT comparing model with different slopes and intercepts with model that has parallel slopes.

[d] Partial F-test comparing model with different slopes and intercepts with simple allometry model.

heavier masses (Guillemette 1994; Norberg 1995; Nudds and Bryant 2002).

Birds and nonflying mammals had comparable small intestine masses, yet small birds had shorter small intestines, and birds generally had less nominal surface area of the small intestine. What might account for this discrepancy? One possibility is that birds may have thicker small intestines. For example, birds may have thicker layers of musculature lining their alimentary canal. Interestingly, birds also have a shorter mean retention time of fluids and particles (Lavin 2007); perhaps a thicker, more muscular gut allows more mixing or mechanical digestion, or birds may propel digesta more quickly through the canal as a means to minimize the digesta load at any given time. In birds, a greater surface area due to villi or microvilli per unit villus area may also account for similar small intestine masses despite length differences. Accordingly, while we did not note a body mass trend for the villus amplification ratio, we did find a significant difference between taxa; however, measurements of this ratio were conducted using varying techniques that can produce dramatically different results even within species (Kisielinski et al. 2002). The magnitude of the difference in villus amplification ratio between birds and nonflying mammals, however, was only about 15% (Table 14), which suggests that even if birds have villus area per unit nominal area significantly greater than that of mammals, this increase is not sufficient to compensate for a 50% reduction in nominal surface area. The only measurements of microvilli in any avian species that we found in the literature were for chickens (e.g., Smith et al. 1990). Thus, future studies could use electron microscopy to measure microvilli surface area enhancement factors to make comparisons between taxa.

Linear dimensions are expected to scale with the 0.33 power of body mass (Schmidt-Nielsen 1984), and we indeed found scaling for intestine length far below 1.0 and somewhat close to expectation (birds: 0.468 ± 0.0169; mammals: 0.395 ± 0.0160; Table 1). Our observations of small intestine nominal surface area scaling to the 0.733 ± 0.0255 (birds) and the 0.703 ± 0.0172 (mammals) powers of body mass are what we would expect from an organ that delivers nutrients to fuel metabolic rate, scaling to approximately the 0.68 power in birds and the 0.73 power in mammals (Nagy 2005). Additionally, we estimated that small intestine volume in birds and mammals scaled with body mass to the 0.891 ± 0.0455 and the 0.964 ± 0.0276 powers, respectively, which is slightly less than the isometric scaling with body mass that was reported by Parra (1978).

In our study, diet was a significant factor affecting some small intestine dimensions in birds and/or mammals (Tables 6, 7, 10, 12). In some cases, the effect was complicated, as for small intestine nominal surface areas of both birds (Table 6) and mammals (Table 7), where diet categories differed in both allometric slopes and intercepts. Generally, we found that within

Table 10: Statistical tests of the effect of diet (carnivore, omnivore, or herbivore) on the allometric relation between small intestine mass and body mass in mammals ($N = 60$)

Simple Allometry			
Model	d	ln Maximum Likelihood	AIC
OLS		−41.0	88.1
PGLS		−50.0	106.0
RegOU	.386	−37.2[a]	82.3

ANCOVA with Same Slope but Different Intercepts (Diet)							
Model	d	ln Maximum Likelihood	AIC	χ^2 for LRT vs. Simple Allometry	P for LRT (df = 2)	Partial F for Diet	P for Partial F (df = 2, 56)
OLS		−30.6	71.3[b]	20.78	<.0001	11.59	<.0001
PGLS		−49.9	109.8	.19	.9094	.09	.9141
RegOU	7.63 E -9	−30.6	73.3	13.04	.0015	11.59	<.0001

ANCOVA with Different Slopes (Diet × Mass) and Intercepts (Diet)							
Model	d	ln Maximum Likelihood	AIC	χ^2 for LRT vs. Different Intercepts	P for LRT (df = 2)	Partial F for Diet and Diet × Mass Interaction	P for Partial F (df = 4, 54)
OLS		−30.4	74.9	.44	.8025[c]	5.73	.0006[d]
PGLS		−47.3	108.5	5.28	.0714[c]	1.29	.2855[d]
RegOU	7.629 E -9	−30.4	76.9	.44	.8025[c]	5.73	.0006[d]

Note. See Table 2 note for additional information and definitions of variables and abbreviations.

[a] On the basis of LRTs, the RegOU model is statistically significantly better than the PGLS model and the OLS model.

[b] Best model by criterion of lowest AIC.

[c] LRT comparing model with different slopes and intercepts with model that has parallel slopes.

[d] Partial F-test comparing model with different slopes and intercepts with simple allometry model.

birds, herbivores had the largest small intestine nominal surface area and volume and nectarivores had the smallest. Within mammals, the small intestine nominal surface area, volume, and mass of carnivores and herbivores were slightly larger than those of omnivores.

Importantly, we sometimes found major differences between the results of conventional and phylogenetic analyses. For example, the OLS models indicated a significant effect of diet on small intestine length for birds, whereas the phylogenetic models, which fit the data much better, did not (Table 3). This is but one of many examples in which diet effects have been found for various morphometric or physiological traits when phylogenetic information is not incorporated into statistical analyses (e.g., Garland et al. 1993; Rezende et al. 2004).

In the analyses of diet effects, we sometimes found that the phylogenetic models (PGLS and/or RegOU) fit significantly better than the OLS models, thus indicating statistically significant phylogenetic signal (Tables 3, 8, 9), but we also sometimes found that this was not the case (Tables 6, 7, 10, 12, 13). We also found cases for which the model selection criterion of lowest AIC versus examination of LRTs or partial F-tests would suggest using different models; the best model selected by the AIC is not statistically significantly better than a simpler model. For example, in the diet analysis of small intestine volume in mammals (Table 13), the model with the lowest AIC for both OLS and RegOU was the ANCOVA with the same slope but

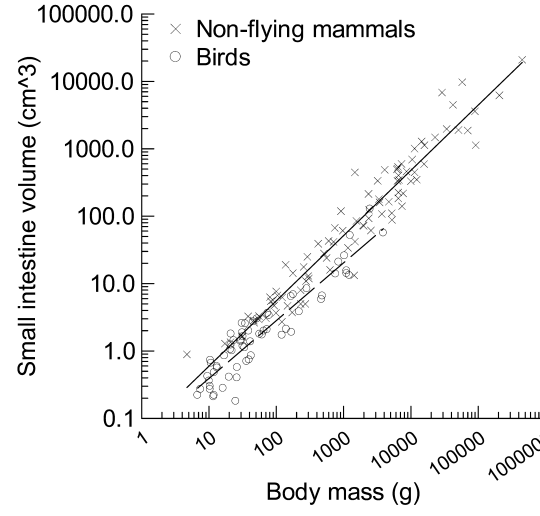

Figure 4. Small intestine volume (cm^3) versus body mass (g) in birds and nonflying mammals on a double-logarithmic scale. Nonflying mammals are depicted by crosses and solid line (regression statistics in Table 1), and birds are depicted by circles and dashed line. For birds, volumes of small intestine were significantly smaller than those of mammals (by 32%; bird slope, 0.891 ± 0.0455; mammal slope, 0.964 ± 0.0276). See text for statistical comparisons.

Table 11: Statistical tests of the effect of clade (bird vs. mammal) on the allometric relation between small intestine volume and body mass ($N = 156$)

Simple Allometry			
Model	d	ln Maximum Likelihood	AIC
OLS		−153.4	312.8
PGLS		−175.7	357.5
RegOU	.229	−150.7[a]	309.4

ANCOVA with Same Slope but Different Intercepts (Clade)							
Model	d	ln Maximum Likelihood	AIC	χ^2 for LRT vs. Simple Allometry	P for LRT (df = 1)	Partial F for Clade	P for Partial F (df = 1, 153)
OLS		−137.4	282.8	32.22	<.0001	34.83	<.0001
PGLS		−175.3	358.5	.95	.3297	.94	.3338
RegOU	.129	−135.9	281.9[b]	29.54	<.0001	32.44	<.0001

ANCOVA with Different Slopes (Clade × Mass) and Intercepts (Clade)							
Model	d	ln Maximum Likelihood	AIC	χ^2 for LRT vs. Different Intercepts	P for LRT (df = 1)	Partial F for Clade and Clade × Mass Interaction	P for Partial F (df = 2, 152)
OLS		−136.1	282.2	2.55	.1103[c]	18.84	<.0001[d]
PGLS		−174.9	359.9	.66	.4166[c]	.79	.4557[d]
RegOU	.109	−135.0	282.1	1.80	.1797[c]	17.36	<.0001[d]

Note. See Table 2 note for additional information and definitions of variables and abbreviations.

[a] On the basis of LRTs, the RegOU model is statistically significantly better than the PGLS model and the OLS model.

[b] Best model by criterion of lowest AIC. See Table 1 for separate allometric equations for birds and mammals.

[c] LRT comparing model with different slopes and intercepts with model that has parallel slopes.

[d] Partial F-test comparing model with different slopes and intercepts with simple allometry model.

different intercepts. However, for this model neither the LRT nor the partial F-test indicated that the model was significantly better than a model of simple allometry (i.e., no effect of diet). This apparent discrepancy is caused by the different conceptual objectives of the two approaches. In model selection, models are compared according to some criterion (such as AIC), and the best is selected. When applying an LRT or a partial F-test, the simpler model is a priori identified as preferred, and it is overthrown by a more complex model only if the probability that the observed data could have been generated by the simple model is small (e.g., 5%) compared with the more complex model. Given this preference for the simple model in LRTs and partial F-tests, it is not surprising that these tests would sometimes lead one to accept a simpler model than model selection based on AIC (e.g., Ludden et al. 1994).

Using a subset of our data, we compared the ratio of hindgut (large intestine + ceca) volume with small intestine volume. Dietary trends were also evident; carnivores and omnivores had smaller ratios, while herbivores and omnivores had larger ratios, indicating that the hindguts of herbivores tended to be relatively larger compared with those of carnivores. Indeed, Chivers and Hladik (1980) also found extensive overlap among diet groups for regression lines of small intestine volume and body size in mammals, but they found that herbivores had more voluminous hindguts compared with those of carnivores. The proportion of the total volume of the gut attributed to the small intestine was large for carnivores, moderate for frugivores, and small for herbivores (Chivers and Hladik 1980). It seems, then, that animals have comparably sized small intestines within taxa and that adaptations to particular diets (e.g., plants) relate primarily to hindgut additions such as an extensive large intestine and/or ceca for microbial fermentation of digesta after it has cleared the small intestine. We also noted that the majority of the whole-gut volume was the small intestine in birds (~80% small intestine; in mammals, ~50%); accordingly, our conclusion of less intestine in birds would be magnified if we compared the whole gut between taxa (but very few data on the whole gut are available for birds). The storage of leafy material to enable time for cellulose digestion via symbiotic microbes is a major disadvantage for flying organisms (Morse 1975). Therefore, it is far more likely for a nonflying mammalian species to possess a considerable amount of hindgut than it is for an avian species to possess a large hindgut, which makes sense from the argument of the costs of carrying extra mass.

Implications for Avian Species Having Less Gut

Among terrestrial vertebrates, bird species have the highest feeding rate, in order to obtain metabolizable energy for use in their natural habitats. For example, a typical bird eats 30%–45% more food than does a typical comparably sized mammal (Nagy 2001). Given the reduced size of their small intestine,

Table 12: Statistical tests of the effect of diet (carnivore, omnivore, herbivore, nectarivore, or frugivore) on the allometric relation between small intestine volume and body mass in birds ($N = 65$)

Simple Allometry			
Model	d	ln Maximum Likelihood	AIC
OLS		−52.8	111.6
PGLS		−67.6	141.2
RegOU	.245	−51.3	110.6

ANCOVA with Same Slope but Different Intercepts (Diet)							
Model	d	ln Maximum Likelihood	AIC	χ^2 for LRT vs. Simple Allometry	P for LRT (df = 4)	Partial F for Diet	P for Partial F (df = 4, 59)
OLS		−45.9	105.8	13.84	.0078	3.50	.0125
PGLS		−61.7	137.3	11.87	.0183	2.96	.0269
RegOU	.216	−44.4	104.8[a]	13.79	.0080	3.44	.0136

Note. See Table 2 note for additional information and definitions of variables and abbreviations.

[a] Best model by criterion of lowest AIC.

how do birds satisfy relatively high energy needs with relatively low absorptive surface area? Theoretically, birds might compensate for a reduced small intestine with a longer gut retention time of food, an increased mucosal surface area via a greater villus and/or microvillus area, increased reaction rates (increased rate of mediated transport and/or enzyme hydrolysis), and/or an alternative means of nutrient transport. Otherwise, birds may operate with a narrower spare capacity (a reduced digestive capacity to meet an increase in metabolic needs) compared with that of nonflying mammals (for a review of digestive spare capacity, see Karasov and McWilliams 2005). We review each of these possibilities.

The amount of energy extracted from a meal is positively related to the retention time of food in the gastrointestinal tract, up to the point where most absorbable material is extracted. Because the mean retention time of fluids and particles was significantly shorter in birds than in mammals (Lavin 2007), birds do not retain food in their gastrointestinal tract for extended periods of time, in order to extract more nutrients with less gut. We found some evidence that the villus amplification ratio was greater in avian species compared with mammalian species, but this finding could simply be an artifact of differences in methodology. Furthermore, the magnitude of the difference in villus area between taxa (15% greater area in birds) was not sufficiently large to completely compensate for a 50% reduction in nominal surface area.

Is there a faster rate of nutrient breakdown and/or absorption in birds versus mammals? If so, it may explain how birds manage to obtain sufficient energy with a reduced gut. There do not appear to be fundamental differences between birds and mammals in the primary enzymes and nutrient transporters of the intestinal brush border membrane (Karasov and Hume 1997). Also, there was no significant difference between omnivorous birds and mammals in transporter-mediated uptake rate of D-glucose or total amino acid L-proline uptake per unit nominal surface area of small intestine ($n = 7$ bird species, and $n = 8$ mammal species; Karasov and Hume 1997). Measurements of nutrient uptake standardized per unit nominal intestine area inherently take differences in surface amplification into account and do not differ significantly between birds and mammals, suggesting that total capacity for mediated uptake is lower in birds. Thus, the difference in total intestinal surface area between birds and nonflying mammals is not likely to be counterbalanced by greater digestive surface amplification by villi in birds, at least in the case of mediated nutrient uptake. There was also no significant difference in small intestine hydrolase-specific (sucrase, aminopeptidase-N, maltase, and isomaltase) activity per gram protein between birds and mammals ($n = 6$–15 bird species, and $n = 4$–24 mammal species, depending on enzyme; T. J. McWhorter, personal communication), suggesting that autoenzymatic reaction rates also do not compensate for the smaller intestinal surface area of birds relative to that of mammals.

In yellow-rumped warblers (*Dendroica coronata*), rainbow lorikeets (*Trichoglossus haematodus*), house sparrows (*Passer domesticus*), and northern bobwhites (*Colinus virginianus*) but not in mice, rats, and rabbits, the capacity for mediated transport of D-glucose measured in vitro underestimated the total glucose uptake at the whole-animal level (Ferraris and Diamond 1989; Karasov and Cork 1994; Caviedes-Vidal and Karasov 1996; Karasov et al. 1996; Afik et al. 1997*a*). Might these small birds use a different transport pathway that accounts for D-glucose absorption differences seen in vitro versus in vivo, as well as one that compensates for having a reduced gut? Absorption of carbohydrates can occur with the aid of specific protein carriers that transport these compounds across the apical and basolateral membranes of intestinal cells. But also, absorption of these compounds may occur through junctions between adjacent enterocytes (paracellularly) rather than across their apical membrane, and this absorption does not exhibit saturation kinetics; thus, paracellular nutrient absorption capacity is matched to dietary load (Pappenheimer 1993; Karasov and

Table 13: Statistical tests of the effect of diet (carnivore, omnivore, or herbivore) on the allometric relation between small intestine volume and body mass in mammals ($N = 91$)

Simple Allometry							
Model	d	ln Maximum Likelihood	AIC				
OLS		−82.9	171.9				
PGLS		−105.1	216.2				
RegOU	.037	−82.8	173.6				
ANCOVA with Same Slope but Different Intercepts (Diet)							
Model	d	ln Maximum Likelihood	AIC	χ^2 for LRT vs. Simple Allometry	P for LRT (df = 2)	Partial F for Diet	P for Partial F (df = 2, 87)
OLS		−80.4	170.7[a]	5.12	.0773	2.52	.0847
PGLS		−104.7	219.3	.91	.6344	.44	.6455
RegOU	.013	−80.4	172.7	4.85	.0885	2.37	.0995
ANCOVA with Different Slopes (Diet × Mass) and Intercepts (Diet)							
Model	d	ln Maximum Likelihood	AIC	χ^2 for LRT vs. Different Intercepts	P for LRT (df = 2)	Partial F for Diet and Diet × Mass Interaction	P for Partial F (df = 4, 85)
OLS		−79.3	172.5	2.23	.3279[b]	1.79	.1383[c]
PGLS		−104.4	222.8	.54	.7634[b]	.34	.8503[c]
RegOU	.004	−79.3	174.5	2.21	.3312[b]	1.71	.1553[c]

Note. See Table 2 note for additional information and definitions of variables and abbreviations.
[a] Best model by criterion of lowest AIC.
[b] LRT comparing model with different slopes and intercepts with model that has parallel slopes.
[c] Partial F-test comparing model with different slopes and intercepts with simple allometry model.

Cork 1994). While paracellular absorption offers a relatively inexpensive means of nutrient uptake, because it is not active and does not involve synthesis of transporters, it is less specific than transcellular absorption; consequently, animals that have extensive paracellular absorption may be exposed to greater systemic concentrations of water-soluble toxins found in the diet (Diamond 1991).

Paracellular absorption, measured as the bioavailability of small, inert carbohydrate probes (L-rhamnose, L-glucose, mannitol; molecular weight 164–180 Da), was relatively low in 11 species of nonflying mammals, averaging 0.13 ± 0.03 (Caviedes-Vidal et al. 2007). Consequently, paracellular absorption was found to contribute little (<7%) to total glucose absorption in mammals of various sizes (Fine et al. 1993; Schwartz et al. 1995; Uhing and Kimura 1995; Lane et al. 1999; Lavin et al. 2007). In contrast, bioavailability of the same probes averaged more than four times higher in 10 avian species (0.56 ± 0.09; $P < 0.001$; Caviedes-Vidal et al. 2007), and paracellular absorption has been estimated to account for >50% of glucose absorption in several birds (Karasov and Cork 1994; Caviedes-Vidal and Karasov 1996; Levey and Cipollini 1996; Afik et al. 1997*b*; Chang and Karasov 2004; McWhorter et al. 2006; Lavin et al. 2007; Lavin and Karasov 2008). Paracellular absorption may offer an alternate means of nutrient assimilation in birds compared with nonflying mammals.

Birds may, however, be operating with a narrower spare capacity, particularly immediate spare capacity. The relative difference between the current or absolute maximal digestion rate and the current food intake is a measure of an animal's so-called safety margin (Diamond 1991; but see Garland 1998). Because bird species tend to have reduced guts, have shorter mean retention times, and do not have increased mediated transport or enzyme hydrolysis rates (compared with mammals), their immediate capacity for increasing nutrient assimilation (due to an increase in energy demands) may be more limited than that of mammals. Furthermore, birds seem to exhibit less modulation of transporters than do mammals: in

Table 14: Nonphylogenetic regression analyses for small intestine villus area and nominal surface area for birds (data from Ricklefs 1996) and mammals (data from Snipes 1997)

	Y-intercept	Slope
Birds ($n = 26$):		
Villus area	2.24 ± .08	.70 ± .04
Nominal surface area	.52 ± .06	.59 ± .09
Mammals ($n = 20$):		
Villus area	1.89 ± .11	.71 ± .04
Nominal surface area	.54 ± .10	.74 ± .03

Note. Both data sets were ln transformed. Slopes for villus area and nominal surface area were not significantly different in birds ($F = 0.58$, df = 1, 48, $P = 0.45$) or mammals ($F = 0.31$, df = 1, 36, $P = 0.58$).

Table 15: Statistical tests of the effect of body mass or clade (bird vs. mammal) on small intestine villus amplification ratio ($N = 69$)

Simple Allometry for Birds and Mammals Combined					
Model	d	ln Maximum Likelihood	AIC	F for Effect of Body Mass	P for Body Mass (df = 1, 67)
OLS		−51.1	108.3[a]	.68	.4125
PGLS		−60.4	126.8	.61	.4375
RegOU	.518	−50.3	108.6	.06	.8072
Test for Effect of Clade (Bird vs. Mammal; Body Mass Not in Model)					
Model	d	ln Maximum Likelihood	AIC	F for Effect of Clade	P for Clade (df = 1, 67)
OLS		−48.0	102.0[a]	7.12	.0096
PGLS		−60.7	127.3	.12	.7301
RegOU	.316	−48.0	103.9	5.24	.0252

Note. See Table 2 note for additional information and definitions of variables and abbreviations.
[a] Best model by criterion of lowest AIC.

contrast to mammals, several species of birds did not increase mediated glucose absorption rates when switched to a higher-carbohydrate diet (Karasov 1992; Levey and Karasov 1992; Caviedes-Vidal and Karasov 1996; Afik et al. 1997*a*). Also, growing chickens fed a carbohydrate-free diet were smaller than birds fed a carbohydrate-containing diet, possibly because the former were not capable of increasing levels of membrane-bound proteinases and amino acid transporters per unit nominal area of intestine; instead, birds on a carbohydrate-free diet exhibited gastrointestinal tract hypertrophy, presumably to increase nutrient assimilation rates (Biviano et al. 1993). Paracellular transport could mitigate constraints on upregulation of nutrient transporters. As birds become more acclimated to an increase in energy demands (long-term spare capacity), however, the phenotypic plasticity of the digestive system (e.g., increased gut surface area, volume) may lessen the effects of the digestive limitations imposed on birds, but the increase in gut size may still be restricted by physiological constraints associated with flying.

Caveat, Conclusions, and Suggestions for Future Research

A limitation of this comparative study and of all broadly based literature compilations is that the various species were not measured under common-garden conditions (Garland and Adolph 1991, 1994; Garland et al. 2005). Thus, it is impossible to know how much of the variation among species may be attributable to acute effects of the different foods they ate. On the other hand, it would not be possible to rear all of the included species under identical conditions because, for example, some could not survive on a particular common diet that might be imposed. This is a conundrum for many comparative studies, and one can only hope that most of the differences among species represent evolved, genetically based differences.

Using a phylogenetically informed analysis on a large data set (493 species), we found that small birds have shorter small intestines and that birds, in general, have nominal surface areas and volumes relatively smaller than those of similarly sized nonflying mammals. A potential consequence of this is reduced intestine area for nutrient breakdown and transport potentially resulting in a smaller/lower spare digestive capacity. Are these findings a consequence of load limitations imposed by flight? Interestingly, there is evidence of both enhanced villus surface area and high passive absorption in bats, which may also have a reduced gut size as a consequence of flight (Barry 1976; Mayhew and Middleton 1985; Makanya et al. 1997; Caviedes-Vidal et al. 2007; Tracy et al. 2007). We do not find evidence to support potential compensatory mechanisms in birds, such as increased digesta retention time or faster reaction rates of nutrient breakdown and absorption. One plausible means of compensation for a limited capability of active transport of nutrients is passive absorption of these compounds. From an evolutionary perspective, both costs and benefits would be associated with an increased permeability to water-soluble chemicals. Passive absorption in flying vertebrates may confer an advantage because it allows the assimilation of nutrients with little energy, but because this pathway is relatively less selective, increased intestinal permeability may result in increased detoxification requirements in certain species. These opposing costs and benefits may be the reason for variations in the extent of passive absorption among species, particularly minimal passive absorption in nonflying mammals, because nonflying species can afford to have a lengthy and voluminous gut with which to actively and selectively absorb nutrients.

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Appendix A

Phylogenetic Regression and the MATLAB Regressionv2.m Program

This appendix provides a brief historical treatment to place the analytical methods used in this article in appropriate context relative to other phylogenetic comparative methods. It also gives the primary documentation for the MATLAB program Regressionv2.m (available on request from T. Garland Jr.), with which all analyses were performed.

Historical Context

As reviewed by Martins and Hansen (1997), when analyzing comparative data one must first translate an evolutionary hypothesis into a statistical model, which will usually include a mean structure and an error term. This is familiar from ordinary multiple regression analysis, in which the dependent variable is continuously distributed. (When the dependent variable is not continuously and/or not normally distributed, general linear models [GLMs] involving link functions can be employed, and these also have phylogenetic versions; Martins and Hansen 1997; Paradis and Claude 2002; Paradis et al. 2004; A. R. Ives and T. Garland Jr., unpublished manuscript.) For example, we might propose that

$$Y = b_0 + b_1X_1 + b_2X_2 + \varepsilon, \tag{A1}$$

where Y is ln intestine length, X_1 is ln body mass diet, and X_2 is diet. Diet might be a continuous-valued trait, such as percent meat (e.g., Muñoz-Garcia and Williams 2005). Alternatively, as in this article, diet might be represented by $k - 1$ dummy variables (having values 0 or 1) that code for k different diet categories, such as omnivore, carnivore, herbivore, nectarivore, or frugivore (see also Garland et al. 1993). In that case, we would have b_2, b_3, b_4, and b_5 indicating the amount by which ln intestine length deviates for four of the diet categories as compared with one that is chosen arbitrarily as the basis for comparison. This constitutes ordinary multiple regression with dummy variables and is equivalent to an ANCOVA model in which the slopes for the relation between ln intestine length and ln body mass are constrained to be parallel.

In ordinary regression, the residual errors (ε) are assumed to be independent and identically distributed (constant variance, irrespective of the values of any of the independent variables). Estimation of the regression parameters (b_0, b_1, b_2, etc.) and their associated standard errors (which then allows tests of the hypothesis that a particular parameter differs from 0 or some other value and requires an assumption of normality of residuals) is typically accomplished by the method of ordinary least squares (OLS), which minimizes the sum of the squared vertical deviations (residuals). These residuals will sum to 0, and the least squares regression line passes through the mean value for each of the independent variables. The OLS estimates are also the maximum likelihood (ML) estimates.

Felsenstein (1985) proposed the first fully phylogenetic statistical method, independent contrasts, for continuous-valued traits based on a Brownian motion (random walk in continuous time) model of character evolution and discussed how it could be applied to regression, correlation, or such related multivariate methods as principal components analysis. The first publication to use the method was that of Sessions and Larson (1987), but the first completely worked numerical example was shown by Garland and Adolph (1994; for another worked example, see Garland et al. 2005). Felsenstein (1985, p. 13) cautioned that "all of the above has been predicated on the acceptance of the Brownian motion model as a realistic statistical model of character change. There are certainly many reasons for being skeptical of its validity."

Felsenstein (1988, pp. 464–465) was also the first to suggest that the Ornstein-Uhlenbeck (OU) process might be used as a model in phylogenetically based statistical methods: "The OU process is a good model for the motion of a population which is wandering back and forth on a selective peak under the influence of genetic drift. Natural selection plays the role of the elastic band. … The OU process could also serve as the model for the wanderings of an adaptive peak in the phenotype space, where the optimum remains within a relatively confined region. If the peak itself wanders according to an OU process, and the population mean is wandering by genetic drift while tethered to the peak, the resulting movement of the population will itself not be an OU process but can be well-approximated by one. A major feature of an OU model of character change is that it gradually 'forgets' past history. … Older history of the species becomes less and less relevant, its influence erased by the steady pull toward the central point. … No one has yet discovered how to carry out statistical comparative methods in the case of the OU process, but when this is done, it will be found that comparisons between distantly related species should be accorded much less weight than those between closely related species. The Brownian motion model instead argues that both should get equal weight. However, in another respect the OU model is inadequate. Under it, there is no way to recover information about events of the distant past. All record of the ancient phenotypes is expected to have been erased by the pull toward the central point. Systematists believe, with apparent justification, that morphology does allow us access to information on ancient evolutionary events. So an OU model cannot be the whole story, any more than a Brownian motion model can be." Garland et al. (1993) implemented an OU model in their PDSIMUL program.

Grafen (1989) first proposed several statistical approaches for comparative data that are now widely used. His standard regression was a generalization of Felsenstein's (1985) phylo-

genetically independent contrasts (IC) that used techniques of generalized least squares (GLS). In GLS, multiple regression can be performed in the usual way if the phylogenetic correlations among residuals are specified. Grafen pointed out that all GLS needs is a hypothesis about the variance-covariance matrix of the error terms, ε, that describes the pattern of relatedness among species. For example, if the evolution of a character's residuals (i.e., the deviations that remain after controlling for relations with independent variables) is assumed to follow a Brownian motion model with phylogenetic branch lengths proportional to time, then it is possible to translate directly from a phylogenetic tree to the expected variance-covariance matrix of the residuals. Specifically, the branch length distance from the root (basal node) of the tree to each tip specifies the variance, whereas the distance from the root to the last common ancestor of a pair of species specifies the expected covariance of the residuals for those two species (for a worked example, see Garland et al. 2005, p. 3,031). Although Grafen recognized that his GLS approach was identical to Felsenstein's IC approach, the link between them was only formally made later (Hansen and Martins 1996; Garland and Ives 2000; Rohlf 2001, 2006). In regression, the IC approach gives the same results as the GLS approach but only when dependent and independent variables are transformed by use of the same phylogenetic tree (topology and branch lengths); when this is not the case, IC and GLS will differ.

The translation of a phylogeny into a variance-covariance matrix is easiest to conceptualize in univariate models where the only interest is in the relationship among species in values of a single trait. In a multivariate regression context, some care is needed in interpreting this variance-covariance matrix. The variance-covariance matrix used in regression analyses is the variance-covariance matrix of ε, not of the dependent variable Y (Grafen 1989, 1992). Because values of Y depend not only on ε but also on the independent variable X, values of Y might be phylogenetically related if values of X are, even when values of ε are independent. Conversely, values of Y might show no phylogenetic resemblance even though values of ε do if the phylogenetic resemblance in the residuals is masked by the values of X. Hansen and Orzack (2005) present a formal statistical model derived from an evolutionary process in which phylogenetic correlation among ε arises when values of Y imperfectly track changes in values of X through evolutionary time. Alternatively, phylogenetically correlated residuals will likely arise if one or more phylogenetically related traits affect the dependent variable Y but are not included in the regression model; unmeasured traits thus create phylogenetic correlations in the unexplained residual variation in Y (Grafen 1989, p. 144; A. R. Ives and T. Garland Jr., unpublished manuscript).

Moving beyond the standard regression, Grafen dealt with the problem of unrecognized phylogeny, also known as soft polytomies (Purvis and Garland 1993). Many biologists presume that speciation usually occurs in a dichotomous fashion such that the true tree of life should be mainly dichotomous. However, many estimates of phylogenetic trees (termed "working phylogenies" by Grafen) contain multifurcations that represent insufficient resolving power. If so, then this should be accounted for. Grafen's (1989) phylogenetic regression did this in a particular way that will not be discussed here (see also Grafen 1992). A simpler way of dealing with soft polytomies involves subtraction of degrees of freedom when hypothesis testing (Purvis and Garland 1993; Garland and Diaz-Uriarte 1999; see also Housworth and Martins 2001); although this approach has been found to produce adequate results in simulation studies, it is nonetheless statistically ad hoc and should be studied with simulations for each separate application. In general, however, questions about uncertainty in the topology of phylogenetic trees are treated separately from questions regarding the rate of evolutionary divergence among species on a phylogenetic tree with a known topology (but see Huelsenbeck and Rannala 2003).

Importantly, Grafen (1989, p. 124) noted that the positions of the internal nodes of the phylogenetic tree proposed for analysis (a working phylogeny) could be pulled or stretched, relative to the root and terminal nodes (tips). A whole family of relative branch lengths (as dictated by the value of a transformation parameter, which he termed ρ) can then be compared, and the best-fitting set of branches and the best-fitting set of regression parameters can be simultaneously estimated by ML or restricted maximum likelihood (REML). In this mode of operation, it is possible that the best-fitting tree will be one in which the internal nodes have been pulled very close to (or even all the way to) the root, such that it is almost (or exactly) a star. (The tips will remain contemporaneous only if the starting tree had contemporaneous tips.) If so, then the estimated regression parameters (and any associated statistical tests) will be almost (or exactly) the same as those obtained from an OLS analysis that ignores phylogenetic information (Grafen 1989; Purvis and Garland 1993). Grafen called these procedures the standard regression because they involved only standard statistical techniques, although the application to comparative data was novel. He stated that "it is the least that can reasonably be done when performing a regression on comparative data" (Grafen 1989, p. 124).

The simultaneous estimation of a suitable branch length transformation parameter is important (see also Martins and Hansen 1997; Freckleton et al. 2002) because it means that one can avoid overcorrecting or undercorrecting for covariances of residuals that are related to descent with modification along a hierarchical phylogenetic tree (phylogenetic signal sensu Blomberg et al. 2003). As noted by Felsenstein (1985, 1988) and many others (e.g., review in Garland et al. 2005), various evolutionary processes (such as rapid adaptation to changing environmental conditions) or even high measurement error (Ives et al. 2007) could result in a comparative data set that is better fit by a star phylogeny (see also Freckleton et al. 2003; Freckleton and Harvey 2006). Given that the results of conventional and phylogenetic analyses often differ in important ways, it thus seems natural to adjudicate between them by choosing a model with a value of ρ (or other such transform) that is

optimal as judged by ML or a similar criterion. To quote Grafen (1992, p. 417), "It will rarely be sensible to assume in advance that we know the strength of phylogeny."

Grafen (1989, p. 146) noted that "the estimate of ρ has very poor statistical properties. I believe that it is asymptotically biased and that its sampling variance does not decrease to zero asymptotically. … The poor statistical properties of the estimate of ρ are not of direct concern, as ρ is essentially a nuisance parameter. But it is important to realize that caution should be taken if the estimate of ρ is interpreted in any way." As discussed below, although the ML estimates of ρ are likely to be biased, other estimation methods (e.g., REML) may have better statistical properties. Grafen's (1989) standard regression procedures did not become widely used, perhaps largely because they were implemented in GLIM, although an SAS implementation is now available (http://users.ox.ac.uk/~grafen/phylo/index.html). In addition, ρ is not tied to a particular model of (residual) character evolution, and some biologists prefer to avoid strictly phenomenological statistical models.

Martins and Hansen (1997, p. 650) proposed a general approach for the statistical analysis of comparative data from an evolutionary perspective. They stated that "our own approach here might be viewed as an extension of Grafen's method in which we show how error structures can be more precisely determined for a particular set of data, phylogenies, and evolutionary question and incorporated using GLS procedures." They suggested that transformation parameters that could be related to models of stabilizing selection, such as the OU model, would enhance evolutionary interpretations (see also Butler et al. 2000; Butler and King 2004). They also discussed various more complicated statistical models, including incorporation of measurement error (see Ives et al. 2007), that can be analyzed by GLMs. For the more complicated models, they suggested ML estimation procedures, as also used by Grafen (1989) for his standard regression as well as the phylogenetic regression. They also noted that GLS produces transformed residuals, and these can be tested for normality, heteroscedasticity, and other diagnostics, much like residuals from OLS (Grafen [1989] also discussed some diagnostics for residuals).

Various models of evolution can be used to generate a variance-covariance matrix from a phylogenetic tree, including simple Brownian motion, Grafen's (1989, 1992) ρ transform, the OU transform (Hansen 1997; Martins and Hansen 1997; Blomberg et al. 2003), Pagel's (1997, 1999) λ transform, and the accelerating-decelerating (ACDC) transform derived by Blomberg et al. (2003). As we previously described, Grafen's ρ transform stretches the variance-covariance matrix using a mathematically convenient formula rather than a biologically motivated one, allowing relatively greater or lesser weight to the covariances dictating phylogenetic correlations among species. The OU transform was motivated by the biological process of stabilizing selection (Felsenstein 1988); the greater the force of stabilizing selection, the less "memory" of ancestral trait values and hence the lower covariances among species. Although based on a biological model, the OU transform can also be used without any reference to the biological process that underlies its derivation. Pagel's λ effectively produces proportional reductions in the covariances among species. This transform can be derived by assuming that there is one component of the residuals, ε, that experiences Brownian motion evolution, while another additive component shows no phylogenetic correlation (Housworth et al. 2004). Finally, the ACDC transform (Blomberg et al. 2003) assumes that the rate of evolution for a trait increases or decreases through time. For phylogenies with contemporaneous tips, the ACDC and OU transforms (as derived in Blomberg et al. 2003) are identical, but they differ when tips are not contemporaneous.

Deriving a sensible terminology to discuss estimation under these different transform models requires making a distinction between statistical models and estimation techniques used to fit models to data. All of these transform models are statistical models in that they describe a statistical distribution and its parameters. In contrast, OLS and GLS are estimation techniques. Several authors refer to one or more of the transform models as GLS models (or PGLS models, for phylogenetic GLS models), although GLS is an estimation procedure. This is particularly confusing because GLS actually cannot be used to estimate the parameters of these transform models because they all contain parameters in the variance-covariance matrix that must be estimated. To reduce confusion, our preference is to refer to transform models as distinct from the estimation approaches that can be applied to them. We will break this convention only when using the established monikers "OLS model" and "GLS model" (i.e., the Brownian motion model) because in these cases there is a one-to-one match between the structure of the model and the appropriate estimation technique.

Estimation for all of the transform models can be performed using ML or REML approaches. In addition, estimated GLS can be used with Pagel's λ transform model, along with some models that incorporate measurement error in the species' values (Ives et al. 2007). Bayesian methods are also possible, but in general the statistical and numerical problems arising in phylogenetic analyses are simple enough that Bayesian methods have no advantages.

Pagel (1997, 1999) used ML estimation for the analysis of continuous-valued traits (as well as other types of traits) using the λ transform model but did not refer to Grafen's (1989) earlier work in this context. Freckleton et al.'s (2002) extension of Pagel's approach used a direct search to find the ML value of λ in combination with likelihood ratio tests to determine whether λ differed from 0 (a star phylogeny), 1 (the original input tree), or even some other value that might be of a priori interest. Freckleton et al. (2002) used simulations to compare the error rates of λ and Grafen's ρ in distinguishing between the OLS and GLS models. They concluded that λ worked better and so performed regression analyses on a large number of comparative data sets using this transformation parameter. They found that in 23 out of 26 phylogenies (88%), at least one character showed significant phylogenetic correlation (i.e., $\lambda > 0$). Several subsequent articles have used estimation of λ

in multiple regression analyses, using either Pagel's CONTINUOUS program or routines in the R language (e.g., Gage and Freckleton 2003; McKechnie et al. 2006; Duncan et al. 2007).

Blomberg et al. (2003) used a similar approach to test for phylogenetic signal in univariate comparative data sets. Rather than the λ or ρ transformations, they used a simple version of an OU model and also a new ACDC model of character evolution, both of which were estimated by ML. In addition, they developed a randomization test that can be implemented either via independent contrasts or GLS methods. Consistent with Freckleton et al. (2002), simulations showed that the tests had good power for comparative data sets comprising 20 or more species. For trees with 20 or more species, 49 of 53 (92%) traits exhibited significant phylogenetic signal by the randomization test. Rather than using likelihood ratio tests, Blomberg et al. (2003) developed additional randomization procedures to test whether the parameters for OU (d) or ACDC (g) transforms differed significantly from 0 (no phylogenetic signal) or 1 (Brownian motion evolution). An advantage of randomization tests is that they are generally robust to small sample sizes when likelihood ratio tests may fail. On the basis of these procedures, the null hypothesis that d or g was equal to 0 was rejected ($P < 0.05$) for 47 of 53 traits and for 49 of 53 traits, respectively.

Statistical Documentation for MATLAB Program Regressionv2.m

We performed all of the analyses in this article using a new MATLAB (MathWorks 1996) program, Regressionv2.m, that can perform multiple regression using OLS and GLS, as well as the four transform models: Grafen's ρ, Pagel's λ, OU (as derived in Blomberg et al. 2003), and ACDC. Respectively, we refer to these models as RegGrafen, RegPagel, RegOU, and RegACDC. Because we generally prefer analyses based on models that have a clear biological interpretation, only the RegOU model is used in the accompanying analyses of small intestine morphometric traits. For simplicity, we will refer to the transform parameter for all four models (ρ, λ, d, and g) collectively as θ. The regression models for all of these transforms have the form of equation (A1) but differ in the variance-covariance matrix $E\{\boldsymbol{\varepsilon}\boldsymbol{\varepsilon}'\} = \sigma^2\mathbf{V}(\theta)$, where the elements of the variance-covariance matrix are for Grafen's ρ, Pagel's λ, OU, and ACDC, respectively,

$$\begin{aligned} v_{ij} &= 1 - (1 - c_{ij})^{\rho}, \\ v_{ij} &= \lambda c_{ij}\ (i \neq j);\ v_{ii} = c_{ii}, \\ v_{ij} &= \frac{d^{(c_{ii}+c_{jj}-2c_{ij})}(1 - d^{2c_{ij}})}{(1 - d^2)}, \\ v_{ij} &= \frac{(1 - g^{-c_{ij}})}{(1 - g^{-1})}, \end{aligned} \tag{A2}$$

where c_{ij} is the ijth element of the starter (untransformed) variance-covariance matrix derived under the Brownian motion evolution assumption.

For the four transform models, Regressionv2.m uses REML to estimate parameters. REML estimation is a variant of ML estimation in which the likelihood function is partitioned into components, allowing estimation of variance parameters in the model, θ, independently from the parameters involving means (Patterson and Thompson 1971; Cooper and Thompson 1977; Smyth and Verbyla 1996; Housworth et al. 2004). The estimates of the regression parameters, b_i, are just the GLS (or equivalently ML) estimates conditional on the estimate of the variance parameter. Specifically, the marginal log-likelihood function from which variance parameters are estimated is (Harville 1974)

$$\begin{aligned} L_{\mathrm{R}}(\sigma^2, \theta) = &-\frac{N-p}{2}\ln(2\pi) + \frac{1}{2}\ln[\det(\mathbf{X}'\mathbf{X})] \\ &- \frac{1}{2}\ln\{\det[\sigma^2\mathbf{V}(\theta)]\} - \frac{1}{2}\ln(\det\{\mathbf{X}'[\sigma^2\mathbf{V}(\theta)]^{-1}\mathbf{X}\}) \\ &- \frac{1}{2}(\mathbf{Y} - \mathbf{X}\hat{\mathbf{b}}_{\mathrm{GLS}})'[\sigma^2\mathbf{V}(\theta)]^{-1}(\mathbf{Y} - \mathbf{X}\hat{\mathbf{b}}_{\mathrm{GLS}}), \end{aligned} \tag{A3}$$

where θ is the transform parameter, p is the number of regression parameters in $\mathbf{b}$, N is the number of species (tips of the phylogenetic tree), and $\hat{\mathbf{b}}_{\mathrm{GLS}}$ is the GLS estimate of the vector $\mathbf{b}$ containing parameters b_0, b_1, …, b_p. The marginal log-likelihood function can be concentrated to remove the term σ^2 (e.g., Judge et al. 1985), so the REML estimate of the transform parameter can be obtained by maximizing the concentrated marginal log-likelihood function

$$\begin{aligned} L_{\mathrm{RC}}(\theta) = &-(N-p)\ln\left[\frac{1}{N-p}(\mathbf{Y} - \mathbf{X}\hat{\mathbf{b}}_{GLS})'\mathbf{V}(\theta)^{-1}(\mathbf{Y} - \mathbf{X}\hat{\mathbf{b}}_{\mathrm{GLS}})\right] \\ &- \ln\{\det[\mathbf{V}(\theta)]\} - \ln\{\det[\mathbf{X}'\mathbf{V}(\theta)^{-1}\mathbf{X}]\}. \end{aligned} \tag{A4}$$

Across a wide range of statistical models, REML produces less biased estimates of variance parameters than does ML, and simulations (not presented here) show that the same is true for the phylogenetic regression models analyzed by Regressionv2.m.

Although REML has good estimation properties, it does not directly give standard errors of the estimates. To obtain standard errors of the regression coefficients, we used GLS formulas with the variance-covariance matrix given with the REML estimate of θ. This ignores the uncertainty in the variance-covariance matrix caused by the uncertainty in the estimate of θ, although generally this introduces little additional uncertainty in the estimates of regression coefficients beyond that given by GLS formulas. Similarly, Regressionv2.m uses standard GLS formulas to compute the t scores and F scores for regression coefficients under the null hypothesis that they are 0.

For model comparision, we use ML approaches. The values of the marginal log-likelihood function (A3) used in REML can be used to compare fit of the variance components of

models, θ, in equation (A2). Nonetheless, they cannot be used to compare the fit of models including coefficients (b_0, b_1, b_2, etc.), although values of the likelihood function obtained from ML estimation can. The concentrated log-likelihood function used to obtain ML estimates of the transform parameter θ is

$$L_C(\theta) = -N\ln\left[\frac{1}{N}(\mathbf{Y} - \mathbf{X}\hat{\mathbf{b}}_{GLS})'\mathbf{V}(\theta)^{-1}(\mathbf{Y} - \mathbf{X}\hat{\mathbf{b}}_{GLS})\right] - \ln\{\det[\mathbf{V}(\theta)]\}. \quad \text{(A5)}$$

This likelihood function can be used to test for the statistical significance of the inclusion of parameters in nested models using likelihood ratio tests. For comparing nonnested models with different numbers of parameters (Burnham and Anderson 2002), we also compute the Akaike Information Criterion (AIC), which equals $-2\ln L(\theta_{ML}) + 2$(no. parameters), where $L(\theta_{ML})$ is the log likelihood at the ML estimates of θ and σ^2:

$$L(\sigma^2, \theta) = -\frac{N}{2}\ln(2\pi) - \frac{1}{2}\ln\{\det[\sigma^2\mathbf{V}(\theta)]\} - \frac{1}{2}(\mathbf{Y} - \mathbf{X}\hat{\mathbf{b}}_{GLS})'[\sigma^2\mathbf{V}(\theta)]^{-1}(\mathbf{Y} - \mathbf{X}\hat{\mathbf{b}}_{GLS}). \quad \text{(A6)}$$

The AIC is becoming widely used in phylogenetically based statistical methods (e.g., Butler and King 2004; McKechnie et al. 2006; O'Meara et al. 2006; Boyle and Conway 2007; Chown et al. 2007; Duncan et al. 2007).

Likelihood-based phylogenetic comparative methods can have poor statistical properties when sample sizes are small (e.g., Freckleton et al. 2002; Blomberg et al. 2003). Therefore, Regressionv2.m incorporates bootstrap methods (Efron and Tibshirani 1993) to obtain confidence intervals for all parameters, including the transform parameter θ. Bootstrapping is performed by resampling (with replacement) the transformed residuals, given by

$$\mathbf{e} = \mathbf{D}(\mathbf{Y} - \mathbf{X}\mathbf{b}),$$

where $\mathbf{D}$ is the Cholesky decomposition of $\sigma^2\mathbf{V}(\theta)$. Other orthogonal decompositions of $\sigma^2\mathbf{V}(\theta)$ could be used (Housworth et al. 2004), such as the singular-value decomposition, although the Cholesky decomposition as implemented by MATLAB maintains the ordering of decomposition axes in a more convenient fashion than other decompositions. Under the assumption that the fitted model is correct, the transformed residuals, $\mathbf{e}$, are independent. For each resampled set of transformed residuals, a bootstrap data set is reconstructed using the regression model. The bootstrap data set is then fit using the model, with all parameters estimated using REML. Repeating this procedure many times (2,000 is recommended for statistical tests with $\alpha = 0.05$) produces a collection of parameter estimates, and the distribution of these estimates approximates the distribution of the REML parameter estimator. For small sample sizes, the bootstrap confidence intervals and other tests of statistical significance are the gold standard. Bootstrapping also provides statistical diagnostics. For example, if the mean of the bootstrap estimator for a parameter is different from the parameter value estimated from the original data, this indicates that the REML estimations are biased (see also Ives et al. 2007).

Regressionv2.m also allows the user to identify interaction terms and categorical variables. For categorical independent variables (e.g., diet coded as 1–5 to represent omnivore, carnivore, herbivore, nectarivore, or frugivore, respectively) and categorical interaction terms, the program automatically codes dummy variables, and F values are provided to test the statistical significance of inclusion of each categorical variable (i.e., all dummy variables treated as a group). It is also possible to test for significance of categorical variables using a likelihood ratio test, and the results should be very close to those from F-tests (e.g., see tables in this article). Regressionv2.m allows missing values to be specified in the tip data matrix by entering "−9999" or "NaN" (in MATLAB, ver. 6 and higher). When the program encounters missing values, it automatically drops that species from the analysis, removing the corresponding row and column from the phylogenetic variance-covariance matrix. This is useful, as in this article, when different dependent or independent variables vary in sample size. One first constructs a phylogenetic tree for the maximum number of species in the entire data set and then creates the phylogenetic matrix from this full tree (see main text for description of how this is done). Note that if the branch lengths for the full phylogenetic tree have been specified by some arbitrary method (e.g., setting all segments equal to 1.0, Pagel's arbitrary method, Grafen's [1989, fig. 2] arbitrary method; see Garland et al. 1992, 2005), then they are not reset to that method by Regressionv2.m. Resetting to such arbitrary values would require that the user delete species manually from the full tree and then resave it and re-create the phylogenetic matrix.

In GLS, RegOU, and other models that include covariance among residuals, we need to reiterate the caution given by Judge et al. (1985, p. 31), that "the potential for misusing R^2 is high." The formula that we use for R^2 (eq. [2.3.16] in Judge et al. 1985) takes values between 0 and 1 and is monotonically related to the F statistic used to test the null hypothesis that regression slopes differ from 0. Nonetheless, because the residuals are not orthogonal, it is difficult to ascribe portions of the explained variation to different independent variables, and therefore we do not report partial R^2 values. Moreover, we remind readers that the R^2 values for OLS models are not comparable with those from GLS, RegOU, and related models.

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