Title: Rapid gastrointestinal tract transit time in volant vertebrates, with implications for gut microbiome convergence: Results from a meta-analysis

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Availability of data and material
The datasets generated and the code used for analysis for the present study are available at: https://github.com/brooklabteam/git-transit-time.git

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ABSTRACT:

Flying birds and bats have simplified gastrointestinal tracts (GITs) and low intestinal mass to support flight. While previous work showed reduced GIT passage times in birds relative to other vertebrates, GIT passage times have never been collectively quantified for bats. We conducted a meta-analysis of published digesta passage times across vertebrates, comparing volant and non-volant vertebrates, while considering the effects of body mass and diet. We hypothesized that, like flying birds, bats have significantly faster digesta passage times relative to nonvolant vertebrates, likely due to their adaptations for flight. Our study supports this, revealing significant differences in passage times among flying and non-flying groups, with bats exhibiting faster transit times compared to non-volant vertebrates. Using a phylogenetic comparative analysis, we show that flight and diet have a strong effect on GIT transit times across diverse taxa, while body mass plays a more limited role. Accelerated transit times in bats likely promote the rapid turnover of gut contents, which may contribute to their distinct GIT microbe compositions. Unique among mammals, bat GIT microbiomes are dominated by Pseudomonadota bacteria, a pattern also observed in flying birds. We hypothesize this convergence may result from rapid GIT transit times quantified here for volant taxa.

INTRODUCTION

Flying vertebrates (e.g., birds and bats) have unique and convergent gastrointestinal physiologies, likely evolved to facilitate powered flight [1]. As one such adaptation, flying birds are known to have rapid gastrointestinal tract (GIT) passage times [2,3] (i.e., the time taken for food to pass through the stomach, small and large intestine, and colon prior to excretion [4]), a strategy hypothesized to help them maintain low digesta volume during flight [3]. The passage of digesta through the GIT is affected by many factors, several of which converge in flying birds and bats. In flying birds, rapid GIT transit is facilitated by short intestine lengths and elevated defecation and consumption rates to meet energy demands [2,5]. Bats share the short intestine lengths [6,7] and high defecation rates of flying birds [8,9]; in turn, faster digesta passage times also enable an increase in consumption rates during foraging [8]. Despite this, digesta passage times have never been collectively quantified for bats. We explore whether convergence in GIT physiology may be mirrored by convergence in digesta passage time in these two taxonomic groups.

Anatomically, longer and more complex GITs (e.g., multiple stomach chambers such as those found in herbivorous ungulates) tend to increase digesta passage time [10,11], whereas shorter, simpler GITs may decrease transit time [6,12]. Nonetheless, very few studies have quantified the correlation between GIT length (which scales allometrically with body size [13,14]) and transit time (but see [15–17]). As previously reviewed [12], relative to non-flying mammals, both small birds and bats have short, simple digestive tracts, with reduced nominal surface area and intestinal tissue [6,18]. Most bat GITs also lack a cecum and appendix, organs with specialized digestive properties in other taxa [18–21]. To compensate for the loss of intestinal absorptive surface area important for nutrient uptake in the GIT, both flying birds and

bats have greater villous amplification (i.e., increase in digestive surface area) in the GIT epithelium and exhibit greater reliance on paracellular absorption (e.g., the passive uptake of water-soluble nutrients in the GIT) for meeting energetic demands [6,7].

In addition to anatomy, diet composition is correlated with differences in digesta passage times—though these differences may be most directly modulated by ingestion rate [22], which, in turn depends on nutrient composition (e.g., lower nutrient diets necessitate higher ingestion rates, which, consequently, yield more rapid GIT transit) [23]. Nonetheless, some evidence indicates that the water and fat content, consistency, and hardness of ingested food (e.g., digestibility) can directly impact passage time [24–26]. For instance, insectivorous bats retain food longer than frugivorous bats, and some studies attribute this lengthier retention to a diet of harder food—though, notably, differences in intake volume may confound this relationship [27–29]. Evidence from pollen-eating bats indicates that the degree of dietary specialization is related to the efficiency of digestion as well: for example, flower-specialist bats digest pollen into usable protein resources more rapidly than generalist species [30,31]. Similarly, among insectivorous bats, the shortest GIT passage times have been recorded for trophic specialists [28]. It is also hypothesized that the microbiome may play a larger role in nutrient extraction for diet specialist bats and could explain some of the differences in the efficiency of digestion and gastrointestinal design, as well [32].

Interactions between the GIT microbial community and the host diet are modulated by digesta passage rates [12,33,34]. In humans, GIT transit is directly, positively correlated with total bacterial count [35,36] and positively associated with the presence of slow-growing gut microbes, such as methanogens [37,38]. In migratory bats, the core phyla of the GIT microbiome remain relatively stable despite major diet shifts due to long distance migration, possibly due to

rapid digesta passage limiting microbiome restructuring [39]. Digesta passage times may also impact the capacity in which GIT microbes produce metabolites for their hosts. For example, short chain fatty acids (SCFAs) are produced through fiber breakdown by GIT microbes. SCFA production, in turn, stimulates intestinal motility for more rapid transit. High SCFA concentrations are also typically associated with lower GIT pH, which is associated with reduced transit time [34].

While most mammalian GIT microbiomes that have been surveyed to date (mainly those of Orders Artiodactyla, Carnivora, Primates, and Xenarthra) are primarily comprised of bacteria in the phyla Bacillota or Bacteroidota [40], both flying bird and bat GIT microbiomes are dominated by bacteria in the highly diverse phylum Pseudomonadota (previously Proteobacteria) [41–43]. This convergence of unusual gut microbiome composition between bats and birds suggests a possible shared mechanism; as Pseudomonadota are well known for their success in rapidly changing (and often aerobic) GIT environments [44–46], it is possible that similarly rapid GIT transit times in bats and flying birds may contribute to their proliferation in these two clades. Nonetheless, GIT transit has never been collectively summarized for bats.

In this study, we compiled GIT passage times from the literature for a wide range of volant (bats and flying-birds) and non-volant (non-flying birds, carnivores, ungulates, rodents, primates, reptiles) vertebrate taxa. Using a phylogenetic comparative analysis, we tested the effects of flight, mass and diet on digesta passage times across taxa. We show that flying vertebrates (e.g., bats) do indeed have significantly faster GIT passage times than non-volant vertebrates, offering one possible explanation for the congruent GIT microbiomes of bats and flying birds.

METHODS

Literature survey and statistical approach

We collected GIT passage times from the primary literature across a wide range of vertebrate taxa to assess the impact of flight on the duration of time required for food passage in the GIT. We constructed our dataset of GIT passage times by first conducting a strict keyword search for primary literature. First, we searched Web of Science for publications which cited the following search criteria in the title or abstract: “gut OR gastrointestinal OR food” AND “transit OR passage” AND “rate OR time”. To focus our study on non-model animal systems, we excluded articles that referenced human and laboratory mouse and/or rat studies using the “NOT” function for the following off-target terms: human[s], [wo]men, obesity, diabetes, adult, child[ren], patient[s], volunteer[s], disease, mice/mouse, rat[s]. This yielded 1169 studies.

We next individually reviewed each of the 1169 studies and retained any study (106) that reported “minimum gut transit” (MGT), “mean retention time” (MRT), “transit time”, or “gut passage time” for any vertebrate taxon in our final database. We then manually curated the database, adding 20 other published studies on digestive passage cross-referenced in the original database. We examined each study to confirm that the value reported corresponded to a measure (e.g., transit time, MRT) of the duration of time between food consumption to first passage in feces, to ensure these values were comparable. Though MRT, the average period of time food is retained in the gut, offers an arguably more accurate representation of the digesta passage rates relevant to the establishment of GIT microbial communities [47], we found transit time (sometimes referenced as MGT) to be more widely reported in the literature across multiple vertebrate taxa and offer a larger pool of species for comparison than MRT. Thus, we report results for GIT transit time in the main analysis and results for MRT in the supplemental

analysis, using the same statistical approach for both datasets (appendix 1). Of the studies reviewed, we identified 119 publications that reported GIT transit times for 131 species, and 49 publications that reported MRT for 74 species for inclusion in our final database (https://github.com/brooklabteam/git-transit-time).

Several factors may influence digesta passage time. Dry matter intake (DMI), or the quantity of food consumed, has been proposed as an influential predictor of digesta passage [48–50]. However, we did not include DMI in our analysis as these data were unavailable across many studies, and we aimed to prioritize species richness in our dataset. Despite our thorough review, we caveat that our database may have under- or overrepresented some taxa, and it is possible that some species were missed (in particular see Abraham et al. 2021 [51]).

From each study, we extracted the following information for each animal surveyed and recorded it in the final database (Supplementary Dataset 1): (1) Order/Family/Genus/Species/Common Name, (2) body mass (grams), (3) food included in feeding trial ("experimental diet", a broad description), (4) mean or median GIT transit time (or MRT) and, when available, minimum/maximum, and standard error/deviation of reported time, and finally (5) number of individuals and number of trials included in survey. We caveat that our conclusions may be limited by discrepancies between the experimental diet recorded in the trial and an organism's natural diet outside captivity; however, manipulation of experimental diet was most likely to directly influence the GIT passage results surveyed here. We categorized experimental diets as dead, fiber/foliage, fruit/nectar/pollen, meat, and protein. The experimental diet for animals that were sampled lethally (n=3 species) was recorded as "dead." Experimental diets including dog or cat food, dairy, eggs, insects, and peanut butter were recorded as "protein," whereas diets including beef, chicks, fish and mice were recorded as "meat." In cases

where a single study reported passage times for multiple individuals of the same species, we averaged the passage time per species reported from that study prior to further statistical analyses. When the transit time or MRT values were only reported in a figure, not as values in a table, we used PlotDigitizer (htts://plotdigitizer.com) to extract data from graphs. When some of the species-level information (i.e., body mass) was missing from the original study, we searched the literature to find average values for the taxon in question. We recorded the source (if not the original study) and the method of data summarization for each entry in our database, adjacent to the metric in question.

We next compared GIT transit times across the different taxonomic groups surveyed. First, we used the unique transit time value for each entry in the database, preferentially selecting the mean reported value, then the median if mean was not reported. We then calculated an average transit time value for each species in our database, weighting database entries by the number of individuals surveyed and the number of trials conducted in each original study. When this original sample size information was not included in the study (n=7), we assumed a sample size and trial size of only one individual in the weighting. We used the same approach to summarize average mass for each species reported in our database. Once the dataset was collapsed to a single transit time (and MRT when available) and mass for each species, we then compared digesta passage times across disparate taxa.

Due to small sample sizes, we elected to categorize all non-mammalian vertebrates taxonomically by Class (specifically, Birds, Fish, Amphibians, Reptiles) and all mammals by Order. We separated birds into “Flying” and “Non-Flying” categories, though transit time was only reported for two species of non-flying birds and MRT for an additional three species of non-flying birds. After assigning these groupings, we subset the database to include only those

taxonomic groupings with more than two reported species and those for which diet fed during the experiment was also reported. We removed two additional taxa (*Uromacer oxyrhynchus* and *Atheris squamigera)* because we could not find their masses reported in the literature. This reduced our database for comparison down to GIT transit times for 112 species and MRTs for 60 species.

Phylogenetic structure

To account for the possible role of phylogenetic relatedness in driving species-level patterns in transit time and MRT, we used the program TimeTree: The Timescale of Life [52] to construct a time-scaled phylogeny. All species represented in the cleaned database were available on TimeTree, except for n=5 species for transit time (*Eptesicus innoxius, Acanthagenys rufogularis, Cercopithecus albogularis, Cercopithecus mitis, Cercopithecus pogonias*) and n=2 species for MRT (*Cercopithecus mitis, Cercopithecus pogonias*). We removed these species from the final database for comparison, leaving our final numbers at n=107 species for transit time analysis and n=58 for MRT analysis (Table 1).

Phylogenetic signal

Before conducting our main analyses, we tested for a phylogenetic signal (phylosig) [53] for both transit time and MRT using the phytools package in R [54]. We quantified the strength of the phylogenetic signal by Pagel's lambda, which can vary from no signal (0) to a strong signal (1) [51]. Because lambda values recovered were high (see Results), we conducted all subsequent model comparison including the phylogenetic signal appropriate for the data, using the nlme package in R (version 2023.9.1) [55].

Model Structures and Comparisons: Predictors of Transit Time in Vertebrates

We constructed a series of candidate generalized least squares (GLS) models to test how flight, experimental diet, and body mass influence gastrointestinal transit time and mean retention time, while accounting for phylogenetic relatedness. All models were fit by maximum likelihood (ML) using nlme functions (gls, lme) [56]. For GIT transit, we compared model fits assuming λ=1 (full phylogenetic signal equivalent to Brownian motion) or λ=0 (no phylogenetic signal). For MRT, we compared model fits assuming λ=1, 0, or 0.89 (as estimated for our data by phylosig; see results).

We first tested for the effect of flight (flyer vs. non-flyer) on the response variable of transit time and MRT, without accounting for mass or diet, but comparing model structures accounting for phylogenetic structure (λ=1 and also λ=0.89 for MRT) against those which did not (λ=0). We next asked whether inclusion of the additional predictor variable of experimental diet improved model fit to the data. For this second analysis, we compared structures modeling diet as a fixed or a random effect, again testing models accounting for phylogeny against those which did not. We next evaluated whether the addition of taxon mass as a fixed effect improved model fit to both GIT transit and MRT data, again comparing models accounting for phylogeny against those which did not. We considered disparate model structures accounting for mass and flight exclusively, in addition to those considering mass, flight, and transit time simultaneously. Finally, we also tested whether mass alone (with no consideration of flight) was significantly predictive of transit time, both when accounting for phylogeny and otherwise.

In all cases, models were compared using Akaike Information Criterion (AIC) [57], with ΔAIC $\geq$ 2 considered support for selection of one model over another. In cases where models

yielded equal support by AIC, we preferred the simplest model. For specific model structures see Table 2.

RESULTS

Phylogenetic structure

We identified a significant phylogenetic signal in the GIT transit time data, at $p<0.0001$ with Pagel's lambda = 1.007 (logL = -731.72, LR = 54.724; Figure 1), and in the MRT data, at $p<0.0001$ with Pagel's lambda = 0.89 (logL = --234.72, LR = 22.43; Figure S1). The higher estimate for Pagel's lambda indicated a stronger phylogenetic signal in the GIT transit vs. MRT data, likely due to larger sample size (n= 107 species for GIT transit vs. n=60 species for MRT). Subsequently, we constructed models for GIT transit and MRT data which incorporated both $\lambda=1$ and $\lambda=0$ and analyzed MRT data with an additional model that specified $\lambda=0.89$.

Comparative Predictors of GIT Transit Time in Vertebrates

Phylogenetic comparative analyses revealed strong effects of flight and diet on GIT transit time, while body mass played a more limited role. Across all models, incorporating phylogenetic structure ($\lambda = 1$) consistently improved fit, as indicated by lower AIC values compared to models without phylogenetic structure (Table 3; Table S1).

In models that included flight as the sole fixed predictor, both with and without phylogenetic structure, flying species had significantly shorter transit times relative to non-flying taxa ($p = 0$ for non-phylogenetic flight-only model; $p = 0.015$ for phylogenetically-informed flight-only model; Table S1). Because the effect of flight retained significance even after accounting for phylogeny, we concluded that flight itself—independent of phylogeny—has a significant negative relationship with GIT transit.

Incorporation of experimental diet as a fixed effect (but not as a random effect), in addition to flight, improved the fit of a phylogenetically-informed model over the flight-only model (Table 3; Tables S1; flight + diet models). Here, an experimental diet of fruit, nectar, or pollen was associated with reduced transit time, though only with marginal significance (Table S1; p=0.052). By contrast, inclusion of body mass as a fixed effect did not improve the fit of a phylogenetically-informed model over either a Flight-only model or a model including fixed effects of both flight and experimental diet (Table 3; flight + mass models and flight + mass + diet models). Nonetheless, body mass demonstrated a marginally significant positive correlation with GIT transit in flight + mass models (Table S1, p=0.10) but was not significant when also accounting for experimental diet (flight + mass + diet models); in this latter case, experimental diet retained largely the same significance as in flight + diet models not including mass. This result suggests that any effect of mass on GIT transit, additional to flight and phylogeny, can be largely explained by experimental diet. Intriguingly, when we tested for the effects of body mass on transit time without considering flight (mass-only models), we found that body mass was a significant positive predictor of transit time only when phylogeny was ignored (Table S1, p = 0) but was not a significant predictor in a phylogenetically-informed model (p = 0.2). These results suggest that the association between mass and GIT transit time is better attributed to phylogenetic structure than mass itself. In summary, our analyses identified flight as the most consistent significant predictor of reduced transit time, but models improved when diet was additionally included as a fixed effect.

The phylogenetic distribution of GIT transit time further illustrates these trends (Figure 1). Flying clades, including birds and bats, consistently cluster toward lower trait values (e.g., faster transit times; orange-red branches), while non-flying mammals and reptiles exhibit longer

transit times (e.g., slower transit times; green-blue branches). Frugivorous bats and birds (i.e., *Manacus vitellinus* and *Pteropus rufus*) exhibit the lowest (fastest) GIT transit times, consistent with model results suggesting a dietary effect.

Comparison of digesta passage time metrics: GIT transit time and MRT

Across both metrics of digesta passage, GIT transit time and MRT, the best supported models were phylogenetically informed and included flight as a key predictor. However, in contrast to GIT transit models, inclusion of diet as either a fixed or random effect, in addition to flight, did not improve fit in phylogenetically-informed MRT models; rather, the flight-only and flight + mass models offered the best fit to the data (Table S1). Consistent with GIT transit time models, in MRT models, we observed that body mass alone was a significant positive predictor of MRT only when phylogenetic structure was ignored (Table S2, Table S3, mass-only models, $p = 0$) but not when phylogenetic structure was modeled at either $\lambda = 1$ or $\lambda = 0.89$, as estimated by phylosig. These results again suggested that the positive association between mass and transit time (here, MRT) can be largely explained by phylogeny; nonetheless, we did recover equivalent support for flight-only and flight + mass models of MRT data. All else being equal, we preferred the simplest model construct and concluded that flight is the strongest predictor of MRT in a phylogenetically-informed context (Fig. S1; Fig. S2). Taken together, both GIT transit time and MRT analyses demonstrate a significant association between flying vertebrate taxa (bats and birds) and faster digesta passage than exhibited by non-flying vertebrates.

DISCUSSION

Understanding the factors influencing digesta passage times across vertebrate taxa is crucial for unraveling the intricate relationship between digestive physiology, diet, and

evolutionary adaptations [48,51]. In this study, we aimed to compare digesta passage times among volant (bats and flying birds) and non-volant vertebrates while considering the effects of flight, experimental diet, and body mass. Our findings shed light on the unique digestive adaptations of flying mammals – i.e., bats – and draw attention to the impacts that these adaptations may have on GIT microbial communities.

Consistent with previous research [10,16], our study reveals significant differences in digesta passage times among flying vertebrate groups. In our analyses, flight was the most influential predictor of digesta passage time across both GIT transit time and MRT, with flying species demonstrating rapid digesta passage compared to non-flyers, after controlling for effects of phylogeny, experimental diet, and mass. Including experimental diet as a fixed effect improved model performance over flight-only models for GIT transit time, suggesting that frugivory, nectarivory, or pollen consumption may be associated with faster GIT transit. This dietary effect may be driven by the frugivorous and nectivorous bats, which have some of the fastest transit times in our dataset. Because flight imposes exceptionally high metabolic demands, birds and bats have evolved to process food swiftly and extract energy-rich compounds with great efficiency. These rapid passage times may also reflect high defecation frequencies through which quickly clearing undigested waste (i.e. feces) minimizes excess body mass to better support highly mobile lifestyles [9,58].

We found that body mass had a more limited effect on digesta passage time relative to flight, especially after taking phylogeny into account. Generally, larger-bodied species exhibited longer transit times, consistent with some allometric scaling theory equating larger mass and longer gastrointestinal tracts [13]. Nonetheless, we observed that the effects of mass on both GIT transit and MRT vanish when accounting for phylogeny, in keeping with prior literature that

suggests that the universality of predictable allometric scaling of GIT length with body size is inconclusive [48,51,59]. Although we observed GIT transit time to scale positively with body mass across most vertebrates, volant species, regardless of size, consistently exhibit reduced baseline transit times relative to non-volant species. In general, flying vertebrates demonstrate both reduced body mass and shorter intestines compared to most non-volant mammals [3,14], which we here show to be associated with rapid GIT transit. This pattern indicates an ecological trade-off imposed by flight, as rapid GIT transit likely reduces opportunities for nutrient uptake in flighted taxa and may contribute to convergence in microbiome composition across flying vertebrates. This hypothesis is supported by the convergent dominance of Pseudomonadota in volant bats and birds, in contrast to non-flying taxa (including flightless birds) [43]. Future research should explore the functional significance of GIT microbiome composition in flying vertebrates, with implications for both host nutrient acquisition and immunity.

DECLARATIONS

IRB statement: Not applicable.

Author's contributions: ECR conducted the final data analysis and wrote the first draft of manuscript. KGM collected data from the literature, helped with the data analysis, and shared in the writing of the first draft of manuscript. DNJS conceptualized the project, collected the original data from the literature, and contributed to writing of the manuscript. NAFR helped with the data analysis and collection. NCD reviewed the data and contributed to writing of the manuscript. CJY helped with the data analysis and writing. RKP conceptualized the project and provided funding. CEB conceptualized the project, collected the original data, helped with data

analysis, funded the project, and contributed to writing of the manuscript. All authors reviewed and provided edits to the final manuscript.

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Table 1. *Species counts per taxonomic group for digesta passage metrics (GIT transit time and MRT).*

Taxonomic Grouping	**Number of species**	
	GIT transit time	**Mean retention time**
Rodents	13	14
Flying Birds (Class Aves)	12	NA
Non-Flying Birds (Class Aves)	NA	3
Bats	35	12
Carnivores	6	NA
Primates	18	17
Ungulates (order Artiodactyla)	7	12
Reptiles	16	NA

Species counts per taxonomic grouping for GIT transit time (n=107) and MRT (n=58) included in the final database for analyses. NA indicates taxonomic groupings that had 2 or fewer species and thus were not included in the final analyses.

Table 2. Outline of models included in our comparative analysis. Models were compared using both λ=1 and λ=0. For MRT, an additional lambda set by phylogenetic structure was used (λ=0.89).

Model Set	Model name	Conceptual Structure	model	R Syntax Example
1. Flight models	GIT_0; GIT_1	Transit time ~ Flight (flyer vs. non-flyer)	gls	log_transit_hrs ~ flyer
2. Flight + Diet models	GIT_0_diet; GIT_1_diet; GIT_0_diet_random; GIT_1_diet_random	Transit time ~ Flight + Diet (fixed effect or random effect)	gls and lme	log_transit_hrs ~ flyer + trial.diet log_transit_hrs ~ flyer, random = ~1 | trial.diet
3. Flight + Mass models	GIT_0_mass; GIT_1_mass; GIT_1_mass_simple	Transit time ~ Flight * Mass (interaction tested; simplified to additive if unsupported)	gls	log_transit_hrs ~ flyer * log10(mass_kg) log_transit_hrs ~ flyer + log10(mass_kg)
4. Flight + Mass + Diet models	GIT_1_mass_simple_diet; GIT_1_mass_simple_diet _random	Transit time ~ Flight + Mass + Diet (Diet as fixed or random)	gls and lme	log_transit_hrs ~ flyer + log10(mass_kg) + trial.diet log_transit_hrs ~ flyer + log10(mass_kg), random = ~1 | trial.diet
5. Mass-only models	GIT_0_mass_only; GIT_1_mass_only	Transit time ~ Mass (tested with and without phylogenetic correlation)	gls	log_transit_hrs ~ log10(mass_kg) log_transit_hrs ~ log10(mass_kg)

Table 3. **Model comparison results for GIT transit.** Top supported models are highlighted in bold– the model that included flight and diet as predictors of GIT transit time had the lowest AIC; however, the model that also included mass (in addition to flight and diet) had nearly the same AIC. Models including the full effects of phylogeny (λ=1) had the most support. Overall, flight was negatively associated with transit time, even after accounting for phylogeny, diet, and mass-effects.

Model name	Predictors	Phylogeny	df	AIC	Interpretation
GIT_0	Flight	λ=0	3	-160.46	Flight is negatively associated with transit time (p=0.015), even accounting for phylogeny.
GIT_1		λ=1	3	-309.68	
GIT_0_diet	**Flight + Diet**	λ=0	7	-184.25	**Flight (p=0.003), fruit/nectar/pollen marginal (p=0.052)**
GIT_1_diet		**λ=1**	**7**	**-314.67**	
GIT_0_diet_random	Flight + 1|Diet	λ=0	4	194.81	Diet is better as a fixed effect
GIT_1_diet_random		λ=1	4	197.33	
GIT_0_mass	Flight*Mass	λ=0	5	-160.8	Flight only marginally significant (p=0.1)
GIT_1_mass		λ=1	5	-308.95	
GIT_1_mass_simple	Flight + Mass	λ=1	4	-310.4	Mass marginally significant (p=0.1); flight remains significant **As AIC is nearly equivalent, prefer the simplest model.*
GIT_1_mass_simple_diet	**Flight + Mass + Diet**	**λ=1**	**8**	**-313.1**	**Nearly equivalent to diet-only model GIT_1_diet**
GIT_1_mass_simple_diet _random	Flight + Mass + 1|Diet	λ=1	5	192.00	Not supported
GIT_0_mass_only	Mass	λ=0	3	-110.62	Mass appears significant without phylogeny (p<0.01)
GIT_1_mass_only	Mass	λ=1	3	-307.12	Mass weak when accounting for phylogeny (p=0.065)

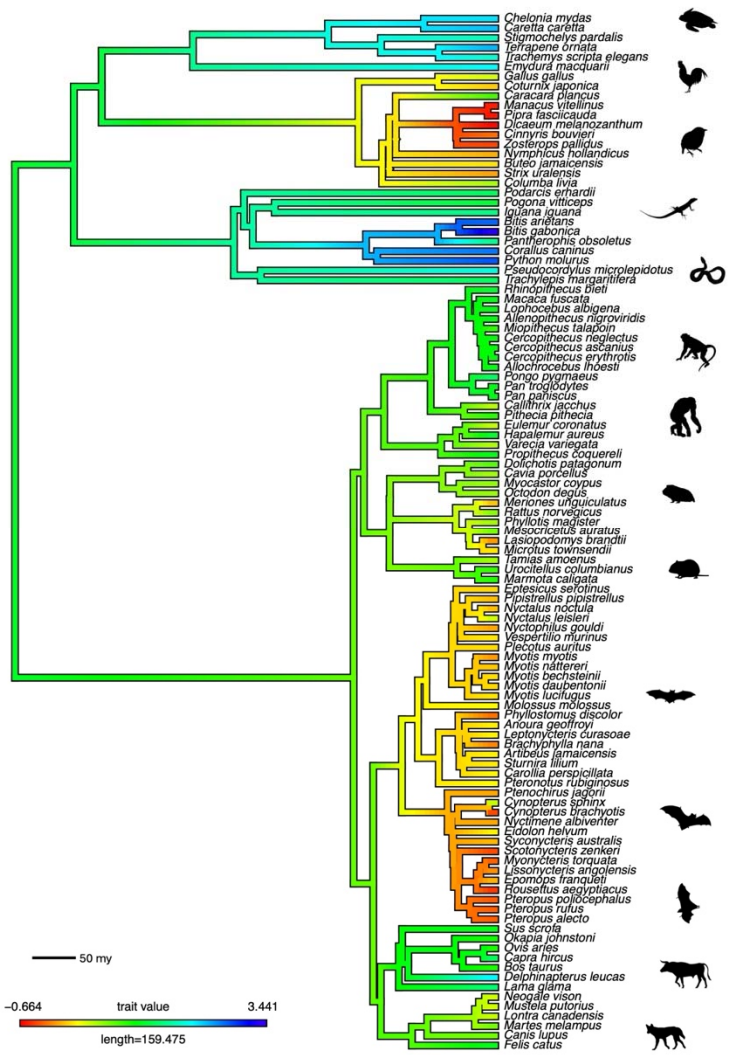

Figure 1: *Phylogenetic mapping of GIT transit time across vertebrate taxa.* A continuous trait map illustrating the evolution of $\log_{10}$-transformed GIT transit time across the phylogenetic tree of species included in our analysis. Branch colors represent reconstructed ancestral state trait values for relative GIT transit time, using a continuous gradient from slow (blue) to fast (red) transit times. The ancestral trait reconstruction was estimated using maximum likelihood, with tip labels corresponding to species names. The scale bar indicates relative branch length (in millions of years), and the color legend shows the range of trait values included in the reconstruction. Turtles (e.g., *Terrapene ornata*, *Caretta caretta*) and snakes (e.g., *Bitis gabonica*, *Python molurus)* show the slowest transit times, whereas bats (e.g., *Pteropus rufus*) and flying birds (e.g., *Pipra fasciicauda*) show the fastest transit times.

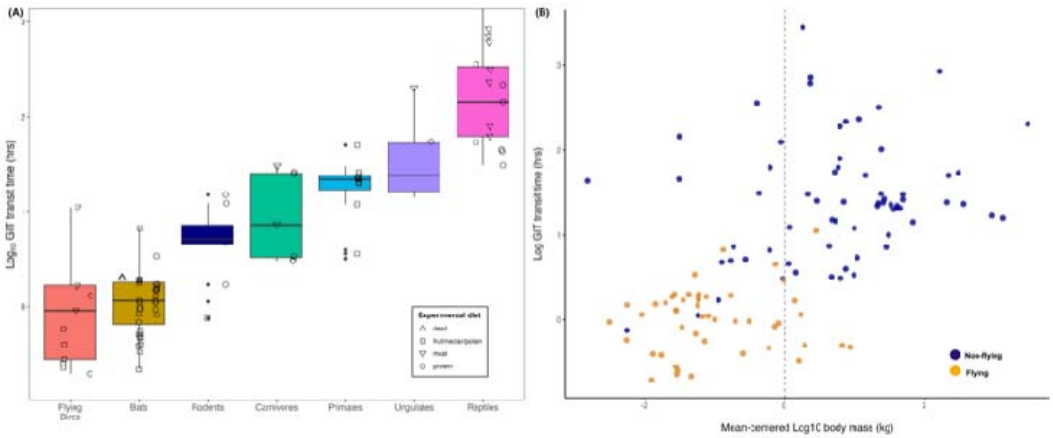

Figure 2: *GIT transit times from experimental studies across volant and nonvolant vertebrates*. **(a)** Boxplots show the interquartile range of GIT transit times (y-axis, $\log_{10}$ scale) recovered from studies across diverse vertebrate taxa (x-axis). Shapes correspond to experimental diet (according to the legend). Flying vertebrates (e.g., flying birds and bats) have significantly faster GIT transit times relative to all non-flying vertebrates; additionally, fruit/nectar/pollen diets are associated with more rapid GIT transit (Table S2). **(b)** GIT transit time (y-axis, $\log_{10}$ scale) for flyers (orange) and non-flyers (blue) relative to their body mass (in kg; x-axis, $\log_{10}$ scale). Though flight + diet models offered the best fit to the GIT transit data, flight + mass models also

performed well (Table 3) and demonstrated a weak positive association between body mass and GIT transit time.

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Gastrointestinal tract (GIT) transit time (hrs)
Taxonomic grouping
GIT transit time (hrs)
Mass (g)
Non-flying
Flying