

Galliformes exhibit reduced cardiorespiratory morphology yet similar skeletal mass compared with other gamebirds

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ABSTRACT—Flight is one of the most effective yet energetically demanding means of movement. Its energy costs are normally associated with interspecific variation in efficiency, size of organs, and physiological systems that reflect different flight capacities. Adaptive morphological variation may be constrained by physical demands that vary with body size, but work remains to place allometry associated with flight mode in an ecological and evolutionary context. We predicted that heart, lung, and skeletal masses, as well as tracheal diameter, should show a reciprocal positive correlation and have reduced size in species with poor flight capacities. We measured tracheal diameter together with lung, heart, and skeletal mass in 21 species of medium to large body sized game birds. We then compared 7 of these species categorized as short flyers belonging to the order Galliformes with 14 other species capable of sustained flapping flight. Our comparative analyses incorporating phylogeny revealed that once accounting for body mass, Galliformes had smaller heart mass compared to other species, as shown in few previous studies. Additionally, we detected reduced tracheal diameter and lung mass in Galliformes, suggesting the presence of morphological pleiotropy predicted under the concept of symmorphosis. These organs all showed different levels of reciprocal correlations due to their functional connection. Residual skeletal mass was independent of flight capacity and showed no relationship with heart mass, thus indicating that selection for reduced skeletal mass may have already maximized how light a bird skeleton can be, constraining mass-independent variation in this trait. Our results suggest that cardiorespiratory organs have evolved symmorphic variation in size that reflects costs and benefits associated with different flight capacities. However, our observed differences are confounded with phylogenetic history, so additional comparative studies are needed to rigorously test this hypothesis. *Received 18 June 2020. Accepted 1 October 2021.*

Key words: Anseriformes, avian anatomy, body mass, organs size, symmorphosis.

Los Galliformes tienen una morfología cardiorespiratoria más reducida y masa esquelética similar a la de otras aves de caza

RESUMEN (Spanish)—El vuelo es una de las formas de movimiento más efectivas y energéticamente más demandantes. Sus costos energéticos están normalmente asociados con variación interespecífica en eficiencia, tamaño de órganos y sistemas fisiológicos que reflejan diferentes capacidades de vuelo. La variación morfológica adaptativa podría estar limitada por las demandas físicas que varían con el tamaño corporal; sin embargo, queda pendiente el trabajo que ubique la alometría asociada con el modo de vuelo en un contexto ecológico y evolutivo. Nuestra predicción es que el corazón, pulmones y masas esqueléticas, así como el diámetro traqueal, debieran mostrar una correlación recíproca positiva y hayan reducido su tamaño en especies con escasas capacidades de vuelo. Medimos diámetro traqueal junto con pulmón, corazón y masa esquelética en 21 especies de aves de caza medianas a grandes. A continuación comparamos 7 de estas especies categorizadas como voladoras en corto y pertenecientes al orden Galliformes con otras 14 especies capaces de vuelo activo sostenido. Nuestros análisis comparativos que incorporan la filogenia revelan que una vez que controlamos el tamaño corporal, las Galliformes tenían un corazón de menor masa comparadas con otras especies, como han mostrado un pequeño número de estudios previos. Adicionalmente, detectamos diámetros traqueales reducidos y masa de pulmones en Galliformes, lo que sugiere la presencia de una pleiotropía morfológica prevista bajo el concepto de simorfosis. Estos órganos mostraron diferentes niveles de correlación recíproca debido a su conexión funcional. La masa esquelética residual fue independiente de la capacidad de vuelo y no mostró relación con la masa del corazón, indicando con ello que la selección para una masa esquelética reducida podría haber maximizado qué tan ligero puede ser el esqueleto de un ave, limitando la variación de masa independiente en esta característica. Nuestros resultados sugieren que los órganos cardiorespiratorios han evolucionado una variación simórfica que refleja los costos y beneficios asociados con diferentes capacidades de vuelo. Sin embargo, las diferencias observadas se confunden con su historia filogenética, por lo que se necesitan estudios comparativos adicionales para someter rigurosamente a prueba esta hipótesis.

Palabras clave: anatomía aviar, Anseriformes, masa corporal, simorfosis, tamaño de órganos.

While most birds fly, not all species practice flight with the same frequency and intensity

(Rayner 1988). This difference is germane when comparing species capable of sustained active flight to those that are not. Most species in the order Galliformes fall in the latter category. Galliformes are mostly medium-sized to large birds similar to the domestic chicken (*Gallus gallus*) that are generally terrestrial and show short and rapid takeoff followed by little flapping and gliding for short distances. These birds predominantly use flight to move between feeding and

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roosting areas or to escape predators (Davis et al. 2014). In contrast, most members of other orders such as Anseriformes, Charadriiformes, or Columbiformes use flight for daily and seasonal movements at a scale that researchers have compared with a human trip to the moon (Guglielmo 2010) and that can lead to the evolution of traits associated with a “migratory syndrome” (Piersma et al. 2005). These different modes of locomotion are associated with different energetic costs (Gwinner 2012) that are under intense selection (Newton 2007), leading to behavioral (Alerstam 2011), physiological (Hedenström 2008), and morphological (Marsh 1983) adaptations.

Adaptive differences in organ size are particularly interesting to explore because they must occur within stringent constraints imposed by body mass (Schmidt-Nielsen 1984). Mass-independent residual variation in organ size may still occur given strong enough selective advantages. Yet, such modifications may also be limited because evolution has already optimized these traits (Hedenström 2008). Indeed, highly efficient circulatory and respiratory systems and extremely light skeletons that allow flight in birds are among the best-known examples of adaptive evolution in biology (Gill 2007). Alternatively, evolution may prioritize the emergence of wing traits by modulating lift:drag ratio, rather than influencing the size of organs that are constrained by body size and can be energetically expensive to maintain (Vágási et al. 2016). Still, species showing sustained flight often have larger hearts and higher hematocrit density controlling for body size, while short flyers such as Galliformes show the opposite (Magnan 1922, Bishop 2005, Krause et al. 2016, Nespolo et al. 2018). These studies support the *heart-size hypothesis*, which suggests that since larger hearts can pump a higher volume of blood with each stroke (Grubb 1983), they are able to provide more oxygen to fuel the metabolic costs of active sustained flight. However, current support comes from mathematical models, or meta-analyses of data collected with different methodologies, and may benefit from studies adopting standardized organ measurements collected within a restricted geographic area and time of the year. More importantly, a deeper understanding of the evolution of physiological systems requires careful study of other organs and morphological traits functionally related to flight performance.

Hearts may also evolve in synchrony with a wide range of organs and tissues that function together to affect metabolic performance. For example, higher blood volumes from larger hearts need to be oxygenized at a higher rate (Bishop 1997). As a consequence, the size of tracheas, lungs, and skeletons may evolve in concert (Grubb 1983) to match functional demands of the organism as predicted under the concept of symmorphosis (Weibel et al. 1991). The emergence of this morphological pleiotropy may be limited by ecology, phylogeny, and altitude that can influence respiratory physiology (Carey and Morton 1976, Maina 1993, Scott 2011). These factors may confound the variation associated to flight modes and the expected correlations among organs but direct interspecific tests of this hypothesis are lacking.

Skeletons provide attachment sites for muscles and experience a wide range of physical stressors during flight. Structure, morphology, and composition of the avian skeleton are comprehensively adapted for flight (Feduccia 1999), and flight-capable birds have lighter bones compared to flightless species (Habib and Ruff 2008) and higher investment in forelimbs relative to hindlimbs (Wright et al. 2016). However, whether skeletons have also undergone further adaptive evolution as a function of different flight modes also remains unclear. Previous studies show additional differences in the skeletal morphology of sedentary versus migratory songbirds (Singh et al. 2015), and point to anatomical variation of the sternum based on flight mode (Düzler et al. 2006). While these results suggest that a lighter skeleton may favor reduced energy budget during long flights, other studies indicate that a lighter skeleton may also be selected against because it may increase the probability of structural damage (Biewener and Dial 1995, Dumont 2010). Thus, species that undertake sustained active flight may have evolved lighter skeletons to reduce energy costs during locomotion compared to poor flyers such as Galliformes, but given a tradeoff with skeletal strength, lighter skeletons may not be adaptive. This possibility is largely untested.

Here we conducted an interspecific study using a phylogenetically informed analysis to compare heart, lung, and skeletal mass, as well as tracheal diameter, between poor-flying species belonging to the order Galliformes, and species of birds from 3

other orders that regularly engage in sustained flights. We tested whether (1) Galliformes have smaller hearts, lungs, and tracheal diameters compared to species that undertake long, sustained, and flapping flight (*heart-size hypothesis*; Nespolo et al. 2018); (2) mass-independent size of hearts, lungs, tracheas, and skeletons may be reciprocally correlated due to their functional connections (*symmorphosis hypothesis*; Weibel et al. 1991); and (3) sustained flight may favor the evolution of lighter skeletons compared with more sedentary species.

Methods

Study system and sample size

Between 2011 and 2014 we collected 202 adult individuals of 21 species of game birds in the state of Montana (USA). Ninety-eight of these samples were from 14 species that are considered sustained flapping flyers while the remaining 104 were from 7 species defined as short flyers following the categorization adopted by Nespolo et al. (2018). Individuals were sexed based on plumage, external morphology, or dissection. We were able to confidently sex 62% of all individuals used in our analysis. Mean sample size per species (\pm SD) was 9.6 ± 10.3 and ranged between 3 and 36 (Supplemental Table S1). All specimens were harvested between 1 September and 31 December by hunters in possession of Montana state hunting permits for migratory and upland game birds. We sampled game birds legal to hunt in Montana because they include species that represent a good dichotomy between poor and good flying capacities and because they span a broad range in body masses (82–4,501 g), thus providing a useful comparison. Furthermore, most of these species are abundant and widespread, making collection easier. Lastly, most of the species considered here share vertical burst take-off strategies that allows us to control for potential anatomical differences due to variation in this behavioral trait (Provini et al. 2012, Watanabe 2016).

Bird processing

Once retrieved, each specimen was tagged with a unique alphanumeric code, kept in a cooler, and dissected within 24 h. First, we made a small incision on the neck and removed food from the

crop. This step excluded potential biases on measures of body mass from accumulation of food that can be substantial, especially in Galliformes. We recorded whole body mass using a digital scale operating between a 0.1 to 5,000 g range (Shenzen Amput Electronic Technology Co. Ltd., Shenzhen, China). We then removed internal organs from the carcass, cleaned them of residual blood or connective tissue, and measured wet heart and lung mass using a GemPro 250 portable electronic scale (MyWeigh, Phoenix, Arizona, USA) with an accuracy of ± 0.001 g. We also measured tracheal diameter above the syrinx in the mid-section between the caudal mouth and the bifurcation leading to the lungs using a digital caliper (0.01 mm resolution; model 500-196-30, Mitutoyo, Aurora, Illinois, USA). With a scalpel, we then cleaned the skeleton as much as possible of muscles, skin, and connective tissue. The rest of the organic matter was removed in the dermestarium of the Philip L. Wright Zoological Museum at University of Montana. Using hide beetles (*Dermestes maculatus*) for bone cleaning is time consuming but allows precise measurement of skeletal mass while preventing structural alterations caused by chemicals (Onwuama et al. 2012). At the end of the process skeletons were further cleaned from eventual residual skin or tissues and weighed using the aforementioned scales. Damaged organs or skeletons were omitted from all analyses.

Statistical analysis

To produce species-specific estimates for all parameters of interest we used a linear mixed-effects model that captured within- and among-species variation in the size of heart, lung, trachea, and skeleton. All measurements were \log_{10} transformed to meet assumptions of normality. The phylogenetic contrast in our sample is relatively weak because it encompasses only 4 orders of birds (i.e., Anseriformes, Charadriiformes, Columbiformes, and Galliformes). Nonetheless, we decided to test for possible phylogenetic effects (Felsenstein 1985), and conducted a phylogenetic generalized least squares (PGLS) analysis using the *Caper* package in R (Orme 2013). We produced a majority-rules consensus phylogenetic tree (Supplemental Fig. S1) using the program Mesquite (Maddison and

Maddison 2001). This tree was created from 1,000 trees containing our study species sampled from www.birdtree.org (Jetz et al. 2012), using the Hackett backbone (Hackett et al. 2008). While controlling for phylogeny, we aimed to test the effect of body mass and flight capacity (short versus flapping flight) on heart, lung, trachea, and skeleton size variation. Additionally, we tested whether the measured organs showed reciprocal correlation once introducing body mass as a covariate in our models to control for allometric effects. We did not include sex in our analyses because further tests using linear mixed models (LMM) did not show significant effects of sex related to our question. This result may be the consequence of our ability to sex only part of our sample and of an uneven sex ratio for certain species due to hunting regulations. All statistical analyses were done in R 3.4.2 (R Core Team 2018). Data and R code needed to replicate our main results are available in the supplement (Supplementary Table S2).

Results

Once accounting for body mass, mean heart size of Galliformes was 30.4% (3.27 ± 1.24 g) lighter compared to those of other species ($t = 2.62$, $P = 0.023$, Fig. 1a). Also, in Galliformes, mass-independent variation of lung mass and trachea diameter was 34.4% (4.34 ± 1.22 g, $t = 3.56$, $P = 0.004$) and 36.5% (1.01 ± 0.31 mm, $t = 3.22$, $P = 0.008$) lower, respectively, than in birds showing sustained flight abilities (Fig. 1b–c). We found no difference in skeletal mass between the 2 flight modes compared ($t = 1.34$, $P = 0.206$, Fig. 1d).

Heart mass, lung mass, and tracheal diameter all showed reciprocal correlations independent from body mass. Residual lung mass alone explained 84% of the variation in heart mass ($t = 10.12$, $P < 0.001$, Fig. 2a). Residual tracheal diameter showed weaker correlations with heart mass ($R^2 = 29\%$, $t = 2.82$, $P = 0.01$) and lung mass ($R^2 = 24\%$, $t = 2.46$, $P = 0.02$, Fig. 2b–c). Skeletal mass showed no correlation with heart mass ($R^2 = 9\%$, $t = 1.42$, $P = 0.172$, Fig. 2d).

Body mass explained 64% of variation in heart mass, 74% in lung mass, 75% in tracheal diameter, and 95% in skeletal mass. Heart and lung mass both had scaling exponents of 0.93, while tracheal

diameter scaled to the 0.32 power with body mass. Skeletal mass had a scaling exponent of 0.98. Therefore, all the organs measured departed from isometry with body mass, meaning that for a unit increase in body mass there is a nonequivalent proportional increase in organ mass.

Discussion

Our findings add robustness and taxonomic support to patterns detected in few previous studies showing that selection shapes heart size functionally to the flight capacities of different orders of birds (Bishop 2005, Nespolo et al. 2018). The substantial differences in size of cardiovascular and respiratory organs detected here and in other studies may be the result of several agents. One possible cause is migratory behavior. Internal organs become hypertrophic as a plastic reaction in preparation for migration or seasonal changes within species (Piersma et al. 1999, Guglielmo and Williams 2003, King et al. 2015). Other investigators, however, have found a negative correlation between heart size and migration distance (Vágási et al. 2016). Yet, migration strategies may vary among species with some sustaining long extended flights, while others progress in their journey by shorter flight bouts and increased stopovers (Warnock 2010). Indeed, some Galliformes, such as sage grouse, can make seasonal migrations of up to 240 km without relying on sustained flight (Newton et al. 2017). Therefore, studies quantifying distance as continuous predictor of organ size, while yielding additional insight, may provide different results than when comparing species that are sedentary short flyers, with other migrants showing long sustained flight.

An alternative explanation to migratory behavior for our results may be found in the phylogenetic history of Galliformes and Anseriformes, which are the 2 most represented orders in our study and are sister clades. As a consequence, there is only one nodal change at the base of their migratory trait that also corresponds with the split of the ancestral lineage in 2 different orders. This suggests that some reasons for the differences in organ morphology between these 2 orders may be rooted in the ancestry of Galliformes. Studies that incorporate a wider range of species that show

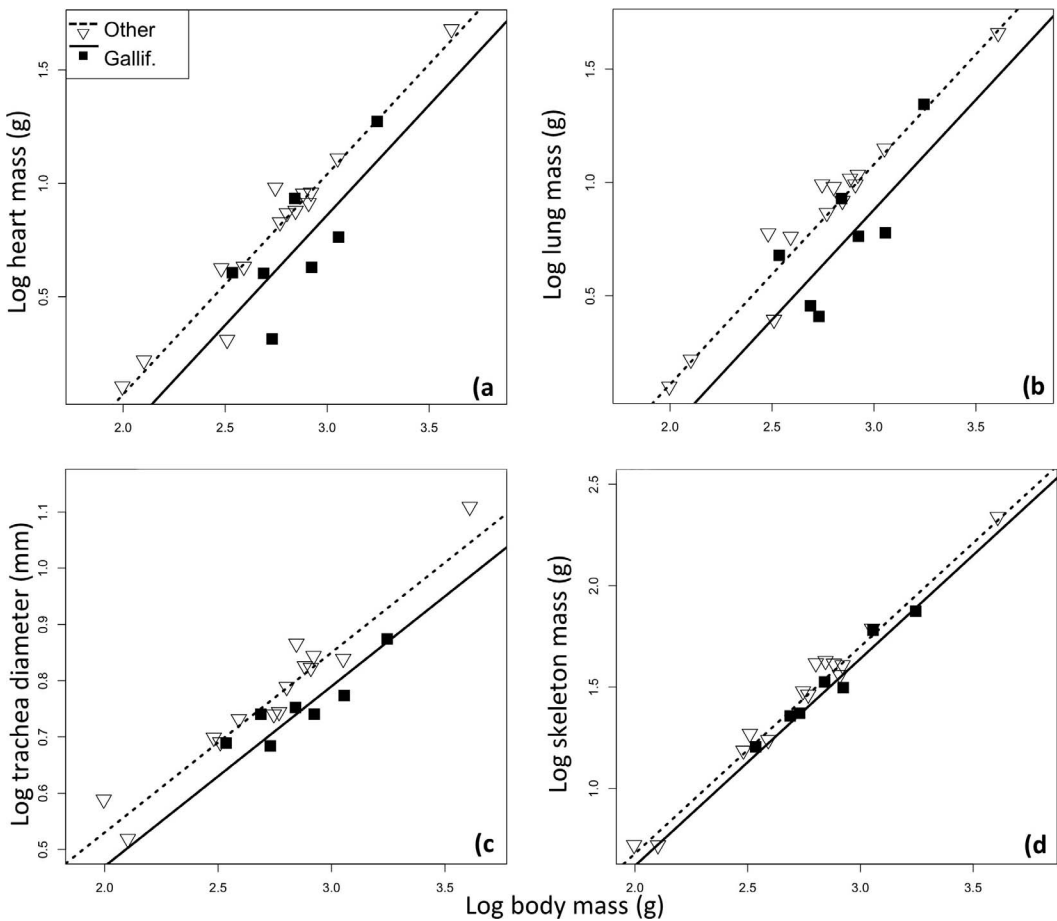


Figure 1. Regression of \log_{10} -transformed body mass on (a) heart mass, (b) lung mass, (c) trachea diameter, (d) skeleton mass, for 7 species of the order Galliformes and 14 species belonging to 3 other orders as obtained from our phylogenetic generalized least square model.

substantial variation in flight mode within orders are needed to test this idea.

Smaller internal organs such as lungs and hearts in Galliformes may also reflect adaptive variation to their lifestyle and environment. Indeed, the size of internal organs contributes to substantial differences in metabolism (Gallagher et al. 1998), and for a given body size, metabolic rate is proportional to heart mass (Bishop 1997). Among our samples, Mallard (*Anas platyrhynchos*) and Ring-necked Pheasant (*Phasianus colchicus*) have almost identical body size but heart mass of the former is more than twice the latter. These differences in heart size are also associated with qualitative variation in flight muscles of the 2 species. Mallards have smaller pectoralis muscles

than pheasants but have aerobic tissues with high myoglobin content and therefore high oxygen binding capacities (Williamson et al. 2001). In contrast, pheasants have largely anaerobic flight muscles with low myoglobin. Thus, smaller heart mass and reduced aerobic scope lead to lower mass-specific metabolic rates and reduced energy expenditures in pheasants rather than Mallards. Because the Galliformes considered here experience harsh lean seasons, with reduced food availability and extended periods of freezing temperatures, low energy expenditure may enhance their survival by reducing daily demands (Swanson 2010). This may be particularly true for highly sedentary species of Galliformes that are known to spend winter months within restricted

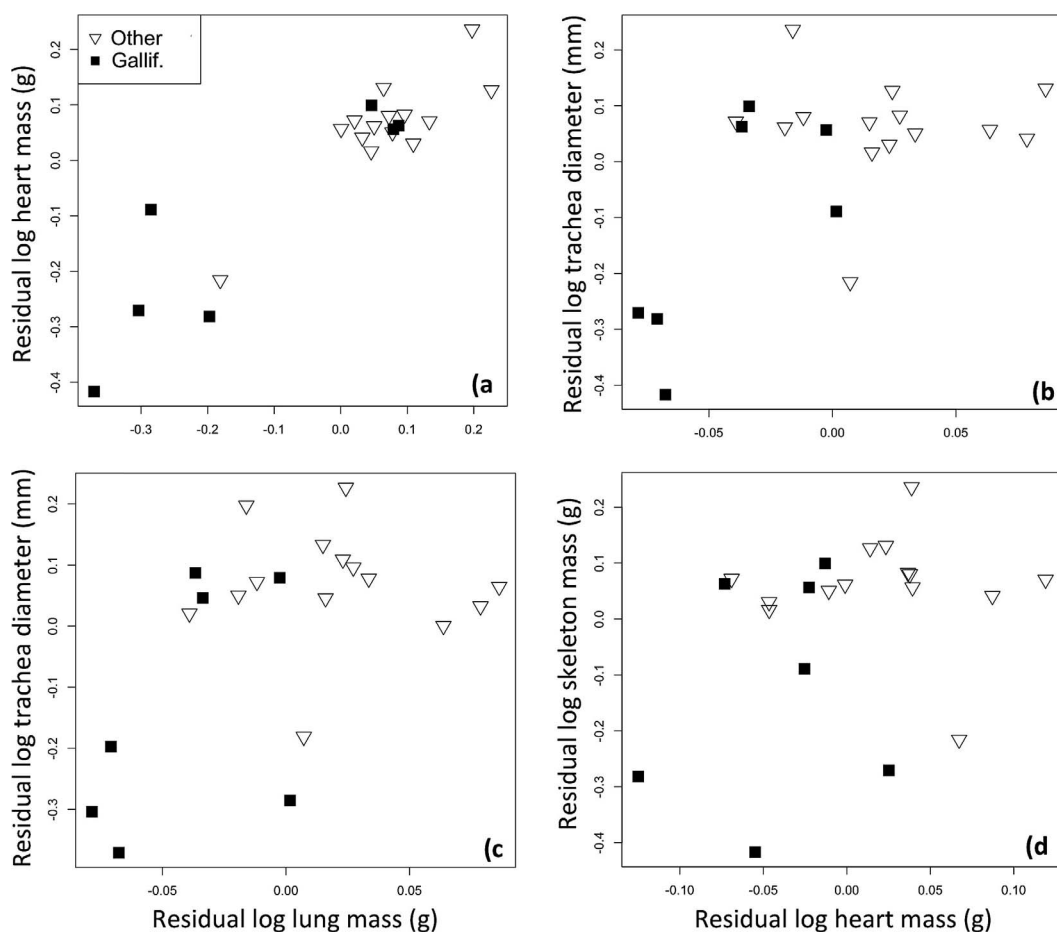


Figure 2. Regression of residual \log_{10} -transformed (a) heart and lung mass, (b) trachea diameter and heart mass, (c) trachea diameter and lung mass, (d) skeleton mass and heart mass for 7 species of Galliformes and 14 species belonging to 3 other orders as obtained from our phylogenetic generalized least square model.

home ranges of a few hectares and limited availability of trophic resources (Whitaker et al. 2007). Conversely, larger organs, despite being more costly to maintain, may favor survival of the other species considered here by allowing access to energy sources that are out of reach for the limited flight abilities of Galliformes.

We also found that larger hearts were associated with heavier lungs and larger tracheas, likely due to their functional linkage. In particular, lung mass explained the vast majority of variation in heart mass and part of the variation in tracheal diameter. These relationships follow from theoretical predictions predicated under the concept of symmorphosis (Weibel et al. 1991), where structural design is expected to match functional demand.

Heart and lung mass were more strongly associated with each other than with tracheal diameter. This could suggest that tracheal diameter is only weakly linked to the capacity to oxygenate blood, compared to the stronger influence of heart and lung size. Indeed, oxygenation also depends on hematocrit content and lung efficiency (Krause et al. 2016). In addition, air flow through the trachea is important for making vocalizations in the syrinx and may face sexual or other social selection pressures in addition to the functional requirements of the respiratory and circulatory systems. We also found no correlation between heart and skeleton mass indicating that heart and skeleton are functionally dissociated unlike organs within the cardiovascular system.

Variation in organ mass is only possible within the boundaries of body size, and body mass explained 64% of differences in heart mass among species. Hooded Merganser (*Lophodytes cucullatus*) is considered a flapping flyer and showed smaller heart size than predicted by its body mass, and 3 of 7 species of Galliformes had larger hearts compared to values predicted by the allometric relationship (Fig. 1a). This subsample includes prairie birds like Sharp-tailed Grouse (*Tympanuchus phasianellus*), sage grouse, and Gray Partridge (*Perdix perdix*). Some populations of these species are known to move up to 240 km seasonally (Robel et al. 1972, Davis et al. 2014, Newton et al. 2017), substantially more than most members of the same order. These results highlight the importance of ecological and evolutionary factors other than flight capacity and body mass in determining residual variation in organ mass.

Our test of the hypothesis that selection may favor lighter skeletons to allow energy savings for sustained flight failed to detect differences between Galliformes and all other species. Instead, there was a nonsignificant tendency for skeletons of species practicing long sustained flapping flight to be heavier. These results may suggest that selection has already minimized skeletal mass among flighted species of birds (Habib and Ruff 2008). Body mass explained almost all the variation in skeletal mass, suggesting that residual differences may be inhibited by stringent isometric limitations (Fig. 1b). Alternatively, species practicing long sustained flights may experience selection for heavier and stronger skeletons due to the intense and prolonged stresses and strains (e.g., aerodynamic and muscle forces) put on skeletal elements during flight (Biewener and Dial 1995, Dumont 2010).

In conclusion, our study suggests that the direction and magnitude of organ size variation may change based on the costs and benefits associated with flight mode. However, as flight capacity and phylogeny were confounded in our study, it is important to consider nonadaptive evolutionary history like “phylogenetic inertia” (Hansen and Orzack 2005) as an explanation for the patterns we observed. Hearts, lungs, and tracheas that are energetically costly to maintain and carry are smaller than expected by body mass in short flyers such as Galliformes (Fig. 1a–c). Costs of larger organ size instead may be offset by

the benefits of long-distance sustained flights in the other species measured. In contrast, further skeletal mass reductions may incur more costs due to excessive fragility than benefits due to energy savings, independent of flight mode. We also detected a functional and morphological relationship among the cardiorespiratory organs (Fig. 2a–c). While this correlation seems intuitive, evidence is rare of morphological coordination among species consistent with the hypothesis of symmorphosis (Weibel et al. 1991). Instead, the skeleton and heart lacked reciprocal correlation (Fig. 2d), suggesting that those 2 systems may experience different selective pressures and intrinsic constraints. This evidence provides further support to previous findings (Bishop 2005, Nespolo et al. 2018), and casts light on other less explored anatomical changes and their reciprocal correlations, indicating that heart size is a primary, yet not a sole, target of selection associated with different phylogenetic lineages, lifestyles, and flight capacities.

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