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Genome size and wing parameters in passerine birds

Chandler B. Andrews[1], Stuart A. Mackenzie[2] and T. Ryan Gregory[1,*]

[1]*Department of Integrative Biology, University of Guelph, Guelph, Ontario, Canada N1G 2W1*
[2]*Long Point Bird Observatory, Bird Studies Canada, Port Rowan, Ontario, Canada N0E 1M0*

Despite their status as the most speciose group of terrestrial vertebrates, birds exhibit the smallest and least variable genome sizes among tetrapods. It has been suggested that this is because powered flight imposes metabolic constraints on cell size, and thus on genome size. This notion has been supported by analyses of genome size and cell size versus resting metabolic rate and other parameters across birds, but most previous studies suffer from one or more limitations that have left the question open. The present study provides new insights into this issue through an examination of newly measured genome sizes, nucleus and cell sizes, body masses and wing parameters for 74 species of birds in the order Passeriformes. A positive relationship was found between genome size and nucleus/cell size, as well as between genome size and wing loading index, which is interpreted as an indicator of adaptations for efficient flight. This represents the single largest dataset presented for birds to date, and is the first to analyse a distinctly flight-related parameter along with genome size using phylogenetic comparative analyses. The results lend additional support to the hypothesis that the small genomes of birds are indeed related in some manner to flight, though the mechanistic and historical bases for this association remain an interesting area of investigation.

Keywords: cell size; *C*-value; flight; image analysis; wing aspect ratio; wing loading index

1. INTRODUCTION

(a) *Genome size diversity in birds*

It has been recognized since the first surveys of avian genome sizes that they contain a limited quantity of DNA relative to those of other terrestrial vertebrates (e.g. Bachmann *et al.* 1972; Tiersch & Wachtel 1991). Available genome size estimates for roughly 200 species of birds reveal a range of only approximately twofold (approx. 1–2 pg in mass, where 1 pg equals 978 mbp) (Gregory 2008). Based on the positive association between genome size and red blood cell size observed across vertebrates (Olmo 1983; Gregory 2001*a*,*b*), a common explanation for the apparent constraint on genome size diversity among birds has been that small cells with high relative surface areas for gas exchange—and hence small genomes—are required to meet the metabolic demands of powered flight (Tiersch & Wachtel 1991; Wachtel & Tiersch 1993; Hughes & Hughes 1995; Hughes 1999; Gregory 2002*a*,*b*).

The notion that flight capability and genome size are linked is supported by the observation that large genomes occur in flightless birds, whereas strong fliers have the smallest genomes (Hughes 1999; Gregory 2005). Likewise, bats have small genomes (Burton *et al.* 1989; Van Den Bussche *et al.* 1995; Gregory 2005). Comparisons of data compiled from the literature have revealed inverse, mass-corrected relationships between genome size and resting metabolic rate, both within a small sample (11 species) of passerines (Vinogradov 1997) and across 50 species of birds from 16 orders (Gregory 2002*a*), as well as between genome size and nucleus size, cell size and body mass across birds (Gregory 2002*a*,*b*). In addition, Vinogradov & Anatskaya (2006) reported an inverse correlation between genome size and relative heart mass, which they took as another indicator of metabolic intensity. While these studies provide important insights into the possible metabolic constraint acting on genome size among birds, most suffer from a lack of taxonomic focus, the introduction of error through compiling data from different studies, a lack of phylogenetic control in statistical analyses, the fact that the parameters under consideration are not directly linked to flight, or some combination of these issues.

(b) *Wing parameters and flight*

The size and shape of a bird's wings provide an indication of its specialization for a particular mode of flight (Norberg 1995). Wing span and wing area indicate the total size of the wings and can be used to calculate composite characters such as wing aspect ratio (WAR) which provide information on the type of flight that is common to particular birds. A high WAR, for example, generally indicates long, narrow wings used for flight specializations such as gliding, whereas a low WAR indicates short, broad wings that allow high manoeuvrability (figure 1). Wing shape alone provides only a partial picture, however, and it has been suggested that comparisons that incorporate information on wing size and body mass are good predictors of the cost of flight (e.g. Castro & Myers 1988). A single parameter, wing loading index (WLI), incorporates both body mass and wing size by providing a measure of the amount of mass carried per unit wing area. Specifically, a low loading index indicates a reduction in body mass per unit wing area, an increase in wing size per unit mass or both. Birds with higher WLI

* Author for correspondence (rgregory@uoguelph.ca).

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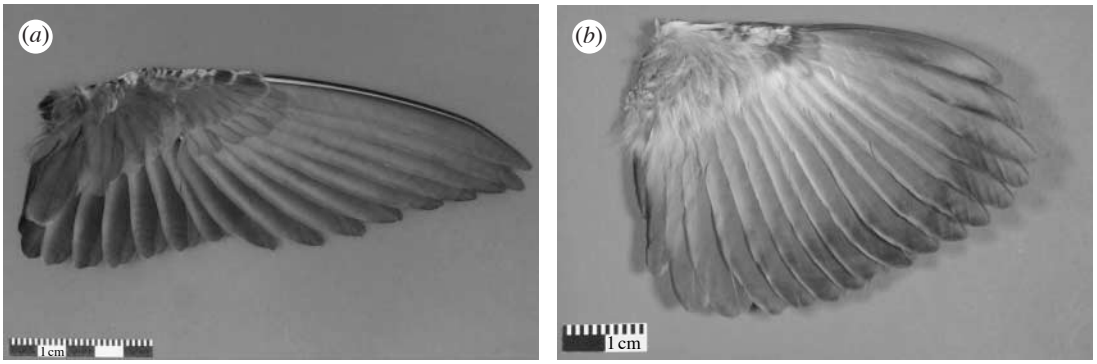

Figure 1. Representative images of wings from (*a*) an adult female purple martin (*Progne subis*) and (*b*) an adult female eastern towhee (*Pipilo erythrophthalmus*). Both images are of ventral views of the left wing. Note the different scale bars. This shows the range in wing shape among species of passerines, as reflected by their different aspect ratios (7.08 and 3.47, respectively). Reproduced by permission of the Slater Museum of Natural History, University of Puget Sound.

must fly faster per unit mass to generate sufficient lift (e.g. Norberg 1995), and it has been shown that WLI scales with flight speed with an exponent of 0.32 (Alerstam *et al.* 2007).

The interpretation of differences in WLI varies somewhat across birds, in part because the scaling of WLI to body mass differs across groups (Videler 2005). For example, ducks have comparatively large body masses but small wings (and hence high WLI), presumably as an adaptation for short take-offs from the water surface, but they remain strong fliers by compensating with a high wing-beat frequency (Videler 2005). However, within a more focused taxonomic sampling, WLI may provide a useful metric of adaptation for efficient flight that can be compared with genome size. Notably, Costantini *et al.* (2008) has recently reported a significant relationship between genome size and an estimate of WLI among 24 species of parrots.

(c) *The present study*

With approximately 5700 described species, the order Passeriformes is the largest within the class Aves (Dickenson 2003; Jønsson & Fjeldså 2006). To date, only 61 genome size estimates have been published for members of this order, covering approximately 1 per cent of known passerine diversity (Gregory 2008). In order to both improve the coverage of this most diverse avian order and to provide a taxonomically fine-scale assessment of relationships between genome size and indicators of flight adaptations, the present study focused entirely on the Passeriformes. The resulting dataset covering 74 species from 51 genera and 18 families represents the largest single contribution of avian genome sizes published to date (Gregory 2008). In addition to genome size estimates, original data on nucleus size, cell size, body mass, WAR and WLI are presented. This large, taxonomically focused and internally consistent dataset provides further insights into the relationship between avian genome size and flight.

2. MATERIAL AND METHODS

(a) *Mist-netting of birds*

Samples were collected from after-hatch-year birds using mist-netting at Long Point Bird Observatory (LPBO) near Port Rowan, Ontario, Canada, from 5 May to 7 June 2007 and 31 August to 8 September 2007. The LPBO is located on a peninsula that extends 40 km into the deepest part of Lake Erie (42°33′ N, 80°10′ W). Three field sites were sampled, covering three different habitats: predominantly coniferous forest (Old Cut), deciduous forest (Breakwater), and cottonwood (the Tip). Avian species (and sex where possible) were identified with morphological keys in the *Identification guide to North American passerines* (Pyle *et al.* 1987). All bird capturing and handling were completed in accordance with University of Guelph animal care policies (Animal Utilization Protocol no. 06R093) and under permits from the Ontario Ministry of Natural Resources (Permit no. 1038033) and the Canadian Wildlife Service (Permit no. CA0189 and CA0190).

(b) *Blood sampling*

Toenail clipping was the preferred method for phlebotomy, as it posed little risk of lysing erythrocytes or causing subcutaneous bleeds. The birds were gently restrained for blood sampling and their nails were cleaned with alcohol swabs. Next, the tip of one toenail was clipped with a small pair of sanitized nail-clippers (J.W. Pet Company, Cat. no. 65026) to sever the quick and produce blood flow. Thin blood smears were prepared as described by Hardie *et al.* (2002), with roughly 20 µl of blood per microscope slide. After the site of blood extraction was cauterized with a silver nitrate stick (Fisher Scientific, Cat. no. 360994378), the birds were released back into the wild. Whenever possible, two or more individuals per species (either male or female, or both if available) were sampled. In total, blood samples were drawn from 230 individual birds representing 74 species of passerines.

(c) *Genome size, nucleus size and cell size*

Genome size estimates were performed with Feulgen image analysis densitometry using the best practice guidelines described by Hardie *et al.* (2002). The blood smears were post-fixed overnight in 85 methanol : 10 formalin : 5 glacial acetic acid, rinsed in tepid tap water and hydrolyzed for 120 min in 5N HCl at room temperature before being stained for 120 min in freshly prepared Schiff reagent and passed through a series of bisulfite and distilled water rinses. Integrated optical densities (IODs) were measured for a minimum of 200 nuclei per individual using the Bioquant Life Science software package and an Optronics DEI-750 CE three-chip CCD camera mounted on a Leica DM LS microscope with a 100× lens. IODs were converted to genome sizes in picograms by comparison with IODs from chicken (*Gallus domesticus*) erythrocytes ($1C = 1.25$ pg) stained in each run along with the passerine slides.

Erythrocyte nucleus and cell sizes (dry areas) were measured using the same image analysis system following Wright staining of slides from one individual per species. At least 50 nuclei and cells were measured per slide. Measurements were converted from pixels to absolute areas in square micrometres using an image analysis calibration slide.

(d) *Body mass*

Average body mass was computed for each species using original measurements stored in the banding archive at the LBPO. A minimum of 19 body mass records from after-hatch-year birds was used to calculate the average body mass for each species, and an equal number of males and females was used to remove as much sexual bias as possible. All data for species included in this study were measured in the spring. One species, the American crow (*Corvus brachyrhynchos*), was donated after the completion of the field season at the LBPO and its body mass was obtained from the Cornell Lab of Ornithology (2008).

(e) *Wing parameters*

Images of spread wings were retrieved from a digital collection available online from the Slater Museum of Natural History (2008) and assessed for size parameters using the Leica Application Suite v. 3.0.0. Values in pixels were converted to absolute units (mm) using a scale bar within each of the original images (figure 1). The area of the wing was defined as the region from the shoulder joint to the tip of the outermost primary remex, then from the distal primary remex along the trailing edge of the wing to the to the tip of the most proximal secondary remex, and finally along the proximal edge of the tertiary remex back to the shoulder joint. As recommended by Pennycuick (1989), wing area was measured by tracing the outline of the wing and excluding any gaps between emarginated remiges with the area tool. Once the area of the wing was defined, the software output the wing area (mm^2) and the maximum length (mm) of the wing. Doubling these values gave total wing area and an estimate of the wing span. For each species, WLI was calculated by dividing body mass by the total wing area ($g\,mm^{-2}$) and WAR was calculated by dividing wing span squared by the total wing area. All wing measurements were conducted using adult specimens. Wherever possible, this included an average of values for male and female individuals (approx. 1/3 of species). However, availability of data meant that in many cases only one male, one female or an individual of an unknown sex could be examined. Based on a comparison of 22 species, male wing areas were on average 10 per cent larger than those of females, though the two were strongly correlated ($r=0.95$, $p<0.0001$). Thus, although differences between sexes do introduce some error, this is comparatively minor relative to interspecific variation and in any case is random with respect to the patterns being evaluated.

(f) *Statistical analyses*

Direct correlations were conducted using Pearson correlation analysis of log-transformed data. To correct for body mass, both parameters to be compared were first regressed against mass and the residuals of the two relationships were saved and compared against one another. In addition, Felsenstein's (1985) phylogenetically independent contrasts (PICs) were calculated using log-transformed data using the PDAP module (Midford *et al.* 2002) in Mesquite v. 2.5 (Maddison & Maddison 2008). The phylogenetic hypothesis used was the supertree of passerine birds of Jønsson & Fjeldså (2006), with some details appended from other studies for the genera *Dendroica* (Lovette & Bermingham 1999) and *Vireo* (Murray *et al.* 1994) (appendix 1, electronic supplementary material). Branch lengths were set to 1 because the tree did not contain branch length information. However, all significant relationships were repeated following each of the branch length methods of Grafen, Nee and Pagel as given in Mesquite (Maddison & Maddison 2008). One degree of freedom was subtracted for each branch in soft polytomies (Purvis & Garland 1993; Garland & Díaz-Uriarte 1999). Mass-corrected PICs were conducted in Mesquite by first performing independent contrast correlations between parameter 1 (e.g. WLI) and body mass and saving the residuals, repeating for parameter 2 (e.g. genome size) versus body mass, and then analysing these residuals together by Pearson correlations forced through the origin.

3. RESULTS

(a) *Summary of the dataset*

In total, new genome size, nuclear and cell area, body mass and wing parameter data were generated for 74 species of passerines (figure 2; appendix 2, electronic supplementary material). The genome sizes of the species analysed ranged from 1.15 pg in the eastern wood pewee (*Contopus virens*) to 1.62 pg in the bay-breasted warbler (*Dendroica castanea*). Geographically disparate populations were not sampled explicitly, but it is likely that the conspecifics converging on Long Point are derived from different regions in many cases. Nonetheless, intraspecific variation in genome size was minimal (appendix 2, electronic supplementary material). For 24 species for which both males and females were clearly distinguished, there was an average difference of less than 3 per cent, though the direction of this variation was not consistent (i.e. in some cases estimated values were larger for males and sometimes for females). There were no significant differences between males and females across the species (paired *t*-test, $p>0.75$). It is likely that the small amount of variation reflects simple measurement error rather than chromosome-level differences between sexes which can be detected using methods such as flow cytometry (cf. Nakamura *et al.* 1990; Tiersch & Mumme 1993). The mean genome size estimate for the passerines surveyed in this study is 1.32 ± 0.08 pg. Of these genome size estimates, 59 are new and 15 overlap with previous studies. In general these agreed well (usually to within 0–8%), but two estimates, one from Bachmann *et al.* (1972) and the other from De Smet (1981), were larger than the present values by 20 per cent. The latter study, at least, is known to include less reliable results due to the method used (Gregory 2002*a*,*b*).

(b) *Genome size, nucleus size and cell size*

Genome size was positively related to nucleus and cell size, and nucleus and cell size were positively correlated with each other, in both Pearson and phylogenetically independent correlations, the latter being generally stronger (table 1). A positive relationship was also found between genome size and percentage of dry erythrocyte area occupied by the nucleus, as was an inverse relationship between genome size and percentage of the cell occupied by cytoplasm (table 1), indicating that cells containing

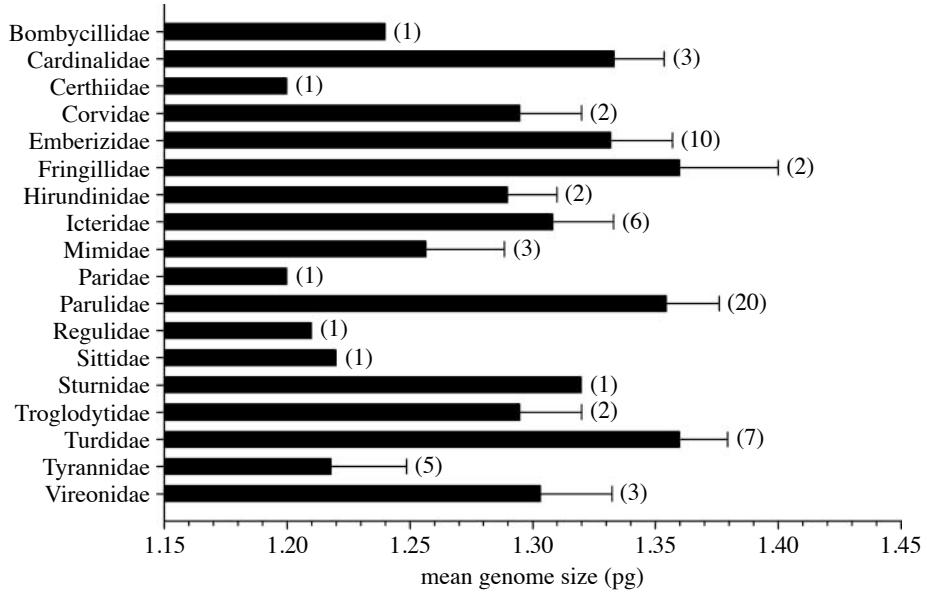

Figure 2. Mean genome sizes for the 18 families of passerines included in this study. Error bars represent standard error. Numbers in parentheses indicate the number of species sampled for that family. The scarlet tanager (*Piranga olivacea*) was not included in this figure as its familial classification remains uncertain. Note that the scale starts at 1.15 pg.

Table 1. Summary of cell-level relationships for 74 species of passerines. (Data were log-transformed prior to analyses using Pearson correlations and phylogenetically independent contrasts (PICs) with branch lengths set to 1 (see appendix 3, electronic supplementary material for results using estimates of branch lengths).)

parameters	correlation	r	p-value
nucleus area versus genome size	Pearson	0.3249	0.005
	PICs	0.4742	<0.0001
cell area versus genome size	Pearson	0.1970	0.09
	PICs	0.3176	0.006
cell area versus nucleus area	Pearson	0.5876	<0.0001
	PICs	0.6000	<0.0001
% nucleus area per cell versus genome size	Pearson	0.2795	0.02
	PICs	0.3618	0.002
% cytoplasm area per cell versus genome size	Pearson	−0.2781	0.02
	PICs	−0.3673	0.001

more DNA are larger but also are filled disproportionately by the nucleus, with an associated reduction in relative cytoplasmic volume (figure 3).

(c) *Wing parameters*

Without log-transformation, the WLI and body mass measurements in the present study were related to each other with an exponent ($y=ax^b$) of 0.228 ($r=0.72$, $p<0.0001$), which is in very good agreement with previous estimates for passerines (Videler 2005). Log-transformed data showed positive correlations between body mass and wing span ($r=0.91$, $p<0.0001$, $n=74$), wing area ($r=0.94$, $p<0.0001$, $n=74$), WAR ($r=0.25$, $p<0.04$, $n=74$) and WLI ($r=0.72$, $p<0.0001$, $n=74$), but not with genome size ($p=0.78$). Wing span and wing area were strongly related to each other ($r=0.97$, $p<0.0001$, $n=74$), but WAR and WLI were not correlated with each other ($p>0.71$; cf. Alerstam *et al.* 2007 for a broader taxonomic sample of birds).

Genome size and wing span were inversely correlated among the birds included in this study only following both

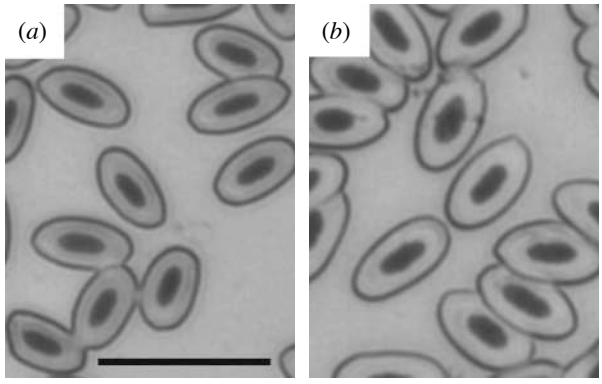

Figure 3. Images of Wright-stained erythrocytes from (*a*) Lincoln's sparrow (*Melospiza lincolnii*, 1.23 pg) and (*b*) purple martin (*Progne subis*, 1.46 pg). A positive association was found between genome size, nucleus size and cell size within the order Passeriformes, although larger cells have proportionately larger nuclei and smaller relative cytoplasmic areas (table 1). Scale bar equals 20 µm.

mass- and phylogenetic correction ($r=-0.34$, $p=0.004$, $n=73$; all other $p=0.2$–0.8). Wing area was not related to genome size without mass correction ($p=0.5$–0.8), but was negatively correlated with genome size after correction for body mass (Pearson: $r=-0.32$, $p=0.005$, $n=74$; PICs: $r=-0.42$, $p=0.0002$, $n=73$). Genome size was positively correlated with WAR only at the family level without mass correction ($r=0.53$, $p=0.02$, $n=19$) and was marginal at this level after correction for body mass ($r=0.44$, $p=0.06$, $n=19$). No relationships were found between WAR and genome size at the genus or species levels with Pearson correlations with or without mass correction (all $p=0.09$–0.28), nor with or without mass correction at the species level with PICs (all $p>0.7$).

A positive relationship was found between genome size and WLI across passerines using either Pearson correlations ($r=0.25$, $p<0.04$, $n=74$) or PICs ($r=0.42$, $p<0.001$, $n=73$). This relationship persisted when the data were corrected for body mass using regression

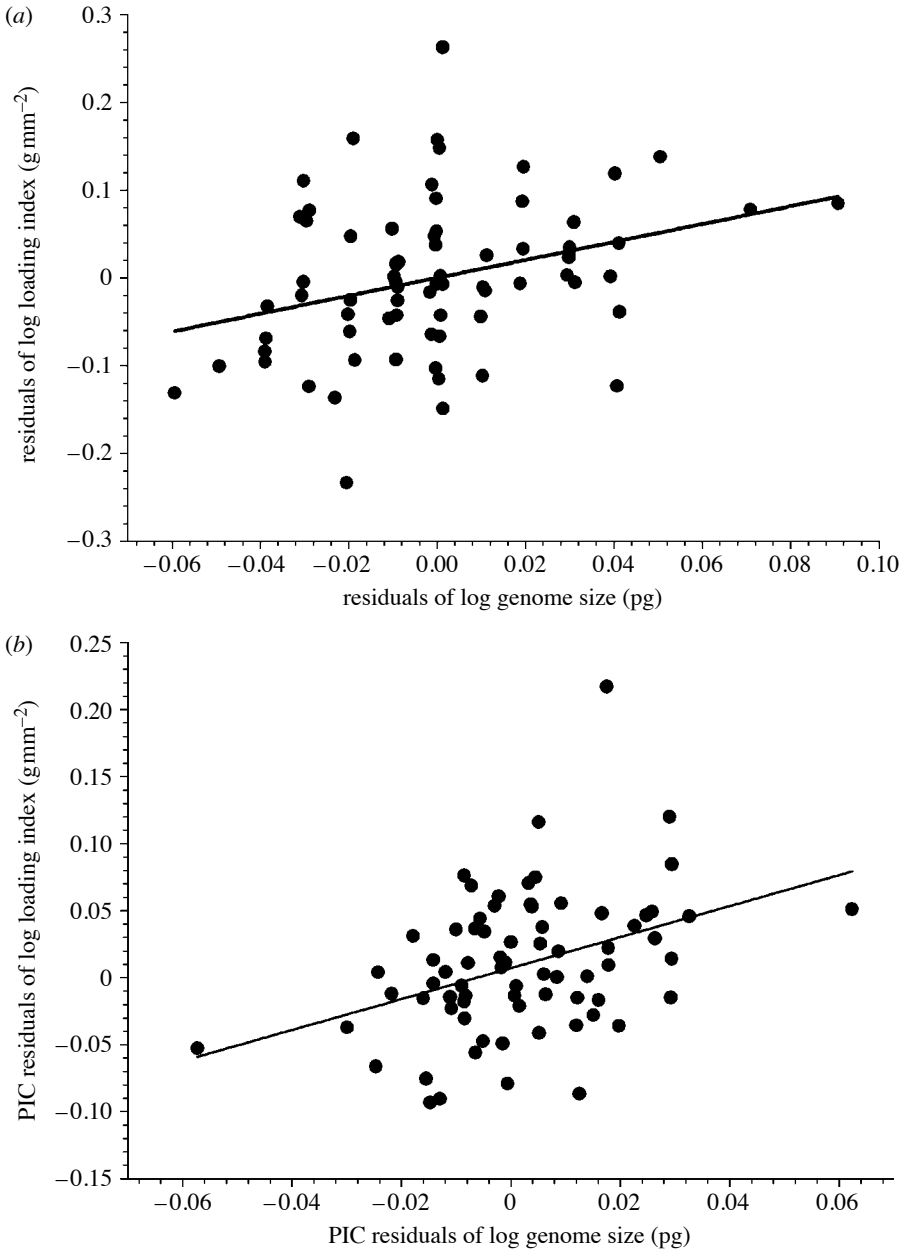

Figure 4. The mass-corrected relationship between WLI and genome size in 74 species of passerine birds, which is significant following (*a*) direct Pearson correlation analysis and (*b*) PICs.

residuals in Pearson correlations at the species ($r=0.32$, $p<0.005$, $n=74$) and genus ($r=0.31$, $p<0.03$, $n=51$) levels, and showed the same correlation coefficient at the family level, though this became non-significant due to the smaller sample size ($r=0.31$, $p=0.2$, $n=19$). The relationship was significant and positive at the species level when correcting for body mass using PICs ($r=0.39$, $p<0.001$, $n=73$) (figure 4).

4. DISCUSSION

The mean genome size of 1.32 pg estimated here for the order Passeriformes is slightly smaller than that for the available data from birds in general (1.45 pg; Gregory 2008), and not surprisingly the range within this single order is smaller than that across the entire assemblage of birds studied to date (approx. 1.4-fold versus 2-fold). Nevertheless, significant positive relationships can still be identified between genome size, nucleus size and cell size within this single order, including after phylogenetic correction. Genome size is also positively related to WLI, which is taken here as an indicator of specializations for efficient flight (see also Costantini *et al.* 2008). This relationship persists following mass- and phylogenetic correction, meaning that it is not a by-product of any link between genome size and body size (Gregory 2002*b*). Indeed, genome size is not positively correlated with body size within the present dataset, but is inversely related to wing area after correction for body mass. That is, the relationship between genome size and WLI derives from the presence of smaller relative wings rather than higher body masses in species with larger genomes. (Larger genome sizes are not postulated to cause differences in wing size, of course—rather, relative wing size and genome size appear to reflect independent features linked to efficient flight.) Other wing parameters including wing span and aspect ratio, by contrast, do not appear to be linked to genome size in any convincing way, suggesting that the type of flight (e.g. gliding versus manoeuvrability) is not

related to constraints on DNA content, at least across the relatively limited extremes in passerine birds.

These results enhance the interpretation that small genome size and generalized flight efficiency are related mechanistically in some fashion within birds. This has usually been explained with regard to the effects of genome/nucleus size on cell surface area to volume ratios, which can influence gas exchange efficiency (e.g. Szarski 1983; Gregory 2001*a*,*b*). However, the present dataset has revealed another, possibly complementary effect on cellular cytoplasm content. As genomes become larger, nucleus sizes increase in absolute terms and also with regard to the percentage of the cell area that they occupy. The net effect is that a larger genome results in a lower relative amount of cytoplasm per unit cell size. Without a compensatory change in haemoglobin concentration or haematocrit, this could be expected to result in a lower amount of haemoglobin per volume of blood. As Lutz (1980) pointed out, 'any variation in haemoglobin component ratios…would be reflected as wide differences in whole blood oxygen affinity characteristics'.

The question of whether the small genomes of birds are ancestral or derived (Tiersch & Wachtel 1991)—that is, 'whether they have jettisoned their genomic baggage or whether it was never loaded in the first place' (Gregory 2002*a*,*b*)—is central to determining whether small genomes represent an adaptation to flight *per se*. New light has been recently shed on this issue by Organ *et al.* (2007) who analysed the sizes of fossil osteocytes in dinosaurs and concluded that birds inherited already diminutive genomes from ancestral theropod dinosaurs (see also Ellegren 2007; Zimmer 2007). This would place partially reduced genome sizes among other features such as bipedal locomotion, feathers and possibly endothermy that appeared prior to, but then became important in, the evolution of avian flight. However, just as feathers subsequently became specialized for flight in birds, the fact that the estimated mean genome size for theropod dinosaurs (Organ *et al.* 2007) is notably larger than that for the average modern bird (Gregory 2008) implies that genome sizes continued to shrink along with specializations for flight.

Wing parameters provide a novel perspective to this relationship between genome size and flight capability. These may be more reliable than resting metabolic rate as indicators of flight adaptation, given that resting metabolic rates are not necessarily linked directly to active flight metabolism; indeed, resting metabolic rate and active metabolic rate do not scale in the same way with body mass (e.g. Bennett & Harvey 1987; Trevelyan *et al.* 1990). Moreover, resting metabolic rate is difficult to standardize even within studies and may vary by season (McKechnie & Wolf 2004; McKechnie 2008). However, comparisons of wing parameters must be interpreted carefully, as major differences in wing and/or body shape can influence the biological significance of features such as WLI. Even within a single order, there may be notable differences in wing shape (figure 1), and this is a much more significant issue in cross-order analyses. For broader comparisons, other factors such as wing beat frequency should also be considered if possible.

In the future, it should be possible to investigate other metabolic parameters more likely to be involved in constraints on cellular and genomic parameters in birds. For example, data are becoming available for such characteristics as field metabolic rate, active metabolic rate, maximum or sustained flight speed, metabolic scope, maximum oxygen consumption and mechanical power (e.g. Bennett & Harvey 1987; Anderson & Jetz 2005; Bishop 2005; Alerstam *et al.* 2007); but these datasets will need to be expanded and matched with reliable genome size estimates for the same species before analyses can be conducted. Data bearing on migration capabilities also would be of interest, but it would be important to distinguish between long-distance direct flights and more gradual seasonal range shifts. Finally, data on haematocrit and total blood haemoglobin concentration would be useful for testing cell-level explanations for such relationships that extend beyond cell surface area to volume ratio impacts.

Overall, it is becoming increasingly apparent that the small, constrained genome sizes of birds are relevant in the light of their demanding metabolic lifestyles. The precise evolutionary and physiological explanations for this link between the size of the genome and powered flight remain to be worked out, and it will be important in this regard to generate consistent, reliable datasets of genome size and flight parameters including a large diversity of bird species.

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