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ARTICLE

Patterns of genome size diversity in bats (order Chiroptera)[1]

Jillian D.L. Smith, John W. Bickham, and T. Ryan Gregory

Abstract: Despite being a group of particular interest in considering relationships between genome size and metabolic parameters, bats have not been well studied from this perspective. This study presents new estimates for 121 "microbat" species from 12 families and complements a previous study on members of the family Pteropodidae ("megabats"). The results confirm that diversity in genome size in bats is very limited even compared with other mammals, varying approximately 2-fold from 1.63 pg in *Lophostoma carrikeri* to 3.17 pg in *Rhinopoma hardwickii* and averaging only 2.35 pg ± 0.02 SE (versus 3.5 pg overall for mammals). However, contrary to some other vertebrate groups, and perhaps owing to the narrow range observed, genome size correlations were not apparent with any chromosomal, physiological, flight-related, developmental, or ecological characteristics within the order Chiroptera. Genome size is positively correlated with measures of body size in bats, though the strength of the relationships differs between pteropodids ("megabats") and nonpteropodids ("microbats").

Key words: Chiroptera, genome size, C-value, flight, metabolism.

Résumé : Bien qu'elles constituent un groupe présentant un intérêt particulier pour l'étude des relations entre la taille du génome et les paramètres métaboliques, les chauves-souris n'ont pas été bien étudiées sous cet angle. Dans ce travail, les auteurs présentent des estimés pour 121 espèces de "microchiroptères" appartenant à 12 familles et ceci vient compléter une étude antérieure sur des membres de la famille des Pteropodidae ("mégachiroptères"). Les résultats confirment que la diversité de la taille des génomes chez les chauves-souris est limitée par rapport à d'autres mammifères, variant environ du simple au double entre 1,63 pg chez le *Lophostoma carrikeri* à 3,17 pg chez le *Rhinopoma hardwickii* pour une moyenne de 2,35 pg ± 0,02 (contre 3,5 pg globalement chez les mammifères). Cependant, contrairement à ce qui est observé chez d'autres vertébrés, peut-être en raison de la faible variation observée, la taille du génome ne semblait pas corrélée avec des caractéristiques chromosomiques, physiologiques, liées au vol, développementales ou écologiques chez les chiroptères. La taille du génome était cependant positivement corrélée avec divers paramètres de la taille du corps chez les chauves-souris, bien que le degré de corrélation différait chez les ptéropodidés ("mégachiroptères") et les non-ptéropodidés ("microchiroptères"). [Traduit par la Rédaction]

Mots-clés : Chiroptera, taille du génome, valeur C, vol, métabolisme.

Introduction

The order Chiroptera is the second largest in mammals after rodents, and with >1100 species in 18 families it represents more than one-fifth of all mammalian diversity (Wilson and Reeder 2005). While bats share many commonalities with other mammals, they are the only mammals capable of true flight and, along with birds and the extinct pterosaurs, one of only three vertebrate taxa to have evolved this highly specialized mode of locomotion. Despite their diversity, abundance, and unique biology, bats remain poorly studied from several important perspectives. Notably, research into the diversity of genome size in bats is one such area that is lacking. Prior to recent work by the current authors (J.D.L. Smith and Gregory 2009; present study), estimates available in the Animal Genome Size Database covered only 62 species from 7 families (Gregory 2013).

Genome size constraint

The genome sizes of animals as a whole range more than 7000-fold. Even among vertebrates, genome sizes vary by a factor of more than 350. Not surprisingly, many studies on genome size in animals have focused on groups with large amounts of variability, such as amphibians (1C = 0.95 – 120.60 pg) and "fishes" (1C = 0.35 – 132.83 pg), especially with the objective of identifying phenotypic correlates that could help to explain this astounding diversity (Gregory 2013). However, increasing attention has been paid more recently to explaining the apparent constraint within some otherwise diverse vertebrate taxa, in particular among birds (Andrews et al. 2009).

It has been hypothesized that genome size may be constrained in some vertebrate groups with high metabolic rates. This is particularly relevant in those taxa subject to the extreme metabolic demands of powered flight owing to the relationship between genome size, cell size, and mass-specific metabolic rate (Hughes and Hughes 1995; Gregory 2002*a*; Organ and Shedlock 2009). Specifically, smaller cells—which are associated with small genomes—have a higher surface area to volume ratio, allowing for improved gas exchange to meet metabolic demands (Szarski 1983; Gregory 2001*b*).

In one analysis, Hughes (1999) used a comparatively small dataset for birds to suggest that strong flyers have smaller genomes than weak flyers or flightless taxa (see also Gregory (2005*a*)). More recently, Andrews et al. (2009) used a much larger, newly generated dataset to examine the relationship between genome size and flight using specific wing parameters. In this case, genome size was positively correlated with wing loading index (an inverse

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Corresponding Editor: Jillian Bainard.

J.D.L. Smith and T.R. Gregory. Department of Integrative Biology, University of Guelph, 50 Stone Road E., Guelph, ON N1G 2W1, Canada.
J.W. Bickham. Battelle Memorial Institute, 10777 Westheimer, Suite 1105, Houston, TX 77042, USA.
Corresponding author: T. Ryan Gregory (e-mail: rgregory@uoguelph.ca).

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indicator of flight efficiency) in passerine birds, lending support to the flight constraint hypothesis (Andrews et al. 2009). Fossil evidence also shows that avian dinosaurs and pterosaurs had small genomes relative to nonflying lineages (Organ et al. 2007; Organ and Shedlock 2009). In light of these studies on other flying vertebrates, it seems that flight may indeed impose a constraint on genome size. Clearly, a study of bats also represents a critical component of this line of inquiry.

Explaining genome size diversity within bats

While genome size in bats appears constrained based on the limited sampling conducted to date, little is known about the causes and consequences of this variation. Flight is a proposed constraint on genome size; however, previous studies have not considered relationships between genome size and flight or any other organism level traits within Chiroptera, such that work on this group lags behind that focusing on other vertebrates (reviewed in Gregory 2005*a*). Mammals, like birds, demonstrate strong positive links between genome size and cell size and inverse relationships between genome size and metabolic rate (Vinogradov 1995; Gregory 2000). In some cases, relationships can be identified within individual orders, such as a correlation between genome size and body size in rodents (Gregory 2002*c*) or between genome size, cell size, and flight parameters within the avian order Passeriformes (Andrews et al. 2009). It, therefore, remains an important question whether cell- and organism-level traits, especially those relevant to flight, are related to genome size diversity among bats; and whether this can help to explain patterns within this highly diverse mammalian order, in addition to assessing the larger issue of flight-related constraints across bats generally.

While bats and birds share the feature of powered flight and high relative metabolisms, mammals differ from other vertebrates in that they possess enucleated red blood cells. This expulsion of nuclei during red blood cell development results in exceptionally compact erythrocytes that allow for efficient gas exchange without necessarily requiring extremely reduced genome sizes (Gregory 2000). This further emphasizes the need to study questions relating to genome size constraints in bats, because this critical difference between birds and mammals could decouple, or at least weaken, the relationship between genome size and metabolism.

Of course, flight is not the only characteristic that could be responsible for influencing genome size in birds and bats. The link between genome size, cell division, and cell size can lead to associations with other biological features, and it has been suggested that organ complexity, development, and ecological lifestyle have been important in the evolution of genome size among other vertebrates (e.g., Gregory 2002*c*, 2005*a*; Andrews and Gregory 2009). For example, Roth et al. (1994) discovered that amphibians exhibit a negative correlation between genome size and brain complexity because fewer, less well differentiated neurons can be fit within the braincase when the cells are larger and divide more slowly. This is important, as brain complexity and function are highly associated with many aspects of animal behaviour and ecology. Similarly, a negative relationship exists between genome size and relative brain size in parrots (Andrews and Gregory 2009). By extension, it is possible that genome size diversity is associated with feeding, habitat, or social behaviour as these can be influenced by cognitive capabilities (Andrews and Gregory 2009). In bats, brain size has been linked to foraging ecology, feeding type, and habitat complexity in a number of studies (e.g., Eisenberg and Wilson 1978; Safi and Dechmann 2005; Ratcliffe et al. 2006).

A negative relationship between genome size and cell division rate has long been observed in many animal groups as well as in plants (e.g., Van't Hof and Sparrow 1963; Grosset and Odartchenko 1975*a*, 1975*b*). This association suggests that developmental traits may also be linked to genome size at the whole organism level. In fact, an inverse relationship has been found between genome size and developmental rate in amphibians (Bachmann 1972*b*; Gregory 2003). Developmental traits have also been studied in fishes, birds, and mammals (including within primates and rodents), although reports have been conflicting with no clear indication of its importance to genome size (Morand and Ricklefs 2001, 2005; Gregory 2002*c*; E.M. Smith and Gregory 2009).

It might be tempting to assume that because links have been found between genome size and one or more adaptive characters, that this alone may be responsible for observed diversity in DNA amount among species. However, there are nonadaptive possibilities that should also be considered. For example, it is possible that mutational processes at the level of whole chromosomes play an important role. Indeed, a link has been found between genome size and chromosomal number in teleost fishes (Mank and Avise 2006; E.M. Smith and Gregory 2009). Whether genome size is driven by chromosomal duplications or losses, or whether more DNA is associated with higher rates of breakage, remains an open issue. It is, therefore, worth examining these associations in bats and other groups.

Another intriguing possibility relates genome size to mutation rate, genetic drift, and population size. From the perspective of population genetics, smaller populations are more susceptible to the influence of genetic drift, and thus the genomes of these animals are more likely to retain mildly deleterious mutations. Lynch and Conery (2003) proposed that animals have larger genomes relative to prokaryotes and unicellular organisms because their smaller population sizes passively allowed for the accumulation of gene duplications and mobile genetic elements. While empirical support for this model has been limited (Gregory and Witt 2008), this remains a question worthy of analysis. Though population size estimates are very difficult to obtain, body size has been suggested as a suitable proxy (Lynch and Conery 2003).

Taxonomy

Bats are interesting targets for genome size study owing to their incredible diversity and apparent genomic contraint, but they also present an interesting case because of ongoing controversey over phylogenetic relationships within order Chiroptera. Early classifications grouped all bats together based on their flight ability, with later separation of bats into two groups, the Megachiroptera (family Pteropodidae) and the Microchiroptera (all other bats). This division was initially based largely on overall body size; however, with time many additonal morphological and ecological differences supporting the separation were found between the two groups. Monophyly was traditionally assumed but was challenged in the 1980s with the hypothesis that megabats were more closely related to primates, and thus that flight evolved twice within mammals (Pettigrew 1986). Cladistic analysis of morphological characteristics appeared to support this hypothesis; however, since the application of molecular phylogenetics, the monophyly of bats has once again become widely accepted (Bailey et al. 1992).

Nonetheless, debate persists regarding the validity of the megabat–microbat division. Some recent classifications have not supported this distinction, instead dividing bats into the Yangochiroptera and Yinpterochiroptera. In this case, "megabats" are nested within the "microbat" families, being more closely related to the families Rhinolophidae, Megadermatidae, and Rhinopomatidae in the Yinpterochioptera (Teeling et al. 2002). By contrast, a recent study of the *prestin* gene, which is involved in hearing and suggested to be very important for echolocation, has provided support for the traditional megabat–microbat division; although this evidence has been criticized based on the nature of the gene (Li et al. 2008). At present, this issue remains unresolved. However, it is clear that the Pteropodidae are morphologically, physiologically, and ecologically unique among the bats, and as such the present study includes analyses across all bats as well as within pteropodids ("megabats") and nonpteropodids ("microbats") treated as functionally (if not phylogenetically) distinct categories.

Questions and predictions

There are many intriguing questions to be asked regarding genome size diversity in vertebrates in general, of which biologically unique but previously overlooked groups like bats represent a particularly interesting case. In an effort to fill this gap and to shed light on the evolution of bats and their genomic characteristics, this study poses and attempts to answer the following specific research questions:

(1) What is the extent of genome size diversity in bats, and is this universally constrained relative to other mammals?
   If flight constrains genome size in vertebrates, then all new estimates for bats are predicted to be small relative to nonflying mammals.
(2) Are cytogenetic features related to genome size diversity among bats?
   If chromosome-level processes are responsible for the diversity in genome size observed among bats, then a relationship between chromosome number and genome size is expected to exist.
(3) Are differences in genome size among bats linked to body size?
   If cell size, and not only cell number, affects body size in bats then genome size is predicted to be positively correlated with body size among bats as it is among rodents and birds. Alternatively (or in addition), body size may represent a proxy for population size, which has been proposed to correlate with genome size. This latter prediction can be tested by investigating other measures of population size, such as roost size.
(4) Can constraints related to flight explain the observed diversity of genome size within bats?
   If flight constraints determine patterns of genome size diversity within bats, and not just relative to other mammals, then bats are predicted to have small cells and to exhibit correlations between genome size and metabolic rate and wing parameters related to flight ability (e.g., wing loading).
(5) Are neurological constraints relevant to genome size diversity among bats?
   If the metabolic expense of the brain influences genome size diversity (due to investment in brain tissue, and (or) constraints on brain complexity based on neuron size vs. number), then bats should display relationships between genome size and indicators of investment in brain tissue (e.g., relative brain volume).
(6) Are developmental parameters related to diversity in genome size among bats?
   If genome size is linked to cell division in bats, then this may be predicted to produce relationships between genome size and parameters related to reproduction and development, such as gestation time or number of litters per year.
(7) Is diversity in genome size among bats associated with any ecological features?
   If genome size is constrained by (or influences) body size, metabolism, cognition, and (or) development, then it might be expected that genome size will be related to ecological features such as feeding, habitat, or sociality.

Materials and methods

The dataset used in this study is composed of novel genome size estimates for nonpteropodid bats ("microbats") (Table 1), 43 estimates for pteropodid bats ("megabat") produced by the same methodology (J.D.L. Smith and Gregory 2009), and a series of biological parameters collected from the literature[2] (Smith 2009).

Genome size data

In total, 650 samples from 448 individuals representing 121 species and 12 families were obtained from the Lubee Bat Conservancy (Gainesville, Florida), the Royal Ontario Museum (Toronto, Ontario), the University of Alaska Museum of the North (Fairbanks, Alaska), and the collection of one of the authors (J.W.B., Purdue University). Samples contributed by the Lubee Bat Conservancy consisted of air-dried blood smears taken from captive bats during routine veterinary care. Samples from Purdue University, the Royal Ontario Museum, and the Museum of the North were prepared on site from frozen kidney, liver, and (or) spleen tissues stored at −80 °C with no added preservatives or media.

Genome size (GS) estimation was conducted using Feulgen image analysis densitometry (FIA), following best practice methods as described in detail by Hardie et al. (2002). Briefly, slides were fixed overnight at room temperature in a methanol–formalin–glacial acetic acid solution of 85:10:5 (by volume), followed by a 2 h hydrolysis in 5.0 N HCl and subsequent staining for 2 h in Schiff reagent; a series of bisulphate and water rinses was used to terminate staining. A minimum of 50 nuclei was measured per sample using the Bioquant Life Science version 8.00.20 software package and an Optronics DEI-750 CE three-chip CCD camera mounted on a Leica DM LS microscope at 100× magnification. The resulting integrated optical densities (IODs) were converted to genome size in picograms using the IODs of two standards of known genome size: *Sus scrofa* (2.91 pg) and *Bos taurus* (3.56 pg) (Gregory 2013). Standards were stained in tandem and were of the same tissue type and preservation method (fresh or frozen) as the samples. Both standards were used for all estimates; averages of the two estimates gave the final genome size estimate. The additional biological parameters compared with these genome size data are summarized below (Smith 2009).

Chromosomal data

- The diploid number of chromosomes.
- The fundamental number of chromosome arms, an indicator of chromosome structure; calculation assumes metacentric, submetacentric, and subtelocentric chromosomes have two arms, while telocentric or acrocentric have one arm.
- The ratio of diploid chromosome number to the fundamental number of chromosome arms.

Cell size data

- Mean dry cell diameter of erythrocytes in micrometers (μm).
- Mean corpuscular volume in femtoliters (fL): calculated by dividing the hematocrit (L) by the red blood cell count (millions/μL).

Body size data

- Body mass in grams (g): recorded primarily from Smith et al. (2003) with missing values obtained from alternate sources when available.
- Head and body length in millimeters (mm) (distance from the tip of the nose to the base of the tail).

Physiological data

- Absolute basal metabolic rate (BMR) in milliliters of oxygen per hour (ml.O_2/hr).
- Body mass (g): recorded from the individuals used to measure basal metabolic rate.
- Relative basal metabolic rate (RBMR) in milliliters of oxygen per hour per gram of body mass (ml.O_2/hr.g): calculated by dividing the absolute basal metabolic rate by the mass of the individuals measured.

[2]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/gen-2013-0046.

Table 1. New mean haploid genome size estimates (GS, in pg) for chiropteran species, including the standard error for estimates obtained from more than one individual.

Family	Species	Common Name	GS (pg)	SE	*n* (F/M/U)	Tissue type	Tissue source
Emballonuridae			**2.67**	**0.49**	**2**		
	Saccopteryx bilineata	Greater sac-winged bat	3.16		1F	SP	1
	Taphozous sp.	Tomb bat sp.	2.18	0.03	2U	KC	1
Hipposideridae			**2.57**	**0.07**	**4**		
	Hipposideros caffer	Sundevall's leaf-nosed bat	2.44	0.05	2F, 2M	KC, SP	1
	Hipposideros commersoni	Commerson's leaf-nosed bat	2.48	0.10	2M	KC	1
	Hipposideros cyclops	Cyclops leaf-nosed bat	2.72		1M	KC	1
	Hipposideros ruber	Noack's leaf-nosed bat	2.65		1F	KC	1
Megadermatidae			**2.54**	**0.19**	**2**		
	Cardioderma cor	Heart-nosed bat	2.72	0.09	2F, 1M	KC	1
	Lavia frons	Yellow-winged bat	2.35		1M	KC	1
Molossidae			**2.53**	**0.08**	**6**		
	Chaerephon nigeriae	Nigerian free-tailed bat	2.47	0.06	1F, 1M	KC	1
	Chaerephon pumilus	Little free-tailed bat	2.40		1M	KC	1
	Eumops perotis	Greater bonneted bat	2.84		1F	LV	1
	Molossus molossus	Pallas's mastiff bat	2.41		1F	KC	1
	Molossus rufus	Black mastiff bat	2.72		1F	SP	1
	Sauromys petrophilus	Roberts's flat-headed bat	2.32	0.15	1M, 1U	KC, LV	1
Mormoopidae			**2.45**	**0.10**	**5**		
	Mormoops megalophylla	Peters's ghost-faced bat	2.83		1M	SP	1
	Pteronotus davyi	Davy's naked-backed bat	2.28	0.06	1F, 1M	SP	1
	Pteronotus parnellii	Common mustached bat	2.35	0.04	3F, 2M	LV, SP	1
	Pteronotus personatus	Wagner's mustached bat	2.43	0.00	2M	SP	1
	Pteronotus personatus psilotis	Wagner's mustached bat	2.35		1F	SP	1
Natalidae			**2.30**		**1**		
	Natalus sp.	Greater funnel-eared bat	2.30		1M	KC	1
Noctilionidae			**2.33**	**0.05**	**2**		
	Noctilio albiventris	Lesser bulldog bat	2.28		1U	KC	1
	Noctilio leporinus	Greater bulldog bat	2.37		1F	SP	1
Nycteridae			**2.77**	**0.05**	**3**		
	Nycteris arge	Bates's slit-faced bat	2.83		1M	KC	1
	Nycteris hispida	Hairy slit-faced bat	2.81		1F	KC	1
	Nycteris major	Dja slit-faced bat	2.66	0.09	2M	KC	1
Phyllostomidae			**2.43**	**0.04**	**34**		
	Artibeus cinereus	Gervais's fruit-eating bat	2.44	0.03	2M	KC	1
	Artibeus intermedius	Intermediate fruit-eating bat	2.43	0.03	1F, 2M	SP	1
	Artibeus hirsutus	Hairy fruit-eating bat	2.28	0.07	2U	KC, LV	1
	Artibeus jamaicensis	Jamaican fruit-eating bat	2.65	0.07	3F	LK, SP	1, 2
	Artibeus lituratus	Great fruit-eating bat	2.57	0.02	3F, 5M	LK, LV, SP	1, 2, 3
	Artibeus obscurus	Dark fruit-eating bat	2.44	0.01	26U	KC, LV	4
	Carollia brevicauda	Silky short-tailed bat	2.48	0.04	1F, 1M	LV	1
	Carollia castanea	Chestnut short-tailed bat	2.54		1F	LV	1
	Carollia perspicillata	Seba's short-tailed bat	2.63	0.01	30U	KC, LV	1, 3, 4
	Centurio senex	Wrinkle-faced bat	2.73		1F	SP	1
	Chiroderma villosum	Hairy big-eyed bat	2.67	0.01	2F	SP	1
	Choeronycteris mexicana	Mexican long-tongued bat	2.35		1U	SP	1
	Chrotopterus auritus	Woolly false vampire bat	2.53	0.10	2F, 2M	LV, SP	1, 3
	Desmodus rotundus	Common vampire bat	2.39	0.11	2F, 1U	SP	1
	Glossophaga longirostris	Miller's long-tongued bat	2.22	0.06	2M	KC, LV	1
	Glossophaga soricina	Pallas's long-tongued bat	2.34	0.04	2F, 1M	LV, SP	1
	Leptonycteris nivalis	Mexican long-nosed bat	2.36		1M	SP	1
	Lichonycteris obscura	Dark long-tongued bat	2.72		1F	KC	1
	Lophostoma carrikeri	Carriker's round-eared bat	1.63		1M	KC, LV	1
	Macrotus waterhousii	Waterhouse's leaf-nosed bat	2.67		1M	SP	1
	Micronycteris hirsuta	Hairy big-eared bat	2.24	0.04	1F, 2M	KC	1
	Mimon cozumelae	Cozumelan golden bat	2.30	0.04	2F, 2M	SP	1
	Mimon crenulatum	Striped hairy-nosed bat	2.19	0.04	1F, 1M	KC, LV	1
	Phylloderma stenops	Pale-faced bat	2.22	0.03	2M	KC	1
	Phyllostomus discolor	Pale spear-nosed bat	2.58		1F	LV	1
	Platyrrhinus helleri	Heller's broad-nosed bat	2.63		1U	KC	1
	Platyrrhinus lineatus	White-lined broad-nosed bat	2.43	0.05	2F, 2M	LV	3
	Pygoderma bilabiatum	Ipanema broad-nosed bat	2.47	0.10	2M	LV	3
	Sturnira lilium	Little yellow-shouldered bat	2.61	0.08	3F, 3M	LV, SP	1, 3

Table 1 (*continued*).

Family	Species	Common Name	GS (pg)	SE	*n* (F/M/U)	Tissue type	Tissue source
	Sturnira tildae	Tilda's yellow-shouldered bat	2.53		1M	LV	1
	Tonatia bidens	Greater round-eared bat	2.27	0.06	1F, 1M	KC, LV	1
	Trachops cirrhosus	Fringe-lipped bat	2.22	0.04	1F, 1M	KC, LV	1
	Trinycteris nicefori	Niceforo's bat	2.32		1M	KC	1
	Uroderma bilobatum	Common tent-making bat	2.42	0.08	1F, 1U	KC, SP	1
Rhinolophidae			**2.30**	**0.04**	**2**		
	Rhinolophus darlingi	Darling's horseshoe bat	2.33	0.04	1F, 3U	KC, LV	1
	Rhinolophus landeri	Lander's horseshoe bat	2.26		1M	LV	1
Rhinopomatidae			**3.17**		**1**		
	Rhinopoma hardwickii	Lesser mouse-tailed bat	3.17		1M	KY	1
Vespertilionidae			**2.33**	**0.02**	**59**		
	Antrozous pallidus	Pallid bat	2.67	0.05	1F, 3M	KC, LV	1
	Bauerus dubiaquercus	Van Gelder's bat	2.62	0.14	1M, 1U	LV, SP	1
	Chalinolobus gouldii	Gould's wattled bat	2.29		1F	KC	1
	Chalinolobus morio	Chocolate wattled bat	2.45	0.09	3F, 2M	KC, LV	1
	Eptesicus bottae	Botta's serotine	2.46	0.02	2F, 1M	KC, LV	1
	Eptesicus brasiliensis	Brazilian brown bat	2.36		1F	SP	1
	Eptesicus furinalis	Argentinian brown bat	2.34	0.03	2F, 2M	LV	1
	Eptesicus fuscus	Big brown bat	2.32	0.04	2F, 1M	LV	1
	Eptesicus hottentotus	Long-tailed serotine	2.37	0.04	2F, 2M	KC	1
	Eptesicus serotinus	Common serotine	2.38	0.00	2M	KC, LV	1
	Glauconycteris beatrix	Beatrix butterfly bat	2.13		1F	KC	1
	Harpiocephalus harpia	Lesser hairy-winged bat	2.41		1M	KC	1
	Hesperoptenus blanfordi	Blanford's bat	2.54		1F	SP	1
	Hesperoptenus tickelli	Tickell's bat	2.83	0.05	1F, 1U	KC, SP	1
	Histiotus macrotus	Big-eared brown bat	2.37		1F	KC, LV	1
	Kerivoula argentata	Damara woolly bat	2.30		1M	KC	1
	Laephotis botswanae	Botswanan long-eared bat	2.19		1M	KC, LV	1
	Laephotis namibensis	Namibian long-eared bat	2.28	0.04	5F, 1M	KC, LV	1
	Laephotis wintoni	De Winton's long-eared bat	2.03		1M	KC	1
	Lasionycteris noctivagans	Silver-haired bat	2.22		1F	LV	3
	Lasiurus ega	Southern yellow bat	2.46	0.05	2F, 3M	KC, LV	1
	Lasiurus insularis	Cuban yellow bat	2.54		1U	LV, SP	1
	Lasiurus intermedius	Northern yellow bat	2.43	0.05	2F, 2M	KC	1
	Lasiurus xanthinus	Western yellow bat	2.37	0.07	1F, 2M	KC	1
	Lasiurus borealis	Eastern red bat	2.31	0.06	2F, 1M	KC, LV	1
	Lasiurus cinereus	Hoary bat	2.37	0.06	1F, 1M	LV	1
	Lasiurus seminolus	Seminole bat	2.46	0.04	1F, 2U	KC, LV	1
	Miniopterus fraterculus	Lesser long-fingered bat	1.91	0.00	1F, 1M	KC, LV	1
	Miniopterus inflatus	Greater long-fingered bat	2.04	0.05	1M, 2U	KC, LV	1
	Miniopterus schreibersii	Schreibers's long-fingered bat	1.93	0.00	1F, 2M	KC	1
	Murina florium	Flores tube-nosed bat	2.00		1M	LV	1
	Myotis bocagii	Rufous myotis	2.35	0.13	2F	LV	1
	Myotis keenii	Keen's myotis	2.21	0.04	3M	KC, LV	1, 3
	Myotis lucifugus	Little brown myotis	2.26	0.07	2F, 4M	KC, LV	1, 3
	Myotis riparius	Riparian myotis	2.20		1U	KC	1
	Myotis septentrionalis	Northern myotis	2.20	0.07	2F, 2M	KC	1
	Myotis velifer	Cave myotis	2.30	0.07	2F, 2M	KC, LV	1
	Myotis yumanensis	Yuma myotis	2.30	0.02	3F	KC, LV	1
	Neoromicia capensis	Cape serotine	2.14	0.05	2F, 2M	KC	1
	Neoromicia guineensis	Guinean serotine	2.61		1F	KC	1
	Neoromicia somalicus	Somali serotine	2.19	0.05	2F, 2M	KC, LV	1
	Neoromicia helios	Samburu pipistrelle	2.26	0.06	1F, 2M	KC, LV	1
	Neoromicia nanus	Banana pipistrelle	2.26	0.11	1F, 2M	KC	1
	Nycticeinops schlieffeni	Schlieffen's twilight bat	2.32	0.14	2U	KC	1
	Nycticeius humeralis	Evening bat	2.19	0.01	2F, 1M, 1U	KC, LV	1
	Nyctophilus gouldi	Gould's long-eared bat	2.34	0.04	1F, 2M	LV	1
	Otonycteris hemprichii	Hemprich's desert bat	2.47		1U	KC	1
	Pipistrellus subflavus	Eastern pipistrelle	2.61	0.05	2F, 1M	KC	1
	Pipistrellus coromandra	Indian pipistrelle	1.99	0.03	2F, 2M	LV	1
	Pipistrellus hesperus	Western pipistrelle	2.29		1U	KC	1
	Pipistrellus kuhlii	Kuhl's pipistrelle	2.04	0.05	1F, 1M	KC	1
	Pipistrellus rusticus	Rusty pipistrelle	2.31		1M	LV	1
	Plecotus sp.	Long-eared bat	2.23		1F	KC	1
	Scotoecus hindei	Hinde's lesser house bat	2.41	0.07	1F, 3M	LV	1

Table 1 (*concluded*).

Family	Species	Common Name	GS (pg)	SE	*n* (F/M/U)	Tissue type	Tissue source
	Scotophilus nigrita	Giant house bat	2.41		1M	LV	1
	Scotophilus nux	Nut-colored house bat	2.54	0.06	2F	KC, LV	1
	Scotophilus viridis	Green house bat	2.56	0.05	2F, 2M	KC, LV	1
	Vespadelus darlingtoni	Large forest bat	2.43	0.04	2F, 1M	KC, LV	1
	Vespadelus regulus	Southern forest bat	2.42	0.05	1F, 2M	LV	1

Note: Genome size estimates were measured using Feulgen image analysis densitometry with at least 50 nuclei measured per sample with two samples being measured from most individuals. The number of individuals sampled is given as the number of females, males, or individuals of unknown sex (*n* (F/M/U)). The tissue type used for measurements is indicated as kidney (KC), leukocytes (LK), liver (LV), or spleen (SP). Standards were the same tissue type from *Bos taurus* (GS = 3.56 pg) and *Sus scrofa* (GS = 2.91 pg) for all samples. Chiropteran tissues were collected from 1: John Bickham, Purdue University, West Lafayette, Indiana; 2: Lubee bat Conservancy, Gainesville, Florida; 3: University of Alaska Museum of the North, Fairbanks, Alaska; 4: Royal Ontario Museum, Toronto, Ontario.

- Body temperature in degrees Celsius (°C): taken from animals at rest at their usual ambient temperature.

Flight data

The defining study on bat flight and wing parameters by Norberg and Rayner (1987) was used for most wing parameters. When necessary, corrections were made to additional values using the methods described by Norberg and Rayner (1987).

- Wingspan in metres (m): the total span of both wings from tip to tip while fully extended.
- Wing area in square meters (m^2): the total surface area of both wings including the intervening body area and area of the tail membrane.
- Wing aspect ratio: calculated as the square of the wingspan divided by wing area.
- Body mass (g): recorded from the individuals used to measure wing parameters.
- Wing loading (N/m^2): calculated as the mass in kilograms (kg) multiplied by *g*, the standard acceleration due to gravity (~9.80665 N/Kg) divided by the wing area.

Brain data

- Total brain mass in milligrams (mg).
- Total brain volume in cubic millimeters (mm^3).
- Neocortex volume (mm^3).
- Body mass (g): recorded from the individuals that were used to measure brain weights and volumes.
- Relative brain mass, and relative brain and neocortex volumes: calculated by dividing the values by body mass converted to milligrams (mg).
- Relative neocortex volume to brain volume: calculated by dividing the neocortex volume by the total brain volume.

Reproduction, development, and longevity data

- Gestation time: the length of uterine development in days. In species with delayed embryonic implantation or delayed development post implantation, only the time of continuous embryonic growth until parturition is recorded.
- Birth weight: the weight in grams (g) of neonates; typically defined as newly born young and occasionally near term embryos.
- Time to weaning: the number of days that neonates are breast fed.
- Time to sexual maturity: typically the age of first reproduction in both males and females; however, occasional data report the age at which functional sexual organs are present; values are given for males and females separately.
- Maximum recorded longevity for captive or wild individuals, in years.

Ecological data

- Feeding categories: bats are divided categorically by diet:
  1: Insectivore
  2: Frugivore/Nectarivore
  3: Omnivore
  4: Carnivore/Sanguivore
- Geographic distribution: bats are divided categorically into biogeographic regions:
  1: Nearctic
  2: Palearctic
  3: Neotropic
  4: Afrotropic
  5: Indo-Malay
  6: Australasian
- Roost size: the largest reported roost size for the species, defining them by categories:
  1: Solitary (1–3 bats)
  2: Small groups (4–20 bats)
  3: Moderate groups (21–99 bats)
  4: Large groups (100–500 bats)
  5: Highly gregarious (more than 500 bats)

Data analysis

The relationships between genome size and continuous parameters were tested using Pearson correlations on log-transformed data. Where appropriate, body mass correction was employed by regression of both parameters against body mass and subsequent Pearson correlations on the residuals. Categorical parameters (feeding categories, geographic distribution, and roost size) were analyzed using ANCOVA with body mass as the covariable. Where the covariable is highly nonsignificant ($p > 0.20$), ANOVA was completed. Significant differences between categories were identified using Tukey HSD tests. In addition, correlation analysis was performed on genome size and principal component 1 (PC1), representing all body size parameters (body mass, head and body length, wingspan, and wing area). Given the large number of correlations in this study, Bonferroni correction was also used to adjust the *p* value to lower the risk of type I error. Given that many similar parameters were used, correction was employed by counting groups of parameters as correlations (e.g., Body size = body mass, head and body length, etc.), giving a total of 10 grouped comparisons. The significance level can be adjusted by Bonferroni correction in several ways depending on how the dataset is considered to be partitioned. If considering all grouped parameters at all taxonomic levels (species, genus, and family) and functional division (bats, megabats, and microbats) ($n = 10 \times 3 \times 2 = 80$), the correction sets $p = 0.0006$. Taken as independent datasets according to taxonomic level or functional division ($n = 10 \times 3 = 30$), $p = 0.002$; while considering just the grouped parameters ($n = 10$), $p = 0.005$.

Accounting for phylogeny

To account for nonindependence of phylogenetically related species, analyses were completed using hierarchical taxonomic correlations (Pagel and Harvey 1988; Vinogradov 1995; Gregory 2000) using nested averages at the species, genus, and family levels for all bats and microchiropterans, and at the species

and genus level for megachiropterans. Additionally, though the current species-level phylogeny for Chiroptera is poorly resolved even at the genus level, phylogenetically independent contrasts (PICs) were attempted using the supertree presented by Jones et al. (2002) and Bininda-Emonds et al. (2007) for all significant or near significant relationships found using Pearson correlations. PICs were conducted using the PDAP module (Midford et al. 2008) in Mesquite v2.5 (Maddison and Maddison 2009), with one degree of freedom subtracted for each branch in a polytomy and branch lengths set to 1.

Results

Overview of genome size in bats

Genome size estimates for 121 species of microbats are presented in Table 1. Of these, 94 represent novel genome size estimates, and 27 species have been previously studied (J.D.L. Smith and Gregory 2009). Of those previously reported (most >20 years ago), our estimates were on average lower (2.40 pg vs. 2.54 pg; *t* test, $p < 0.0008$).

Genome size varied less than 2-fold in the species analyzed (including the megabats surveyed by J.D.L. Smith and Gregory 2009), ranging from 1.63 pg in Carriker's round-eared bat, *Lophostoma carrikeri*, to 3.17 pg in the lesser mouse-tailed bat, *Rhinopoma hardwickii*, with an average of 2.35 pg ± 0.02 SE. Thus, it remained the case in this larger sample that all bats examined possess genomes much smaller than those typical of most mammals (average ~3.50 pg) (*t* test, $p < 0.0001$; Fig. 1A) (Gregory 2013). The bat data were non-normally distributed around a mean of 2.35 pg ± 0.02 SE (Shapiro–Wilk test, $W = 0.97$, $p < 0.05$); however, when analyzed separately the data for megabats were normally distributed around a mean of 2.20 pg ± 0.02 SE (Shapiro–Wilk test, $W = 0.98$, $p > 0.80$), whereas the data for microbats were non-normally distributed, averaging 2.40 ± 0.02 SE (Shapiro–Wilk test, $W = 0.97$, $p < 0.05$). Megabats appear to be even more strongly constrained to small genome sizes than other bats in terms of both mean values (2.20 pg vs. 2.40 pg; *t* test, $p < 0.0001$; Fig. 1B) and variance (*F* test, $F_{[120,42]} = 3.61$, $p < 0.0001$) (J.D.L. Smith and Gregory 2009).

Chromosomal features

As might be expected, chromosome number and fundamental number of arms were correlated within and across bat groups for genus and species level analyses (all $r > 0.55$; $p < 0.0037$), though nonsignificant at the family level. However, no significant relationship was found between genome size and chromosome number, fundamental number of arms, or the ratio between the two (all $p > 0.32$).

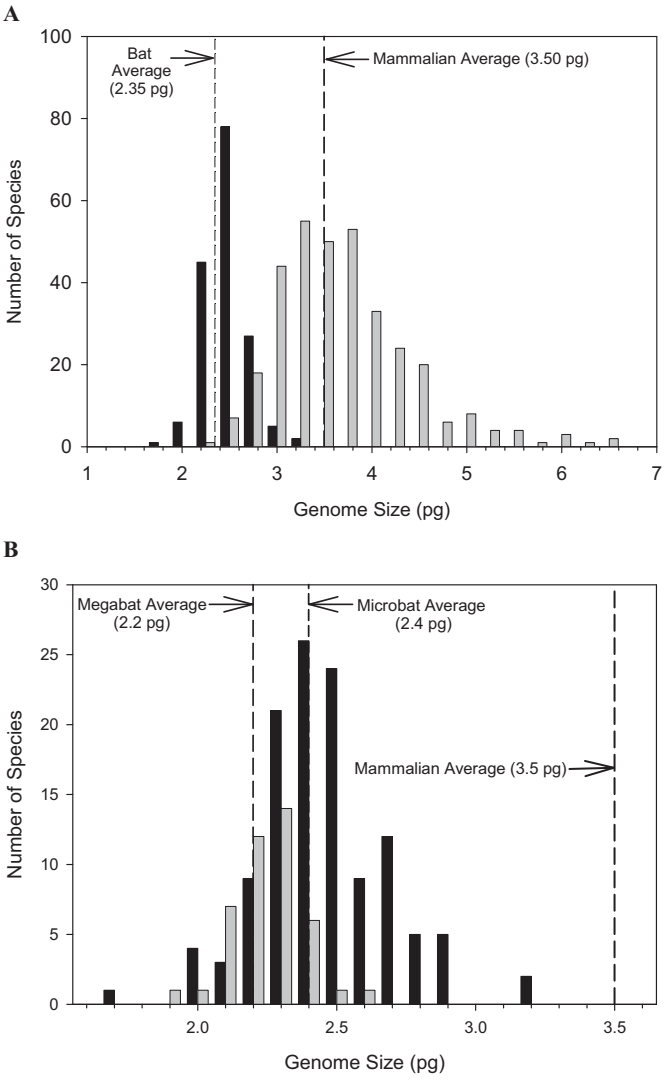
Cell size

While sufficient cell size data were not available for statistical analyses within bats, it was possible to compare bats versus other mammals in terms of cell size. Figure 2 shows the relationship between genome size and erythrocyte size given as mean dry diameter and mean corpuscular volume in mammals, including the few bats for which these data were available. Bats appear at the lower end of the plot with minimal deviation from the main line, as would be expected if genome size and cell size do correlate in this group as among mammals in general (Gregory 2001*a*).

Body size

Body size can be measured using a variety of parameters, all of which are highly intercorrelated. Principal component analysis was used on several morphometric parameters (body mass, head and body length, wingspan, and wing area) to produce a single parameter (PC1) that accounted for ~96% of the variation in the combined dataset, while a second component (PC2) accounted for an additional 3%. Figure 3A plots PC1 versus PC2,

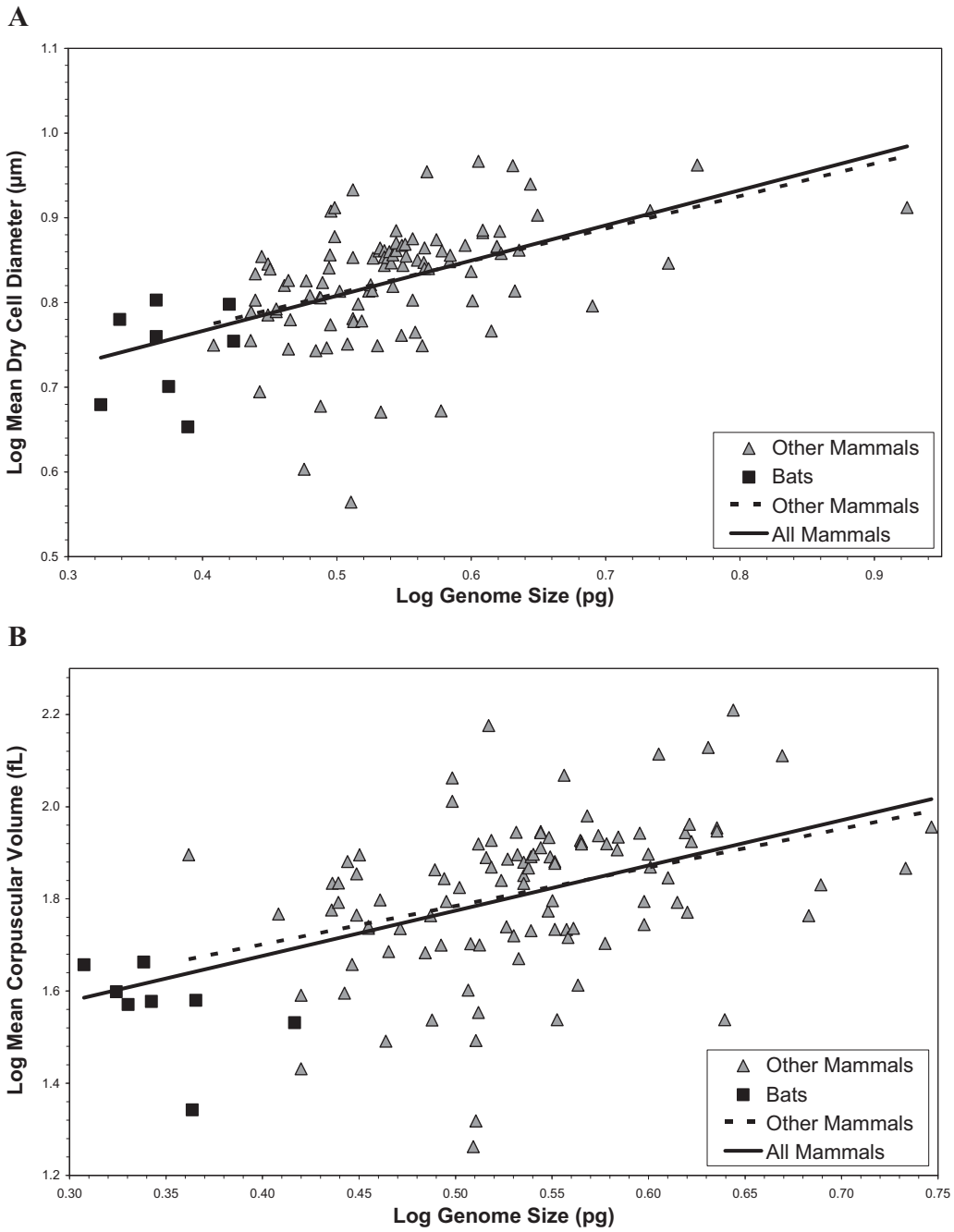
Fig. 1. (A) Summary of haploid genome size diversity in bats (black bars; data from J.D.L. Smith and Gregory (2009) and the present study) relative to other mammalian species (grey bars; data from the Animal Genome Size Database; Gregory (2013)). Bats have a smaller average genome size relative to the mammalian average of ~3.5 pg and show a distribution of genome sizes near the low end of the mammalian dataset. (B) Summary of genome size diversity in 43 species of bats in the family Pteropodidae (megabats; grey bars; data from J.D.L. Smith and Gregory (2009)) and 121 species of nonpteropodids (microbats) from 12 families (black bars; data from the present study).



highlighting the differences between morphology in pteropodids ("megabats") and nonpteropodids ("microbats") (but note that the two do not form clear clusters as in some other animals; e.g., Ardila-Garcia and Gregory (2009)). Pearson correlations of genome size with PC1 (Fig. 3B) revealed no relationship for all bats ($r = -0.1640$, $p = 0.2227$, $n = 56$) or within microbats ($r = 0.0343$, $p = 0.8359$, $n = 39$), or megabats ($r = 0.4656$, $p = 0.096$, $n = 17$). Employing PICs weakened the relationship in megabats ($r = 0.3429$, $p = 0.1829$, $n = 16$), whereas the relationship remained insignificant in microbats ($r = 0.2423$, $p = 0.1408$, $n = 38$).

Body mass was found to correlate with genome size at the species level in both microbats ($r = 0.2733$, $p = 0.0042$, $n = 108$) and megabats ($r = 0.4847$, $p = 0.0027$, $n = 36$), but this was not so when all bats were analyzed together ($r = -0.0341$, $p = 0.6846$, $n = 144$). With PICs, the

Fig. 2. Relationships between genome size and cell size in mammals, measured as (A) mean dry diameter (μm) and (B) mean corpuscular volume (fL). Although data are not sufficient to examine correlations within bats, it is clear that their cell sizes fall along the same line (at the lowest end) as those of mammals in general.



relationship seen in megabats disappears ($r = 0.0329$, $p = 0.8498$, $n = 35$), whereas it persists in microbats ($r = 0.2947$, $p = 0.0024$, $n = 106$).

Head and body length correlated with genome size within megabats (species level; $p = 0.0043$; genus level, $p = 0.0509$); however, after mass correction the relationship was not significant; PICs revealed no relationship ($r = 0.1910$, $p = 0.3535$, $n = 16$).

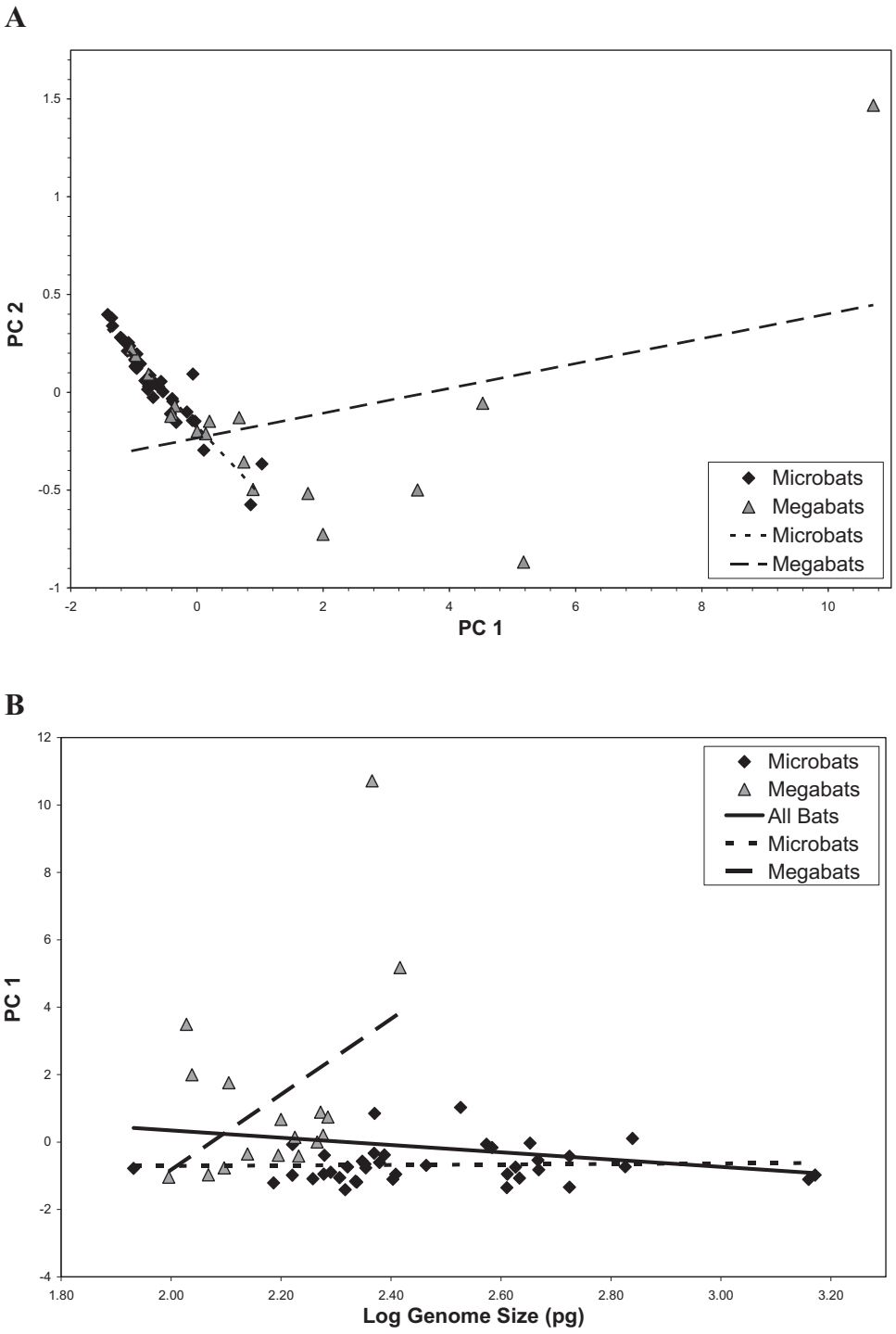
Physiology

Absolute (BMR) and relative (RBMR) basal metabolic rates did not correlate with genome size at any level for all bats or within microbats. Relationships between genome size and BMR and RBMR were only significant without mass correction at the species level for BMR (uncorrected: $r = 0.632$, $p = 0.0153$, $n = 14$; mass-corrected: $r = -0.0077$, $p = 0.9792$, $n = 14$) and genus level for RBMR (uncorrected: $r = -0.8141$, $p = 0.0076$, $n = 9$; mass-corrected: $r = -0.5597$, $p = 0.1171$, $n = 9$). No correlations were found between genome size and body temperature at any taxonomic level among all bats, within microbats, or within megabats. PICs could not be performed on significant BMR correlations; data spread yielded contrasts that could not be correlated by Mesquite.

Flight

No significant relationships were found between genome size and wing parameters among all bats or within microbats at any taxonomic level. Wingspan, wing aspect ratio, and wing loading index were all correlated with body mass and highly intercorrelated (all

Fig. 3. (A) Relationship between principal components 1 and 2 for all morphometric parameters, illustrating the separation and difference between pteropodids (megabats) and nonpteropodids (microbats). (B) Relationship between principal component 1 and genome size among all bats, within microbats and within megabats.

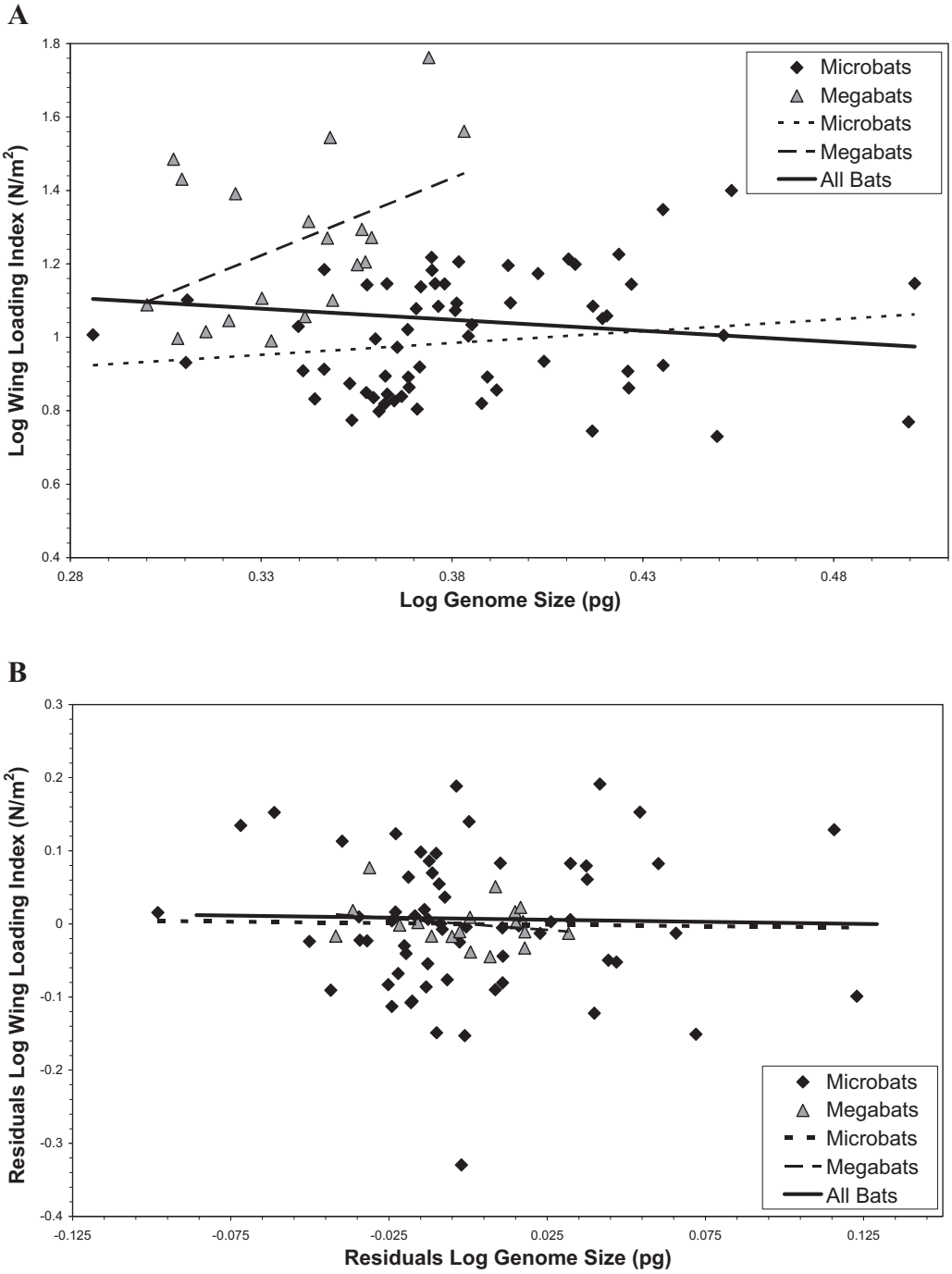


$r > 0.45$, most $p < 0.0001$). Within megabats there was a significant correlation with wing loading index at the species level prior to mass correction (uncorrected: $r = 0.4507$, $p = 0.0461$, $n = 20$; mass-corrected: $r = -0.2234$, $p = 0.2234$, $n = 19$; Fig. 4). Prior to mass correction, relationships between genome size and with wingspan, wing area, and aspect ratio were marginal with all $p < 0.1$, with PICs; after mass correction these relationships were much weaker within megabats, showing no relationship.

Brain size

Using multiple brain size parameters, no obvious relationships were found with genome size. Megabat genome size correlated with all brain parameters prior to mass correction at the species level (all $p < 0.0172$, all $r > 0.49$ for brain volume, neocortex volume, BRBM and neocortex to brain volume and all $r < -0.45$ for relative brain mass, and relative brain and neocortex volumes); however, all relationships disappeared af-

Fig. 4. Relationship between genome size and wing loading index within pteropodids (megabats; gray triangles, long-dashed lines), among nonpteropodids (microbats; black diamonds, short-dashed lines), and across all bats analyzed together (solid line), (A) before and (B) after mass correction.



ter mass correction (all $p > 0.15$; Fig. 5); PICs yielded similar results. All brain parameters were highly intercorrelated as might be expected (all $r > 0.9$, $p < 0.0001$).

Reproduction, development, and longevity

No relationships were found between genome size and any developmental parameters (gestation time, birth weight, time to weaning, time to sexual maturity, and longevity).

Ecology

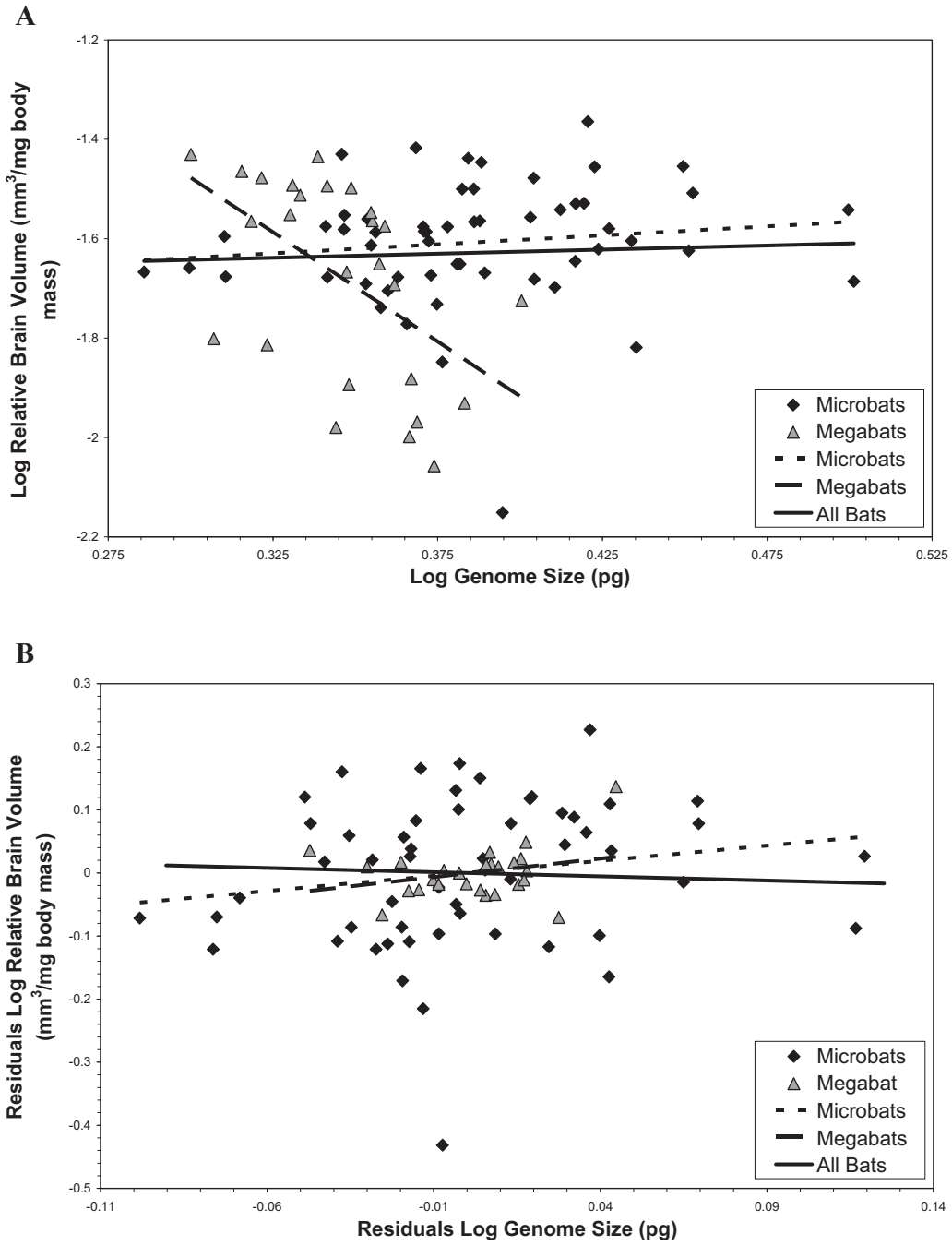
No significant differences were found between genome size and roost size or genome size and feeding categories. Analysis of genome size based on biogeography revealed significant differences between Indomalayan bats and Neotropical bats; however, this reflects the differences in genome size between megabats and microbats.

Discussion

(1) What is the extent of genome size diversity in bats, and is this universally constrained relative to other mammals?

Previous studies have indicated that genome size estimates for bats are much lower on average than other mammalian groups. The suggested reason for this apparent genomic con-

Fig. 5. Relationship between genome size and relative brain volume within pteropodids (megabats; gray triangles, long-dashed lines), among nonpteropodids (microbats; black diamonds, short-dashed lines), and across all bats analyzed together (solid line), at the species level, (A) before and (B) after mass correction.



straint is the evolution of flight in bats, which precludes large genomes (and cells) owing to the metabolic intensity of flight. This study greatly expanded the available dataset for bats, generating genome size estimates for 121 species from 12 families. In agreement with previous work, all of these genome size estimates are constrained relative to other mammals (Fig. 1).

Of the mammals studied to date, bats do indeed possess the smallest genomes. However, there is overlap with the low end of the distribution of some other orders. For example, some rodents display genome sizes as small as those at the higher end of the bat distribution. In keeping with the hypothesis that metabolic constraints are relevant to patterns of genomic diversity, this overlap tends to occur in groups with high metabolic rates such as shrews.

Within bats, the megabats (family Pteropodidae) also have small genomes (J.D.L. Smith and Gregory 2009), and, surprisingly, they seem to have even more constrained genome sizes than microbats in terms of both average and variance. While this may be expected since megabats comprise only one family whereas microbats represent many families, megabats have a lower average genome size and equal or lower variance than other bat families taken individually (Table 1).

Genome size estimates within bat species tended to be consistent, usually varying less than 0.2 pg (i.e., within 8%), with standard errors

less than ±0.05. In general, agreement with previous estimates was good in terms of upholding similar interspecies differences. However, the new estimates were on average lower than estimates reported in the past for the same species. Differences in current estimates and previous studies' are likely due to differences in methodologies (Hardie et al. 2002); previous estimates came from several different research groups, all of which used differing methodologies (Capanna and Manfredi Romanini 1971; Bachmann 1972*a*; Manfredi Romanini et al. 1975; Kato et al. 1980; Burton et al. 1989; Redi et al. 2005).

(2) Are cytogenetic features related to genome size diversity among bats?

Chromosome number can be highly variable among animal groups, in terms of both absolute number and the range of diversity across related species. Chromosome numbers can be altered by events such as deletions, duplications, inversions, and translocations, which can not only alter the overall structure of the chromosome complement but may in some cases result in a change in DNA content. Furthermore, given that necessary structural components such as centromeres and telomeres are composed of repetitive DNA, it might be expected that some of this variability would be related to total DNA content. These factors may account for the reported correlation between genome size and diploid chromosome number in ray-finned fishes (Mank and Avise 2006; E.M. Smith and Gregory 2009).

As with genome size, bats have somewhat lower diploid chromosome numbers (average: $2n \approx 38$, range: $2n = 16$ to 62) than the mammalian average ($2n \approx 44$) (Neuweiler 2000; Ruvinsky and Marshall Graves 2004). However, within bats, no relationship was found between genome size and diploid chromosome number, fundamental number of chromosome arms, or the ratio between the two parameters, suggesting that chromosome level alterations are not responsible for overall genome size variation within this order.

While chromosome-level mechanisms do not seem to play a major role in determining genome size diversity among bats, there are other genomic features that may be relevant. Transposable elements (TEs) in particular represent a substantial portion of mammalian genomes—45% of the human genome is comprised of TEs, for example (International Human Genome Sequencing Consortium 2001). Differences in TE composition between species could explain a large amount of the genome size variation. Notably, megabats experienced an extinction of the most common long interspersed nuclear element in mammals (LINE-1) early in their ancestry, which may help to explain the reduction in absolute numbers of these elements and a correlated decrease of genome size in the megabats (Cantrell et al. 2008). This may be additionally relevant because short interspersed nuclear elements (SINEs) are dependent on LINEs for their mobility. On the other hand, recent evidence in the little brown bat (*Myotis lucifugus*) has suggested that while most TEs are thought to be inactive in mammals, there has been more recent activity in some species of bats (Ray et al. 2007, 2008). This is similar to the situation found in pufferfishes, in which TEs are not abundant but are diverse and active, perhaps reflecting divergence of elements under strict competition imposed by limited insertion sites in small genomes (Neafsey and Palumbi 2003; Gregory 2005*b*).

(3) Are differences in genome size among bats linked to body size?

Initial hypotheses relating genome size to body size stem from invertebrate groups such as copepods and flatworms whose body size is determined by changes in cell volume, rather than changes in cell number (Gregory et al. 2000). Such relationships are not typically expected in groups like mammals, where genome size ranges 4-fold but body sizes differ by several orders of magnitude. While examining mammals at higher taxonomic levels, this expectation of a decoupling of genome size and body size is met. However, in more closely related groups with much less body size variability such as rodents, body size and genome size may be positively related (Gregory 2002*c*).

Principal component analysis to assess the relationship between genome size and body size revealed a marginal relationship within megabats but not in microbats (except after employing PICs). Unfortunately, multivariate techniques have the limitation of requiring data for every measure of body size assessed, resulting in very small sample sizes in instances where only some parameters are available for species. Using Pearson correlations revealed relationships between genome size and body mass in microbats and megabats and head and body length. Although the relationships were not universal (with Bonferroni correction some would be considered nonsignificant), they did not appear randomly. Rather, the relationships appeared at different taxonomic levels and both in microbats and megabats, not as would be expected if the correlations were due to chance when completing large numbers of correlations. Although not strongly related, measures of body size do correlate with genome size, particularly in megabats where body size differences are greater and detectable relationships are stronger. With PICs similar results were found, although the relationship between genome size and body size in megabats may be related to phylogeny. Unfortunately, the best tree available for bats is still unresolved at the species level for many taxa, with a large number of polytomies (~35% of the tree) making it difficult to draw any firm conclusions.

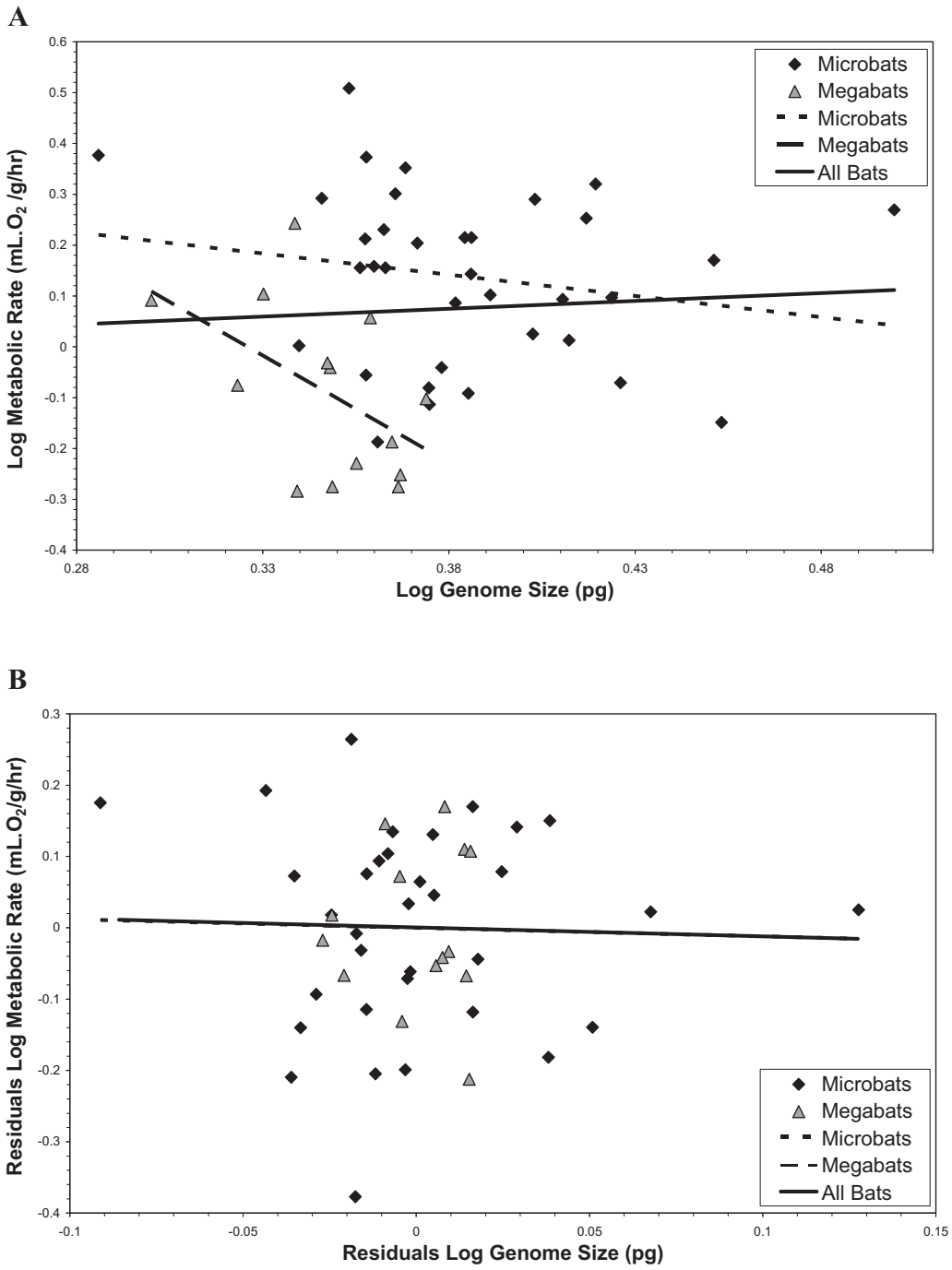
The relationship between genome size and body size could be explained by changes in cell volume, as has been found in some invertebrates. However, it is also possible that the relationship is somewhat spurious and results from links of both genome size and body size to an additional trait. Body size is related to a large number of potentially adaptive biological features. This relationship makes it somewhat difficult to determine which traits are acting adaptively to modulate genome size because mass correction removes so much variation that often no residual relationships can be found. While adaptive mechanisms may lead to a relationship between genome size and body size, it is also possible that nonadaptive mechanisms are involved. Lynch and Conery (2003) proposed that genome size can evolve through accumulation of slightly deleterious duplications and TE insertions fixed by genetic drift in small populations. Body mass has been used as an estimator of population size and relationships between genome size and body size may simply reflect passive accumulation of DNA (Lynch 2007). This study did examine roost size in bats, finding no relationship between genome size and population size; however, whether roost size can be taken as a measure of effective population size is uncertain, leaving this possibility open. The link between genome size and body size in bats (and rodents) requires further investigation to tease apart the underlying mechanisms.

(4) Can constraints related to flight explain the observed diversity of genome size among bats?

Flight is thought to be a constraining factor on genome size in birds and bats through a link between genome size, cell size, and metabolic rate. Flight is a metabolically expensive activity leading to very high mass-specific metabolisms in bats. Genome size and cell size positively correlate within mammals (Gregory 2000); however, in bats, too few cell size data were available to examine the link between genome size and cell size (and hence metabolism). Nonetheless, the few data that were available fit at the lower end of the relationship found between cell size and genome size in other mammals, as would be expected (Fig. 2).

Previous studies have found that in both mammals and birds there is a relationship between mass specific metabolic rate and genome size (Vinogradov 1995, 1997; Gregory 2002*a*). Relationships were seen in megabats between genome size and absolute and

Fig. 6. Relationship between genome size and relative metabolic rate within pteropodids (megabats; gray triangles, long-dashed lines), among nonpteropodids (microbats; black diamonds, short-dashed lines), and across all bats analyzed together (solid line), (A) before and (B) after mass correction.



relative basal metabolic rates but not in microbats. This might be expected as absolute and relative metabolic rates scale with body mass, and megabats have a much greater variation in body size (White and Seymour 2003). However, absolute basal metabolism and relative basal metabolic rate in bats were not correlated with genome size in bats when the influence of body mass was removed statistically (Fig. 6).

The lack of relationship between genome size and metabolism can be interpreted in many ways. The first possibility would be that while a high metabolism may have been important in an early reduction in genome size in the ancestors of bats, it may not be sufficiently influential to determine the narrow range of genome size in modern bats. The second possibility is that BMR does still relate to genome sizes among bats but that a relationship was not found for a number of reasons including the following: (*i*) Measurements of basal metabolism are particularly sensitive to variation in experimental technique, but they were necessarily sourced from several different references. It cannot be guaranteed that cross comparison is accurate as this level of error may interfere with detection of relationships with such a narrow range in genome size. (*ii*) Considering basal metabolism as the informative metabolic measurement, rather than active metabolism or meta-

bolic scope (for which data are unavailable) may not be the most informative. (*iii*) Bats have unique features that confound studies on energetics; for example, they are capable of long-term hibernation and daily torpor to reduce energy usage. These unique patterns of energy distribution make it difficult to interpret single measures of metabolism. (*iv*) The relationship between genome size and body mass may remove so much variation that after mass correction there is insufficient variation to yield a significant relationship with current sample sizes. A third interpretation relates to the fact that, like all mammals, bats have enucleated erythrocytes, which allows for more compact cells compared with other vertebrate groups with similar genome sizes. Erythrocyte size still correlates with genome size in mammals (Gregory 2000); however, it is possible that this mechanism of achieving small cells and high metabolism allows for less stringent selection on genome size than is seen for example in nucleated erythrocytes in birds.

While bats have high metabolisms and low genome size, similar to birds, they differ as no correlation exists between metabolism and genome size among bats. Notably, parameters that indicate specialization for flight such as wing loading index are related to genome size in birds but not in bats (Andrews et al. 2009). So while bats have low genome size relative to other mammals, suggesting that small genomes may in some way be associated with flight, current levels of variation in genome size among bats do not seem to be due to adaptations for flight. Again this could be related to novel innovations of erythrocytes, which may decouple the link between genome size, cell size, and metabolism that can be seen in birds. Another possibility is that small genomes and high metabolic rates evolved together early in the bat lineage and have been maintained since, leading to minimal variation that can correlate strongly among modern bats.

(5) Are neurological constraints relevant to genome size diversity among bats?

Brain size in bats has been found to relate to flexibility in feeding behaviours, whereby bats with larger brains have the ability to utilize more dispersed feeding areas, can occupy more complex habitats, and can feed using different mechanisms (Eisenberg and Wilson 1978; Safi and Dechmann 2005; Ratcliffe et al. 2006). Similarly, in birds larger brains appear to increase survival in novel environments (Sol et al. 2005). Recently, relative brain size was found to negatively correlate with genome size in parrots, indicating a higher relative investment in brain tissue and (or) complexity (Andrews and Gregory 2009). Unlike in birds, relative brain size does not correlate with genome size in bats independent of body mass. However, relative brain volume does correlate with body mass, suggesting that brain size is increased to improve brain function or give equivalent brain power to larger animals, as would be expected, accomplished by increasing brain size, not by decreasing cell size.

(6) Are developmental parameters related to diversity in genome size among bats?

Developmental rate is often thought to correlate with genome size because of corresponding influences of DNA amount on cell cycle duration. Relationships between genome size and developmental rate and intensity of metamorphosis have been found within amphibians (Gregory 2002*b*); however, in mammals, a developmental relationship is only apparent within rodents (Gregory 2002*c*) and was not observed in primates (Morand and Ricklefs 2005).

Based on the present analysis, genome size does not correlate with any developmental parameters or longevity in bats. As genome size in bats is quite constrained, it is possible that small changes in genome size may not be enough to cause appreciable differences in cell cycle length. However, it is probable that bats are uniquely constrained in terms of their reproduction and development owing to their volant lifestyle and the burden of carrying young.

(7) Is diversity in genome size among bats associated with any ecological features?

Many of the reasons for expecting genome size to correlate with ecological features are dependent on relationships with metabolism, cognition, and development. These characters do not appear to be related to genome size in bats, so it may not be surprising that roost size, diet, and biogeography were unrelated to genome size in this study.

Conclusions

Based on the results of the present research, the following three major conclusions can be drawn regarding genome size diversity in bats:

(1) Genome size in both bat groups is constrained relative to other mammals as expected; however, while they might both be expected to be similarly constrained on the basis of flight, there are some interesting differences between microbats and megabats illustrated by the relationships of genome size with various parameters.
(2) Genome size in bats does not appear to be related to most biological parameters that have been found to correlate in other vertebrate taxa, with the exception of a relationship with body size. While it is possible that genome size relates to body size owing to changes in cell volume, it is also possible (and perhaps more likely) that genome size is sculpted by adaptive parameters that correlate with body size. It is also possible that this reflects the role of nonadaptive processes relating to population size. The significance of the body size correlation, therefore, remains an open question.
(3) The hypothesis that flight was a constraining factor on genome size early in bat evolution is strongly supported, but variability in flight intensity does not explain the small differences in DNA amount observed in modern bat species.

Future directions

Although the present study has contributed significantly to information on bat genome size, many interesting topics remain to be explored in this group. Some of these are outlined as follows:

(1) While the fossil record is limited for bat species, it might be worthwhile to estimate genome size from extinct species. Transitional forms would be ideal to test the hypothesis that genome size must be small for flight to evolve; however, even extinct flighted species could be informative. If bats follow the patterns seen in dinosaurs/birds and pterosaurs, then there should be signs of genome size decrease early in bat evolution.
(2) Transitional forms of bats are predicted to be similar to modern day colugos or flying squirrels, with membranes stretched between limbs for gliding. Studies concerning the relative metabolisms and genome size of gliding mammals and their closest nongliding relatives would perhaps illustrate the importance of small genomes for prevolant lifestyles.
(3) The relationship between genome size and cell size has been examined almost entirely on the basis of erythrocytes among vertebrates. It would be helpful to examine other cell types in mammals and other taxa. This could be especially informative for determining the cause of the relationship between genome size and body size in bats.
(4) The notion that passive accumulation of DNA in small populations is a determinant of diversity in genome size remains to be tested for most groups. Reliable genetic methods for estimating long-term effective population size could be use-

ful, especially in groups such as bats where population sizes vary widely.

Bats have highly constrained genomes relative to other mammals, overlapping in genome size with many avian species. While clear comparisons can be drawn between birds and bats in terms of their genomes, the biological impact of this diversity may differ between the two groups. Thus, while flight may have constrained genome size in the early ancestry of all three lineages of flying vertebrates (bats, birds, and pterosaurs), flight-related effects are not important in terms of patterns within bats in the manner that they are in birds. Instead, other factors (particularly those directly or indirectly linked to body size) represent the most significant areas for further exploration in airborne mammals.

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