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The genome sizes of megabats (Chiroptera: Pteropodidae) are remarkably constrained

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It has long been recognized that bats and birds contain less DNA in their genomes than their non-flying relatives. It has been suggested that this relates to the high metabolic demands of powered flight, a notion that is supported by the fact that pterosaurs also appear to have exhibited small genomes. Given the long-standing interest in this question, it is surprising that almost no data have been presented regarding genome size diversity among megabats (family Pteropodidae). The present study provides genome size estimates for 43 species of megabats in an effort to fill this gap and to test the hypothesis that all bats, and not just microbats, possess small genomes. Intriguingly, megabats appear to be even more constrained in terms of genome size than the members of other bat families.

Keywords: bats; long interspersed element-1; LINE-1; transposable elements

1. INTRODUCTION

Diversity in genome size (DNA content per haploid nucleus) among eukaryotes has been a subject of study since the late 1940s. However, growth in interest in this topic has been particularly marked over the past decade, prompted by several factors: (i) the rise of large-scale comparative genomics, for which genome size data have practical importance (e.g. Gregory 2005*a*), (ii) the identification of relationships between genome size and key biological parameters such as cell size, cell division rate and related organism-level traits (e.g. Bennett & Leitch 2005; Gregory 2005*b*), (iii) the launch of online databases now containing genome size estimates for more than 10 000 species (Gregory *et al.* 2007), and (iv) the advent and/or refinement of efficient technologies for genome quantification.

Various mechanisms capable of increasing and/or decreasing genome size are now recognized, ranging from small-scale replication slippage to whole-genome duplication. In particular, the spread and loss of transposable elements—sequences that (at least initially) behave as parasites of the host genome—are increasingly understood to be a dominant mode of genome size change in animals and plants (e.g. Kidwell & Lisch 2001; Lynch & Conery 2003; Gregory 2005*a*). In combination, these processes have generated a more than 7000-fold range in genome size among animals (Gregory 2009). Not surprisingly, much of the foregoing discussion of genome size evolution has focused on the enormous differences that occur among taxa. On the other hand, there is an increasing interest in groups that are diverse both taxonomically and ecologically but which exhibit only a limited range in genome size. As a notable example, amphibians (approx. 5000 species) range in genome size by 120-fold, whereas birds (approx. 10 000 species) differ by approximately twofold (Gregory 2009).

One hypothesis that has been the subject of much recent discussion is that groups of vertebrates with especially high metabolic demands—in particular, those that have evolved powered flight—are constrained at the genome level due to the links between genome size, cell size and metabolic rate (Hughes & Hughes 1995; Gregory 2002; Organ & Shedlock 2008). It has recently been shown, for example, that wing loading (an index of flight ability) is correlated with genome size among perching birds (Andrews *et al.* 2009). Studies of fossil cell sizes have also indicated that, as with feathers and bipedal locomotion, theropod dinosaurs already exhibited some reduction in genome size prior to the evolution of birds (Organ *et al.* 2007). Likewise, it has recently been reported that pterosaurs had smaller genomes than their non-flying relatives (Organ & Shedlock 2008).

In keeping with this pattern, it has been recognized for several decades that bats possess genome sizes much smaller than the average for mammals (e.g. Capanna & Manfredi Romanini 1971, 1973; Bachmann 1972; Burton *et al.* 1989; Redi *et al.* 2005). Unfortunately, all but one of the more than 60 species of bats that have been assessed thus far are 'microbats' (Gregory 2009). The only 'megabat' studied, the flying fox *Pteropus giganteus*, also exhibits a small haploid genome size (2.2 pg; Manfredi Romanini *et al.* 1975), but it is not possible to draw conclusions on the basis of so few data. The almost total lack of data from megabats therefore represents a glaring omission in the current animal genome size dataset.

2. MATERIAL AND METHODS

This study marks the first major survey, to our knowledge, of megabat genome size diversity and the single largest study of bats conducted to date. In total, 215 samples from 144 individuals in 43 species were obtained from the Lubee Bat Conservancy (Gainesville, FL, USA), Dr John Bickham at Purdue University (West Lafayette, IN, USA) and the Royal Ontario Museum (Toronto, Ontario, Canada). 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 and the Royal Ontario Museum were prepared from frozen kidney and/or liver tissues stored at −80°C.

Genome size estimates were conducted by Feulgen image analysis densitometry following best practice methods as described in detail by Hardie *et al.* (2002). A minimum of 50 nuclei was measured per sample and integrated optical densities were converted to genome size in picograms (1 pg = 978 Mbp) using two standards: *Sus scrofa domesticus* (2.91 pg) and *Bos taurus* (3.56 pg). The standards used in calculating genome size were of the same tissue type as the relevant sample.

Body mass data were taken from Nowak (1994) and Smith *et al.* (2003). The relationship between genome size and body mass was tested using Pearson's correlations on log-transformed data. While the current species-level phylogeny for the family Pteropodidae remains poorly resolved, phylogenetically independent contrasts (PICs; Felsenstein 1985) were attempted using the supertree presented by Jones *et al.* (2002) and Bininda-Emonds *et al.* (2007). These were conducted using the PDAP module (Midford *et al.* 2003) in Mesquite v. 2.5 (Maddison & Maddison 2008), with one degree of freedom subtracted for each branch in a polytomy.

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Table 1. Haploid genome size estimates for 43 species of megabats (family Pteropodidae). Information regarding the number and sex of specimens (F, female; M, male; U, unknown), tissue type (KC, kidney cells; LK, leucocytes; LV, liver) and sources of specimens is also included. Taxonomy and common names follow Wilson & Reeder (2005). Note: 1 pg = 978 Mbp. (Sources of tissues: 1, the Royal Ontario Museum; 2, the Lubee Bat Conservancy; 3, Dr John Bickham, Purdue University.)

genus	species	common name	genome size (pg)	s.e.	*n* (F/M/U)	tissue type	tissue source
Aethalops	*alecto*	pygmy fruit bat	2.15	0.03	1F, 4M	LV	1
Balionycteris	*maculata*	spotted-winged fruit bat	2.07	0.04	3F, 1M	LV	1
Chironax	*melanocephalus*	black-capped fruit bat	2.10		1M	KC, LV	1
Cynopterus	*brachyotis*	lesser short-nosed fruit bat	2.14	0.03	3F, 5M	KC, LK, LV	1, 2
Cynopterus	*horsfieldii*	Horsfield's fruit bat	2.28	0.11	2F	LV	1
Cynopterus	*sphinx*	greater short-nosed fruit bat	2.27	0.04	2F, 2M	KC, LV	1
Dobsonia	*moluccensis*	Moluccan naked-backed fruit bat	2.23		1M	LV	3
Dyacopterus	*spadiceus*	Dyak fruit bat	2.27		1F	LV	1
Eidolon	*helvum*	African straw-coloured fruit bat	2.03	0.03	3F, 2M	LK, LV	2, 3
Eidolon	sp.	straw-coloured fruit bat	1.86		1U	KC	3
Eonycteris	*spelaea*	lesser dawn bat	2.23	0.02	3F, 3M	LV	1, 3
Epomophorus	*gambianus*	Gambian epauletted fruit bat	2.04		1M	KC	3
Epomophorus	sp.	epauletted fruit bat	2.18		1F	KC	3
Epomops	sp.	epauletted fruit bat	2.18	0.01	2U	KC, LV	3
Hypsignathus	*monstrosus*	hammer-headed fruit bat	2.42		1M	LV	3
Lissonycteris	*angolensis*	Angolan soft-furred fruit bat	2.30	0.03	2F, 2M	LV	3
Macroglossus	*minimus*	dagger-toothed long-nosed fruit bat	2.00	0.03	2F, 3M	KC, LV	1
Macroglossus	*sobrinus*	greater long-nosed fruit bat	2.15	0.03	1F, 4M	LV	1
Megaerops	*ecaudatus*	Temminck's tailless fruit bat	2.20		1F	LV	1
Megaerops	*kusnotoi*	Javan tailless fruit bat	2.03		1F	KC	1
Megaerops	*niphanae*	Ratanaworabhan's fruit bat	2.26	0.03	3F, 2M	LV	1
Megaloglossus	*woermanni*	Woermann's long-tongued fruit bat	2.18	0.02	2F, 1M, 1U	KC, LV	1, 3
Melonycteris	sp.	fruit bat	2.27		1M	LV	3
Micropteropus	*pusillus*	Peters's lesser epauletted fruit bat	2.23	0.03	2F, 2M	LV	3
Myonycteris	sp.	collared fruit bat	2.19		1U	KC	3
Nanonycteris	*veldkampii*	Veldkamp's dwarf epauletted fruit bat	2.27	0.04	3F, 2M, 1U	LV	1, 3
Penthetor	*lucasi*	Lucas's short-nosed fruit bat	2.23	0.02	2F, 3M	LV	1
Pteropus	*conspicillatus*	spectacled flying fox	2.34		1F	LK	2
Pteropus	*giganteus*	Indian flying fox	2.18	0.02	2M, 1U	KC, LK	2, 3
Pteropus	*hypomelanus*	variable flying fox	2.33	0.03	3F, 2M	LK	2
Pteropus	*lylei*	Lyle's flying fox	2.51	0.02	3F, 2M	KC, LV	1
Pteropus	*neohibernicus*	great flying fox	2.21		1F	LV	3
Pteropus	*poliocephalus*	grey-headed flying fox	2.32	0.03	2F, 2M	LK	2
Pteropus	*pumilus*	little golden-mantled flying fox	2.32	0.03	2F, 2M	LK	2
Pteropus	*rodricensis*	Rodrigues flying fox	2.23	0.10	2M	LK	2
Pteropus	*vampyrus*	large flying fox	2.37	0.02	3F, 3M	KC, LK	2, 1
Rousettus	*aegyptiacus*	Egyptian rousette	2.11	0.02	6F, 3M	LK, LV	1, 2, 3
Rousettus	*amplexicaudatus*	Geoffroy's rousette	2.29	0.04	4F, 1M	LV	1

(*Continued*.)

Table 1. (*Continued.*)

genus	species	common name	genome size (pg)	s.e.	*n* (F/M/U)	tissue type	tissue source
Rousettus	*lanosus*	long-haired rousette	2.20	0.03	1F, 1M	LV	3
Rousettus	*leschenaultii*	Leschenault's rousette	2.20	0.05	4F	LV	1
Scotonycteris	*zenkeri*	Zenker's fruit bat	2.14	0.02	2M, 2U	KC, LV	1, 3
Sphaerias	*blanfordi*	Blanford's fruit bat	2.08	0.06	1F, 2M	LV	1
Syconycteris	sp.	blossom bat	2.07		1M	LV	3

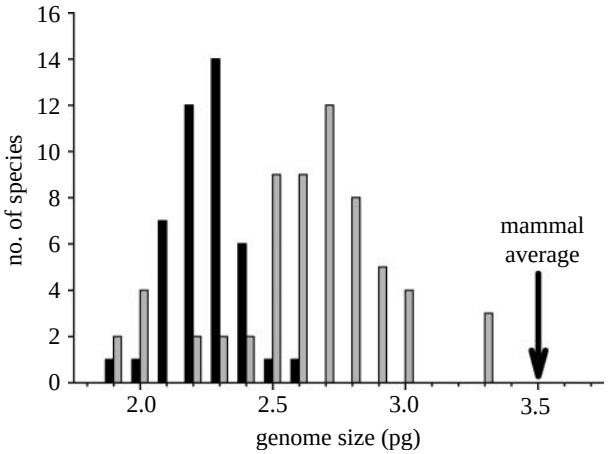

Figure 1. Summary of genome size diversity in 43 species of megabats of the family Pteropodidae (black bars, present study) and 62 species from six families of microbats (grey bars, Gregory 2009).

3. RESULTS AND DISCUSSION

Genome size estimates for 43 species of megabats are presented in table 1. These ranged from 1.86 pg in the straw-coloured fruit bat *Eidolon* sp. to 2.51 pg in Lyle's flying fox *Pteropus lylei*, all of which are well below the mammalian average of 3.5 pg (Gregory 2009). The data for megabats were normally distributed around a mean of 2.20 pg $\pm$ 0.02 s.e. (Shapiro–Wilk test, $W = 0.98$, $p > 0.80$). Interestingly, megabats appear to be even more strongly constrained to small genome sizes than other bats in terms of both mean values (2.20 versus 2.58 pg; *t*-test, $p < 0.0001$; figure 1) and variance (*F*-test, $F_{61,42} = 6.72$, $p < 0.0001$).

The results of this study raise three important questions: (i) why are all bat genome sizes small relative to other mammals, (ii) why are megabat genome sizes smaller than those of microbats, and (iii) why do species of megabats differ (albeit modestly) in genome size from one another as they do?

An answer to the first question is coming into clearer focus, thanks to recent studies of all three groups of vertebrates that independently evolved powered flight. Overall, the patterns now documented in pterosaurs, birds and both major bat groups support the notion that some factor(s)—most probably including high metabolic rate—has imposed a limit on genome size in each lineage (Organ & Shedlock 2008; Andrews *et al.* 2009). It has recently been hypothesized that genome sizes began shrinking prior to the evolution of flight in all three groups (Organ & Shedlock 2008), which seems plausible. However, this may be difficult to test in bats (cf. dinosaurs/birds and pterosaurs; Organ *et al.* 2007; Organ & Shedlock 2008), as data from non-volant bat ancestors will be difficult to acquire due to a paucity of pre-flight fossils in the lineage.

The question of why megabat genome sizes are smaller and less variable than those of microbats is intriguing, particularly in the light of the recent discovery that megabats experienced an extinction of the long interspersed element-1 (LINE-1) transposable element early in their ancestry (Cantrell *et al.* 2008).

This element constitutes 15–20 per cent of the human genome and is thought to be the most common LINE element in mammals. A lineage-specific loss of LINE-1 transposition could explain why megabats experienced a more severe reduction in genome size (or deviated less from an initially small ancestral genome) than other bats. This may have been accentuated by additional limitations on the duplication of short interspersed elements and processed pseudogenes, both of which appear to be dependent on LINEs (Cantrell *et al.* 2008).

A loss of LINE-1 activity alone would not explain why more DNA was lost from megabat genomes than in other bats, but two mutually compatible explanations can be offered in this regard: natural selection operating at the organism level for reduced genome size and/or fixation of deletion mutations in inactive elements by drift. At the least, a megabat-specific loss of LINE-1 activity means that even if selection is involved, it is not necessary to assume stronger selective pressures favouring small genome size in megabats than in microbats.

The question regarding the small amount of variation that does exist among megabats also remains an open one. Again, this could be explained in part by differential selection pressures for small genome size, differences in the strength of upward mutation pressure, historical patterns in which small ancestral genomes tend to remain small (Oliver *et al.* 2007) and/or neutral loss of DNA as influenced by features such as population size (Lynch & Conery 2003). As a test of the latter, Organ & Shedlock (2008) compared the genome and body sizes (taken as an inverse proxy for population size) across diverse vertebrates and found no relationship. In the present study, genome size was positively correlated with body size using Pearson's correlations ($r=0.48$, $p<0.003$, $n=36$); this was not significant using PICs ($p>0.7$), but probably reflects the limited resolution of the available tree. Assuming that body size is linked strongly to population size, the neutral hypothesis (Lynch & Conery 2003) cannot be ruled out when considering patterns within megabats. Of course, body size is also associated with an array of physiological and ecological parameters that could be relevant in influencing selection on genome size. Moreover, megabats are much larger than microbats in terms of body mass, but their genome sizes differ in the opposite direction.

Overall, it is clear that flying vertebrates are of particular interest in studies of genome size evolution. The data reported here for megabats help to close a significant gap in the dataset for these groups, but they also raise additional questions that should be addressed in future studies. Indeed, a full understanding of the factors that influence genome size must not only account for the enormous variability observed across many groups, but also for the remarkably limited ranges observed within some of the most diverse vertebrate taxa.

Sampling of bats was undertaken in accordance with animal use policies of the respective institutions from which material was acquired.

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