Evolution of Body Mass in Bats: Insights from a Large Supermatrix Phylogeny

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Abstract

Bats are atypical small mammals. Size is crucial for bats because it affects most aerodynamic variables and several key echolocation parameters. In turn, scaling relationships of both flight and echolocation have been suggested to constrain bat body size evolution. Previous studies have found a large phylogenetic effect and the inclusion of early Eocene fossil bats contributed to recover idiosyncratic body size change patterns in bats. Here, we test these previous hypotheses of bat body size evolution using a large, comprehensive supermatrix phylogeny (+800 taxa) to optimize body size and examine changes reconstructed along branches. Our analysis provides evidence of rapid stem phyletic nanism, an ancestral value stabilized at 12 g for crown-clade Chiroptera followed by backbone stasis, low-magnitude changes inside established families, and massive body size increase at accelerated rate in pteropodid subclades. Total variation amount explained by pteropodid subclades was 86.3%, with most changes reconstructed as phyletic increases but also apomorphic decreases. We evaluate these macroevolutionary patterns in light of the constraints hypothesis, and in terms of both neutral and adaptive evolutionary models. The reconstructed macroevolution of bat body size led us to propose that echolocation and flight work as successive, nested constraints limiting bat evolution along the body size scale.

Keywords Chiroptera · Optimization · Body mass reconstruction · Macroevolution · Nested constraints

Introduction

Body mass is the single most important variable affecting the biology of animals, especially mammals (see McNab 2007 and citations therein). Mass is even more important for one group of mammals, the bats (Chiroptera), for two major reasons: flight and echolocation. Bats are the only mammals

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capable of powered flight. Flight is presumed to have had an immediate impact on the great evolutionary success of bats: by the early Eocene, shortly after their origination, bats were the first mammalian group to reach a nearly cosmopolitan distribution, a fact documented in the fossil record of all continents except Antarctica (Gunnell and Simmons 2005; Eiting and Gunnell 2009; Smith et al. 2012). A spectacular diversification ensued: with >> 1200 bat species in 21 currently recognized families, chiropteran diversity is only second to rodents among extant mammals (Simmons 2005). Bats are undisputedly monophyletic, as indicated by all recent phylogenies (van den Bussche and Hoofer 2004; Eick et al. 2005; Teeling et al. 2005; Meredith et al. 2011; O'Leary et al. 2013; Amador et al. 2018). Key Eocene fossils appeared as successive sister taxa to a crown group that comprised all extant families (Simmons et al. 2008), which in turn split into two major clades, the Yinpterochiroptera, or Pteropodiformes (including fruitbats and five families of echolocating bats), and the Yangochiroptera, or Vespertilioniformes (including the remainder of echolocating bat families; see also Teeling et al. 2005). "Microbats" or echolocating bats are thus

paraphyletic, so echolocation either was lost in fruitbats, or convergently evolved twice, once in each "microbat" lineage (Teeling et al. 2012). Early Eocene fossils either show clear morphological signatures of echolocation (e.g., Novacek 1987), or lack them completely, as in *Onychonycteris finneyi*, the most functionally primitive bat known (Simmons et al. 2008, 2010; cf. Veselka et al. 2010). Although not universally accepted (cf. Fenton and Ratcliffe 2004; Carter and Adams 2015), the lack of echolocation in *Onychonycteris* and the nested position of pteropodids among echolocating bats imply that flight evolved first, and echolocation soon after in more derived bats, to be secondarily lost in fruitbats (Simmons et al. 2008, 2010; cf. Veselka et al. 2010). New developmental (Wang et al. 2017) and sensory organ (Thiagavel et al. 2018) evidence supports this latter view.

Powered flight is the most strenuous mode of locomotion (Rayner 1986; Norberg and Rayner 1987; Biewener 2011). Carrying the least weight up in the air is thus naturally favored, so all flying animals, including bats, are relatively small (Norberg 1990). All bats figure in the lowest quartile of the mammalian body mass distribution (Safi et al. 2013). The distribution of body mass in bats themselves (Fig. 1) shows that half of bat species weigh 14 g or less (median value), and most (some 70% of species), weigh <30 g (see Giannini et al. 2012). A minority of bat species, most of them in the megabat family Pteropodidae, exceeds 100 g, and a couple of species exceptionally reach 1600 g: the flying foxes *Pteropus vampyrus* and *Acerodon jubatus* in the Pteropodidae (Kunz and Pierson 1994). The largest flying foxes have gone a long way in the path of size evolution: not only are they many times larger than a hypothetical median bat, they also may double the size of similarly frugivorous flying animals (e.g., the largest toucans; Aves: Ramphastidae; Norberg and

Norberg 2012). Some key aerodynamic variables are typically affected by body mass (Norberg 1994) and may constitute a strong constraint to body size evolution in all vertebrates, including bats (see Smith et al. 2013).

The second major factor influencing body size in bats is echolocation—a sophisticated sonar system that enables bats to navigate without visual input (e.g., in complete darkness; Kalko and Schnitzler 1998 and citations therein). Bats emit ultrasonic calls and receive air pressure waves—echoes—from objects impacted by sound; then bats process elapsed time and several information parameters contained in the returning echoes to compose instantaneous acoustic images of the surrounding space as they rapidly fly through it, or as they receive echoes from a stationary position (Kalko and Schnitzler 1998). Several key parameters of the echolocation system are expected to scale either positively or inversely with body size (see review in Jones 1999), and these scaling relationships have been suggested to constrain body size in bats (Barclay and Brigham 1991; Jones 1999).

In addition, flight and echolocation interact in ways that are relevant for the evolution of size in bats, and vice versa. For instance, synchronization of echolocation call emission and wingbeat (specifically, the downstroke) enables bats to echolocate at search-phase frequency with no additional cost while flying (e.g., Speakman and Racey 1991; Jones 1999; Parsons et al. 2010). This coupling likely represents an important selective pressure on size to keep both flight and echolocation costs as low as possible (Speakman and Racey 1991).

Clearly, body mass has the potential to affect key evolutionary variation in the bat lineage via various functional and scaling effects. Safi et al. (2013) demonstrated that most interspecific body mass variation is phylogenetically structured,

Fig. 1 Distribution of body mass in bats based on records for 751 extant species from several specific sources (see Methods Section and SI.1). Mode and median values are indicated
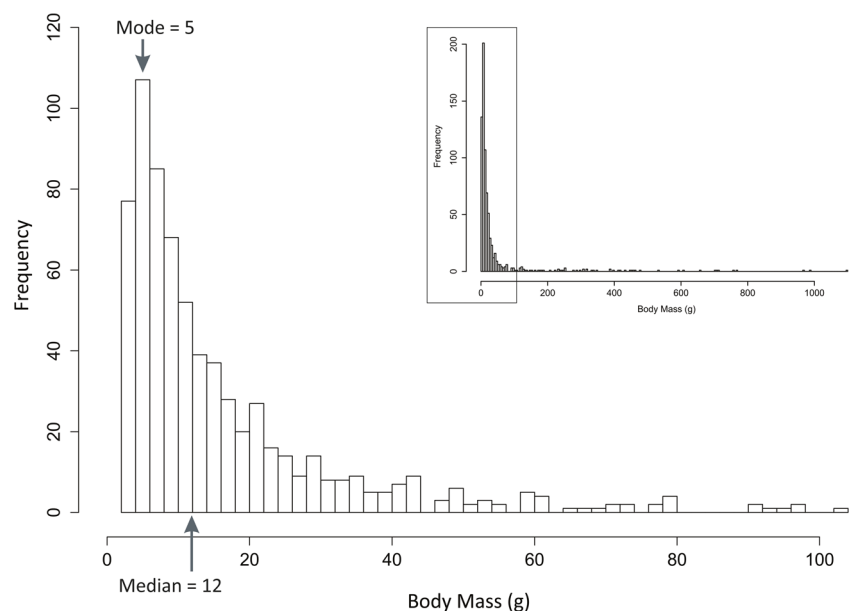
although little evidence exists that body mass per se exerted any influence in the rapid diversification of bats (Isaac et al. 2005). Hutcheon and Garland (2004) estimated ancestral body mass of the crown group of extant bats between 20 and 23 g, but with exceedingly large confidence intervals (between 9 and 51 g); similarly, Safi et al. (2005) obtained a close point estimate at 19 g. More recently, Giannini et al. (2012), using optimization of continuous characters, reported a narrower and lower-valued interval (10–14 g) that included their observed median for extant bats (at ca. 14 g), and Thiagavel et al. (2018) reported 20 g. Despite methodological differences, these studies have all converged on a small value for the ancestral node of extant bat groups, with point estimates bracketed between 10 and 23 g. Examination of changes in all the family-level nodes of the bat phylogeny in analyses including key early Eocene fossil bats (Giannini et al. 2012) revealed potentially highly relevant patterns of body size change, including: initial rapid phyletic nanism, i.e., continued size reduction along successive branches of a phylogeny, sensu Gould and MacFadden (2004); stasis around the global median value along the backbone of the crown chiropteran group; evolutionary change (increases or decreases) inside major clades (established families); more net size increases than decreases; and phyletic giantism sensu Gould and MacFadden (2004), i.e., continued size increase along successive branches of a phylogeny, in several families, particularly in Old World fruit bats (Pteropodidae).

These patterns were consistent with the hypothesis of an initial evolutionary adjustment lowering the range of body mass values to first refine flight (nanism phase), next a stabilization of body mass change to establish functional echolocation parameters in all "modern" bat groups of aerial hawking bats (backbone stasis phase), and escape from stasis whenever echolocation constraints were released (e.g., giantism in Old World fruit bats; Giannini et al. 2012). This particular scenario depended on a critically small sample of just 44 extant terminals (tree from Teeling et al. 2005 and Simmons et al. 2008). In addition, release from constraint, whatever its nature, was not supported as a general explanatory mechanism driving body size evolution in mammals as a group (Baker et al. 2015), and so such finding would make of Chiroptera a notable exception among mammals.

Here, we test the hypotheses of body size evolution in bats advanced by Giannini et al. (2012) using our large-scale supermatrix phylogeny (Amador et al. 2018). This phylogeny resulted from unconstrained searches of a nine-genes supermatrix, with ca. 800 bat terminals at the species level with curated and updated taxonomy, no chimeras, and a varied placental outgroup set (see details in Methods). We intensely searched body size data from the literature and optimized the data as a continuous character on our phylogeny (see Methods). Next, we examined changes reconstructed along branches of the large bat tree, quantifying changes as net

increases or decreases, phyletic (along successive branches) or apomorphic (at single branches) following Gould and MacFadden (2004), in order to characterize the nature of body size changes in the entire bat lineage. We fit models of phenotypic evolution and detected shifts in the rate of body size change in Chiroptera and in functionally significant bat subgroups. In light of these results, we evaluate hypotheses of body size evolution in bats that may result from the combined constraints of flight and echolocation (Giannini et al. 2012). In addition, we evaluated in bats hypotheses of body size trends discovered in mammals, both in terms of neutral (e.g., Bokma et al. 2016) and adaptive (e.g., Baker et al. 2015) models of character change. Our detailed analysis questions current explanations of body size evolution in mammals as extended to bats, and singles out bats as a group experiencing unique evolutionary pressures. We propose a new hypothesis of bat body size evolution based on nested constraints from echolocation and flight.

Materials and Methods

Body Mass Data Compilation

We obtained body size data, in grams, for 751 of 799 bat species plus a few distinct subspecies (making up 804 bat terminals) represented in our phylogenetic hypothesis, from specific sources (Schober and Grimmberger 1997; Bonaccorso 1998; Churchill 1998; Smith et al. 2003; Gannon et al. 2005; Goodman et al. 2006; Aguirre 2007; Decher and Fahr 2007; dos Reis et al. 2007; Garbutt 2007; Pacheco et al. 2007; Zhang et al. 2007; Magalhāes and Costa 2009; Lim et al. 2010; Harvey et al. 2011; Mendes 2011; Threlfall et al. 2011; Hasan et al. 2012; Patterson and Webala 2012; Thong et al. 2012; Kruskop 2013; Monadjem et al. 2013; Dammhahn and Goodman 2014; Huang et al. 2014; Kawai et al. 2014; Lu et al. 2014; Sedlock et al. 2014; Table SI.1). For each species, we averaged the values from all available sources, excluding some data judged unreliable (e.g., extreme values, juveniles, etc.). In addition, for species lacking data from literature sources we searched in one museum database (National Museum of Natural History, NMNH) and, when available, averaged weight values of ten specimens (males and females; Table SI.2). For extinct bat species, we used body mass values provided by Giannini et al. (2012) from reverse estimate of mass from humerus least mid-shaft diameter using ordinary least squares regression fit with extant species.

Phylogenetic Framework

We used a pruned version of the phylogenetic hypothesis proposed by Amador et al. (2018) with, 751 out of 799 species, as a cladistic framework to search for body size evolutionary

patterns (see below). This phylogeny was based on a comprehensive data matrix with updated taxonomy and no chimeric terminals, 11,489 aligned bp from nine nuclear and mitochondrial genes, and intense, unconstrained analyses including parsimony, maximum likelihood, and Bayesian inference for dating the phylogeny; the last analysis used 44 calibration points from undisputed fossil bats. The 21 currently recognized bat families were represented in the matrix, with ca. 90 and 64.4% of extant diversity at the genus and species level, respectively; i.e., 198 genera and 799 currently recognized species. The point estimate of age for crown clade Chiroptera in this phylogeny was 62.6 mya.

Also, we composed a tree adding five key Eocene bat species: *Onychonycteris finneyi* (Onychonycteridae), *Icaronycteris index* (Icaronycteridae), *Archaeonycteris trigonodon* (Archaeonycteridae), *Hassianycteris messelensis* (Hassianycteridae), and *Paleochiropteryx tupaiodon* (Palaeochiropterygidae), following the topology proposed by Simmons et al. (2008), with *O. finneyi* as the most basal taxon, and the other Eocene terminals as successive stem branches sister of the crown clade of extant terminals. This wide taxonomic sampling allowed us to test different aspects of the hypothesis of physical constraints proposed by Giannini et al. (2012) for explaining the evolution of body mass in Chiroptera (see above).

Evolution of Body Mass

We approached the macroevolutionary analysis of body mass in Chiroptera using two contrasting methodologies. First, we examined body size data applying a local, node-by-node descriptive approach that allowed an appropriate identification of evolutionary changes in the framework proposed by Gould and MacFadden (2004). Body size data were arranged in TNT data matrix format and mapped onto the phylogeny of Amador et al. (2018) as a continuous character (see Goloboff et al. 2006) with a single value (the species average) per terminal, as implemented in the phylogenetic program TNT (Goloboff et al. 2008), freely available at www.lillo.org.ar/phylogeny/tnt/. Following Giannini et al. (2012) and Amador and Giannini (2016), we chose to interpret nodal location, sign (increase or decrease), and magnitude of change, with the last being the net change or the amount of body mass increase or decrease common to all reconstructions for a given branch. We quantified frequency and magnitude of net increases and decreases along the complete phylogeny, and within the most specious families. We looked for macroevolutionary patterns in body size variation, in terms of phyletic or apomorphic changes, giantism or nanism, and their corresponding combinations (see Gould and MacFadden 2004).

To specifically test, by means of a comprehensive taxonomic sampling, the validity of patterns of body mass evolution in Chiroptera recovered by Giannini et al. (2012), we

compared our results in the following aspects (Table 1): 1. value of reconstructed body mass for the ancestral node of extant bats; 2. presence of a pattern of rapid phyletic nanism in the early evolution of Chiroptera; 3. presence of stasis along the backbone of extant chiropterans; 4. amount of evolutionary net change (increases or decreases) occurring inside established families, as compared with total change; 5. relative frequency and magnitude of body mass increases and decreases; and 6. occurrence of phyletic giantism in the group that loss echolocation, the Old World fruitbats (Pteropodidae).

We assessed the global phylogenetic effect on body mass in the group, by means of estimating the fraction of total variance in body size explained by phylogeny. Previous estimates indicated ca. 39% variation explained by phylogeny (Safi et al. 2013) based on a supertree topology (from Bininda-Emonds et al. 2007). To re-evaluate this assessment, we choose a topological method, Canonical Phylogenetic Ordination (hereafter CPO; Giannini 2003). CPO considers the comparative data as dependent variable(s), here the vector of body mass data for each chiropteran species (univariate version of CPO). Tree partitions represent the independent variables in the type of analysis applied, Redundancy Analysis (Rao 1964; Ter Braak 1995), which is appropriate for continuously-varying data as body size (see Giannini 2003). CPO treats the tree as a network whose partitions are equivalent to clades of a rooted tree. Tree partitions are binary coded to represent membership of terminals (0 applied to terminals belonging to one side of the tree partition, 1 applied to the other side), and each constitutes an explanatory variable that is tested by means of an F-value using unrestricted Monte Carlo permutations (here 4999 permutations were used). These tests of individual tree partitions are followed by a forward stepwise selection procedure that seeks to produce a maximally explanatory, but non-redundant model that is a linear combination of selected partitions (clades). As a

Table 1 Comparison between this study and Giannini et al. (2012)

Comparison	Giannini et al. 2012	This study
# outgroup terminals	5	20
# fossil bat terminals	5	5
# extant bat terminals	38 (38)	804 (751)
Ancestral size: stem	39–41 g	39–41 g
Ancestral size: crown	11–16 g	12 g
Phyletic nanism	present*	present*
Chiropteran backbone stasis	present	present
Amount of net size change	1211 g	9314 g
Net change per terminal	28 g	12 g
Raw decrease-to-increase ratio	0.75	1.00
Phyletic giantism	present**	present**

*in stem Chiroptera
**in Pteropodidae

result, CPO allows the identification of major tree partitions that best explain the main patterns in the data, and provides a non-redundant estimate of total variation explained by those tree partitions. We kept groups in the model using two criteria. First, we included all the groups that individually explained 5% or more of total variation. This is a conservative approach, so we also fit the model successively adding groups that remained significant at a predefined alfa = 0.01 (see Giannini 2003). These analyses were executed using the program CANOCO version 4.0 (Ter Braak and Smilauer 1998).

We tested predictions from mammalian neutral models of body size evolution on the bat lineage. According to Bokma et al. (2016), mammalian lineages are expected to increase size on average by ca. 4.1% every 5 my with respect to the size of a given ancestor. We applied this prediction to Chiroptera and compared it to our observed values. We calculated the expected mean of bats as a group, pteropodids only, echolocating bats only, and the two clades of echolocating bats separately, i.e., Yinochiroptera (composed of families Rhinopomatidae, Megadermatidae, Craseonycteridae, Rhinolophidae, Hipposideridae and Rhinonycteridae) and Yangochiroptera (composed of Emballonuridae, Nycteridae, Myzopodidae, Mystacinidae, Thyropteridae, Furipteridae, Noctilionidae, Mormoopidae, Phyllostomidae, Natalidae, Molossidae, Miniopteridae, Cistugidae, and Vespertilionidae) sensu Amador et al. (2018). We used point estimates of crown age of each of these clades from our supermatrix phylogeny (Amador et al. 2018) and size of each ancestor from this study as departing point to apply the expected increase predicted in Bokma et al. (2016).

We used the package *OUwie* for R (Beaulieu et al. 2012) to determine the model of phenotypic evolution that best describes the evolution of weight in pteropodid and non-pteropodid bats. *OUwie* implements the Hansen model, in which a quantitative character evolves according to an Orstein-Uhlenbeck (OU) process. The model describes the change of a character through time, depending on the character evolutionary optimum (parameter θ), the strenght of selection towards this optimum (parameter α), and a variable that denotes the rate of stochastic evolution (parameter σ^2). In the absence of selection ($\alpha = 0$), the model resembles the Brownian motion. *OUwie* allows the fit of different models to a phylogeny with two predetermined regimes (or set of clades). These models differ on how the parameter values (α, θ, and σ^2) vary between those two regimes. We then applied a method that identifies shifts in the evolution of a trait without the need of predetermined regimes (e.g. echolocating, non-echolocating bats). The methods implemented in the package *bayou* for R (Uyeda et al. 2014) identify possible shifts in phenotypic (here weight) optimum along a phylogeny using Bayesian methods (Uyeda and Harmon 2014). A MCMC chain using default, relaxed priors with 6 million generations was conducted. Parameters were sampled every

100 generations and results were summarized after discarding the first 24,000 samples (40%). A second run with the same characteristics was done to check for convergence, which was done using the Gelman and Rubin's R statistics and plots of branch posterior probabilities.

Results and Discussion

Our analysis, based on optimization of body size as a continuous character on our large supermatrix phylogeny (from Amador et al. 2018), and models of phenotypic evolution, provides strong evidence of rapid stem phyletic nanism, followed by stasis along the backbone of the crown chiropteran subtree, considerable amount of low-magnitude change in many bat groups, and massive body size increase at accelerated pace in a few, but chiefly in one, group of bats, the Pteropodidae (see below). These results differ with respect to predictions of body size evolution in mammals as a group, either based on neutral or adaptive models, and they agree with aspects of previous hypotheses of bat body size evolution. These aspects can be linked directly with functional demands of flight, echolocation, and their interaction. In the following we dissect these results to discuss in depth bat body size evolution.

Magnitude, Location and Pattern of Bat Body Size Evolution

Optimization of body size as a continuous character data in our phylogeny of Chiroptera yielded 9.314 kg of total net changes, of which almost three quarters (72.6%) were increases (Fig. 2). In terms of frequency, stasis (no change) was dominant with 41% of nodes, while net increases and net decreases had each 16% share of total nodes; ambiguity (nodes with assignments that depend on particular reconstructions) represented 27% (Fig. 2). Distribution of changes as mapped on our tree is shown in Fig. 3 for the backbone topology of Chiroptera. The root state was assigned the interval of 30–40 g and in subsequent branches was assigned 30 g. Stem bats included in our study (five genera in five families, all extinct) varied greatly in size, from average 12 to 90 g, as estimated from humerus least shaft diameter (Giannini et al. 2012). However, as optimized in the phylogeny, a pattern of phyletic nanism (see below) was apparent along stem branches, given that size decreased from ca. 40 to 12 g (Fig. 3). Two strongly diverging changes were optimized in branches involving stem bats (Eocene fossils): the branch leading to the large *Hassianycteris messelensis* (+60 g), and its sister branch leading to *Palaeochiropteryx tupaiodon* + all extant bats (−18 g). This steep descent established a root body size (ancestral value) of 12 g for crown-clade Chiroptera (see below). This value is only 30% of the value estimated in the

Fig. 2 Results for body mass optimization of 751 bat species in the phylogeny of Amador et al. (2018). Relative values (%) for frequency of changes and total amount of net increases and decreases for the magnitude of change

Frequency

Magnitude (Kg)

□ Ambiguity ▨ Stasis ■ Net increases ▨ Net decreases
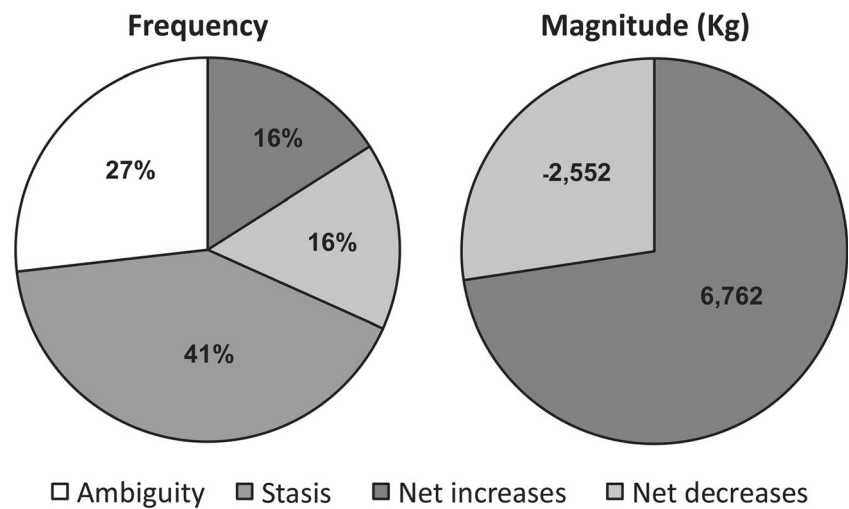
oldest fossils considered here (specimens of *Onychonycteris finneyi*, sister to all bats), and it remained stable along the entire chiropteran backbone were no net changes were reconstructed (bold branches in Fig. 3). Remarkably, these changes,

and the changes toward the stem terminals, occurred from around the K–Pg boundary, through the Paleocene, to the early Eocene, given that the fossils were dated 52–49 my (see Gunnell and Simmons 2005, and citations therein) and
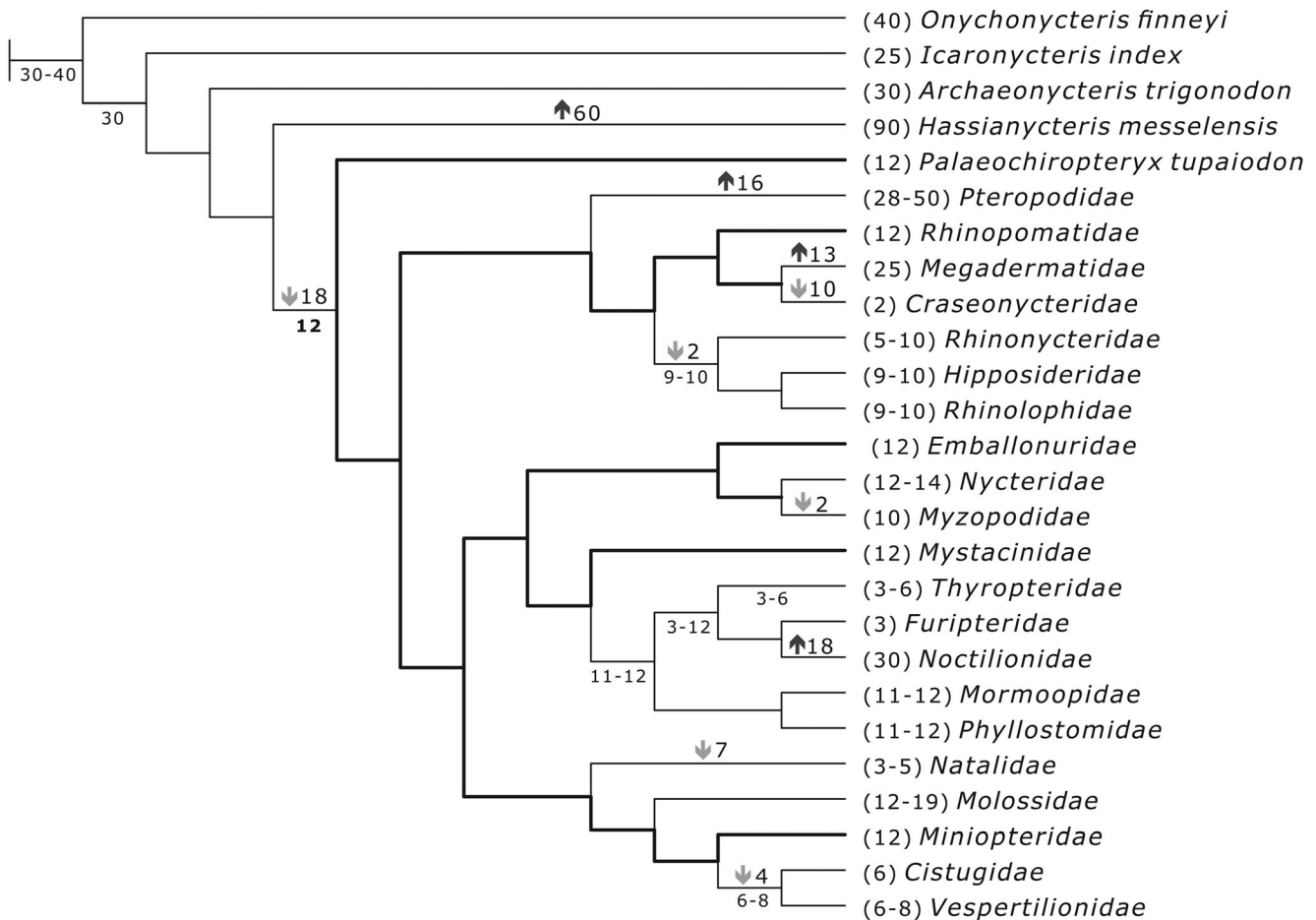

Fig. 3 Optimization of body mass in the backbone of bats phylogenetic tree of Amador et al. (2018), including basal extinct taxa reconstructed following Simmons et al. (2008). Black arrows denote increases and gray ones, decreases. Body mass values (in grams) indicated in brackets refers

to the estimated body mass value for fossils and for the ancestral of each extant family. Ancestral reconstructed values are indicated below branches. Bold line denotes backbone stasis (i.e., no net changes)

the stem and crown age of Chiroptera were ca. 67 and 62 mya, respectively (Amador et al. 2018).

The reconstructed size of crown-clade Chiroptera and its median value, estimated from >750 terminals, were the same at 12 g (cf. Figs. 1 and 3). This suggests highly conserved body size evolution, which is reflected in a pattern of virtual backbone stasis that accumulated <2% of total size changes in Chiroptera (see below; Fig. 3), and in the very close optimum size fitted for echolocating bats from an Ornstein-Uhlenbeck model with varying parameters (see below). Then, net changes were reconstructed independently in nodes representing the base of eight families or superfamilies, most notably in Pteropodidae, which doubled the size of the reconstructed backbone value, and continued evolving size to accumulate most of the >9 kg total change reconstructed (see below). These results match, both in pattern and magnitude, previous estimates by Giannini et al. (2012), who used the same fossil estimates, but with an extant sample limited to only 44 terminals (versus 751 terminals from Amador et al. (2018) in the present study).

Patterns of evolutionary change in extant bats can be reliably estimated with a representatively large sample of species-level terminals. Here we focus our interpretation on the effect of our large sample on the major patterns of body size evolution in bats as a group (specific patterns inside families will be dealt with elsewhere). Shifts in the interval range were mapped to the Neotropical noctilionoid branch, with intervals successively changing from 12 g (backbone) to 11–12 g and 3–12 g in noctilionoids. Net changes included three increases in the nodes corresponding to the families Pteropodidae (+16 g), Megadermatidae (+13 g), and Noctilionidae (+18 g). Five net decreases were reconstructed in the nodes corresponding to Craseonycteridae (−10 g), rhinolophoids sensu stricto (Rhynonycteridae + Hipposideridae + Rhinolophidae, −2 g), Myzopodidae (−2 g), Natalidae (−7 g), and vespertilionids sensu lato (Cistugidae + Vespertilionidae, −4 g). Total change in the backbone chiropteran tree (as seen in Fig. 3) was only 150 g. This low level of body size change is especially relevant because it is along this backbone that most of the bat divergence occurred, as all recognized bat families originated from these branches. Therefore, the majority of changes were reconstructed inside families, as summarized in Table 2. In fact, 73.4% of total magnitude of net change, or ca. 6.8 kg, was concentrated in one family, the Pteropodidae. Remarkably, only a comparatively modest increase of +16 g was reconstructed in the root of the pteropodid subtree; still, basal pteropodids were at least twice as big as the backbone ancestral bat species (28–50 g vs. 12 g; Fig. 3 and Fig. 7 from supplementary information). The greatest fraction of change occurred in more derived branches of pteropodids, principally in those leading to large or very large flying foxes (see below). Giannini et al. (2012) reconstructed phyletic increases in the pteropodid clade. In our study, pteropodids accumulated ca. 4.8 kg of increases, but

also decreases were important with ca. 2.1 kg or ca. 30% of all net changes in the family (Table 2); this latter aspect is missing in Giannini et al. (2012) given the small sample of extant pteropodids included. Other aspects also reveal the peculiar evolution of size in pteropodids; the ratio of the magnitude of change to the frequency of change (which measures the average amount of change allocated to one evolutionary event), and to the number of nodes in the clade (expected amount of change per node), is one or two orders of magnitude larger in pteropodid bats than in any other bat family, both considering increases and decreases, albeit more so in the case of increases (Table 2). The "microbat" family Phyllostomidae occupied a distant second place with 708 g of total magnitude of net change (Table 2). Analysis of the nine more speciose bat families indicates patterns of increases, decreases, stasis, and ambiguity that are generally similar to that of Chiroptera as a group, with few exceptions like Miniopteridae, in which the frequency of decreases outnumbered increases, and Phyllostomidae (Table 2).

The dominant patterns of change in Chiroptera as a group were phyletic increases and apomorphic decreases (Table 3). Average phyletic vs. apomorphic total changes across families were nearly balanced (51% of phyletic changes; Table 3). This lineage-level pattern is replicated inside the five more speciose families (Vespertilionidae, Pteropodidae, Phyllostomidae, Hipposideridae, and Rhinolophidae). In Phyllostomidae there were three times more phyletic increases than decreases, and two families (vespertilionids and rhinolophids; Table 3) had 1.6–1.7 times as many increases as decreases. In Pteropodidae the pattern of apomorphic nanism dominated, in spite of the sheer magnitude of phyletic increases detected in the family (see above). In the next four diverse families (Molossidae, Miniopteridae, Emballonuridae, and Mormoopidae), all four types of change (the combination of phyletic and apomorphic increases and decreases) were nearly balanced (Table 3).

Predictions from Giannini et al. (2012) are shown in the comparative Table 1, in the context of a wider comparison between that study and this one. Methodologically, the studies included the same five fossil taxa, and differed in the number of non-bat outgroup terminals (5 vs. 20) and more crucially in the number of ingroup (bat) terminals (38 vs. 804, 751 terminals used here; Table 1). In terms of results, our study confirmed the basic trends reconstructed in Giannini et al. (2012) of initial phyletic nanism, backbone stasis, considerable amount of low-scale size evolution inside families, and phyletic giantism chiefly in pteropodids (Table 3). As expected, the total amount of size change was many times greater in our study due to the greater taxonomic sample, but this translated into a different average change, estimated as net change per terminal; in our study, this proportion was less than half the estimate in Giannini et al. (2012), indicating a dilution of perceived change in a larger sample. Put in another way, small taxonomic samples may distort this value, so comprehensive

Table 2 Results from body mass optimization in the most specious clades of Chiroptera

Clades	Number of nodes	Frequency								Total net change (Kg)	Magnitude (Kg)			
		Increases		Decreases		Stasis		Ambigüity			Increases		Decreases	
		a.v.	%	a.v.	%	a.v.	%	a.v.	%		a.v.	%	a.v.	%
Chiroptera*	1510	241	15.96	238	15.76	626	41.46	405	26.82	9.314	6.762	72.60	−2.552	27.40
Hipposideridae	84	12	14.29	15	17.86	30	35.71	27	32.14	0.270	0.218	80.74	−0.052	19.26
Rhinolophidae	102	17	16.67	19	18.63	40	39.22	26	25.49	0.163	0.11	67.48	−0.053	32.52
Pteropodidae	218	33	15.14	45	20.64	71	32.57	69	31.65	6.836	4.762	69.66	−2.074	30.34
Phyllostomidae	308	61	19.81	46	14.94	123	39.94	78	25.32	0.708	0.551	77.82	−0.157	22.18
Mormoopidae	18	3	16.67	4	22.22	4	22.22	7	38.89	0.029	0.02	68.97	−0.009	31.03
Emballonuridae	76	14	18.42	11	14.47	31	40.79	20	26.32	0.112	0.095	84.82	−0.017	15.18
Miniopteridae	36	4	11.11	7	19.44	13	36.11	12	33.33	0.022	0.01	45.45	−0.012	54.55
Vespertilionidae	472	69	14.62	62	13.14	217	45.97	124	26.27	0.489	0.386	78.94	−0.103	21.06
Molossidae	90	16	17.78	14	15.56	29	32.22	31	34.44	0.366	0.309	84.43	−0.057	15.57

Frequencies of increases, decreases, stasis and ambigüity and magnitude of increases and decreases are indicated for each main Chiroptera clade, both in absolute (a.v.) and relative terms (%)

samples of the known diversity, such as the one included in the phylogeny of Amador et al. (2018), are expected to approximate this estimate of change in a more reliable fashion. Raw decrease-to-increase ratio is also different between studies, with balanced (ratio ≈ 1) estimate in our study as compared with a predominance of increases (and hence, a < 1 ratio) in Giannini et al. (2012).

In Pteropodidae in particular, the fact that the most common pattern of body size change was apomorphic decrease contrasts sharply with previous results in which only phyletic giantism was reconstructed in the family (see Giannini et al. 2012). This pattern indicates that evolution of size was very active in the group, with sharp increases in magnitude toward the largest pteropodids, but in fact most commonly changing toward decreasing relative large size in other pteropodids,

likely in response to local demands or as a result of opportunistic evolutionary change (e.g., Baker et al. 2015).

Phylogenetic Effects and the Case of Pteropodidae

Results from Canonical Phylogenetic Ordination (CPO) are summarized in Table 4. Forward stepwise selection retained 20 tree partitions, all but one belonging in the single family Pteropodidae. Amount of total variation explained by these tree partitions was 86%. Deletion of pteropodid terminals did not improve fit of the remaining "microbat" groups (not shown). If we allow inclusion of partitions that explain no less than 5% of total variation, the analysis would retain only the first two groups, "pteropines" (i.e., *Pteropus* + *Acerodon*) and pteropodids except cynopterines and rousettines (i.e., the

Table 3 Patterns of body mass evolution in therms of phyletic giantism (PG), phyletic nanism (PN), apomorphic giantism (AG), and apomorphic nanism (AN)

Clades	Total net changes	Phyletic changes					Apomorphic changes				
		PG		PN		Total	AG		AN		Total
		a.v.	%	a.v.	%		a.v.	%	a.v.	%	
Hipposideridae	27	9	33.3	8	29.6	17	3	11.1	7	25.9	10
Rhinolophidae	36	10	27.8	6	16.7	16	7	19.4	13	36.1	20
Pteropodidae	78	20	25.6	19	24.4	39	14	17.9	25	32.1	39
Phyllostomidae	108	45	41.7	14	13.0	59	17	15.7	32	29.6	49
Mormoopidae	7	–	–	2	28.6	2	3	42.9	2	28.6	5
Emballonuridae	25	8	32.0	9	36.0	17	5	20.0	3	12.0	8
Miniopteridae	11	–	–	4	36.4	4	4	36.4	3	27.3	7
Vespertilionidae	131	40	30.5	25	19.1	65	29	22.1	37	28.2	66
Molossidae	30	7	23.3	6	20.0	13	9	30.0	8	26.7	17
TOTAL	442	139	31.4	89	20.1	228	87	19.7	127	28.7	214

Numbers indicate absolute (a.v.) and relative (%) terms

Table 4 Results of Canonical Phylogenetic Ordination, univariate case with body size as dependent variable and tree partitions as explanatory variables

Tree Partition (clade)	F-value	P-value	Variation explained (%)	Cumulative variation (%)
"Pteropines" (*Pteropus* + *Acerodon*)	1179.22	0.0001	0.61	0.610
Pteropodidae except Cynopterinae and Rousettinae	140.12	0.0001	0.06	0.670
Pteropus livingstonii + *vampyrus* + *griseus* species groups	58.41	0.0001	0.02	0.695
Acerodon + *Pteropus personatus**	38.14	0.0025	0.02	0.710
"Dobsonines" (*Aproteles* + *Dobsonia*)	32.24	0.0001	0.01	0.722
"Pteralopines" (*Desmalopex* + *Mimirimi* + *Pteralopex*)	42.59	0.0004	0.02	0.737
Dobsonia	38.05	0.0072	0.01	0.749
Pteropus alecto + *P. conspicillatus*	27.26	0.0080	0.01	0.758
Pteropodidae except Cynopterinae	25.01	0.0001	0.01	0.766
Pteropus cognatus + *P. rayneri*	27.52	0.0078	0.01	0.774
Pteropus griseus species group – *P. neohibernicus*	21.37	0.0020	0.01	0.781
Pteropus griseus species group	88.22	0.0023	0.02	0.804
Pteropus griseus species group + *P. ocularis* (*ocularis* spp. group) + *P. ornatus* (*ornatus* spp. group)	40.94	0.0034	0.01	0.814
Pteropus vampyrus species group – *P. vampyrus* – *P. dasymallus* – *P. pumilus*	22.95	0.0026	0.01	0.820
Pteropus vampyrus species group – *P. dasymallus* – *P. pumilus*	76.18	0.0020	0.02	0.837
Pteropus vampyrus species group	65.72	0.0004	0.01	0.850
Dobsonia – *D. minor*	17.79	0.0090	>0,01	0.850
Pteropus – *Pteropus pelagicus* species group	20.22	0.0080	>0,01	0.860
Pteropus vampyrus species group – *P. rodricensis* – *P. vampyrus* – *P. dasymallus* – *P. pumilus*	19.55	0.0090	>0,01	0.860
Miniopteridae + Cistugidae + Vespertilionidae	9.17	0.0010	>0,01	0.860

Listed are groups that were significant at a predetermined alfa = 0.01. We report F-value, P-value and the amount of variation explained individually by each partition, and accumulated in the ordering that groups entered the model, both as percentage of total variation. Informal groups are in quotes. Formally recognized groups (e.g., subfamilies) and species groups as in Almeida et al. (2014) and Amador et al. (2018). The signs "–" and "+" indicate exclusion or inclusion of a listed taxon in the corresponding group

*The form *personatus* may not belong in *Pteropus* (Amador et al. 2018)

group inclusive of Macroglossinae, Harpyionycterinae, Eidolinae, Nyctimeninae, and Pterodinae). This most conservative approach demonstrates that most variation (now 67%) still is within a few major pteropodid groups.

Safi et al. (2013) determined that bats, like most mammals, have a high degree of phylogenetic signal in body size, and suggested that body size evolution is mainly phylogenetically constrained. We concur in that a strong phylogenetic signal is indeed present, but in quantitative terms, the bulk of phylogenetically structured size variation is concentrated inside just one group, the pteropodids. This perceived pattern is the result of applying CPO (but see also Thiagavel et al. 2018), which unlike other methods, dissects evolutionary change across tree partitions (i.e., monophyletic groups in rooted trees; Giannini 2003). As a consequence, patterns that corresponded with clades (e.g., clade members on average larger or smaller than the pool of all other terminals) may explain a fraction of total variation in a linear model, provided that simulations reject randomness in the data (Giannini 2003). This allows finding those clades, and calculating the amount of variation explained by such partition; because tree partitions are considered natural structures of a given lineage, it is relevant to detect variation associated with them (Giannini 2003). Here almost all significant variation was associated with subclades of megabats (Table 4). Hutcheon and Garland (2004) posed the relevant question, are megabats big? The bimodal distribution of mass in bats (Fig. 1) is explained by some species of

fruitbats being generally much larger than most "microbats" (or echolocating bats). Hutcheon and Garland (2004) concluded that once phylogeny was considered, in consort with certain branch length values and neutral evolutionary models, the "signal" disappeared and Old World fruit bats were no longer distinct with respect to "microbats" (Hutcheon and Garland 2004). CPO (but see below) suggested that not "megabats" as a group, but instead particular groups inside "megabats," explained the most conspicuous portion of chiropteran variation in size.

Predictions from Neutral Models of Size Evolution in Mammals

Predictions of body mass evolution according to a general neutral trend toward body size increase across mammalian lineages (Depéret's, or Cope's, rule, modeled in Bokma et al. 2016) are shown in Table 5. Based on the general average rate in Bokma et al. (2016) of 4.1% increase in size per million year with respect to a given ancestor, bats as a group are predicted to achieve a mean body size of 60.9 g, which is 35% larger than the actual estimate of extant bats at ca. 45 g (Table 5). A finer analysis per bat group, which was mandatory given the functional differences that distinguish these groups, revealed far greater discrepancies between predicted and observed values. Observed mean in pteropodid bats is ca. 213 g; predicted values range (depending on ancestral body

Table 5 Comparison of observed versus expected mean body size in major bats groups. Ancestral size, from this study

Group		Ancestral size (g)	Age (my)	Mean extant terminals (g)	Expected mean (g)	Signed difference (g)
Chiroptera		12	62,61	45,2	60,9	15,8
Pteropodidae	Lower estimate	28	25,93	213,7	60,6	−153,1
	Mean estimate	39	25,93	213,7	91,1	−122,6
	Higher estimate	50	25,93	213,7	123,8	−89,9
Echolocating bats*	All*	12	62,61	15,6	60,9	45,3
	Yinochiroptera	12	51,08	16,8	42,4	25,6
	Yangochiroptera	12	56,04	14,9	49,3	34,4

Age of each group, from Amador et al. (2018). Mean of extant terminals based on terminals used in this study. Expected mean per group calculated as ancestor value increased 4.1% every 5 my as in Bokma et al. (2016). Positive and negative differences mean expected value greater or lesser than observed, respectively, by amount in last column. Calculations in Pteropodidae are based on results of body size optimization (lower, mid-point, and higher values of reconstructed interval) of this study

*paraphyletic, grouped for comparative purposes only

size reconstruction) are far below, between 60 and 123 g (Table 5). Echolocating bats show the reverse pattern. Body size average of all echolocating bats (a paraphyletic array) is 15.6 g; predicted value is as for all Chiroptera (same age and ancestral size) at 60.9 g. Splitting echolocating bats into its two natural constituent groups to avoid paraphyly yielded similar results: expected vs. observed values for Yinochiroptera and Yangochiroptera were 42.4 vs. 16.8 g and 49.3 vs. 14.9 g, respectively (Table 5). In other words, the mean body size of bats as a group is close (just 15 g above) from the predicted mean using the general rate of change for mammals, but decomposing bats into meaningful functional groups (non-echolocating vs. echolocating bats, and the major groups therein) yielded mean size estimates that were too far from the expected means calculated using the general model (90–150 g below in megabats, 25–45 g above in "microbats").

Another model from Venditti et al. (2011) predicts a mean instantaneous increase of 1.02 g per million year, with considerable (up to 52-fold) variation in rate across mammalian groups. In this model, the two more speciose mammalian orders, rodents and bats, exhibited no history of sustained body size increase. However, dinomyid rodents are known to have reached 700–1000 kg in body size (Sánchez-Villagra et al. 2003; Rinderknecht and Blanco 2008), and we estimate that the largest megabats are evolving size at a (linear) rate of 101 g per million year (notice that 101 g is greater than the expected final size of bats evolving at the average 1.02 g per million rate from a starting point at 12 g, but during ca. 62 my!). As with the previous model (from Bokma et al. 2016) there seems to be a critical lack of fit to the variation we reconstruct in bats.

Adaptive Models and Constraints to Body Size Evolution in Bats

Neutral models appear to fit poorly the reconstructed evolution of bat body size (see above). Baker et al. (2015)

have suggested an adaptive basis for mammalian body size evolution. These authors found that bats were no exception to a general trend to increase size over time detected across mammals as a group, as well as within groups. Interestingly, they also detect "rapid evolutionary change toward body size decrease in the branch leading to extant bats", which corresponds nicely with the phase of phyletic nanism that we reconstruct with early Eocene fossils, "although subsequent evolution within this group returned to a general pattern of body size increase" (Baker et al. 2015:5096), which is not obvious in our study (see above and Table 3). Baker et al. (2015) proposed adaptive explanations to these trends, which confirmed Depéret's (= Cope's) rule in mammals and major subclades. However, they considered unlikely that either the overall mammalian trend, or the group-specific trends that they detected, were due to the relaxation of functional unspecified constraints that allowed size to increase with respect to the ancestral condition, and a phylogenetic test failed to recognize any general pattern of evolutionary body size release across mammals. If this is generally true for mammals, then bats represent a macroevolutionary exception. We show next that bat body size evolved around conserved optima and that the "unspecified constraints" can be materialized in the abundant functional information that is available for bats.

The OU model with different optima, drift, and selection parameters for each regime (OUMVA) had a significantly better fit to the evolution of weight across the bat phylogeny as compared with other fitted models (Table 6). The estimated parameters of the OUMVA model are shown in Table 7. All parameters had higher estimates for pteropodids as compared to those of the other bats. Both higher θ and σ² favor phenotypic disparity. Remarkably, the parameter σ² that describes the rate of stochastic evolution of the trait is 100 times higher in Pteropodidae, reflecting the much higher variance of weight

Table 6 Results of the fit of alternative models of weight evolution in bats using OUwie

Model	lnL	AIC	DAIC	AICc
OUMVA	1808.41	−3604.82	0	−3604.71
BMS	1737.49	−3466.98	−137.84	−3466.92
OUMV	1712.96	−3415.92	−188.9	−3415.84
OUMA	1712.94	−3415.88	−188.94	−3415.8
OUM	808.41	−1608.82	−1996	−1608.763
OU1	750.99	−1495.99	−2108.83	−1495.95
BM1	514.99	−1025.99	−2578.83	−1025.97

The best fitting model, OUMVA, favors the Ornstein-Uhlenbeck model with different values of α, σ^2 and θ for Pteropodidae as compared to the echolocating (non-pteropodid) bats

BM1: Brownian Motion (BM) with same σ^2 (rate of stochastic evolution – drift) and θ (trait optimum) for the two groups

BMS: BM with different with different σ^2 and different θ

OU1: Ornstein-Uhlenbeck (OU) with same parameters for all both groups

OUM: OU with different θ but same and σ^2 and α (strength of selection)

OUMV: OU with different θ and σ^2, but same α

OUMA: OU with different θ and α, but same σ^2

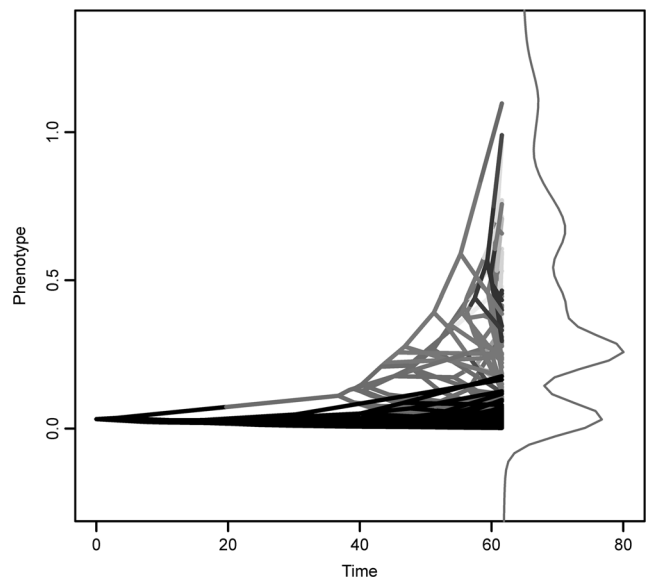OUMVA: OU with different θ, α, and σ^2

Fig. 4 Rate of body mass stochastic evolution according to estimated parameters of the OUMVA model. The pteropodids are shown in gray while the rest of the families are shown in black Curve in the right hand side of the plot indicates posterior density for optima values

in this family (Fig. 4). The Bayesian runs reached convergence (supplementary information) and ESS >100 for all parameters except for root (ESS = 70). The method identified 16 branches with a posterior probability >0.5 of having suffered shifts in the weight optima, most of which are the same as those detected by CPO (see above). All of them were located in the Pteropodidae lineage (Fig. 5 and Fig. 8 from supplementary information).

Besides the possible phylogenetic constraint to size variation discovered by previous authors (particularly Safi et al. 2013), bats face potentially severe physical limitations on size evolution from flight, echolocation, and their combination. Considering flight first, it is regarded as the most physiologically strenuous mode of locomotion in vertebrates, so small size should be strongly favored (Rayner 1986; Norberg and Rayner 1987; Biewener 2011). In addition, several flight parameters have a direct dependence on body size. Flight speed (ms^{-1}) scales positively with body mass (Norberg 1994), which in turn depends on wing loading (Pa), so large bats need to fly faster to produce enough lift (Bullen and McKenzie 2002) and tend to have large home ranges and to commute long distances (Norberg 1990). By contrast,

Table 7 Parameter estimates of the OUMVA model

	Pteropodids	Other bats
α	0.219	0.051
σ^2	0.004	0.00004
θ	0.146 ± 0.03	0.017 ± 0.002

wingbeat frequency (Hz) scales inversely with body mass (Bullen and McKenzie 2002), as expected for frequencies in general (see Calder 1996). This relationship determines several aspects of bat flight; for instance, only small bats can afford the high wingbeat frequency required for hovering flight, which is required for certain life styles such as nectarivory (Rayner 1986; Norberg 1994).

Echolocation also imposes hard limitations on size derived from scaling relationships, especially frequencies. Call frequencies such as peak frequency (kHz), frequency of various harmonics (kHz), and pulse repetition rate (Hz) are expected to scale inversely with body mass (see above), which has been corroborated in some studies (e.g., Jones 1999). One significant consequence of this inverse scaling is that call parameters may constraint size evolution in echolocating bats: as bats evolve larger, their call frequencies drop (Jones 1999). Thus, for scaling reasons, larger bats fly faster at lower wingbeat frequencies while emitting low frequency calls at low repetition rates, a combination that compromises their ability to detect and track small and fast-moving, airborne prey (insects). In addition, in order to avoid call-echo overlap, most bats should hear the upcoming echo before emitting their next call, which further limits pulse repetition rate (Fenton 1990). Finally, pulse duration (ms) is a key echolocation parameter, one that is expected to scale positively with body mass (Jones 1999), but such relationship has been found only in certain bats that use quasi-constant-frequency calls (Jones 1999).

Most of the previous arguments apply chiefly to echolocating, aerial hawking bats—not to all bats. However, from an evolutionary perspective, this reasoning is especially appropriate given the history of echolocation. Simmons et al.

Fig. 6 Body mass evolution in Pteropodidae. Reconstructed values of body mass for ancestral nodes and for the extreme extant species (the lightest *Megaloglosssus azagnyi* and heaviest *Acerodon juvatus*). Values of body mass expected mean (according to neutral model of Bokma et al. 2016) and observed mean, as the theorical upper limit (Norberg and Norberg 2012), are indicated
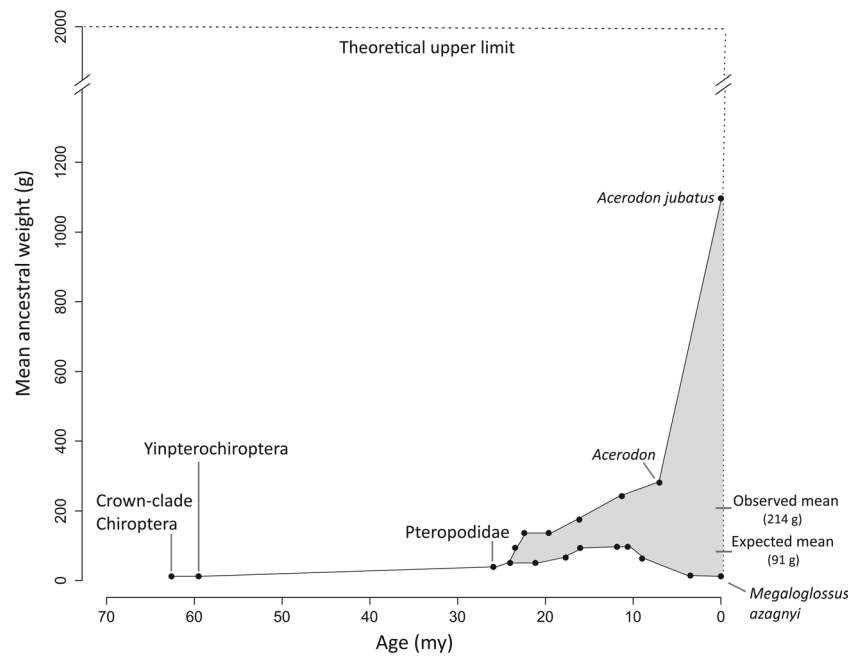
(2008, 2010) proposed that in the bat lineage, flight evolved first and echolocation next (cf. Veselka et al. 2010). The most primitive bat known *Onychonycteris finneyi*, which we placed as sister of all other bats following Simmons et al. (2008), likely was an accomplished flier but lacked the morphological signature of echolocation (Simmons et al. 2008; cf. Veselka et al. 2010); by contrast, all other Eocene fossils were echolocators, as are all non-pteropodid extant bats. Available phylogenetic evidence suggests that megabats are nested inside echolocating bats (e.g., Simmons et al. 2008), so echolocation must have been lost in this group. Recent ontogenetic (late-fetal arrested cochlear development in megabats; Wang et al. 2017) and sensory organ (Thiagavel et al. 2018) evidence supports echolocation loss in megabats. So for most of the bat lineage history, echolocation has been present and presumably acting as strong constraint on size evolution

(optimum from OU model at ca. 17 g) through key inverse scaling relationships associated to frequencies and timings that limit detection of airborne insects in large bats (Barclay and Brigham 1991; Jones 1996, 1999).

Only after echolocation loss, pteropodids evolved size in different directions with great increases occurring in some subclades. This was likely associated with a loss of echolocation capabilities that forced them to change diet; or more likely, a dietary shift from insectivory to frugivory made pteropodids independent of echolocation and its constraints (see also Thiagavel et al. 2018). In other words, megabats may have evolved size in all directions (increases and decreases) in the context of diet-mediated ecological release (Giannini et al. 2012). This hypothesis matches our phylogenetic results, which showed that the bulk of size variation in bats was
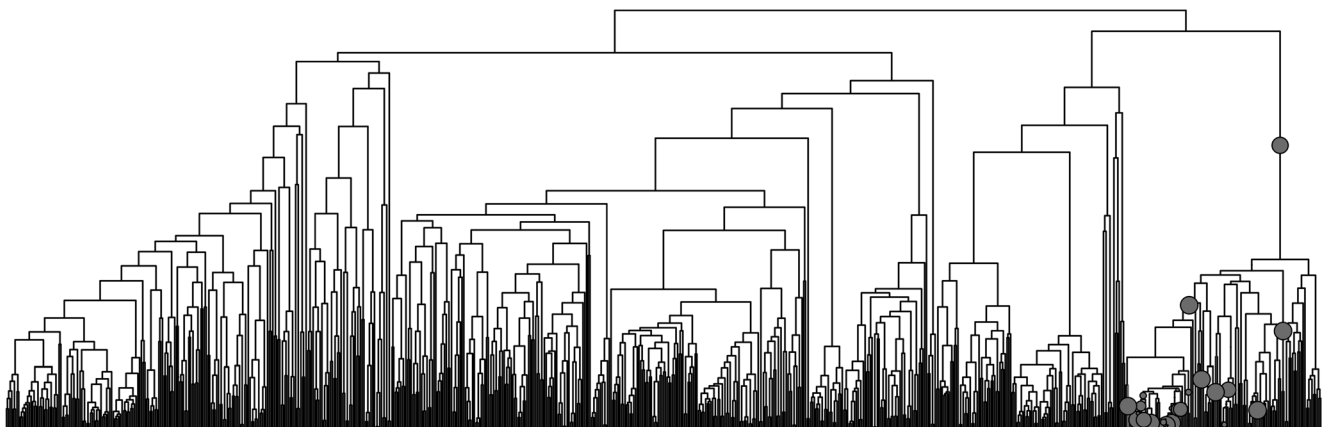

Fig. 5 Detail of the "simmap" figure from the bayou analysis showing, in gray circles, the shift in parameters related to weight evolution according the OU process. All shifts are concentrated in the pteropodids branch (for details see Fig. 8 of supplementary information)

associated with megabat subclades, particularly those of flying foxes and other large pteropodids (Table 4). The impressive evolutionary explosion of size reconstructed in flying foxes can be appreciated in the abrupt widening of the pteropodid size change envelop (shaded area in Fig. 6) and the steep slope of size change in largest megabats (see Figs. 4 and 6). For instance, the reconstructed change from ancestral *Acerodon* to its largest descendant *A. jubatus* represented at least a three-fold increase in some 7 my (the 101 g my^{-1} rate mentioned above).

A New Hypothesis of Nested Constraints

Flight costs limit body size expression in vertebrates in general (Pennycuick 1975; Rayner 1986; Norberg 1994). In addition, in aerial hawking bats, which represent the vast majority of Chiroptera, echolocation has been proposed to impose severe costs from the inverse scaling of frequency parameters (see Jones 1999); these combine with the direct scaling of increasing flight speed (through the wing-loading dependence on body size) required to produce enough lift in larger bats, and the coupling of wingbeat frequency and call emission for energy savings during search-phase echolocation (Jones 1999). As a result, size increase may be severely penalized in most bats (Barclay and Brigham 1994; Jones 1999). Only certain bats are expected to gain any ecological advantage from flight when increasing size: specifically, commuting bats (see above), among which large frugivores figure prominently (Norberg 1994). Echolocating bats rarely exceed 100 g and the largest aerial hawking bat, *Cheiromeles torquatus* (Molossidae; up to 200 g), exhibits a mixture of unusual echolocation features (including calls at two different frequencies, both higher than predicted by its mass; Heller 1995) and usual ones (including a close-to-expected call repetition rate; see Jones 1999 contra Heller 1995).

From scaling relationships, physiological constraints, and aerodynamics, Norberg and Norberg (2012) modeled the theoretical maximum size in bats using mass-specific muscle power, proportion of body mass allocated to flight muscles, and maximum attainable wing beat frequency, which together configure the maximum power output during flight in any bat. Norberg and Norberg (2012) estimated that the body mass of the largest potential bat would be 1.4–2.3 kg; the authors also give the range of 1.1–2.0 kg based on more conservative assumptions, and so we take this latter range as reference to evaluate bat body size evolution in the light of these theoretical predictions. With individual bats recorded at a maximum of 1.6 kg, it is clear that the largest bats fall within the model's range of maximum size (Norberg and Norberg 2012), but also that the lower boundary of that range is a sub-estimation of maximum size. If we then take 2 kg as the maximum size attainable for a bat, as in Norberg and Norberg's (2012) most

conservative model, how long does it going to take for a hypothetical bat to actually start experiencing the flight constraints on maximum body size? Applying our (linear) rate of change reconstructed in large pteropodines (101 g my^{-1}), and extending size increase in these bats with the assumption that everything else remains as in Norberg and Norberg's (2012) model (i.e., no further evolution modifies the physiological conditions used in the model), flying foxes will experience the physical limitations of the upper boundary of body size in about 8 my from now, when these forms would reach the theoretical upper limit of 2 kg. That is, body size evolution in non-echolocating bats presently is not as strongly penalized from flight physiology as to prevent further increases, and thus these bats are expected to continue evolving size for a few million years.

Considering bats altogether, it is clear that body size evolution depends on different aspects of flight, echolocation, and their combination, as factors acting at different levels of the size scale. We propose here that these factors work as nested constraints. Echolocation would impose the strongest physical limitations, keeping most aerial hawking bats small (Barclay and Brigham 1994; Jones 1999). This first level of constraint might have been acting since the very early Eocene when bats first started using echolocation to pursue airborne prey and it might explain part of the size evolution pattern in bats: 1. initial nanism (detected in Giannini et al. 2012; Baker et al. 2015; and in this study), as a phase of adjustment to the evolving echolocating system; 2. the remarkably little variation in size in echolocating bats that is manifested in the backbone stasis; and 3. the little change, over some 60 my, from the reconstructed ancestral size of crown clade Chiroptera (at 12 g) to the average size of living echolocating bats (at 15 g; Table 1; Fig. 1). The last aspect corresponds with the finding of Cooper and Purvis (2010) that the best model of size change in bats is the OU (Ornstein-Uhlenbeck) model; the OU model is selected over other Brownian-like models whenever evolutionary character variation shows a tendency of returning to some optimum—theoretical or empirically determined (Cooper et al. 2016). We found the same result here with the optimum parameter from the OU model at ca. 17 g and we propose that this optimal value of size is determined by echolocation constraints. The optimal value is thus somewhere between the ancestral value (at 12 g) and the estimated optimum (at ca. 17 g), which comprise the mean of extant echolocating bats (at 15 g). As echolocating bats become larger, the strength of the echolocation constraint may increase and eventually limit the aerial hawking behavior to exceptions that depend on at least some energy-expensive especial traits (e.g., in *Cheiromeles*; see Heller 1995). Loss of echolocation may have opened up the size landscape to pteropodids

so these bats accumulated most of the sampled size variation in bats; evidence presented so far indicates that these bats are not favored by size increases unless they can profit from the obligate faster flight that accompanies size increase, as in the case of large commuting frugivores (see Norberg 1994). Thus, factors limiting size evolution in bats can be described as nested constraints from echolocation and flight acting at different levels of the size scale: echolocation at ca. 200 g and flight at ca. 2000 g. For non-echolocating bats, the ultimate constraint in size comes exclusively from flight and meeting the theoretical upper limit of body size appears to be a few million years away at the estimated (linear) rates of evolution in the largest extant species.

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