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Comparative analysis of bat genomes provides insight into the evolution of flight and immunity

Guojie Zhang[1,2,*,†], **Christopher Cowled**[3,†], **Zhengli Shi**[4,†], **Zhiyong Huang**[1,†], **Kimberly A. Bishop-Lilly**[5,†], **Xiaodong Fang**[1], **James W. Wynne**[3], **Zhiqiang Xiong**[1], **Michelle L. Baker**[3], **Wei Zhao**[1], **Mary Tachedjian**[3], **Yabing Zhu**[1], **Peng Zhou**[3,4], **Xuanting Jiang**[1], **Justin Ng**[3], **Lan Yang**[1], **Lijun Wu**[4], **Jin Xiao**[1], **Yue Feng**[1], **Yuanxin Chen**[1], **Xiaoqing Sun**[1], **Yong Zhang**[1], **Glenn A. Marsh**[3], **Gary Crameri**[3], **Christopher C. Broder**[6], **Kenneth G. Frey**[5], **Lin-Fa Wang**[3,7,*], **Jun Wang**[1,8,9,*]

[1]BGI-Shenzhen, Shenzhen, 518083, China

[2]Centre for Social Evolution, Department of Biology, University of Copenhagen, Universitetsparken 15, 2100 Copenhagen, Denmark

[3]CSIRO Australian Animal Health Laboratory, Geelong, Victoria 3220, Australia

[4]State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China

[5]Naval Medical Research Center and Henry M. Jackson Foundation, Fort Detrick, MD 21702, USA

[6]Department of Microbiology and Immunology, Uniformed Services University, Bethesda, MD 20814, USA

[7]Program in Emerging Infectious Diseases, Duke-NUS Graduate Medical School, Singapore 169857

[8]Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, DK-2200, Copenhagen, Denmark

[9]Department of Biology, University of Copenhagen, DK-2200, Copenhagen, Denmark

Abstract

Bats are the only mammals capable of sustained flight and are notorious reservoir hosts for some of the world's most highly pathogenic viruses, including Nipah, Hendra, Ebola and SARS. To identify genetic changes associated with the development of bat-specific traits, we performed

[*]Corresponding authors: Correspondence and requests for materials should be addressed to G.Z. (zhanggj@genomics.org.cn), L.-F.W (Linfa.wang@csiro.au) or J.W. (wangj@genomics.org.cn).

[†]These authors contributed equally to this work

Author contributions J.W., L.-F.W, G.Z., C.C.B and K.A.B.-L conceived the study. M.T., M.L.B., G.A.M., G.C., L.W. and Z.S. prepared the samples. G.Z., Z.H., X.F., Z.X., W.Z., Y. Zhu, X.J., L.Y., J.X., Y.F., Y.C., X.S., Y. Zhang, K.G.F., K.A.B.-L and J.W. performed genome sequencing, assembly and annotation. G.Z. and J.W. supervised genome sequencing, assembly and annotation. G.Z., C.C., Z.H., X.F., J.W.W., Z.X., J.N., W.Z., P.Z., Y. Zhu, M.T. and M.L.B. performed genome analyses. G.Z., Z.H., C.C. and J.W.W. carried out genetic analyses. G.Z., C.C., Z.H., X.F. P.Z., J.N. M.T., J.W.W., M.L.B, and L.-F.W discussed the data. All authors contributed to data interpretation. C.C. and J.W.W. wrote the paper with significant contributions from G.Z., Z.H., P.Z., J.N. M.T., M.L.B, L.-F.W. and input from all authors.

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whole genome sequencing and comparative analyses of two distantly related bat species, fruit bat *Pteropus alecto* and insectivorous *Myotis davidii*. We discovered an unexpected concentration of positively selected genes in the DNA damage checkpoint and *NF-κB* pathways that may be related to the origin of flight, as well as expansion and contraction of important gene families. Comparison of bat genomes with other mammalian species has provided new insights into bat biology and evolution.

Bats belong to the order Chiroptera within the mammalian clade Laurasiatheria (1). While consensus has not been reached on the exact arrangement of groups within Laurasiatheria, a recent study placed Chiroptera as a sister taxon to Cetartiodactyla (whales + even-toed ungulates such as cattle, sheep and pigs) (2). The Black flying fox (*Pteropus alecto)*, and David's Myotis (*Myotis davidii*), represent the Yinpterochiroptera and Yangochiroptera suborders, respectively, and display a diverse range of phenotypes (Fig. 1). Captive colonies, immortalized cell lines and bat-specific reagents have been developed for these two species, however genomic data is currently unavailable.

The most conspicuous feature of bats, distinguishing them from all other mammalian species, is the capacity for sustained flight. Positive selection in the oxidative phosphorylation (OXPHOS) pathway suggests increased metabolic capacity played a key role in its evolution (3), yet the byproducts of oxidative metabolism (such as reactive oxygen species, ROS) can produce harmful side effects including DNA damage (4). We hypothesize that genetic changes during the evolution of flight in bats likely included adaptations to limit collateral damage caused by byproducts of elevated metabolic rate. Another phenomenon that has sparked intense interest in recent years is the discovery that bats maintain and disseminate numerous deadly viruses (5). In this context, we further hypothesize that the long-term coexistence of bats and viruses must have imposed strong selective pressures on the bat genome, and the genes most likely to reflect this are those directly related to the first line of antiviral defense - the innate immune system.

We performed high-throughput whole-genome sequencing of individual wild-caught specimens of *P. alecto* and *M. davidii* using the Illumina HiSeq platform (6). More than 100x coverage high quality reads were obtained for *P. alecto* and *M. davidii* resulting in high quality assemblies (Tables S1–3, Fig. S1). The two bat genomes, at approximately 2 Gb, were smaller in size than other mammals (7) (Fig. S2), while the number of genes we identified was similar to other mammals (21,392 and 21,705 in *P. alecto* and *M. davidii*, respectively) (Fig. S3). Both species displayed a high degree of heterozygosity at the whole genome level (0.45% and 0.28% in *P. alecto* and *M. davidii*, respectively) (Tables S4–5), while repetitive content accounted for slightly less than a third of each genome (Tables S6–7). We identified a novel endogenous viral element derived from *Saimiriine herpesvirus 2* that has expanded to 126 copies in *P. alecto* (Table S8, Fig. S4). Gene family expansion and contraction analysis (Tables S9–12) revealed significant expansion ($p<0.05$) of 71 gene families in *M. davidii* compared to only 13 in *P. alecto*, which may be related to a recent wave of DNA transposon activity (8).

We screened all nuclear-encoded bat genes to identify those for which a single orthologous copy was unambiguously present in both bat species as well as human, rhesus

macaque, mouse, rat, dog, cat, cattle and horse. From this, 2,492 genes were used to perform maximum likelihood and Bayesian phylogenomic analysis (Figs. 2, S5–7). All phylogenetically informative signals including concatenated nucleotides and amino acids vigorously supported bats as a member of Pegasoferae (Chiroptera + Perissodactyla + Carnivora) (9), with the bat lineage diverging from the *Equus* (horse) lineage approximately 88 million years ago (MYA), supported by findings at the transcript level (10). Interestingly, phylogenetic reconstruction with mitochondrial DNA sequences resulted in bats occupying an outlying position in Laurasiatheria (Fig. S8). The incongruence between nuclear and mitochondrial trees likely reflects rapid evolution of the mitochondrial genome of the bat ancestor during the evolution of flight (3).

To identify mechanisms that facilitated the origin of flight in bats, we surveyed genes involved in detection and repair of genetic damage. A high proportion of genes in the DNA damage checkpoint/DNA repair pathway were found to be under positive selection in the bat ancestor, including *ATM*, *DNA-PKc*, *RAD50*, *KU80*, and *MDM2* (Fig. 3A, Table 1). We propose that these changes may be directly related to minimizing/repairing the negative effects of ROS generated as a consequence of flight. Additionally in this pathway, *TP53* (*p53*) and *BRCA2* were shown to be under positive selection in *M. davidii*, while *LIG4* was under positive selection in *P. alecto* (Table 1). Bat-specific mutations in a nuclear localization signal in *p53* and a nuclear export signal in *MDM2* (Figs. 3B, S9) may affect subcellular localization and function in both species (11, 12). Other candidate flight-related genes under positive selection in the bat ancestor included *COL3A1*, involved in skin elasticity, and *CACNA2D1*, which has a role in muscle contraction (Table S13).

We next examined genes of the innate immune system (Table 1). Positively selected genes in the bat ancestor included *c-REL*, a member of the *NF-κB* family of transcription factors, which also contained amino acid changes potentially affecting *IκB* binding (Fig. S10). In addition to diverse roles in innate and adaptive immunity (13), *c-REL* plays a role in the DNA damage response by activating *ATM* (14) and *CLSPN* (15), while *ATM* is also an upstream regulator of *NF-κB* (16). The DNA damage response plays an important role in host defense and is a known target for virus interaction (17), raising the possibility that changes in DNA damage response mechanisms during selection for flight could have influenced the bat immune system.

Intriguingly, both *P. alecto* and *M. davidii* have lost the entire locus containing the PYHIN gene family, including *AIM2* and *IFI16*; both of which are involved in sensing microbial DNA and the formation of inflammasomes (Fig. S11). The association between PYHIN genes and cell cycle regulation in other species (18) hints that loss of the PYHIN family in bats may be connected to changes in the DNA damage pathway; since at least one PYHIN gene is present in all other major groups of eutherian mammals (19). *NLRP3*, triggered by both viral infection and ROS in other mammals (20), plays an analogous role to *AIM2* in inflammasome assembly and was also under positive selection in the bat ancestor (Table 1).

Natural killer (NK) cells provide a first line of defense against viruses and tumors and include two families of NK cell receptors; killer-cell immunoglobulin-like receptors (*KIRs*), encoded by genes in the leukocyte receptor complex (LRC) and killer cell lectin-like

receptors (*KLRs*, also known as *Ly49* receptors), encoded within the natural killer gene complex (NKC). *KLRs* and *KIRs* were entirely absent in *P. alecto* and reduced to a single *Ly49* pseudogene in *M. davidii* (Table S14). *KIR*-like receptors identified in other species (21) were also absent from both *P. alecto* and *M. davidii* genomes, supported by transcript analysis in *P. alecto* (10). This likely indicates that bat NK cells use a novel class of receptors to recognize classical MHC class I molecules. Furthermore, additional LRC members of the immunoglobulin superfamily (including *SIGLECS*, *LILRs*, *CEACAMs* and *LAIRs*) have undergone considerable gene duplication in *M. davidii* and other mammals; yet have almost completely failed to expand in *P. alecto* (Fig. S12). As the genes encoded within the LRC bind a variety of ligands and play multiple roles in immune regulation, these observations have diverse implications for differences in immune function between *P. alecto* and *M. davidii* and between bats and other mammals.

We identified seven complete and two partial copies of the digestive enzyme *RNASE4* in *M. davidii* (Table S15), while *P. alecto RNASE4* has acquired a frame-shift mutation resulting in loss of catalytic residues (Fig. S13). We also identified critical amino acid changes in *M. davidii RNASE4* genes (relative to the mammalian consensus) that suggest diversification of substrate specificity (Fig. S13). With a proven role in host defense against RNA viruses (22), *RNASE4* expansion in *M. davidii* may have implications for virus resistance, but may also reflect the insectivorous diet of *M. davidii*, which contrasts that of *P. alecto* which consumes predominantly fruit, flowers and nectar.

M. davidii also differs from *P. alecto* in aspects including hibernation and echolocation (Fig. 1). Bile salt-stimulated lipase (*BSSL*), capable of hydrolyzing triglycerides into monoglycerides and subsequently releasing digestible free fatty acids, has been specifically expanded in *M. davidii* compared to *P. alecto* and other mammals (Fig. S14). In addition, we observed six candidate genes related to hibernation showing positive selection in *M. davidii* and three other hibernating species, relative to non-hibernators (Table S16). Seven echolocation related genes, including new candidates *WNT8A* and *FOS* (a subunit of the *AP-1* transcription factor) had significantly higher dN/dS in the echolocating *M. davidii* branch relative to non-echolocating branches (Table S17). Of note, the third exon in *M. davidii FOXP2* had even greater variation from the mammalian consensus than two previously identified variable sites (Fig. S15) suggesting a specific transcript variant is involved in echolocation (23).

In summary, comparative analysis of *P. alecto* and *M. davidii* genomes has provided insight into the phylogenetic placement of bats, and has revealed evidence of genetic changes that may have contributed to their evolution. Gene duplication events played a particularly prominent role in the evolution of *Myotis* bats and may have helped contribute to their speciation. Concentration of positively selected genes in the DNA damage checkpoint pathway in bats may indicate an important step in the evolution of flight, while evidence of change in components shared by the DNA damage pathway and the innate immune system raises the interesting possibility that flight-induced adaptations have had inadvertent effects on bat immune function and possibly also life expectancy (24). The data generated by this study will help to address major gaps in our understanding of bat biology and provide new directions for future research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References:

1. Teeling EC et al. Science 307, 580 (Jan 28, 2005). [PubMed: 15681385]
2. Nery MJ, Gonzalez DJ, Hoffmann FG, Opazo JC, Mol. Phylogenet. Evol 10, 210 (2012).
3. Shen YY et al. Proc. Natl. Acad. Sci. U. S. A 107, 8666 (May 11, 2010). [PubMed: 20421465]
4. Barzilai A, Rotman G, Shiloh Y, DNA Repair 1, 3 (Jan 22, 2002). [PubMed: 12509294]
5. Wang LF, Walker PJ, Poon LL, Current Opinion in Virology 1, 649 (2011). [PubMed: 22440923]
6. Materials and methods are available as supplementary material on Science Online.
7. Smith JDL, Gregory TR, Biol. Lett 5, 347 (Jun 23, 2009). [PubMed: 19324635]
8. Pritham EJ, Feschotte C, Proc. Natl. Acad. Sci. U. S. A 104, 1895 (Feb 6, 2007). [PubMed: 17261799]
9. Nishihara H, Hasegawa M, Okada N, Proc. Natl. Acad. Sci. U. S. A 103, 9929 (Jun 27, 2006). [PubMed: 16785431]
10. Papenfuss AT et al. BMC Genomics 13, 261 (2012). [PubMed: 22716473]
11. O'Keefe K, Li HP, Zhang YP, Mol. Cell. Biol 23, 6396 (Sep, 2003). [PubMed: 12944468]
12. Roth J, Dobbelstein M, Freedman DA, Shenk T, Levine AJ, EMBO J. 17, 554 (Jan 15, 1998). [PubMed: 9430646]
13. Gilmore TD, Gerondakis S, Genes Cancer 2, 695 (2011). [PubMed: 22207895]
14. De Siervi A et al. Cell Cycle 8, 2093 (Jul 1, 2009). [PubMed: 19502793]
15. Kenneth NS, Mudie S, Rocha S, EMBO J. 29, 2966 (Sep 1, 2010). [PubMed: 20657549]
16. Brzoska K, Szumiel I, Mutagenesis 24, 1 (Jan, 2009). [PubMed: 18832076]
17. Turnell AS, Grand RJ, J. Gen. Virol 93, 2076 (2012). [PubMed: 22855786]
18. Schattgen SA, Fitzgerald KA, Immunol. Rev 243, 109 (Sep, 2011). [PubMed: 21884171]
19. Cridland JA et al. BMC Evol. Biol 7, 140 (2012).
20. Tschopp J, Schroder K, Nature Reviews Immunology 10, 210 (Mar, 2010).
21. Sambrook JG, Beck S, Curr. Opin. Immunol 19, 553 (Oct, 2007). [PubMed: 17884420]
22. Cocchi F et al. Proc. Natl. Acad. Sci. U. S. A 109, 5411 (Apr 3, 2012). [PubMed: 22431590]
23. Li G, Wang JH, Rossiter SJ, Jones G, Zhang SY, PLoS ONE 2, (Sep 19, 2007).
24. Brunet-Rossinni AK, Austad SN, Biogerontology 5, 211 (Aug, 2004). [PubMed: 15314271]
25. Li RQ et al. Genome Res. 20, 265 (Feb, 2010). [PubMed: 20019144]
26. Li H, Durbin R, Bioinformatics 25, 1754 (Jul 15, 2009). [PubMed: 19451168]

27. Li H et al. Bioinformatics 25, 2078 (Aug 15, 2009). [PubMed: 19505943]
28. Benson G, Nucleic Acids Res. 27, 573 (Jan 15, 1999). [PubMed: 9862982]
29. Chen N, Using RepeatMasker to identify repetitive elements in genomic sequences. Current Protocols in Bioinformatics (John Wiley & Sons, Inc., 2004).
30. Abrusan G, Grundmann N, DeMester L, Makalowski W, Bioinformatics 25, 1329 (May 15, 2009). [PubMed: 19349283]
31. Kent WJ, Genome Res. 12, 656 (Apr, 2002). [PubMed: 11932250]
32. Birney E, Clamp M, Durbin R, Genome Res. 14, 988 (May, 2004). [PubMed: 15123596]
33. Katzourakis A, Gifford RJ, PLoS Genet. 6, (Nov, 2010).
34. Edgar RC, Nucleic Acids Res. 32, 1792 (Mar, 2004). [PubMed: 15034147]
35. Stamatakis A, Bioinformatics 22, 2688 (Nov 1, 2006). [PubMed: 16928733]
36. Stanke M, Waack S, Bioinformatics 19, Ii215 (Sep, 2003). [PubMed: 14534192]
37. Salamov AA, Solovyev VV, Genome Res. 10, 516 (Apr, 2000). [PubMed: 10779491]
38. Trapnell C, Pachter L, Salzberg SL, Bioinformatics 25, 1105 (May 1, 2009). [PubMed: 19289445]
39. Li H et al. Nucleic Acids Res. 34, D572 (Jan 1, 2006). [PubMed: 16381935]
40. Kim EB et al. Nature 479, 223 (Nov 10, 2011). [PubMed: 21993625]
41. Li RQ et al. Nature 463, 311 (Jan 21, 2010). [PubMed: 20010809]
42. Hahn MW, De Bie T, Stajich JE, Nguyen C, Cristianini N, Genome Res. 15, 1153 (Aug, 2005). [PubMed: 16077014]
43. Stamatakis A, Ludwig T, Meier H, Bioinformatics 21, 456 (Feb 15, 2005). [PubMed: 15608047]
44. Yang ZH, Rannala B, Mol. Biol. Evol 23, 212 (Jan, 2006). [PubMed: 16177230]
45. Yang ZH, Mol. Biol. Evol 24, 1586 (Aug, 2007). [PubMed: 17483113]
46. Yang ZH, Comput. Appl. Biosci 13, 555 (Oct, 1997). [PubMed: 9367129]
47. Zhao HB, Yang JR, Xu HL, Zhang JZ, Mol. Biol. Evol 27, 2669 (Dec, 2010). [PubMed: 20573776]
48. Andrews MT, Squire TL, Bowen CM, Rollins MB, Proc. Natl. Acad. Sci. U. S. A 95, 8392 (Jul 7, 1998). [PubMed: 9653197]
49. Holmes RS, Cox LA, Cholesterol 2011, 1 (2011).
50. Kelley J, Walter L, Trowsdale J, PLoS Genet. 1, 129 (Aug, 2005). [PubMed: 16132082]
51. Paulson JC, Macauley MS, Kawasaki N, Ann. N. Y. Acad. Sci 1253, 37 (2012). [PubMed: 22288608]
52. Zebhauser R et al. Genomics 86, 566 (Nov, 2005). [PubMed: 16139472]

Trait	***Myotis davidii***	***Pteropus alecto***
Common name	David's Myotis	Black flying fox
Suborder	Yangochiroptera	Yinpterochiroptera
Distribution	China	Australia, PNG, Indonesia
Habitat	Rock cavities	Trees, mangroves, rainforest
Diet	Insectivorous	Frugivorous, nectarivorous
Hibernation	Hibernates Nov-May	No
Echolocation	Yes	No
Viral reservoir	Potential	Yes

Fig. 1. Comparison of bat biological traits

P. alecto and *M. davidii* represent two distinct Chiropteran suborders and demonstrate diverse evolutionary adaptations. PNG: Papua New Guinea.

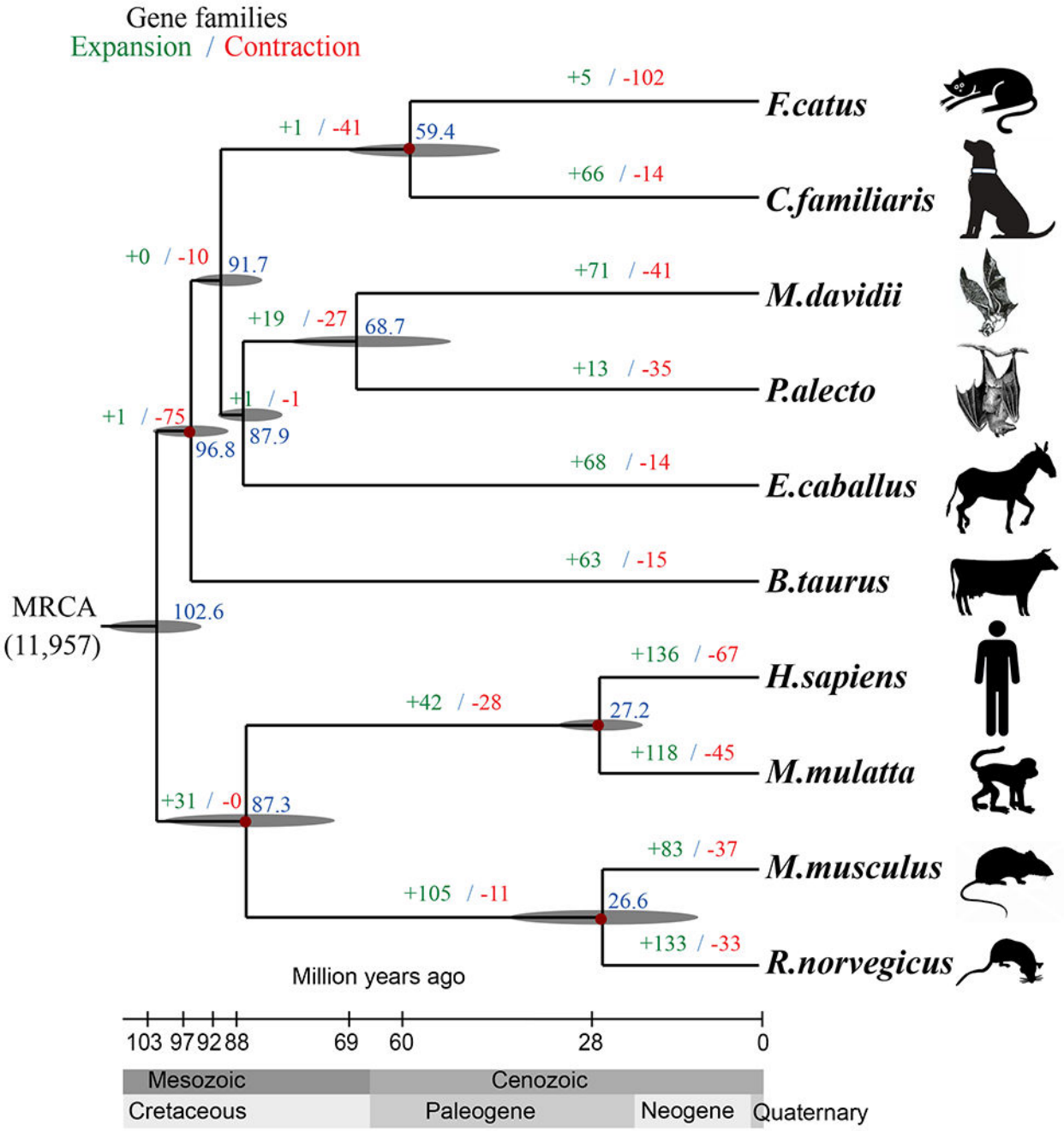

Fig 2. Phylogenomic analysis

Maximum likelihood phylogenomic analysis of 2,492 genes from *M. davidii*, *P. alecto* and eight mammalian species. Divergence time estimates in blue, gene family expansion events in green, and gene family contraction events in red. MRCA: most recent common ancestor.

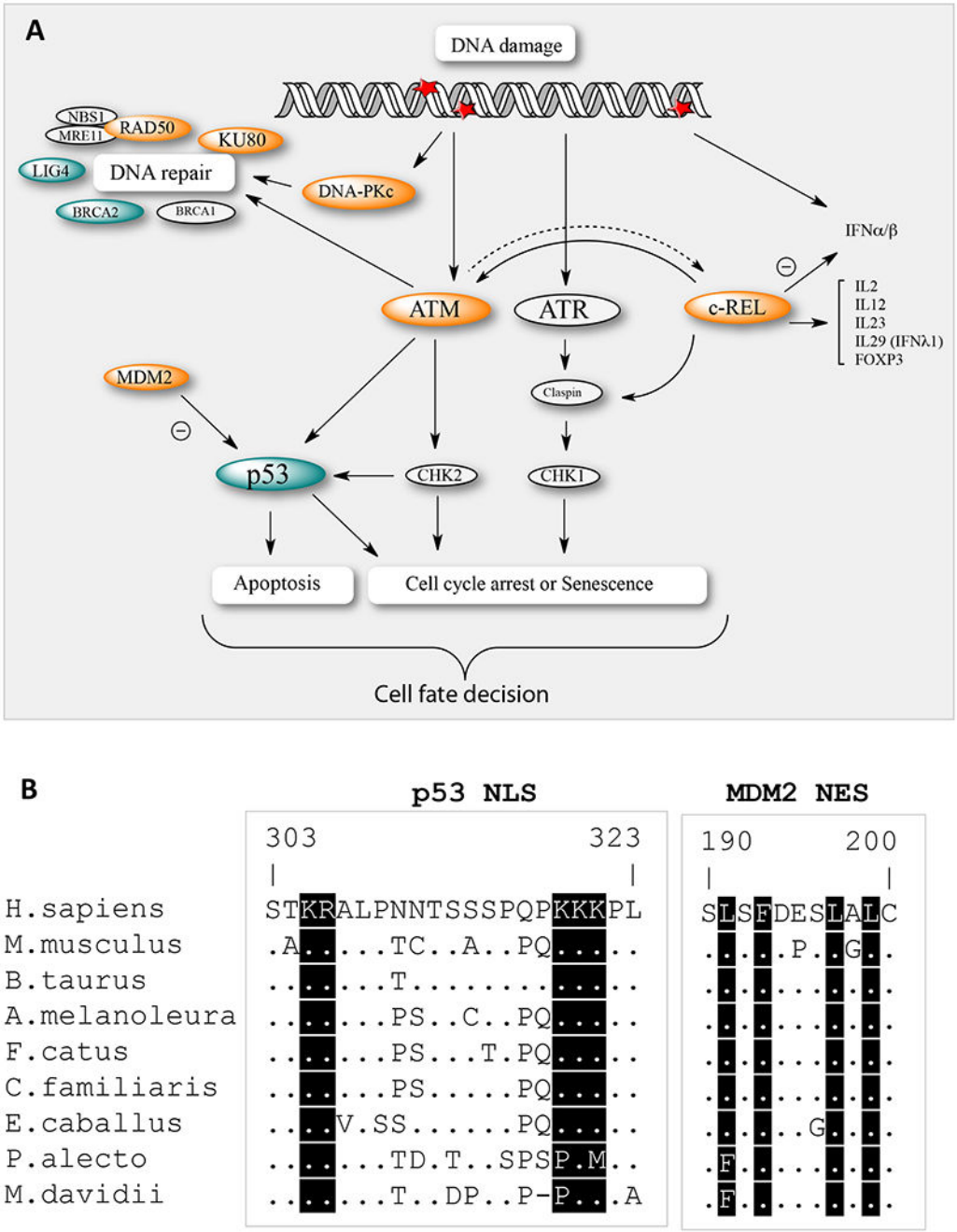

Fig. 3. Accelerated evolution in the DNA damage checkpoint in bats

(A) Positive selection in the DNA damage checkpoint/DNA repair pathway. Genes under positive selection in the bat ancestor are highlighted in orange. Genes under positive selection in *M. davidii* only (*p53*, *BRCA2*) or *P. alecto* only (*LIG4*) are highlighted in blue. (B) Mutations unique to bats were detected in the functionally relevant regions of the *p53* nuclear localization signal (NLS) and *MDM2* nuclear export signal (NES) (black highlight).

Table 1.

DNA damage checkpoint and innate immune genes under positive selection in the bat lineages

Lineage	Symbol	Gene	ω0 (average)	ω1 (other)	ω2 (target)	p-value
Ancestor	*TLR7*	toll-like receptor 7	0.2821	0.2670	2.7778	3.54E-07
	ATM	ataxia telangiectasia mutated	0.20096	0.19595	0.7163	1.34E-05
	MDM2	Mdm2 p53 binding protein homolog (mouse)	0.13358	0.12615	0.81085	4.05E-04
	NLRP3	NLR family, pyrin domain containing 3	0.1788	0.1714	1.1884	1.93E-04
	MAP3K7	mitogen-activated protein kinase kinase kinase 7	0.0216	0.0194	0.4786	8.93E-03
	RAD50	RAD50 homolog	0.09657	0.09343	0.28882	7.95E-03
	PRKDC	protein kinase, DNA-activated, catalytic polypeptide	0.23036	0.22768	0.45155	6.80E-03
	KU80	X-ray repair complementing defective repair in Chinese hamster cells 5	0.31145	0.30436	0.91747	3.75E-02
	c-REL	v-rel reticuloendotheliosis viral oncogene homolog (avian)	0.2495	0.2403	1.5717	1.11E-02
P. alecto	*TBK1*	TANK-binding kinase 1	0.0643	0.0522	0.2930	1.29E-09
	LIG4	ligase IV, DNA, ATP-dependent	0.12033	0.11376	0.24797	8.91E-04
	IL18	interleukin 18 (interferon-gamma-inducing factor)	0.5298	0.4532	1.7647	2.66E-04
	IFNG	interferon, gamma	0.5010	0.4527	1.3282	4.89E-03
	ISG15	ISG15 ubiquitin-like modifier	0.2069	0.1909	0.4387	2.63E-02
	DDX58	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58	0.3040	0.2923	0.4661	1.23E-02
M. davidii	*IFNAR1*	interferon (alpha, beta and omega) receptor 1	0.4954	0.4723	1.0924	7.00E-03
	TP53	tumor protein p53	0.25623	0.23933	0.48123	7.00E-03
	BRCA2	breast cancer 2, early onset	0.49002	0.47732	0.64213	1.31E-03
	IRAK4	interleukin-1 receptor-associated kinase 4	0.1670	0.1583	0.3531	1.96E-02

The rate ratio ω of non-synonymous to synonymous substitutions (dN/dS) was calculated using multi protein alignments of *P. alecto* and *M. davidii* sequences with orthologous sequences from human, rhesus macaque, mouse, rat, dog, cattle and horse. ω0 is the average ratio in all branches, ω1 is the average ratio in non-bat branches, and ω2 is the ratio in the bat branch. A low p-value indicates that the ω2 model fits the data better than the ω1 model.