

LARGE-SCALE GENOME SAMPLING REVEALS UNIQUE IMMUNITY AND METABOLIC ADAPTATIONS IN BATS

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January 4, 2021

Abstract

Comprising more than 1400 species, bats possess adaptations unique among mammals including powered flight, unexpected longevity given small body size, and extraordinary immunity. Some of the molecular mechanisms underlying these unique adaptations includes DNA repair, metabolism and immunity. However, analyses have been limited to a few divergent lineages, reducing the scope of inferences on gene family evolution across the Order Chiroptera. We conducted an exhaustive comparative genomic study of 37 bat species encompassing a large number of lineages, with a particular emphasis on multi-gene family evolution across immune system and metabolic genes. In agreement with previous analyses, we found lineage-specific expansions of the APOBEC3 and MHC-I gene families, and loss of the proinflammatory PYHIN gene family. We inferred more than 1,000 gene losses unique to bats, including genes involved in the regulation of inflammasome pathways such as epithelial defense receptors, the natural killer gene complex and the interferon-gamma induced pathway. Gene set enrichment analyses revealed genes lost in bats are involved in defense response against pathogen-associated molecular patterns and damage-associated molecular patterns. Gene family evolution and selection analyses indicate bats have evolved fundamental functional differences compared to other mammals in both innate and adaptive immune system, with the potential to enhance anti-viral immune response while dampening inflammatory signaling. In addition, metabolic genes have experienced repeated expansions related to convergent shifts to plant-based diets. Our analyses support the hypothesis that, in tandem with flight, ancestral bats had evolved a unique set of immune adaptations whose functional implications remain to be explored.

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Abstract

Comprising more than 1400 species, bats possess adaptations unique among mammals including powered flight, unexpected longevity given small body size, and extraordinary immunity. Some of the molecular mechanisms underlying these unique adaptations includes DNA repair, metabolism and immunity. However, analyses have been limited to a few divergent lineages, reducing the scope of inferences on gene family evolution across the Order Chiroptera. We conducted an exhaustive comparative genomic study of 37 bat species encompassing a large number of lineages, with a particular emphasis on multi-gene family evolution across immune system and metabolic genes. In agreement with previous analyses, we found lineage-specific expansions of the APOBEC3 and MHC-I gene families, and loss of the proinflammatory PYHIN gene family. We inferred more than 1,000 gene losses unique to bats, including genes involved in the regulation of inflammasome pathways such as epithelial defense receptors, the natural killer gene complex and the interferon-gamma induced pathway. Gene set enrichment analyses revealed genes lost in bats are involved in defense response against pathogen-associated molecular patterns and damage-associated molecular patterns. Gene family evolution and selection analyses indicate bats have evolved fundamental functional differences compared to other mammals in both innate and adaptive immune system, with the potential to enhance anti-viral immune response while dampening inflammatory signaling. In addition, metabolic genes have experienced repeated expansions related to convergent shifts to plant-based diets. Our analyses support the hypothesis that, in tandem with flight, ancestral bats had evolved a unique set of immune adaptations whose functional implications remain to be explored.

Key Words

adaptive immunity, gene family evolution, innate immunity, inflammatory pathway, metabolism, viral tolerance

Introduction

Comparative genomics provides a framework for identifying the molecular mechanisms underlying unique organismal adaptations, in their endless forms. To date, comparative genomic approaches have revealed the mechanisms underlying terrestrial adaptations in mudskipper fish (You et al., 2014), heat tolerance in coral (Bay, Rose, Logan, & Palumbi, 2017), cold stress tolerance in *Draba* (Nowak et al., 2020), and extreme longevity in naked mole rats (X. Zhou et al., 2020). In most cases the search for molecular adaptations has focused on orthologous single-copy genes, but gene loss and duplication can also be adaptive and are critical to understanding of how phenotypic adaptations evolve. Analyses based on highly contiguous genome assemblies have uncovered gene expansions likely associated with production of urushiol and anthocyanins in mango (P. Wang et al., 2020), the earliest events of gene duplication in cytoskeletal and membrane-trafficking families in eukaryotic cellular evolution (Vosseberg et al., 2020), pseudogenization in genes associated with testicular descent in afrotherian mammals (Sharma, Lehmann, Stuckas, Funke, & Hiller, 2018), gene losses associated with diving-related adaptations in cetaceans (Huelsmann et al., 2019), and losses associated with physiological and metabolic adaptations in fruit bats (Sharma, Hecker, Roscito, Foerster, Langer & Hiller, 2018). Given the importance of gene family evolution, multiple large-scale genome sequencing consortia such as the Earth BioGenome Project (Lewin et al., 2018), the Vertebrate Genomes Project (Rhie et al., 2020), and Bat1K (Teeling et al., 2018) aim to generate high-quality genome assemblies for species spanning entire clades and even the entire phylogenetic ‘Tree of Life’, thereby enabling greater confidence in analyses of gene loss and gene family evolution.

Gene family expansions and contractions are influenced by selection, including from biological factors such as pathogens. Host-pathogen interactions are shaped by reciprocal selection, an evolutionary arms race which has forced hosts to evolve complex immune defense mechanisms (Papkou et al., 2019; Sironi, Cagliani, Forni, & Clerici, 2015). Vertebrates have two types of immune response: innate immunity, which is non-specific and acts as a first line of defense; and adaptive immunity, which is highly specific and generates immune memory (Delves, Martin, Burton, & Roitt, 2017; Janeway & Travers 2001.). Several immune-related gene families that have experienced substantial evolutionary changes during mammal evolution. While many important facets of the immune system are conserved, immune gene families have high rates of evolution whether measured via substitution rate ratios or birth–death turnover (Bernatchez & Landry, 2003; Goebel et al., 2017; Minias, Pikus, Whittingham, & Dunn, 2019; Santos et al., 2016; Shultz & Sackton, 2019; Van Oosterhout, 2009). This is especially true of the Major Histocompatibility Complex (MHC), which is responsible for generating cell surface proteins that play essential functions in the adaptive immune system (Janeway & Travers 2001).

This combination of highly conserved, and highly variable components of the immune system, is particularly intriguing among bats. Among mammals, bat diversity is second only to that of rodents, and encompasses over 1,400 species that occupy a broad diversity of ecological niches on six continents (Fenton & Simmons, 2015; Nogueira et al., 2018). The success of bats is likely related to a suite of adaptations unique both to the clade as a whole and to various subclades within the Order Chiroptera. The most obvious of these is powered flight, allowing bats to occupy a unique aerial niche not utilized by any other mammal. While this unique niche limits body size, within that constraint bats have been exceptionally successful and have diversified in ways unparalleled among other mammals. For example, bats evolved virtually every mammalian dietary strategy (e.g., frugivory, carnivory, nectarivory, piscivory) and have done so in a relatively short evolutionary time frame (Dumont et al., 2012). Another less obvious but likely more interesting adaptation is the exceptional longevity and increased health span (the period of life during which an organism is in

generally good health) exhibited by many bat species given their body size. Many species such as the Bechstein's bat (*Myotis bechstein*) the little brown bat, Brandt's bat (*Myotis brandtii*), greater mouse-eared bat (*Myotis myotis*) and greater horseshoe bat (*Rhinolophus ferrumequinum*) have unexpectedly long health spans, living 30 - 40 years (Fleischer, Gampe, Scheuerlein & Kerth, 2017; Foley et al., 2018; Podlusty, Khritankov, Ovodov & Austad, 2005; Seim et al., 2013; Wilkinson & Adams, 2019). Such longevity defies the expectation that large species are longer-lived than small species; despite constrained body size, bats live longer than other mammals of similar size (Austad & Fischer, 1991; Healy et al., 2014). Bat longevity and health span may be influenced by their exposure to extrinsic mortality factors. Powered, mostly nocturnal flight may lower bats' exposure to some sources of extrinsic mortality, including predation (Healy et al., 2014). However, bats' propensity to roost in large colonies also increases their exposure to another source of mortality – infections (Brook & Dobson, 2015; H. Han et al., 2015). Thus, to achieve such longevity and decreased senescence, long-lived bat populations must overcome the burden of infectious diseases.

The uniqueness of bats extends to the immune repertoire. Early in the age of whole-genome analyses, it was clear that inflammation-related gene families had expanded or contracted, and certain single-copy genes associated with immunity and cell repair had experienced selection in bats (G. Zhang et al., 2013). There is still debate as to whether bats harbor a disproportionately large number of viruses (Moratelli & Calisher, 2015; Olival et al., 2017), but there is no doubt that several recent viral intrusions into our own species ultimately originated from bat hosts (Drexler et al., 2012; Goldstein et al., 2018; Hu et al., 2017; Memish, Perlman, Van Kerkhove, & Zumla, 2020; Towner et al., 2007). This likely includes the current SARS-CoV-2 pandemic (Boni et al., 2020; Lau et al., 2020). Bats appear to have the ability to tolerate these viruses with few health impacts, hence recent studies have focused on bat comparative genomics (Jebb et al., 2020) and its emphasis on viral response (reviewed in: Gorbunova, Seluanov, & Kennedy, 2020; Hayman, 2019). Although little is known from this perspective, there is a growing body of functional analyses showing that bats are unusual among mammals in how they deal with viruses (Ahn et al., 2019; A. Banerjee et al., 2020; Miller et al., 2016; Schountz, Baker, Butler, & Munster, 2017; Xie et al., 2018).

The 'inflammosome' is typically highly conserved across mammals, but bats exhibit a reduced inflammatory response that may be tied to their ability to cope with viral infection while experiencing minimal impact (Pavlovich et al., 2018). For example, the PYHIN gene family, namely, appears to have been almost completely lost in bats (Ahn, Cui, Irving, & Wang, 2016; G. Zhang et al., 2013) while at least one PYHIN gene can be found in all other eutherians examined. Similarly, in bats, the inflammatory function of interferons (G. Zhang et al., 2013) appears distinct and the APOBEC3 repertoire, which is associated with anti-viral response, is expanded (Jebb et al., 2020; Hayward et al., 2018). All of these functional patterns suggest an overall dampened inflammatory reaction despite a robust immune response to viruses whose origins may lie in the gene repertoires available to bats (A. Banerjee, Rapin, Bollinger, & Misra, 2017; A. Banerjee et al., 2020).

Gene family evolution also likely plays a role in the unique dietary ecology of bats. Several studies have found a variety of mechanisms influencing dietary adaptation. For example, convergent amino acid substitutions in several lineages of frugivorous bats have occurred independently (Gutierrez-Guerrero et al., 2020; Shen, Han, Zhang, Rossiter, & Zhang, 2012; Teeling et al., 2018; K. Wang et al., 2020), and are associated with the shift to a high-sugar diet. Another strategy has been to repurpose a given gene to accommodate such dietary shifts. (Shen, Han, Jones, Rossiter, & Zhang, 2013). With the exception of olfactory receptors (Hayden et al., 2014; Hughes et al., 2018; Tsagkogeorga, Muller, Dessimoz, & Rossiter, 2017), the roles of gene loss and gain in shaping dietary evolution of bats have not been comprehensively explored.

Here we investigate bat gene family evolution related to immunity, metabolism, and dietary adaptations, using the most extensive genomic sampling within bats to date. Despite variability in quality of assemblies, the ecological diversity of lineages for which assemblies are available allows, for the first time, an investigation of gene family evolution across 10 families, two suborders, and a complete coverage of the entire range of diets. We find two major patterns. First, system-wide gene losses related to inflammatory response and selection on genes associated with antiviral immunity appear to have influenced bat lineages. This

suggests that bats— compared to other mammals such as cow, dog, horse, pig, mouse and human— have evolved complex, complementary adaptations across multiple functional pathways to simultaneously reduce inflammatory response while maintaining strong antiviral defenses, potentially underlying their suspected tolerance of viruses. Second, the move from the ancestral arthropod diet to high-sugar nectar and fruit-based diets is associated with t lineage-specific gene family expansions in metabolic gene families.

Materials and Methods

Whole genome sequencing

We generated a whole genome assembly for a male *Phyllostomus hastatus* , PE091, collected in Jenaro Herrera, Peru. Field-collected tissues from *Phyllostomus hastatus* specimen PE091 were lawfully collected under permit #0122–2015–SERFOR–DGGSPFFS, exported under SERFOR permit #0002287, and imported under USFW 3-177 2015MI1694291.

Samples were preserved in RNAlater for one week before flash-freezing in a liquid nitrogen dry shipper, following previously published protocols (Yohe et al., 2019). High molecular weight genomic DNA was extracted from flash-frozen liver using the Qiamp DNA Micro Kit (Germantown, MD, USA) and sequenced on a PromethION instrument (Oxford Nanopore Technologies, New York, NY, USA) at Cold Spring Harbor Laboratory. Additionally, short-read Illumina whole genome sequencing was performed at Novogene, Inc (California, USA). Genomic DNA from lung was randomly fragmented to 350bp, end-repaired, adenylated, ligated with Illumina sequencing adapters, and further PCR-enriched. The final libraries were purified (AM-Pure XP system) and library quality and size verification were assessed on an Agilent 2100 Bioanalyzer (Agilent Technologies, CA, USA). Molar concentration was assessed using real-time PCR.

De novo genome assembly was performed using Flye v.2.7.1 (Kolmogorov, Yuan, Lin, & Pevzner, 2019) using default *-nano-raw* parameterization. The obtained pre-assembly was polished using Illumina short-reads with POLCA tool built-in MaSuRCA genome assembly and analysis toolkit (Zimin et al., 2013).

Genome database construction

Publicly-available genome assemblies for an additional 36 bat species (Supplementary Table 1) were downloaded from open-source databases to maximize bat taxonomic sampling (D. Dong et al., 2017; Eckalbar et al., 2016; Gutierrez-Guerrero et al., 2020; Jebb et al., 2020; Parker et al., 2013; Seim et al., 2013; K. Wang et al., 2020; Zepeda Mendoza et al., 2018; G. Zhang et al., 2013). Assemblies were masked with RepeatMasker v.4.1.0 (Smit, Hubley, & Green, n.d.) using a custom library combining known mammalian transposable elements (TE) from Repbase (v20181026), *ade novo* mammalian TE library generated using assemblies from the Zoonomia Project (Genereux et al., 2020) and the Dfam database, and a custom bat-specific TE library generated by manual curation (Jebb et al., 2020).

All assemblies were annotated or re-annotated with the MAKER annotation pipeline v.2.31.10 (Holt & Yandell, 2011) to avoid bias in downstream analyses caused by differences in genome assembly annotation quality. Two iterations of MAKER were performed for each species. During the first run we provided expressed sequence tags (ESTs) and transcriptomic data as inputs (Davies et al., 2020; Potter et al., n.d.) (Supplementary Table 2). If species-specific transcriptomic data were unavailable, we used information from a related species of the same genus. We used two databases for protein homology the Uniprot/Swiss-Prot protein sequence database (Bateman, 2019) and a bat-specific protein database obtained from high-quality genome annotations for six bat species (Jebb et al., 2020). Repeat evidence was provided using the repeat annotation GFF3 file generated by RepeatMasker. Gene models generated on the first run were used for gene predictions with two gene software packages, SNAP (Korf, 2004) and Augustus (Stanke & Waack, 2003). Only gene models with an AED score < 0.25 and with more than 50 amino acids were retained. For the second run, focusing on re-annotation, the MAKER control file was edited to include the GFF3 output file

from the first run gene predictions generated by SNAP and the Augustus gene prediction species model as inputs. Functional annotation was performed with BlastP (Camacho et al., 2009) using the Uniprot/Swiss-Prot database and protein domain annotation with InterProScan (Jones et al., 2014).

Homology inference

Protein homology was inferred among the proteins of 43 mammals: *Homo sapiens*, *Mus musculus*, *Sus scrofa*, *Bos taurus*, *Equus caballus*, *Canis lupus familiaris*, and the 37 bat species (Supplementary Table 1). Orthologous groups (orthogroups) were assigned with Orthofinder v.2.4.0 (Emms & Kelly, 2019). When no orthologs were inferred for the Chiroptera in a given orthogroup, we independently analyzed the genome data to confirm gene losses in bats. To this end, we performed a BLAST search against the 37 bat genomes using the following criteria: an e-value of 1e-6 and an identity and protein coverage greater than 80%. Then, genomic regions with a BLAST hit were extracted along with 200bp upstream and downstream. Sequences were aligned with the MAFFT aligner tool v.7.402 (Katoh & Standley, 2013) and visualized using Geneious version 11.1.3 (Kearse et al., 2012) to discriminate annotation errors. Additionally, BLAST searches were also performed against transcriptomic data from 22 bat species (Supplementary Table 2) (Potter et al., n.d.). For these searches, potential matches were filtered more strictly, and those with identity and protein coverage [?] 90% were retained. Subsequent blast hit extraction, alignment and visualization were as for the genome searches.

Enrichment in chiropteran gene losses

We conducted pathway enrichment analyses with the final list of genes missing from all bat species using two databases: BioPlanet (R. Huang et al., 2019) and DICE GOnet (Pomaznoy, Ha, & Peters, 2018). In each case, we used the list of gene symbols as input with a cutoff value of 0.05 (BioPlanet) and a similar p-value in the DICE GOnet biological process classification for the mouse model. In both cases, all genes found to be missing were used as input and compared to a reference set of genes annotated in the corresponding database.

Gene family evolution

While previous analyses that included bat species have analyzed signals of positive selection across bats (e.g. Parker et al., 2013), fewer have explicitly centered on gene family evolution (Jebb et al., 2020; Tsagkogeorga et al., 2017). To analyze our comprehensive bat-focused sample, we modeled gene family expansions and contractions using CAFE (Computational Analysis of Gene Family Evolution) v.4.2.1 (M. V. Han, Thomas, Lugo-Martinez, & Hahn, 2013). The effect of genome quality on downstream gene predictions is well documented and leads to an overestimation of gene gains and losses (Denton et al., 2014; Tsagkogeorga et al., 2017). To mitigate the aforementioned bias, only genome assemblies with BUSCO completeness scores over 80%, totaling 34 species (28 bat species and 6 outgroup mammals) were used for CAFE. This smaller subset of protein sequences was filtered, retaining only the longest isoform. Homology clustering was performed with Orthofinder v.2.4.0 (Emms & Kelly, 2019).

CAFE fits a birth and death parameter (λ) to estimate the probability of gene gains or losses across a specified phylogeny (Hahn, De Bie, Stajich, Nguyen, & Cristianini, 2005). We first inferred an ultrametric phylogenomic tree based on 350 single copy orthologous genes (207,551 amino acid sites). All the orthologs were concatenated into a single 207,551-amino acid “contig” and sequence alignment was performed using the MAFFT aligner tool v.7.402 (Katoh & Standley, 2013). We evaluated the best-fit models of protein evolution with ProtTest v.3 (Darriba, Taboada, Doallo, & Posada, 2011) using two criteria: the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC) (distribution JTT, +G +I +I +G and 80% consensus threshold). A maximum likelihood tree was inferred for the concatenated data set with RAxML v.8 (Stamatakis, 2014). Estimation of species divergence times was performed with Bayesian phylogenetic methods using the MCMCtree tool in the PAML v.4.9 package (Yang, 2007). We calibrated

divergence dates using six points based on fossil records: 1) *Icaronycteris*, considered as one of the oldest echolocating fossil bats, dated at 52 Mya (Gunnell & Simmons, 2005; Simmons, Seymour, Habersetzer, & Gunnell, 2008); *Tachypteron*, the oldest known emballonurid fossil from the early Middle Eocene, with an age range of 48.6 to 40 Mya (Storch, Sigé, & Habersetzer, 2002); *Hipposideros africanum*, the oldest fossil record of the family Hipposideridae, its records date at 41.3 Mya (Ravel et al., 2016); Vespertilionidae indet. (41.3 Mya) (Eiting & Gunnell, 2009); Phyllostomidae indet. (30 Mya) (Nicholas J Czaplewski, 2010), and *Palynephyllum* (11.8 Mya) (Nicholas J Czaplewski, Takai, Naeher, & Setoguchi, 2003; Dávalos, Velasco, Warsi, Smits, & Simmons, 2014). Additionally, we included and corroborated the molecular dates for the base of the ingroup root estimated by Teeling et al. (2005).

We filtered the final input for CAFE to reduce systematic bias in inferring gene family evolution. First, we retained only gene families present at the most recent common ancestor of the phylogeny, with at least one gene present in each of the four clades assigned: a) Euarchontoglires (*Homo sapiens* and *Mus musculus*), b) non-Chiroptera Laurasiatheria (*Bos taurus*, *Canis familiaris*, *Equus caballus*, *Sus scrofa*), c) Yungitrochiroptera and d) Yinpterochiroptera. Second, gene families missing in more than 50% of bat species were excluded. Finally, families with large gene copy number variance ($[?]100$ gene copies) were excluded for the global birth and death (λ) rate inference.

To analyze families with at least one gene copy across the taxa sampled, we first estimated a global λ for all branches. The global model was compared against a three multi- λ model that fits each lineage with its own gene family evolution rate. To test which model fits better with our dataset, we performed a likelihood ratio test for 100 gene family evolution simulations. We ran CAFE in error correction mode to account for genome assembly and annotation errors and estimate the global distribution of error with the assumption that all branches share a unique λ rate ($\lambda=0.0033734$) as described in Han et al. (2013). Finally, we used complementary tools; the Protein Analysis Through Evolutionary Relationships (PANTHER v.15) (Mi, Muruganujan, Ebert, Huang, & Thomas, 2019) and Gene Ontology Analysis (GOnet) to annotate genes with gene ontology (GO) terms (Ashburner et al., 2000; Carbon et al., 2019) and assign them to gene families, pathways, and biological process categories.

Selection tests

We identified genes under positive selection by evaluating 268 single-copy genes involved in immune response, based on a curated database of 1,793 genes downloaded from the IMMPORDB repository (Bhattacharya et al., 2014) available at <https://www.immport.org/home>. Gene alignments were built with MAFFT v.7.402 (Katoh & Standley, 2013) and manually filtered to remove sequences with less than 70% of protein coverage based on the homologous human protein. Only alignments represented by at least 30% of the species were used for downstream analysis. For each gene in the codeml analyses, we built a phylogeny with RAxML (Stamatakis, 2014) and a codon alignment for each gene with PAL2NAL (Suyama, Torrents, & Bork, 2006).

We tested for evidence of positive selection among sites along bat lineages using the strict branch-site model (Yang, Wong, & Nielsen, 2005; J. Zhang, Nielsen, & Yang, 2005) with maximum-likelihood estimations implemented in codeml in PAML v.4.9 (Yang, 2007). We implemented model 2 as this allows the dN/dS ratio (ω) to vary across branches and sites and to detect if selection differs in a few amino acid residues in specific lineages (foreground branches). We compared two hypotheses, assigning the 37 bat species as foreground branches: 1) the null hypothesis with a fixed ω ($\omega=1$) for all branches does not allow for positive selection, and 2) an alternative hypothesis assuming that the foreground branches have a greater proportion of sites under positive selection ($\omega > 1$) than the background branches. The null hypothesis was tested against the alternative model with the likelihood-ratio test (LRT); the p-value was calculated under a chi-square distribution with 1 degree of freedom, additionally we adjusted the p-value using the false discovery rate (FDR) correction. To detect sites under positive selection, we used the Bayes Empirical Bayes (BEB) (Yang et al., 2005) approach to calculate posterior probabilities that a site has a significant value of $\omega > 1$. The residues with a high posterior probability ($P > 95\%$) were considered.

To determine how robust the signals of positive selection detected were, we used the adaptive Branch-Site Random Effects Likelihood (aBSREL) (Smith et al., 2015) model, as implemented in HyPhy (Kosakovsky Pond, Frost, & Muse, 2005). The aBSREL model explores whether a proportion of sites have evolved under positive selection in each branch of the phylogeny, and was applied to all alignments using their respective gene trees. The false discovery rate method of multiple testing correction was applied to all p-values generated for each branch and gene.

Results

Genome sequencing

The final assembly for *P. hastatus* comprised 2.1 Gb and has a N50 contig length >39 Mb. Assembly quality completeness was estimated at 95.4%. These values are similar to those observed for bat assemblies inferred using similar methods (Jebb et al., 2020).

Homology inference

BUSCO analysis results indicated that the bat genome assemblies contained between 68.5 and 96.5% of the single-copy orthologs present among mammals (Figure 1). Orthologs were grouped into 42,441 groups, of which 1,193 were single copy. In total, 5,528 orthogroups had at least one representative in each of the entire set of 43 species that were analyzed. In contrast, 1,055 orthogroups were represented in at least 50% of bat species but missing from the six outgroup taxa (Supplementary table 3). To annotate diets, we used the semi-quantitative database compiled by Rojas, Ramos, Fonseca and Dávalos (2018), which focuses on neotropical noctilionoids (Yangochiroptera), supplemented with summaries from Animal Diversity Web (<https://animaldiversity.org/>).

Enrichment in chiropteran gene losses

We inferred the first densely sampled chiropteran phylogeny based on hundreds of loci (Figure 1). Our results confirmed the monophyly of the suborders Yinpterochiroptera and Yangochiroptera but the phylogeny of the neotropical leaf-nosed bats (family Phyllostomidae) differed from previous phylogenies (Dávalos, Velasco, & Rojas, 2020), in the paraphyly of plant-eating lineages. As the obtained phylogeny is the best supported by all genome-scale analyses available thus far (S.J. Rossiter and M. Hiller pers. obs.), we used this phylogeny for gene family evolution analyses.

A total of 1,115 genes (Supplementary Table 4) were identified as missing in bats, even after filtering BLAST searches against the genomes and transcriptomes. Based on this list, we identified eight over-represented pathways in BioPlanet (Supplementary Table 5) and 63 GO terms in GOnet (Supplementary Table 6). While the former included 104 genes, of which 49 were unique, the latter included 339 unique missing genes. As expected, over-represented categories included chemosensory gene losses in the categories of olfactory transduction, G-protein-coupled receptors (GPCR), and signal transduction. BioPlanet pathways were also enriched for less common categories including immune system pathways that include alpha and beta defensins, antigen process and presentation, and graft-versus-host disease (Supplementary Table 5). GOnet analyses also identified the expected enrichments in chemosensory gene losses and general response to stimuli categories, but also included many more immune categories. Of the latter, the categories comprising the most genes were defense response (58 genes), defense response to other organism (54), response to bacterium (53), innate immune response (46), defense response to bacterium (44), humoral immune response (34), adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains (23), lymphocyte mediated immunity (23), and leukocyte mediated immunity (23). Although these categories share many genes across them, a preponderance of immune system losses is evident in Supplementary Table 6. We used BioRender to summarize the immune gene ontology categories and connections, highlighted in Figure 2.

Gene family evolution

To determine branches and gene families with significant gene family expansions and contractions, we analyzed 14,171 orthogroups under two models: a global rate of gene family evolution, and a three multi- λ model. The three-rate model best fit the data ($p < 0.01$), this analysis estimated a higher rate of gene family turnover ($\lambda_{\text{Yangochiroptera}} = 0.0048$) in the ancestral Yangochiroptera lineage than in the Yinpterochiroptera ancestral lineage ($\lambda_{\text{Yinpterochiroptera}} = 0.0024$), with the lowest turnover rate for outgroup lineages ($\lambda_{\text{Outgroups}} = 0.0017$).

With an estimated error distribution of 0.049 (i.e., 4.9% of gene families showed an error in gene size), we identified 2,555 orthogroups with significant expansions or contractions along at least one of the branches in the species tree. Given our focus on immune system and metabolic evolution, we extracted PANTHER annotations for the most frequent (900 orthogroups) biological process categories: immune response, metabolic process, and cellular process. All GOnet annotations were used and binned into immune, metabolic, and two additional processes: response to stress (271 orthogroups) and autophagy (19). PANTHER and GOnet annotations were mostly complementary; orthogroups were often annotated in one database but not the other (1,268 orthogroups). When annotations were available from both databases, these tended to agree on both immune and metabolic categories (594 orthogroups), or to agree on one or the other (404), with only 48 orthogroups disagreeing completely in immune and metabolic annotations between the databases. The remaining 241 were not annotated in either database. Categories, locations, and size of significant gene family changes were summarized using tools in the R package ggtree (Yu, Smith, Zhu, Guan, & Lam, 2017) and are shown in Figure 3. Although several pairs of sister species showed apparently large differences along corresponding tips (e.g., *Rhinolophus*, *Miniopterus*), such variation is common in analyses that include genome assemblies of varying quality (Denton et al., 2014; Tsagkogeorga et al., 2017). Therefore, we focus our discussion on the more robust inference of gene family expansions and contractions for non-sister lineages. Taking advantage of relatively dense taxon sampling within bats (Figure 1), we focused on parallel adaptation to plant-rich diets across suborders Yinpterochiroptera and Yangochiroptera.

Selection tests

Branch-site selection tests identified 37 of 268 single-copy genes with evidence for positive selection, of which 27 remained after false discovery rate correction (Table 1). This subset included genes involved in interferon-gamma (IFNG) signaling, inflammatory response, as well as cytokines, chemokines, and interleukins. A total of 16,979 branches across 268 genes were analysed using the aBSREL model in HyPhy. After FDR correction, 683 branches from 191 gene trees were found to be significant, 25 of which were consistent with CODEML results (Supplementary table 8).

Discussion

Gene losses in inflammation-related gene families and positive selection in single-copy genes associated with immune and cell repair functions in mammalian models have been evident since the very first bat genome assemblies were published (G. Zhang et al., 2013). Although subsequent studies have confirmed those initial results (Ahn et al., 2016; Seim et al., 2013), confidence in assessing both gene losses and gene family expansions has strengthened only recently, with the publication of highly contiguous assemblies for a few bat species (Jebb et al., 2020; Scheben et al., 2020). Examining a comprehensive sample of bat lineages while checking against high quality genome assemblies and multi organ RNA Seq, our analyses reveal system wide gene losses with the potential to modify the sensitivity, targets, and magnitude of immune responses across all bats. These inferred losses are particularly concentrated along inflammasome activation pathways, which are triggered by the innate immune recognition of pathogenic signals through both pathogen-associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs). In contrast with more pathogen-driven PAMPs, DAMPs result from host cellular distress signals such as mitochondrial stress and

reactive oxygen species (ROS) (Zheng, Liwinski, & Elinav, 2020), which bats produce during active flight (Costantini, Lindecke, Petersons, & Voigt, 2019). Bat cells, in turn, display exceptional mechanisms of repair (Pickering, Lehr, Kohler, Han, & Miller, 2014) and resist damage (Harper, Salmon, Leiser, Galecki, & Miller, 2007), connecting molecular signaling and cell processes to extreme longevity (Salmon et al., 2009; Wilkinson & Adams, 2019).

Based on our genomic surveys, immune-related losses can be divided into three categories: the epithelial defense receptors (defensins), the Natural Killer gene complex (NKC) and the interferon-induced pathway (IFI; HIN; PYHIN) (Figure 2). This particular combination of losses in crucial components of immune activation seems surprising, as it would imply that bats mount a low intensity immune response to primarily intracellular pathogens: viruses. But integrating these genomic findings with published functional data suggests complex, systemic adaptation, in line with both previous analyses of bat immune system responses (A. Banerjee et al., 2020; Basler, 2020; P. Zhou, 2020) and the growing body of evidence for cellular mechanisms underlying longevity (Z. Huang, Whelan, Dechmann, & Teeling, 2020; Z. Huang et al., 2019, Kacprzyk et al., 2017). We review these losses in a stratigraphic order, from the outer cellular matrix to the inner cellular pathways, starting with the defensins.

While defensins are the primary barrier of the immune system, with broad antimicrobial activity that covers bacteria, fungi and viruses (Semple & Dorin, 2012; Xu & Lu, 2020), bat defensin losses consist mainly of orthologs of genes localized to epithelial cells. Defensins can function as modulators of the host's cell surface receptors, and α and β defensins genes have pleiotropic effects on the regulation of carcinogenesis and inflammation (Xu & Lu, 2020). By acting as chemokines to alter the adaptive immune response, defensins also serve as a bridge between innate and adaptive immunity (Grigat, Soruri, Forssmann, Riggert, & Zwirner, 2007). In bats, both α and β defensin genes have undergone a rapid evolutionary change through either loss or positive selection (Table 1, Figure 2a, Supplementary Table 4). In humans, defensins can elicit proinflammatory cytokine production (Niyonsaba et al., 2010; Wiens, Wilson, Lucero, & Smith, 2014), but overexpression of certain defensins can actually enhance viral infection (Rapista et al., 2011). We hypothesize that specific defensin losses in bats (Figure 2a) complement several other mechanisms (Ahn et al., 2019; A. Banerjee et al., 2017; Xie et al., 2018) contributing to a dampened inflammatory response, reduced host-driven damage from viral infections, and enhanced longevity (Baker & Schountz, 2018; Brook & Dobson, 2015; Gorbunova et al., 2020). For example, modifying defensin repertoires on epithelial cells would result in fewer instances of both immune cell recruitment and initiation of inflammatory pathways known to damage healthy tissue (e.g., focal necrosis in lungs, spleen and lymph nodes during the inflammatory response during SARS-Cov2 infection (Merad & Martin, 2020)). In humans, loss of β -defensins prevents the inhibition of neutrophil apoptosis and thus averts the production of proinflammatory cytokines and chemokines (Nagaoka, Niyonsaba, Tsutsumi-Ishii, Tamura, & Hirata, 2008), avoiding the amplification of the immune response, and may have a similar effect in bats. Losses of some epithelial surface defensins would thus reduce inflammation without compromising responses to intracellular pathogens.

Another result with inferred implications for reducing proinflammatory reactions involves losses of Natural Killer (NK) receptors that play an important role in the recognition of MHC-I molecules and regulation of cytotoxic activity against virus-infected cells. While killer-cell immunoglobulin like receptors (KIR) and killer cell lectin-like receptors (KLR) receptor losses has been previously reported for *Pteropus alecto* and *Myotis davidii* (Papenfuss et al., 2012; G. Zhang et al., 2013), our analyses confirm these losses across Chiroptera (Supplementary Table 4). Although the *Killer Cell Lectin Like Receptor K1* (KLRK1 or NKG2D) gene is present in bats, its ligands, gene subfamilies *RAET1* and *H60* responsible for binding and activating NKG2D receptors, recruiting natural killer cells, and stimulating them to secrete Interferon gamma (IFN- γ) (Zhi et al., 2010), were absent in all bat species (Figure 2b).

We hypothesize that these losses lead to low recruitment of proinflammatory NK cells and reduce B-cell signaling (Arapović et al., 2009; Stolberg et al., 2014; Takada et al., 2008; Wortham et al., 2012), as they do in mice and humans. Loss of this particular mechanism of activation of the MHC-I pathway prevents proliferation of immune cells, which can be cytotoxic, proinflammatory, and targets of viral infections (Djelloul,

Popa, Pelletier, Raguénez, & Boucraut, 2016; Wortham et al., 2012). For example, NKG2D-deficient mice infected with influenza viruses exhibit less airway damage and reduced inflammation without compromising viral clearance; NKG2D stimulation is a central pathway to viral-mediated NK cell hyperresponsiveness (Wortham et al. 2012), and has been shown to be involved in age-dependent COVID-19 severity (Akbar & Gilroy, 2020). During viral exposure, rarer activation of NKG2D function would therefore lead to less inflammatory exacerbation. Reducing instances of NKG2D activation might also reduce B cell signaling, as it occurs in NKG2D-deficient mice (Lenartić et al., 2017; Zafirova et al., 2009), and complements losses of immunoglobulin heavy chain variable regions IGHV1, IGVH3 and IGHV14 genes that modify the B cell receptor signaling pathway, and thus B lymphocyte differentiation (M. Banerjee, Mehr, Belevovsky, Spencer, & Dunn-Walters, 2002; McHeyzer-Williams, Okitsu, Wang, & McHeyzer-Williams, 2012; Reddy et al., 2010). Based on the roles of both NKG2D and B cell activation in promoting inflammation in viral infection, and since some viral proteins have been shown to specifically target the NKG2D receptor via the RAET1 and H60 loci (Arapović et al., 2009), we propose that these losses resulted from selection during viral infections early in the evolutionary history of bats. While the functional implications for bats need to be tested, in humans, lack of specificity of the T and B cells in children results in a broader immune response to novel viruses (Pierce et al., 2020), and it may confer analogous advantages in bats.

Complementing losses in defensins and NK signaling, the third large group of gene losses involves the IFN- γ pathway (Figure 2c). While representatives of the PYRIN and HIN domain (PYHIN) gene family, immune sensors of cytosolic DNA activating the inflammasome and IFN- γ , are present in all mammals, they have not been found in any of the bat genomes analyzed thus far examined (Ahn et al., 2016; G. Zhang et al., 2013; Jebb et al., 2020). Previous genomic analyses linked losses in this inflammasome pathway to the unique demands of bat flight and in response to increased ROS production (G. Zhang et al., 2013), but there are immune implications as well. In other mammals, the presence of dsDNA, DAMPs and PAMPs, or, especially, bacteria and DNA viruses, induces the (PYHIN) AIM2 inflammasome, while the IFI16 inflammasome (Interferon-inducible protein 16, also missing in bats) recognizes viruses replicating in the nucleus (Zheng et al., 2020). Hence, these bat gene losses could undermine innate defense against viruses. We hypothesize that bats have evolved mechanisms to overcome this potential disadvantage in rapid recognition and response against viruses through expansion of MHC-I class genes (Supplementary Table 7). These genes are involved in the recognition and binding of intra cellular peptides, and previous studies have described a unique 5-amino acid insertion at the exon 2 peptide binding region (PBR) on bats which may allow the host to recognize longer peptides (Ng et al., 2016; Papenfuss et al., 2012). Besides implications for immunity, IFN- γ pathway gene losses also point to changes in autophagy. In mice, loss of the IFN- γ inducible immunity related GTPase gene (IRGM1 and IRGM2) results in an IFN- γ induced autophagic death program in lymphocytes (Feng et al., 2008). Along with the loss of other IFN- γ related genes (IGTO, IIGP, TGTP2), these losses may help achieve apoptosis of infected cells without runaway inflammation.

While some mechanisms of activation of IFN- λ are lost in bats, IFN- γ itself is under positive selection within branches (Table 1 Supplementary Table 7). IFN- γ is a crucial part for the first line of defense against viruses, helps shape adaptive immune memory (Schroder, Hertzog, Ravasi, & Hume, 2004), and its deficiency increases inflammation (Loo et al., 2017). Thus, evolutionary adaptation may have shaped bats' unique ability to induce a rapid antiviral response without triggering runaway inflammation. This fine-tuned response may be achieved by expressing high levels of IFN- γ early on, which recruits broad-spectrum immune cells to the site of injury, while negatively regulating the IFN- γ pathway receptors that trigger inflammation (Ahn et al., 2019; Ferber et al., 1996).

In contrast to a pattern of proinflammatory signal losses, most other variation in gene families in Chiroptera corresponded to cell processes and metabolic functions. Shifts from the ancestral bat insectivorous diet to including nectar and fruit and the resulting mutualistic relationships between bats and plants appear to have led to elevated rates of diversification and the evolution of new morphological traits (Dumont et al., 2012; Jones, Bininda-Emonds, & Gittleman, 2005), but gene family evolution has remained underexplored. Regarding significant expansions (Supplementary Table 7), we identified few —only nine— sets of duplications independently replicated across all pteropodids and phyllostomids with convergent, plant-based diets

(Figure 1). In addition to a trace amine associated receptor (TAAR) of unknown chemosensory function (Liberles & Buck, 2006) and a putative homolog of the yeast protein transport protein YIP1, two genes stand out as candidates for diet-linked adaptive gene family evolution: those encoding homologs of *inositol monophosphatase 1* (IMPA1) and *integrin alpha-D/beta-2* (ITAD). Glycolysis, the metabolic pathway that breaks down glucose to ultimately phosphorylate more ADP into ATP than the reverse, begins with the phosphorylation of glucose into D-glucose 6-phosphate (Berg, Tymoczko, & Stryer, 2002). This metabolite, however, cannot diffuse through the membrane and is thus highly osmotic; its accumulation would cause cells to swell. Through the synthesis of *myo* -inositol from D-glucose 6-phosphate, IMPA1 provides one avenue to protect cells, particularly in the brain (Parthasarathy, Parthasarathy, & Vadnal, 1997), from the osmotic stress of this glucose metabolite (Rafikov et al., 2019). We found independent IMPA1 duplications in the pteropodid ancestor, *A. jamaicensis*, *A. caudifer*, *P. discolor*, and the common ancestor of phyllostomids and *Mormoops*. Except for the aerial insectivore *Mormoops*, all the lineages with IMPA1 duplications include nectar and fruit in their diet (Figure 1), are expected to at least occasionally experience high blood glucose levels (Amitai et al., 2010; Ayala & Schondube, 2011; Kelm, Simon, Kuhlow, Voigh & Ristow, 2011; Welch, Herrera & Suarez, 2008; Meng, Zhu, Huang, Irwin, & Zhang, 2016), and therefore require options for processing metabolites from glycolysis. Although beta integrins, including ITAD, are regulators of leukocyte function and therefore not annotated as directly involved in metabolism, leukocyte adhesion has been found to modulate glucose homeostasis via lipid metabolism (Meakin et al., 2015). Specifically, mice deficient in a paralogous beta-2 integrin become spontaneously obese in old age despite a normal diet (Z. Dong, Gutierrez-Ramos, Coxon, Mayadas, & Wagner, 1997), and when fed a fat rich diet show obesity, inflammation, high neutrophil activity and insulin resistance in skeletal muscle (Meakin et al., 2015). Likewise, mice deficient in this same integrin are unable to respond to fasting by increasing fat uptake and reduce insulin levels slowly compared to normal mice (Babic et al., 2004). We found single ITAD duplications in lineages that include sugar rich foods in their diet: ancestral pteropodids and phyllostomids, as well as *Leptonycteris yerbabuenae*, two each in *Macroglossus*, *Anoura*, and *Tonatia*, and three in *Artibeus jamaicensis*. While the function of these lineage-specific bat paralogs remain unknown, their phylogenetic distribution warrants future exploration and functional analysis.

In summary, our results, grounded on the most comprehensive survey of bat genomes to date, suggest bats have evolved complex mechanisms of inflammasome regulation. These may have evolved to prevent uncontrolled inflammatory response against DAMPs byproducts of the high metabolic rate required for powered flight (Banerjee et al., 2017; Banerjee et al., 2020; Subudhi, Rapin & Misra, 2019; Xie et al., 2018), to better respond against intra-cellular pathogens such as viruses, or some combination of both. Regardless of the ecological origin of selection, compared to mammals such as humans or mice, bat genomes reveal systemwide immune evolution that prevents or dampens aggressive inflammatory responses. In contrast with these gene losses, we found significant expansions in gene families involved with glucose degradation, coinciding with the transition from a diet based mainly on insects to a high-glucose content diet that includes fruit and nectar.

By undertaking large-scale comparative genomic analyses encompassing many ecologically divergent lineages, the present study demonstrates the impact of genomics in non-model organisms. Such analyses allow elucidating the broad evolutionary mechanisms in a given clade, with potential for functional implications. Yet, heterogeneity in assembly quality continues to limit the scope of inference. Hence, the need to generate high quality genomes for future studies endures.

Acknowledgments

For support in long-read Oxford Nanopore sequencing, we thank Dr. Sara Goodwin from Cold Spring Harbor Laboratories. L.M.D. was supported, in part, by NSF-DEB 1838273, NSF-DGE 1633299, with S.J.R. by NSF-DEB 1442142, and with A.M.B. by NSF-IOS 2032063 and 2031906. D.A.R. and D.D.M.S. were supported, in part, by NSF-DEB 1838283, and NSF-IOS 2032006. A.P.C. was supported, in part, by NSF-IOS 2032011 and 2031926. T.M.L. was supported by NSF-PRFB 2010853. L.R.Y. was supported by NSF-IOS 2032073 and NSF-DBI 1812035. E.C.T. was supported in part by an Irish Research Council

Laureate Award IRCLA/2017/58. SCV was supported by a Max Planck Research Group awarded by the Max Planck Gesellschaft, a Human Frontiers Science Program Grant (RGP0058/2016) and a UKRI Future Leaders Fellowship (MR/T021985/1). The authors would like to thank Stony Brook Research Computing and Cyberinfrastructure, and the Institute for Advanced Computational Science at Stony Brook University for access to the high-performance SeaWulf computing system, which was made possible by a \$1.4M National Science Foundation grant (#1531492). The High-Performance Computing Center at Texas Tech University and The Scientific Computing Department at the Instituto de Ecología, Universidad Nacional Autónoma de México provided computational infrastructure and technical support throughout the work.

References

- Ahn, M., Anderson, D. E., Zhang, Q., Tan, C. W., Lim, B. L., Luko, K., ... Wang, L. F. (2019). Dampened NLRP3-mediated inflammation in bats and implications for a special viral reservoir host. *Nature Microbiology*, 4, 789-799. doi: 10.1038/s41564-019-0371-3
- Ahn, M., Cui, J., Irving, A. T., & Wang, L. F. (2016). Unique loss of the PYHIN gene family in bats amongst mammals: Implications for inflammasome sensing. *Scientific Reports*, 6 . doi: 10.1038/srep21722
- Amitai, O., Holtze, S., Barkan, S., Amichai, E., Korine, C., Pinshow, B., & Voigt, C. C. (2010). Fruit bats (Pteropodidae) fuel their metabolism rapidly and directly with exogenous sugars. *The Journal of Experimental Biology*, 213 (215), 2693-2699. doi: 10.1242/jeb.043505
- Arapović, J., Lenac, T., Antulov, R., Polić, B., Ruzsics, Z., Carayannopoulos, L. N., ... Jonjić, S. (2009). Differential Susceptibility of RAE-1 Isoforms to Mouse Cytomegalovirus. *Journal of Virology*, 83 (16), 8198-8207. doi: 10.1128/jvi.02549-08
- Akbar, A . N., & Gilroy, D. W. (2020). Aging immunity may exacerbate COVID-19. *Science*, 369 (6501), 256-257. doi.org/10.1126/science.abb0762
- Ashburner, M., Ball, C. A., Blake, J. A., Botstein, D., Butler, H., Cherry, J. M., ... Sherlock, G. (2000). Gene ontology: Tool for the unification of biology. *Nature Genetics*, 25, 25-29. doi: 10.1038/75556
- Austad, S. N., & Fischer, K. E. (1991). Mammalian aging, metabolism, and ecology: Evidence from the bats and marsupials. *Journals of Gerontology*, 46 (2),47-53. doi: 10.1093/geronj/46.2.B47
- Ayala-Berdon, J., & Schondube, J. E. (2011). A physiological perspective on nectar-feeding adaptation in phyllostomid bats. *Physiological and Biochemical Zoology* . 84, 458-466. doi: 10.1086/661541
- Babic, A. M., Wang, H. W., Lai, M. J., Daniels, T. G., Felbinger, T. W., Burger, P. C., ... Wagner, D. D. (2004). ICAM-1 and $\beta 2$ integrin deficiency impairs fat oxidation and insulin metabolism during fasting. *Molecular Medicine*, 10 (7-12), 72-79. doi: 10.2119/2004-00038.Wagner
- Baker, M. L., & Schountz, T. (2018). Mammalia: Chiroptera: Immunology of Bats. In E. Cooper (Eds.), *Springer International Publishing*(pp. 869-832). doi.org/10.1007/978-3-319-76768-0_23
- Banerjee, A., Baker, M. L., Kulcsar, K., Misra, V., Plowright, R., & Mossman, K. (2020). Novel Insights Into Immune Systems of Bats. *Frontiers in Immunology*, 11, 1-26. doi: 10.3389/fimmu.2020.00026
- Banerjee, A., Rapin, N., Bollinger, T., & Misra, V. (2017). Lack of inflammatory gene expression in bats: A unique role for a transcription repressor. *Scientific Reports* 7, 1-15. doi: 10.1038/s41598-017-01513-w
- Banerjee, M., Mehr, R., Belevsky, A., Spencer, J., & Dunn-Walters, D. K. (2002). Age- and tissue-specific differences in human germinal center B cell selection revealed by analysis of IgVH gene hypermutation and lineage trees. *European Journal of Immunology* , 32 (7), 1947-1957. doi: 10.1002/1521-4141(200207)32:7<1947::AID-IMMU1947>3.0.CO;2-1

- Basler, C. (2020). Innate Immunity in Bats. In Eugenia Corrales-Aguilar & M. Schwemmler (Eds.), *Bats and Viruses: Current Research and Future Trends* (pp. 119–134). Caister Academic Press. doi:10.21775/9781912530144.08
- Bateman, A. (2019). UniProt: A worldwide hub of protein knowledge. *Nucleic Acids Research*, 47 (D1), D506–D515. doi: 10.1093/nar/gky1049
- Bay, R. A., Rose, N. H., Logan, C. A., & Palumbi, S. R. (2017). Genomic models predict successful coral adaptation if future ocean warming rates are reduced. *Science Advances*, 3 (11). doi: 10.1126/sciadv.1701413
- Berg, J., Tymoczko, J., & Stryer, L. (2002). *Biochemistry* (5th ed.). New York: WH Freeman.
- Bernatchez, L., & Landry, C. (2003). MHC studies in nonmodel vertebrates: What have we learned about natural selection in 15 years? *Journal of Evolutionary Biology*, 16 (3), 363–377. doi: 10.1046/j.1420-9101.2003.00531.x
- Bhattacharya, S., Andorf, S., Gomes, L., Dunn, P., Schaefer, H., Pontius, J., ... Butte, A. J. (2014). ImmPort: Disseminating data to the public for the future of immunology. *Immunologic Research*, 58, 234–239. doi: 10.1007/s12026-014-8516-1
- Boni, M. F., Lemey, P., Jiang, X., Lam, T. T. Y., Perry, B. W., Castoe, T. A., ... Robertson, D. L. (2020). Evolutionary origins of the SARS-CoV-2 sarbecovirus lineage responsible for the COVID-19 pandemic. *Nature Microbiology*, 5, 1408–1417. doi: 10.1038/s41564-020-0771-4
- Brook, C. E., & Dobson, A. P. (2015). Bats as “special” reservoirs for emerging zoonotic pathogens. *Trends in Microbiology*, 23 (3), 172–180. doi: 10.1016/j.tim.2014.12.004
- Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., & Madden, T. L. (2009). BLAST+: Architecture and applications. *BMC Bioinformatics*, 10 (421). doi: 10.1186/1471-2105-10-421
- Carbon, S., Douglass, E., Dunn, N., Good, B., Harris, N. L., Lewis, S. E., ... Westerfield, M. (2019). The Gene Ontology Resource: 20 years and still GOing strong. *Nucleic Acids Research*, 47 (D1), D330–D338. doi: 10.1093/nar/gky1055
- Costantini, D., Lindecke, O., Petersons, G., & Voigt, C. C. (2019). Migratory flight imposes oxidative stress in bats. *Current Zoology*, 65 (2), 147–153. doi: 10.1093/cz/zoy039
- Czaplewski, N. J. (2010). Bats (Mammalia : Chiroptera) from Gran Barranca (early Miocene , Colhuehuapian), Chubut Province , Argentina. In Madden, R., Carlini, A. A., Guimar, V. M., & Kay, R. F.(Eds.), *The Paleontology of Gran Barranca. Evolution and environmental change through the middle Cenozoic of Patagonia*. (pp. 240–252). Cambridge University Press.
- Czaplewski, Nicolas J, Takai, M., Naeher, T. M., & Setoguchi, T. (2003). Additional bats from the middle miocene la venta fauna of Colombia. *Revista de la academia colombiana de ciencias*, 27 (103), 263–282.
- Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2011). ProtTest 3: Fast selection of best-fit models of protein evolution. *Bioinformatics (Oxford, England)*. 27 (8), 1164–1165. doi: 10.1093/bioinformatics/btr088
- Davalos, L. M., Velazco, P. M., & Rojas, D. (2020). Phylogenetics and Historical Biogeography. In *Phyllostomid bats: A Unique Mammalian Radiation* (pp. 87–103). Chicago, University of Chicago Press.
- Dávalos, L. M., Velazco, P. M., Warsi, O. M., Smits, P. D., & Simmons, N. B. (2014). Integrating incomplete fossils by isolating conflicting signal in saturated and non-independent morphological characters. *Systematic Biology*, 63 (4), 582–600. doi: 10.1093/sysbio/syu022
- Davies, K. T. J., Yohe, L. R., Almonte, T., Sanchez, M. K. R., Rengifo, E. M., Dumont E. R., ... Rossiter, S. J. (2020). Foraging shifts and visual preadaptation in ecologically diverse bats. *Molecular Ecology* , 28 (10), 1839–1859. <https://doi.org/10.1111/mec.15445>

- Delves, P. J., Martin, S. J., Burton, D. R., & Roitt, I. M. (2017). *Roitt's Essential Immunology* (13th edition). London, UK: Wiley Blackwell.
- Denton, J. F., Lugo-Martinez, J., Tucker, A. E., Schrider, D. R., Warren, W. C., & Hahn, M. W. (2014). Extensive Error in the Number of Genes Inferred from Draft Genome Assemblies. *PLoS Computational Biology*, 10 (12). doi: 10.1371/journal.pcbi.1003998
- Djelloul, M., Popa, N., Pelletier, F., Raguénez, G., & Boucraut, J. (2016). RAE-1 expression is induced during experimental autoimmune encephalomyelitis and is correlated with microglia cell proliferation. *Brain, Behavior, and Immunity*, 58, 209-217. doi: 10.1016/j.bbi.2016.07.147
- Dong, D., Lei, M., Hua, P., Pan, Y. H., Mu, S., Zheng, G., ... Zhang, S. (2017). The genomes of two bat species with long constant frequency echolocation calls. *Molecular Biology and Evolution*, 34 (1), 20-34. doi: 10.1093/molbev/msw231
- Dong, Z. M., Gutierrez-Ramos, J. C., Coxon, A., Mayadas, T. N., & Wagner, D. D. (1997). A new class of obesity genes encodes leukocyte adhesion receptors. *Proceedings of the National Academy of Sciences of the United States of America*, 94 (14), 7526-7530. doi: 10.1073/pnas.94.14.7526
- Drexler, J. F., Corman, V. M., Müller, M. A., Maganga, G. D., Vallo, P., Binger, T., ... Drosten, C. (2012). Bats host major mammalian paramyxoviruses. *Nature Communications*, 3 (796). doi: 10.1038/ncomms1796
- Dumont, E. R., Dávalos, L. M., Goldberg, A., Santana, S. E., Rex, K., & Voigt, C. C. (2012). Morphological innovation, diversification and invasion of a new adaptive zone. *Proceedings of the Royal Society B: Biological Sciences*, 279 (1734). doi: 10.1098/rspb.2011.2005
- Eckalbar, W. L., Schlebusch, S. A., Mason, M. K., Gill, Z., Parker, A. V., Booker, B. M., ... Ahituv, N. (2016). Transcriptomic and epigenomic characterization of the developing bat wing. *Nature Genetics*, 48, 528-536. doi: 10.1038/ng.3537
- Eiting, T. P., & Gunnell, G. F. (2009). Global completeness of the bat fossil record. *Journal of Mammalian Evolution*, 16, 151-173. doi: 10.1007/s10914-009-9118-x
- Emms, D. M., & Kelly, S. (2019). OrthoFinder: Phylogenetic orthology inference for comparative genomics. *Genome Biology*, 20, 238. doi: 10.1186/s13059-019-1832-y
- Feng, C. G., Zheng, L., Jankovic, D., Báfica, A., Cannons, J. L., Watford, W. T., ... Sher, A. (2008). The immunity-related GTPase Irgm1 promotes the expansion of activated CD4+ T cell populations by preventing interferon- γ -induced cell death. *Nature Immunology*, 9, 1279-1287. doi: 10.1038/ni.1653
- Fenton, M. B., & Simmons, N. B. (2015). *Bats: a world of science and mystery*. Chicago, IL: The University of Chicago Press.
- Ferber, I. A., Brocke, S., Taylor-Edwards, C., Ridgway, W., Dinisco, C., Steinman, L., ... Fathman, C. G. (1996). Mice with a disrupted IFN-gamma gene are susceptible to the induction of experimental autoimmune encephalomyelitis (EAE). *Journal of Immunology (Baltimore, Md. : 1950)*, 156 (1), 5-7.
- Fleischer, T., Gampe, J., Scheuerlein, A., & Kerth, G. (2017). Rare catastrophic events drive population dynamics in a bat species with negligible senescence. *Scientific reports*, 7, 7370. doi.org/10.1038/s41598-017-06392-9
- Foley, N. M., Hughes, G. M., Huang, Z., Clarke, M., Jebb, D., Whelan, C. V., ... Teeling, E. C. (2018). Growing old, yet staying young: The role of telomeres in bat's exceptional longevity. *Genetics*, 4. doi: 10.1126/sciadv.aao0926
- Genereux, D. P., Serres, A., Armstrong, J., Johnson, J., Marinescu, V. D., Murén, E., ... Karlsson, E. K. (2020). A comparative genomics multitool for scientific discovery and conservation. *Nature*, 587, 240-245. doi: 10.1038/s41586-020-2876-6

- Goebel, J., Promerová, M., Bonadonna, F., McCoy, K. D., Serbielle, C., Strandh, M., ... Fumagalli, L. (2017). 100 million years of multigene family evolution: Origin and evolution of the avian MHC class IIB. *BMC Genomics*, 18, 460. doi: 10.1186/s12864-017-3839-7
- Goldstein, T., Anthony, S. J., Gbakima, A., Bird, B. H., Bangura, J., Tremeau-Bravard, A., ... Mazet, J. A. K. (2018). The discovery of Bombali virus adds further support for bats as hosts of ebolaviruses. *Nature Microbiology*, 3, 1084-1089. doi: 10.1038/s41564-018-0227-2
- Gorbunova, V., Seluanov, A., & Kennedy, B. K. (2020). The World Goes Bats: Living Longer and Tolerating Viruses. *Cell Metabolism*, 32 (1), 31-43. doi: 10.1016/j.cmet.2020.06.013
- Grigat, J., Soruri, A., Forssmann, U., Riggert, J., & Zwirner, J. (2007). Chemoattraction of Macrophages, T Lymphocytes, and Mast Cells Is Evolutionarily Conserved within the Human α -Defensin Family. *The Journal of Immunology*, 179 (6), 3958-3965. doi: 10.4049/jimmunol.179.6.3958
- Gunnell, G. F., & Simmons, N. B. (2005). Fossil evidence and the origin of bats. *Journal of Mammalian Evolution*, 12, 209-246. doi: 10.1007/s10914-005-6945-2
- Gutiérrez-Guerrero, Y. T., Ibarra-Laclette, E., Martínez Del Río, C., Barrera-Redondo, J., Rebollar, E. A., Ortega, J., ... Eguarte, L. E. (2020). Genomic consequences of dietary diversification and parallel evolution due to nectarivory in leaf-nosed bats. *GigaScience*, 9 (6). doi:10.1093/gigascience/giaa059
- Hahn, M. W., De Bie, T., Stajich, J. E., Nguyen, C., & Cristianini, N. (2005). Estimating the tempo and mode of gene family evolution from comparative genomic data. *Genome Research*, 15 (8), 1153-1160. doi: 10.1101/gr.3567505
- Han, H. J., Wen, H. ling, Zhou, C. M., Chen, F. F., Luo, L. M., Liu, J. wei, & Yu, X. J. (2015). Bats as reservoirs of severe emerging infectious diseases. *Virus Research*, 505, 1-6. doi: 10.1016/j.virusres.2015.05.006
- Han, M. V., Thomas, G. W. C., Lugo-Martinez, J., & Hahn, M. W. (2013). Estimating gene gain and loss rates in the presence of error in genome assembly and annotation using CAFE 3. *Molecular Biology and Evolution*, 30 (8), 1987-1997. doi: 10.1093/molbev/mst100
- Harper, J. M., Salmon, A. B., Leiser, S. F., Galecki, A. T., & Miller, R. A. (2007). Skin-derived fibroblasts from long-lived species are resistant to some, but not all, lethal stresses and to the mitochondrial inhibitor rotenone. *Aging Cell*, 6 (1), 1-13. doi: 10.1111/j.1474-9726.2006.00255.x
- Hayden, S., Bekaert, M., Goodbla, A., Murphy, W. J., Dávalos, L. M., & Teeling, E. C. (2014). A cluster of olfactory receptor genes linked to frugivory in bats. *Molecular Biology and Evolution*. 31 (4), 917-927. doi: 10.1093/molbev/msu043
- Hayman, D. T. S. (2019). Bat tolerance to viral infections. *Nature Microbiology*, 4, 728-729. doi: 10.1038/s41564-019-0430-9
- Hayward, J. A., Tachedjian, M., Cui, J., Cheng, A. Z., Johnson, A., Baker, M. L., ... Tachedjian, G. (2018). Differential evolution of antiretroviral restriction factors in pteropid bats as revealed by APOBEC3 gene complexity. *Molecular Biology and Evolution*, 35 (7), 1626-1637. doi: 10.1093/molbev/msy048
- Healy, K., Guillerme, T., Finlay, S., Kane, A., Kelly, S. B. A., McClean, D., ... Cooper, N. (2014). Ecology and mode-of-life explain lifespan variation in birds and mammals. *Proceedings of the Royal Society B: Biological Sciences*, 281 (1784). doi: 10.1098/rspb.2014.0298
- Holt, C., & Yandell, M. (2011). MAKER2: An annotation pipeline and genome-database management tool for second-generation genome projects. *BMC Bioinformatics*, 12, 491. doi: 10.1186/1471-2105-12-491
- Hu, B., Zeng, L. P., Yang, X. Lou, Ge, X. Y., Zhang, W., Li, B., ... Shi, Z. L. (2017). Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus. *PLoS Pathogens*. doi: 10.1371/journal.ppat.1006698

- Huang, R., Grishagin, I., Wang, Y., Zhao, T., Greene, J., Obenauer, J. C., ... Austin, C. P. (2019). The NCATS BioPlanet – An integrated platform for exploring the universe of cellular signaling pathways for toxicology, systems biology, and chemical genomics. *Frontiers in Pharmacology*, 10, 445. doi: 10.3389/fphar.2019.00445
- Huang, Z., Whelan, C. V., Dechmann, D., & Teeling, E. C. (2020). Genetic variation between long-lived versus short-lived bats illuminates the molecular signatures of longevity. *Aging*, 12 (16), 15962-15977. doi: 10.18632/aging.103725
- Huang, Z., Whelan, C. V., Foley, N. M., Jebb, D., Touzalin, F., Petit, E. J., ... Teeling, E. C. (2019). Longitudinal comparative transcriptomics reveals unique mechanisms underlying extended healthspan in bats. *Nature Ecology and Evolution*, 3 (7), 1110-1120. doi: 10.1038/s41559-019-0913-3
- Huelsmann, M., Hecker, N., Springer, M. S., Gatesy, J., Sharma, V., & Hiller, M. (2019). Genes lost during the transition from land to water in cetaceans highlight genomic changes associated with aquatic adaptations. *Science Advances*, 5 (9), eaaw6671. doi: 10.1126/sciadv.aaw6671
- Hughes, G. M., Boston, E. S. M., Finarelli, J. A., Murphy, W. J., Higgins, D. G., & Teeling, E. C. (2018). The birth and death of olfactory receptor gene families in Mammalian niche adaptation. *Molecular Biology and Evolution*, 35 (6), 1390-1406. doi: 10.1093/molbev/msy028
- Janeway, C. A., & Travers, M. W. (2001). The major histocompatibility complex and its functions. *Immunobiology: The Immune System in Health and Disease* . Retrieved from <https://www.ncbi.nlm.nih.gov/books/NBK10757/>
- Jebb, D., Huang, Z., Pippel, M., Hughes, G. M., Lavrichenko, K., Devanna, P., ... Teeling, E. C. (2020). Six reference-quality genomes reveal evolution of bat adaptations. *Nature*, 538 (7817), 578-584. doi: 10.1038/s41586-020-2486-3
- Jones, K. E., Bininda-Emonds, O. R. P., & Gittleman, J. L. (2005). Bats, clocks, and rocks: diversification patterns in chiroptera. *Evolution; international journal of organic evolution*, 59 (10), 2243-2255. doi: 10.1554/04-635.1
- Kacprzyk, J., Hughes, G. M., Palsson-McDermott, E., Quinn, S. R., Puechmille, S. J., O'Neill, L. A. J. & Teeling, E. C. (2017). A potent anti-inflammatory response in bat macrophages may be linked to extended longevity and viral tolerance. *Acta Chiropterologica*. 19 (2), 219-228. doi: 10.3161/15081109ACC2017.19.2.001
- Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, 30 (4), 772-780. doi: 10.1093/molbev/mst010
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., ... Drummond, A. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics (Oxford, England)*, 28 (12), 1647-1649. doi:10.1093/bioinformatics/bts199
- Kelm, D.H., Simon, R., Kuhlow, D., Voigt, C. C., Ristow M. (2011). High activity enables life on a high-sugar diet: blood glucose regulation in nectar-feeding bats. *Proceedings of the Royal Society B: Biological Sciences*, 278 (1724), 3490-3496. doi.org/10.1098/rspb.2011.0465
- Kolmogorov, M., Yuan, J., Lin, Y., & Pevzner, P. A. (2019). Assembly of long, error-prone reads using repeat graphs. *Nature Biotechnology*, 37 (5), 540-546. doi: 10.1038/s41587-019-0072-8
- Korf, I. (2004). Gene finding in novel genomes. *BMC Bioinformatics*, 5, 59. doi: 10.1186/1471-2105-5-59
- Kosakovsky Pond, S. L., Frost, S. D. W., & Muse, S. V. (2005). HyPhy: Hypothesis testing using phylogenies. *Bioinformatics (Oxford, England)*, 21 (5), 676-679. doi: 10.1093/bioinformatics/bti079
- Lau, S. K. P., Luk, H. K. H., Wong, A. C. P., Li, K. S. M., Zhu, L., Zirong, H., ... Woo, P. C. Y. (2020). Possible Bat Origin of Severe Acute Respiratory Syndrome Coronavirus 2. *Emerging infectious diseases*, 26

(7), 1542-1547. doi: <https://dx.doi.org/10.3201/eid2607.200092>.

Lenartić, M., Jelenčić, V., Zafirova, B., Ožanić, M., Marečić, V., Jurković, S., ... Polić, B. (2017). NKG2D Promotes B1a Cell Development and Protection against Bacterial Infection. *The Journal of Immunology (Baltimore, Md. : 1950)*, 198 (4), 1531-1542. doi: 10.4049/jimmunol.1600461

Lewin, H. A., Robinson, G. E., Kress, W. J., Baker, W. J., Coddington, J., Crandall, K. A., ... Zhang, G. (2018). Earth BioGenome Project: Sequencing life for the future of life. *Proceedings of the National Academy of Sciences of the United States of America*, 115 (17), 4325-4333. doi: 10.1073/pnas.1720115115

Liberles, S. D., & Buck, L. B. (2006). A second class of chemosensory receptors in the olfactory epithelium. *Nature*, 442 (7103), 645-650. doi: 10.1038/nature05066

McHeyzer-Williams, M., Okitsu, S., Wang, N., & McHeyzer-Williams, L. (2012). Molecular programming of B cell memory. *Nature Reviews Immunology*, 12 (1), 24-34. doi: 10.1038/nri3128

Meakin, P. J., Morrison, V. L., Sneddon, C. C., Savinko, T., Uotila, L., Jalic, S. M., ... Fagerholm, S. C. (2015). Mice lacking beta2-integrin function remain glucose tolerant in spite of insulin resistance, neutrophil infiltration and inflammation. *PLoS ONE*, 10 (9), e0138872. doi: 10.1371/journal.pone.0138872

Memish, Z. A., Perlman, S., Van Kerkhove, M. D., & Zumla, A. (2020). Middle East respiratory syndrome. *Lancet (London, England)*, 395 (10229), 1063-1077. doi: 10.1016/S0140-6736(19)33221-0

Meng, F., Zhu, L., Huang, W., Irwin, D. M., & Zhang, S. (2016). Bats: Body mass index, forearm mass index, blood glucose levels and SLC2A2 genes for diabetes. *Scientific Reports*, 6, 29960. doi: 10.1038/srep29960

Merad, M., & Martin, J. C. (2020). Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages. *Nature Reviews Immunology*, 20 (6), 355-362. doi: 10.1038/s41577-020-0331-4

Mi, H., Muruganujan, A., Ebert, D., Huang, X., & Thomas, P. D. (2019). PANTHER version 14: More genomes, a new PANTHER GO-slim and improvements in enrichment analysis tools. *Nucleic Acids Research*, 47 (D1), D419-D426. doi: 10.1093/nar/gky1038

Miller, M. R., McMinn, R. J., Misra, V., Schountz, T., Müller, M. A., Kurth, A., & Munster, V. J. (2016). Broad and Temperature Independent Replication Potential of Filoviruses on Cells Derived from Old and New World Bat Species. *Journal of Infectious Diseases*, 214 (suppl 3), S297-S302. doi: 10.1093/infdis/jiw199

Minias, P., Pikus, E., Whittingham, L. A., & Dunn, P. O. (2019). Evolution of copy number at the MHC varies across the avian tree of life. *Genome Biology and Evolution*, 11 (1), 17-28. doi: 10.1093/gbe/evy253

Moratelli, R., & Calisher, C. H. (2015). Bats and zoonotic viruses: Can we confidently link bats with emerging deadly viruses? *Memorias Do Instituto Oswaldo Cruz*, 110 (1), 1-22. doi: 10.1590/0074-02760150048

Nagaoka, I., Niyonsaba, F., Tsutsumi-Ishii, Y., Tamura, H., & Hirata, M. (2008). Evaluation of the effect of human β -defensins on neutrophil apoptosis. *International Immunology* 20(4), 543-553. doi: 10.1093/intimm/dxn012

Ng, J. H. J., Tachedjian, M., Deakin, J., Wynne, J. W., Cui, J., Haring, V., ... Baker, M. L. (2016). Evolution and comparative analysis of the bat MHC-I region. *Scientific reports*, 6, 21256. doi: 10.1038/srep21256

Niyonsaba, F., Ushio, H., Hara, M., Yokoi, H., Tominaga, M., Takamori, K., ... Okumura, K. (2010). Antimicrobial Peptides Human β -Defensins and Cathelicidin LL-37 Induce the Secretion of a Pruritogenic Cytokine IL-31 by Human Mast Cells. *The Journal of Immunology (Baltimore, Md. : 1950)*, 184 (7), 3526-3534. doi: 10.4049/jimmunol.0900712

Nogueira, R. M. R., Lima, I. P., Garbino, G. S. T., Moratelli, R., Tavares, V. C., Gregorin, R., & Peracchi, A. L. (2018). No Updated checklist of Brazilian bats: version 2018. Comitê da Lista de Morcegos do Brasil-CLMB. Retrieved from Sociedade Brasileira para o Estudo de Quirópteros Retrieved from <https://www.sbeq.net/lista-de-especies>

- Nowak, M. D., Birkeland, S., Mandáková, T., Roy Choudhury, R., Guo, X., Gustafsson, A. L. S., ... Brochmann, C. (2020). The genome of *Draba nivalis* shows signatures of adaptation to the extreme environmental stresses of the Arctic. *Molecular Ecology Resources* . doi: 10.1111/1755-0998.13280
- Olival, K. J., Hosseini, P. R., Zambrana-Torrel, C., Ross, N., Bogich, T. L., & Daszak, P. (2017). Host and viral traits predict zoonotic spillover from mammals. *Nature*, 546(7660), 646–650 . doi: 10.1038/nature22975
- Papenfuss, A. T., Baker, M. L., Feng, Z. P., Tachedjian, M., Cramer, G., Cowled, C., ... Wang, L. F. (2012). The immune gene repertoire of an important viral reservoir, the Australian black flying fox. *BMC Genomics* , 13 (1). doi: 10.1186/1471-2164-13-261
- Papkou, A., Guzella, T., Yang, W., Koepper, S., Pees, B., Schalkowski, R., ... Schulenburg, H. (2019). The genomic basis of red queen dynamics during rapid reciprocal host–pathogen coevolution. *Proceedings of the National Academy of Sciences of the United States of America*, 116 (3), 923–928.. doi: 10.1073/pnas.1810402116
- Parker, J., Tsagkogeorga, G., Cotton, J. A., Liu, Y., Provero, P., Stupka, E., & Rossiter, S. J. (2013). Genome-wide signatures of convergent evolution in echolocating mammals. *Nature*, 502 (7470), 228–231. doi: 10.1038/nature12511
- Parthasarathy, R., Parthasarathy, L., & Vadnal, R. (1997). Brain inositol monophosphatase identified as a galactose 1-phosphatase. *Brain Research*, 778 (1), 99–106. doi: 10.1016/S0006-8993(97)01042-1
- Pavlovich, S. S., Lovett, S. P., Koroleva, G., Guito, J. C., Arnold, C. E., Nagle, E. R., ... Palacios, G. (2018). The Egyptian Rousette Genome Reveals Unexpected Features of Bat Antiviral Immunity. *Cell*, 173 (5), 1098–1110.e18. doi: 10.1016/j.cell.2018.03.070
- Pickering, A. M., Lehr, M., Kohler, W. J., Han, M. L., & Miller, R. A. (2014). Fibroblasts from longer-lived species of primates, rodents, bats, carnivores, and birds resist protein damage. *Journals of Gerontology - Series A Biological Sciences and Medical Sciences*, 70 (7), 791–799. doi: 10.1093/gerona/glu115
- Pierce, C. A., Preston-Hurlburt, P., Dai, Y., Aschner, C. B., Cheshenko, N., Galen, B., ... Herold, B. C. (2020). Immune responses to SARS-CoV-2 infection in hospitalized pediatric and adult patients. *Science Translational Medicine* , 12 (564), eabd5487. doi: 10.1126/scitranslmed.abe8120
- Podlutzky, A. J., Khritankov, A. M., Ovodov, N. D., & Austad, S. N. (2005). A new field record for bat longevity. *The journals of gerontology*, 60 (11), 1366–1368. doi.org/10.1093/gerona/60.11.1366
- Pomaznoy, M., Ha, B., & Peters, B. (2018). GONet: A tool for interactive Gene Ontology analysis. *BMC Bioinformatics*, 19 (1), 470. doi: 10.1186/s12859-018-2533-3
- Potter, J., Davies, K., Yohe, L. R., Struebig, M., Warren, K., Tsagkogeorga, G., ... Rossiter, S. J. (in press). Dietary diversification and specialisation in New World bats facilitated by early molecular evolution. *Molecular Biology and Evolution* .
- Rafikov, R., McBride, M. L., Zemskova, M., Kurdyukov, S., McClain, N., Niihori, M., ... Rafikova, O. (2019). Inositol monophosphatase 1 as a novel interacting partner of RAGE in pulmonary hypertension. *American Journal of Physiology - Lung Cellular and Molecular Physiology*, 316 (3), L428–L444. doi: 10.1152/ajplung.00393.2018
- Rapista, A., Ding, J., Benito, B., Lo, Y. T., Neiditch, M. B., Lu, W., & Chang, T. L. (2011). Human defensins 5 and 6 enhance HIV-1 infectivity through promoting HIV attachment. *Retrovirology*, 8, 45. doi: 10.1186/1742-4690-8-45
- Ravel, A., Adaci, M., Bensalah, M., Charruault, A.-L., Essid, E. M., AMmar, H. K., ... Marivaux, L. (2016). Origine et radiation initiale des chauves-souris modernes : nouvelles découvertes dans l'Éocène d'Afrique du Nord. *Geodiversitas*, 38 (3), 355–434. doi: 10.5252/g2016n3a3
- Reddy, S. T., Ge, X., Miklos, A. E., Hughes, R. A., Kang, S. H., Hoi, K. H., ... Georgiou, G. (2010). Monoclonal antibodies isolated without screening by analyzing the variable-gene repertoire of plasma cells. *Nature*

Biotechnology , 28 (9), 965–969. doi: 10.1038/nbt.1673

Rhie, A., McCarthy, S. A., Fedrigo, O., Damas, J., Formenti, G., London, S. E., ... Friedrich, S. R. (2020). Towards complete and error-free genome assemblies of all vertebrate species. *BioRxiv*, 1–56. doi:10.1101/2020.05.22.110833

Rojas, D., Ramos Pereira, M. J., Fonseca, C., & Dávalos, L. M. (2018). Eating down the food chain: generalism is not an evolutionary dead end for herbivores. *Ecology Letters*, 21 (3), 402–410. doi: 10.1111/ele.12911

Salmon, A. B., Leonard, S., Masamsetti, V., Pierce, A., Podlutzky, A. J., Podlutzkaya, N., ... Chaudhuri, A. R. (2009). The long lifespan of two bat species is correlated with resistance to protein oxidation and enhanced protein homeostasis. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, 23 (7), 2317–2326. doi: 10.1096/fj.08-122523

Santos, P. S. C., Courtiol, A., Heide, A. J., Höner, O. P., Heckmann, I. Nagy, ... Sommer S. (2016). MHC-dependent mate choice is linked to a trace-amine-associated receptor gene in a mammal. *Scientific Reports*, 6, 38490. doi.org/10.1038/srep38490

Scheben, A., Ramos, O. M., Kramer, M., Goodwin, S., Oppenheim, S., Becker, D. J., ... McCombie, R. (2020). Unraveling molecular mechanisms of immunity and cancer-resistance using the genomes of the Neotropical bats *Artibeus jamaicensis* and *Pteronotus mesoamericanus*. *BioRxiv* . doi.org/10.1101/2020.09.09.290502

Schountz, T., Baker, M. L., Butler, J., & Munster, V. (2017). Immunological control of viral infections in bats and the emergence of viruses highly pathogenic to humans. *Frontiers in Immunology*, 8 , 1098. doi: 10.3389/fimmu.2017.01098

Schroder, K., Hertzog, P. J., Ravasi, T., & Hume, D. A. (2004). Interferon- γ : an overview of signals, mechanisms and functions. *Journal of Leukocyte Biology*, 75 (2), 163–189. doi: 10.1189/jlb.0603252

Seim, I., Fang, X., Xiong, Z., Lobanov, A. V., Huang, Z., Ma, S., ... Gladyshev, V. N. (2013). Genome analysis reveals insights into physiology and longevity of the Brandt's bat *Myotis brandtii*. *Nature Communications*, 4 , 2212. doi: 10.1038/ncomms3212

Semple, F., & Dorin, J. R. (2012). β -Defensins: Multifunctional modulators of infection, inflammation and more? *Journal of Innate Immunity*, 4 (4), 337–348. doi: 10.1159/000336619

Sharma, V., Hecker, N., Roscito, J. G., Foerster, L., Langer, B. E. & Hiller, M. (2018) A genomics approach reveals insights into the importance of gene losses for mammalian adaptations. *Nature communications*, 9 (1), 1215. doi: 10.1038/s41467-018-03667-1

Sharma, V., Lehmann, T., Stuckas, H., Funke, L., & Hiller, M. (2018). Loss of RXFP2 and INSL3 genes in *Afrotheria* shows that testicular descent is the ancestral condition in placental mammals. *PLoS Biology*, 16 (6), e2005293. doi: 10.1371/journal.pbio.2005293

Shen, B., Han, X., Jones, G., Rossiter, S. J., & Zhang, S. (2013). Adaptive Evolution of the Myo6 Gene in Old World Fruit Bats (Family: Pteropodidae). *PLoS ONE*, 8 (4), e62307. doi: 10.1371/journal.pone.0062307

Shen, B., Han, X., Zhang, J., Rossiter, S. J., & Zhang, S. (2012). Adaptive evolution in the glucose transporter 4 gene *Slc2a4* in old world fruit bats (family: Pteropodidae). *PLoS ONE*, 7 (4), e33197. doi: 10.1371/journal.pone.0033197

Shultz, A. J., & Sackton, T. B. (2019). Immune genes are hotspots of shared positive selection across birds and mammals. *eLife*, 8, e41815. doi: 10.7554/eLife.41815

Simmons, N. B., Seymour, K. L., Habersetzer, J., & Gunnell, G. F. (2008). Primitive Early Eocene bat from Wyoming and the evolution of flight and echolocation. *Nature*, 451 (7180), 818–821. doi: 10.1038/nature06549

- Sironi, M., Cagliani, R., Forni, D., & Clerici, M. (2015). Evolutionary insights into host-pathogen interactions from mammalian sequence data. *Nature Reviews Genetics*, 16 (4), 224-236. doi: 10.1038/nrg3905
- Smit, A. F. A., Hubley, R. M., & Green, P. (n.d.). RepeatMasker. Retrieved from: <http://Repeatmasker.Org>.
- Smith, M. D., Wertheim, J. O., Weaver, S., Murrell, B., Scheffler, K., & Kosakovsky Pond, S. L. (2015). Less is more: An adaptive branch-site random effects model for efficient detection of episodic diversifying selection. *Molecular Biology and Evolution*, 32 (5), 1342-1353. doi: 10.1093/molbev/msv022
- Stamatakis, A. (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics (Oxford, England)*, 30 (9), 1312-1313. doi: 10.1093/bioinformatics/btu033
- Stanke, M., & Waack, S. (2003). Gene prediction with a hidden Markov model and a new intron submodel. *Bioinformatics (Oxford, England)*, 19, 215-225. doi: 10.1093/bioinformatics/btg1080
- Stolberg, V. R., Martin, B., Mancuso, P., Olszewski, M. A., Freeman, C. M., Curtis, J. L., & Chensue, S. W. (2014). Role of CC chemokine receptor 4 in natural killer cell activation during acute cigarette smoke exposure. *The American Journal of Pathology*, 184 (2), 454-463. doi: 10.1016/j.ajpath.2013.10.017
- Storch, G., Sigé, B., & Habersetzer, J. (2002). Tachypteran franzeni n. gen., n. sp., earliest emballonurid bat from the Middle Eocene of Messel (Mammalia, Chiroptera). *Paläontologische Zeitschrift*, 79 (189-199). doi: 10.1007/bf02989856
- Subudhi, A., Rapin, N., & Misra, V. (2019). Immune system modulation and viral persistence in bats: Understanding viral spillover. *Viruses*, 11 (2), 192 .doi: 10.3390/v11020192
- Suyama, M., Torrents, D., & Bork, P. (2006). PAL2NAL: Robust conversion of protein sequence alignments into the corresponding codon alignments. *Nucleic Acids Research*, 34, 609-612. doi: 10.1093/nar/gkl315
- Takada, A., Yoshida, S., Kajikawa, M., Miyatake, Y., Tomaru, U., Sakai, M., ... Kasahara, M. (2008). Two Novel NKG2D Ligands of the Mouse H60 Family with Differential Expression Patterns and Binding Affinities to NKG2D. *Journal of immunology (Baltimore, Md. : 1950)*, 180 (3), 1678-1685. doi: 10.4049/jimmunol.180.3.1678
- Teeling, E. C., Vernes, S. C., Dávalos, L. M., Ray, D. A., Gilbert, M. T. P., & Myers, E. (2018). Bat Biology, Genomes, and the Bat1K Project: To Generate Chromosome-Level Genomes for All Living Bat Species. *Annual Review of Animal Biosciences*, 6, 23-46. doi: 10.1146/annurev-animal-022516-022811
- Towner, J. S., Pourrut, X., Albariño, C. G., Nkogue, C. N., Bird, B. H., Grard, G., ... Leroy, E. M. (2007). Marburg virus infection detected in a common African bat. *PLoS ONE*, 2 (8), e764. doi: 10.1371/journal.pone.0000764
- Tsagkogeorga, G., Müller, S., Dessimoz, C., & Rossiter, S. J. (2017). Comparative genomics reveals contraction in olfactory receptor genes in bats. *Scientific Reports*, 7 (1), 259. doi: 10.1038/s41598-017-00132-9
- Van Oosterhout, C. (2009). A new theory of MHC evolution: Beyond selection on the immune genes. *Proceedings of the Royal Society B: Biological Sciences*, 276 (1657), 657-665. doi: 10.1098/rspb.2008.1299
- Vosseberg, J., Hoof, J. E., Marcet-Houbé, M., Vlimmeren, A., Wijk, T. G., & Snel B. (2020). Timing the origin of eukaryotic cellular complexity with ancient duplications. *Nature Ecology & Evolution*, 5, 92-100. doi:10.1038/s41559-020-01320-z
- Wang, K., Tian, S., Galindo-González, J., Dávalos, L. M., Zhang, Y., & Zhao, H. (2020). Molecular adaptation and convergent evolution of frugivory in Old World and neotropical fruit bats. *Molecular Ecology*, 29 (22), 4366-4381. doi: 10.1111/mec.15542
- Wang, P., Luo, Y., Huang, J., Gao, S., Zhu, G., Dang, Z., ... Chen Y. (2020). The genome evolution and domestication of tropical fruit mango. *Genome Biology*, 21 (1), 60. doi:10.1186/s13059-020-01959-8

- Welch, K. C., Herrera, M. L. G., & Suarez, R. K. (2008). Dietary sugar as a direct fuel for flight in the nectarivorous bat *Glossophaga soricina*. *Journal of Experimental Biology*, 211, 310-316. doi: 10.1242/jeb.012252
- Wiens, M. E., Wilson, S. S., Lucero, C. M., & Smith, J. G. (2014). Defensins and Viral Infection: Dispelling Common Misconceptions. *PLoS Pathogens*, 10 (7), e1004186. doi: 10.1371/journal.ppat.1004186
- Wilkinson, G. S., & Adams, D. M. (2019). Recurrent evolution of extreme longevity in bats. *Biology Letters*, 15 (4), 20180860. doi: 10.1098/rsbl.2018.0860
- Wortham, B. W., Eppert, B. L., Motz, G. T., Flury, J. L., Orozco-Levi, M., Hoebe, K., ... Borchers, M. T. (2012). NKG2D Mediates NK Cell Hyperresponsiveness and Influenza-Induced Pathologies in a Mouse Model of Chronic Obstructive Pulmonary Disease. *Journal of immunology (Baltimore, Md. : 1950)*, 188 (9), 4468–4475. doi: 10.4049/jimmunol.1102643
- Xie, J., Li, Y., Shen, X., Goh, G., Zhu, Y., Cui, J., ... Zhou, P. (2018). Dampened STING-Dependent Interferon Activation in Bats. *Cell Host and Microbe*, 23 (3), 294-301. doi: 10.1016/j.chom.2018.01.006
- Xu, D., & Lu, W. (2020). Defensins: A Double-Edged Sword in Host Immunity. *Frontiers in Immunology*, 11 (764), 1–9. doi: 10.3389/fimmu.2020.00764
- Yang, Z. (2007). PAML 4: Phylogenetic analysis by maximum likelihood. *Molecular Biology and Evolution*, 24 (8), 1586–1591. doi: 10.1093/molbev/msm088
- Yang, Z., Wong, W. S. W., & Nielsen, R. (2005). Bayes empirical Bayes inference of amino acid sites under positive selection. *Molecular Biology and Evolution*, 22 (4), 1107-1118. doi: 10.1093/molbev/msi097
- Yates, A. D., Achuthan P., Akanni, W., Allen, J., Alvares, J. J., ... Flicek, P. (2020). Ensembl 2020. *Nucleic Acids Research*, 48 (D1), D682-D688. doi:10.1093/nar/gkz966
- Yohe, L. R., Devanna, P. Davies, K. T. J., Potter, J. H. T., Rossiter, S. J., Teeling, E. C., ... , Dávalos L. M. (2019). Tissue Collection of Bats for –Omics Analyses and Primary Cell Culture. *Journal of Visualized Experiments:JoVE*, (152). doi: 10.3791/59505
- You, X., Bian, C., Zan, Q., Xu, X., Liu, X., Chen, J., ... Shi, Q. (2014). Mudskipper genomes provide insights into the terrestrial adaptation of amphibious fishes. *Nature Communications*, 5, 5594. doi: 10.1038/ncomms6594
- Yu, G., Smith, D. K., Zhu, H., Guan, Y., & Lam, T. T. Y. (2017). ggtree: an r package for visualization and annotation of phylogenetic trees with their covariates and other associated data. *Methods in Ecology and Evolution*, 8 (1), 28-36. doi: 10.1111/2041-210X.12628
- Zafirova, B., Mandarić, S., Antulov, R., Krmpotić, A., Jonsson, H., Yokoyama, W. M., ... Polić, B. (2009). Altered NK Cell Development and Enhanced NK Cell-Mediated Resistance to Mouse Cytomegalovirus in NKG2D-Deficient Mice. *Immunity* 31 (2), 270-282. doi: 10.1016/j.immuni.2009.06.017
- Zepeda Mendoza, M. L., Xiong, Z., Escalera-Zamudio, M., Runge, A. K., Thézé, J., Streicker, D., ... Gilbert, M. P. T. (2018). Hologenomic adaptations underlying the evolution of sanguivory in the common vampire bat. *Nature Ecology and Evolution*, 2 (4), 659-668. doi: 10.1038/s41559-018-0476-8
- Zhang, G., Cowled, C., Shi, Z., Huang, Z., Bishop-Lilly, K. A., Fang, X., ... Wang, J. (2013). Comparative analysis of bat genomes provides insight into the evolution of flight and immunity. *Science*, 339 (6118), 456-460. doi: 10.1126/science.1230835
- Zhang, J., Nielsen, R., & Yang, Z. (2005). Evaluation of an improved branch-site likelihood method for detecting positive selection at the molecular level. *Molecular Biology and Evolution*, 22 (12), 2472-2479. doi: 10.1093/molbev/msi237
- Zheng, D., Liwinski, T., & Elinav, E. (2020). Inflammasome activation and regulation: toward a better understanding of complex mechanisms. *Cell Discovery*, 6, 36. doi: 10.1038/s41421-020-0167-x

- Zhi, L., Mans, J., Paskow, M. J., Brown, P. H., Schuck, P., Jonjić, S., ... Margulies, D. H. (2010). Direct interaction of the mouse cytomegalovirus m152/gp40 immunoevasin with RAE-1 isoforms. *Biochemistry*, 49 (11), 2443-2453. doi: 10.1021/bi902130j
- Zhou, P. (2020). Immune (Adaptive) Response in Bats. In Corrales-Aguilar, E & Schwemmler, M. (Eds.), *Bats and Viruses: Current Research and Future Trends* (pp. 135–148). Caister Academic Press. doi: 10.21775/9781912530144.09
- Zhou, X., Dou, Q., Fan, G., Zhang, Q., Sanderford, M., Kaya, A., ... Gladyshev, V. N. (2020). Beaver and Naked Mole Rat Genomes Reveal Common Paths to Longevity. *Cell Reports*, 32 (4), 107949. doi: 10.1016/j.celrep.2020.107949
- Zimin, A. V., Marçais, G., Puiu, D., Roberts, M., Salzberg, S. L., & Yorke, J. A. (2013). The MaSuRCA genome assembler. *Bioinformatics (Oxford, England)*, 29 (21), 2669-2677. doi: 10.1093/bioinformatics/btt476

Declarations

The authors claim no conflicts of interest.

Data Accessibility

-Final genome assembly is currently under submission process to Genbank, this will be completed prior publication.

-Scripts for genome assembly, gene family and selection test are deposited in Dryad repository <https://doi.org/10.5061/dryad.59zw3r265>. However, submission is on hold until this manuscript has a doi assigned.

Author Contributions

LMD, DAR, DDMS conceived of the study; DAR, JHP, LRY, SJR and HZ collected samples; DAR, DDMS, ECT, GM, JHP, KTJD, LMD, LRY, PD, SJR, SV, YTGG and ZH generated data; AMB, APC, DDMS, GH, LMD, TML, YTGG and ZH analyzed data guided, in part, by DAR and FGH; APC, DAR, DDMS, LMD and TML wrote the manuscript. All authors reviewed the manuscript prior to submission.

Tables

Table 1 . Branch-site codeml results for all species on single-copy immune system genes. FDR, false discovery rate; LR, likelihood ratio; P , nominal P -value.

Symbol	Name	Category
Bbc3	BCL2 Binding Component 3	Inflammatory response
BPIFB5	BPI fold containing family B member 4	Antimicrobials
CCL1	C-C motif chemokine 1	Chemokines/Cytokines/Anti
CD3E	CD3e molecule	TCR signaling Pathway
CD79B	CD79b molecule	BCR Signaling Pathway
CD86	CD86 molecule	Antimicrobials
CSF2	colony stimulating factor 2	Cytokines
CXCL13	C-X-C motif chemokine 13	Chemokines/Cytokines/Anti
DEFB129 +	Beta-defensin 129	Antimicrobials
DEFB133	defensin beta 133	Antimicrobials

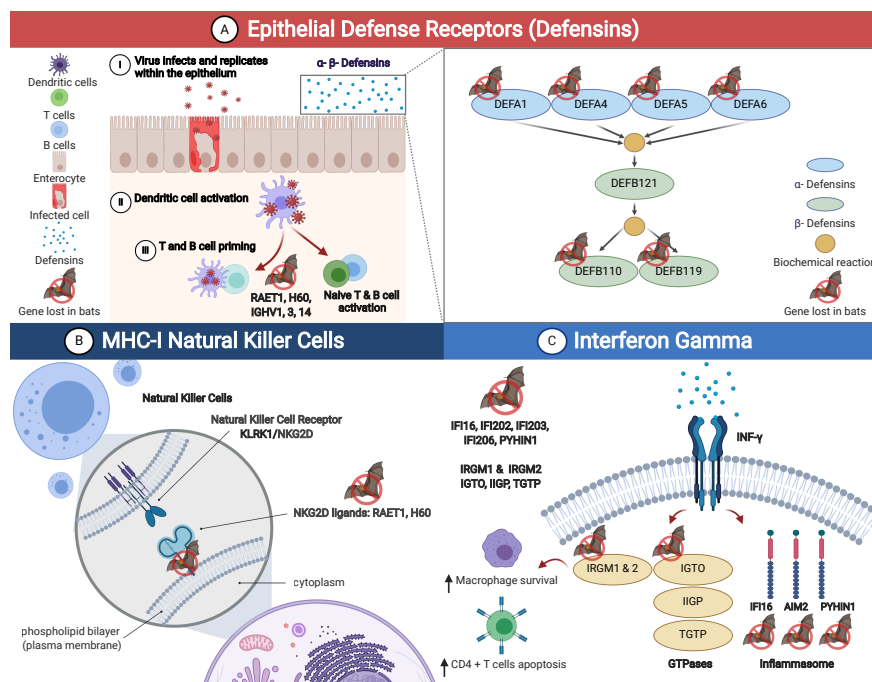


Figure 2 . Graphical summary of the cellular location and biological process categorization for genes involved in the inflammasome activation pathway found to be missing across all bats. A) Gene loss of specific epithelial α and β defensins. B) Gene losses of NKG2D ligands RAET1 and H60, involved in recruiting NK cells and IFN- γ stimulation. C) Loss of the PYRIN and HIN domain (PYHIN) gene family (AIM2, IFI16, PYHIN1), along with the IFN- γ inducible related GTPase genes (IRGM1, IRGM2, IGTO, IIGP, TGTP2); loss of IRGM1 and 2 results in increase macrophage survival and CD4+ T cells apoptosis.

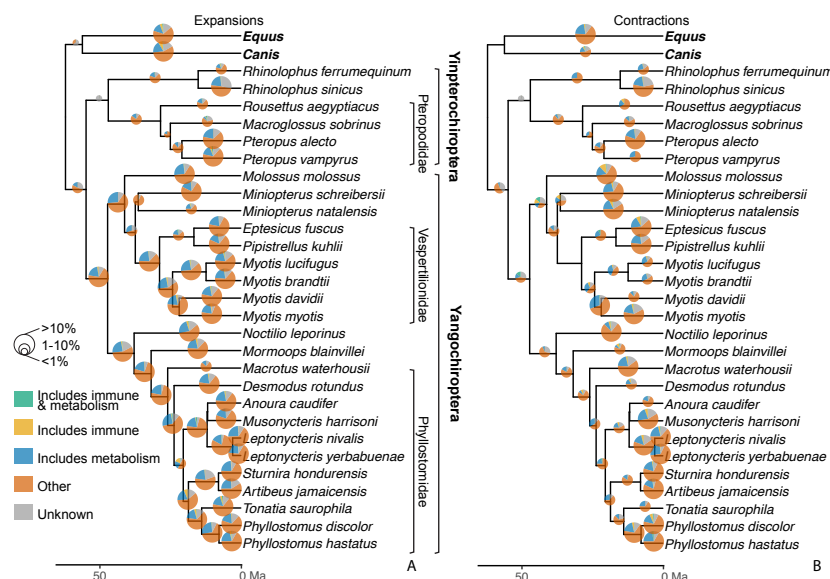


Figure 3 . Gene ontology categories, phylogenetic locations, and relative size of significant gene family expansions (A) and contractions (B) inferred using CAFE. Outgroups are shown in **bold** , “other” category

comprises mostly Panther cellular processes, and GOnet response to stress and autophagy. Pie sizes are relative to a maximum of 594 expansions and 579 contractions.