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Bat Biology, Genomes, and the Bat1K Project: To Generate Chromosome-Level Genomes for all Living Bat Species

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Abstract

Bats are unique among mammals, possessing some of the rarest mammalian adaptations, including true self-powered flight, laryngeal echolocation, exceptional longevity, unique immunity, contracted genomes, and vocal learning. They provide key ecosystem services, pollinating tropical plants, dispersing seeds, and controlling insect pest populations, thus driving healthy ecosystems. They account for more than 20% of all living mammalian diversity, and their crown-group evolutionary history dates back to the Eocene. Despite their great numbers and diversity, many species are threatened

and endangered. Here we announce Bat1K, an initiative to sequence the genomes of all living bat species ($n \sim 1{,}300$) to chromosome-level assembly. The Bat1K genome consortium unites bat biologists (>132 members as of writing), computational scientists, conservation organizations, genome technologists, and any interested individuals committed to a better understanding of the genetic and evolutionary mechanisms that underlie the unique adaptations of bats. Our aim is to catalog the unique genetic diversity present in all living bats to better understand the molecular basis of their unique adaptations; uncover their evolutionary history; link genotype with phenotype; and ultimately better understand, promote, and conserve bats. Here we review the unique adaptations of bats and highlight how chromosome-level genome assemblies can uncover the molecular basis of these traits. We present a novel sequencing and assembly strategy and review the striking societal and scientific benefits that will result from the Bat1K initiative.

INTRODUCTION

Of all living mammals, bats are considered the most extraordinary given their unique and peculiar adaptations. From the largest golden-capped fruitbat (*Acerodon jubatus*), with a wingspan of ~1.5 m and a weight of ~1 kg, to the smallest, ~2-g echolocating bumblebee bat (*Craseonycteris thonglongyai*), the huge diversity and extraordinary adaptive radiations in bat form and function have both fascinated and terrified people for centuries (1–3). Bats account for ~20% of all living mammals (>1,300 species) and are found throughout the globe, absent only from the extreme polar regions. They are the only mammals that can truly fly and likewise have uniquely evolved laryngeal echolocation, or biosonar, which enables them to orient in complete darkness using sound alone (4–5). Using these and other traits, they have evolved to thrive in diverse ecological niches and can feed on insects, small mammals, fish, blood, nectar, fruit, and pollen (5). They perform key ecosystem services, pollinating crop species in the tropics (e.g., bats pollinate the flowers of agave, making possible the distillation of tequila), dispersing seeds, and feeding on crop pests throughout their range (6–9). Without bats, it is estimated that the United States would spend more than $3 billion a year on pesticides alone (10). Bats are suspected reservoirs for some of the deadliest viral diseases [e.g., Ebola, SARS (severe acute respiratory syndrome), rabies, and MERS (Middle East respiratory syndrome coronavirus); 11–14], but they appear to be asymptomatic and survive these infections. This suggests that bats have evolved unique immune systems, and potentially the solution to better tolerate these pathogens may lie in uncovering how bats limit their immunopathology upon infection (15, 16). Bats also exhibit extraordinary longevity—they can live up to 10 times longer than expected given their small body size and high metabolic rate (17). Only 19 mammal species live proportionately longer than humans given their body size, and 18 are bats (17), with Brandt's bat (*Myotis brandtii*) holding the reported record for bat longevity [>41 years, ~7 g (18)]. Bats show few signs of senescence and low to negligible rates of cancer (11), suggesting they have also evolved unique mechanisms to extend their health spans, rendering them excellent models to study extended mammalian longevity and ageing (17). Bats face a variety of global threats that threaten populations with regional or global extinction. The IUCN (International Union for Conservation of Nature) Red List currently classifies 77 bat species as Critically Endangered or Endangered and a further 184 as Vulnerable or Near Threatened due to significant population declines from conservation threats. Lack of knowledge about bat species hampers our ability to assess population stability in many cases, and 222 bat species are considered

Data Deficient by the IUCN, meaning that their status cannot yet be determined, and 105 newly identified species are not yet listed (**http://www.batcon.org**).

The evolutionary history of bats has stimulated some of the most passionate debates in science, some spanning decades, from the initial disbelief among the scientific community when Spallanzani discovered in 1794 that bats were using sound to orient in darkness (19–21); to heated debates regarding whether the order was monophyletic and hence questioning if flight had a single origin in mammals (e.g., the flying primate hypothesis; 22); to current debates over the convergent evolution of laryngeal echolocation in bats and potential loss in the Old World fruit bats (for review, see 23, 24). Molecular phylogenetic analyses have reclassified their position within the mammalian tree (25–27), refuting their position within Archonta (including Primates, Scandentia, and Dermoptera) and placing them within Laurasiatheria (including Carnivora, Perissodactyla, Eulipotyphla, Pholidota, and Cetartiodactyla); and reclassified familial and interordinal relationships (**Figure 1**) (5, 23). Despite these advances, however, the sister taxon to bats remains unresolved (for review, see 23). As a mammalian order, bats have an impoverished fossil record, with an estimated >70% of fossil data missing (5, 28). There are astounding Lagerstätten Eocene fossils, but there is limited fossil representation thereafter until the Pliocene (29), making it difficult to reconstruct bat evolutionary history from fossils alone. Solving these outstanding evolutionary questions is difficult, as convergent homoplastic characters, incomplete lineage sorting, and a fragmented fossil record have limited phylogenetic reconstruction and obscured our current understanding of the evolution and basis of the unique adaptations in bats.

Animal genomes are increasingly revealing the genetic basis of environmental niche specialization and adaptation (30–33), and studying the molecular mechanisms responsible for this diversity has allowed some of the greatest insights into the functioning and evolution of our own genome (32, 34, 35). Some of the most important challenges facing humanity into the next century are biological. These include improving the well-being of our large and rapidly ageing human populations (36), preventing the spread of emergent infectious diseases (37), maintaining agricultural productivity (10), and restoring natural ecosystems worldwide (38). These challenges will require a range of approaches to overcome them, starting with understanding the intrinsic mechanisms that make us vulnerable to disease and the ecological relationships underlying ecosystem maintenance and resilience. To date, insights into these various health-related challenges have primarily come from model organisms such as the fruit fly (*Drosophila melanogaster*) or the house mouse (*Mus musculus*). Having been optimized to be reared and studied in labs, these organisms reproduce in great numbers and live short lives, and thus are ideal experimental organisms. However, insights gained from the behavior and responses of short-lived organisms do not always translate to those that live longer, such as humans (17, 39, 40). Studying bats will enable us to address all of these challenges, as many of their biological features mirror humans and their ecological roles both contribute to and prevent the spread of infectious diseases, and structure functional ecosystems today and into the future.

We announce Bat1K, a global effort to sequence and annotate chromosome-level genome assemblies of all living bat species. Prioritization of bat genomes is not just desirable but indispensable to confront the many challenges to human well-being, ecosystem function, and biodiversity conservation we now face.

Central to the success of Bat1K is wide involvement from bat researchers across diverse research areas. This article aims to provide information to the scientific community about the Bat1K effort; to encourage participation; to set standards for tissue collection and vouchering, assembly quality, and data release; and to outline the major research endeavors that we anticipate will benefit from Bat1K.

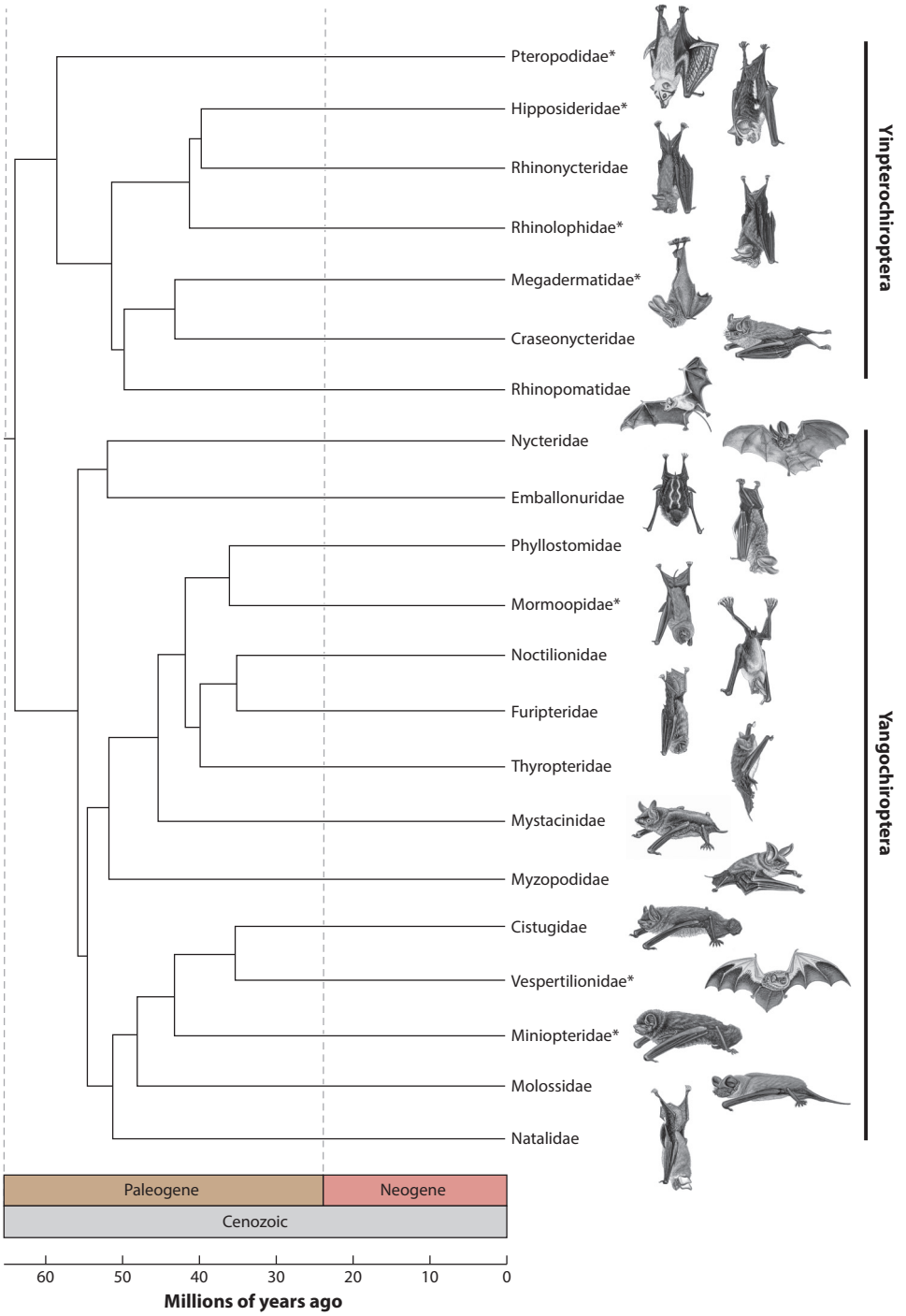

Figure 1

Molecular consensus on bat familial relationships and divergence times (23). Asterisks indicate bat families with genomes available in the National Center for Biotechnology Information. Species are listed in **Table 2**. Bat artwork created by Fiona Reid.

KEY AREAS OF IMPACT

The study of bats and their genomes is likely to have widespread impacts on a range of diverse scientific fields. Below we outline some key areas of study.

Model for Healthy Ageing

Unlike most lab animals, bats are excellent models for understanding human senescence and ageing and to discover the means to improve health into old age. Although there are some merits to mechanistic hypotheses of ageing (e.g., that life span should be inversely related to metabolic rates, as the latter contribute to the accumulation of intracellular debris leading to ageing), the best-supported theory of ageing is evolutionary and linked to life history (41). According to this theory, if adults in a population experience high extrinsic mortality (e.g., from predation), natural selection will favor short-term reproductive success over long-term survival and maintenance, resulting in rapid senescence after reproduction, past the age at which most individuals would have died in a natural population (42). The survivorship and life history of house mice reflect this pattern, with intrinsic mortality and morbidity rising sharply past one year of age, even under highly favorable lab conditions. Conversely, populations with low extrinsic mortality will experience continued selection throughout longer lifetimes, favoring slow senescence and resulting in longer, healthier lifetimes. Because bats are both nocturnal and capable of active flight, they have escaped the attention of most predators. This in turn has led them to evolve the relatively unusual vertebrate combination of long life spans with small bodies (17, 40, 43). As long-lived mammals, in some cases living >41 years, bats offer clues regarding the mechanisms for maintaining high function across internal systems over a long life span, longer than any similar-sized mouse can live (17, 44, 45).

Natural selection over millions of generations for continued health and reproduction throughout a long lifetime has equipped bats with excellent cellular and system-wide mechanisms of maintenance (45). This is particularly impressive considering that the high metabolic rates characteristic of bats are expected to produce reactive oxygen species, typically causing chronic inflammation and hastening senescence (46). However, the maintenance of function alone is not enough, as cells and tissues need constant repair over the course of a multiyear life span. Studies focused on bats have identified suites of cellular repair mechanisms that potentially evolved to support the unusual longevity of bats (11, 40, 44, 45). These genes and variants can be readily compared with human genes to discover specific features that would enable a healthy old age (11, 44, 45).

Model for Enhanced Disease Resistance

Bats have enhanced immune function, coupled with a potentially modulated inflammatory response (11, 16, 47). This holds considerable potential for addressing some of the worst consequences of senescence of humans. Inflammatory disorders associated with autoimmune diseases are among the fastest growing causes of disease worldwide, particularly in ageing populations (48). The ability to modulate inappropriate inflammation in response to stressors without impairing immune function could improve the lives of millions. Hence, detailed exploration of the genomic mechanisms of gene expression in wild bats could hold the key to improving health conditions worldwide (16). High-quality bat genomes will drive a better understanding of molecular bases underlying the resistance/tolerance of European bats to white-nose syndrome (49), which could ultimately be used to inform future bat conservation and management efforts within the United States. White-nose syndrome is a deadly fungal disease recently introduced to North America from Europe (50) that has decimated US bat populations, particularly of little brown bats (*Myotis lucifugus*) and tricolored bats (*Perimyotis subflavus*), and is responsible for an estimated 5–6 million

Table 1 Selected emerging viral pathogens, bat species in which they have been found, geographic locales of infected bats, and bibliographic sources

Emerging viral pathogen	Bat species	Geographic locales	Source
Hendra virus	*Pteropus poliocephalus*	Queensland, Australia	170
Nipah virus	*Pteropus hypomelanus*, *Pteropus lylei*, *Pteropus vampyrus*	Cambodia, Malaysia	171
Australian bat lyssavirus	*Pteropus alecto*, *Pteropus poliocephalus*, *Pteropus conspicullatus*, *Pteropus scapulatus*, *Saccolaimus flaviventris*	Queensland, Australia	172
European bat lyssavirus 1	*Eptesicus serotinus*, *Eptesicus isabellinus*, *Plecotus auritus*, *Pipistrellus pipistrellus*, *Pipistrellus nathusii*	Germany, the Netherlands, Denmark, Poland, France, Spain	173
Marburg virus	*Rousettus aegyptiacus*	Gabon	174
SARS-like coronaviruses	*Rhinolophus sinicus*	Kunming, Yunnan Province, China	175
Relative of Middle East respiratory syndrome coronavirus	*Neoromicia* cf. *zuluensis*	South Africa	176

bat deaths (51). Closely related European bats, such as greater mouse-eared bats (*Myotis myotis*) and Brandt's bat (*Myotis brandtii*), can be colonized by the responsible fungus *Pseudogymnoascus destructans* but do not suffer the same mass mortality (52), suggesting that European bats have evolved the required immune/behavioral response to survive this infection.

As wide-ranging individuals that constantly encounter new environments, bats come into contact with diverse viral pathogens over the course of a life span (15, 53). Again, over eons, bats have evolved to cope with these challenges, often leading to survival despite viral exposure (for a comprehensive list of viruses detected in bats, see 54). Epidemiological studies and field surveys suggest viruses circulate in wild bat populations (12, 55) without causing the great morbidity or mortality observed as a result of viral spillovers into humans (**Table 1**) (14, 56). Although the processes through which bats manage to clear viral infections remain poorly understood, possible explanations for the resilience of individual bats to these infections include not only heightened immune function but modulation of inflammation and mechanisms of repair (16). Genomic analyses have already revealed coevolution with viruses as a long-term consequence of bat evolution (57). High-quality bat genomes (e.g., including regulatory regions) from diverse species are needed to determine both how extensive these interactions between bats and viruses have been and what innate genetic mechanisms bats use to clear these viruses. Coupled with viral monitoring in the wild and viral genomics, bat genomic analyses have the potential to predict and manage future spillover events (56).

Key Species for Ecosystem Functioning

The unique biology of bats is important not only for understanding human health but also for maintaining and improving ecosystem health (7, 58). Bats contribute disproportionately to ecosystem functions, for example, in the regeneration of tropical forests (59–61). The convergent evolution of mutualism with plants in both Old World and New World tropics has led to the dependence of many flowering plant species on bats for their reproduction and dispersal. Pollinating and seed-dispersing bats are keystone species across the largest stands of tropical forests in biodiversity hotspots from Amazonia to the Congo Basin to Southeast Asia (7, 60). As natural forests are increasingly fragmented for human uses, bats play a unique role in dispersing pollen between plants

and seeds between fragments across large distances, maintaining plant genetic diversity and aiding the regeneration of forests after clearing. Breaching ocean barriers through active flight, bats are often the only mammals native to oceanic islands and are important to the survival and maintenance of those isolated ecosystems. As people have brought in predators and commensal species, island bats have become increasingly imperiled, threatening to collapse local trophic chains and disrupt pollination and seed dispersal (61). Bats also feed on many pest insect species and act as a natural and effective pest control (6, 8–10, 58). Insect-eating bats save maize farmers globally an estimated ~US$1 billion a year from crop damage (58). Bat genomes will help elucidate the genetic adaptations enabling bats to both detect flowering plants and act as pollinators and will provide the basic genomic resources and tools for future population-level analyses to investigate and understand the relationships between their dwindling populations and the ecosystems on which they rely and that rely on them.

Model for the Evolution of Sensory Perception

Sensory perception plays one of the most important roles in the survival of an individual and is responsible for many key behaviors (e.g., foraging, predator avoidance, mate recognition, and communication) that drive evolution. Therefore, genes involved in sensory perception should show signs of adaptation and selection, as these genes are at the frontline of evolutionary pressures. Indeed, spectacular evidence of molecular convergence has been uncovered in hearing genes in echolocating whales and certain echolocating bats (62–65). Olfactory receptor genes that are directly involved in olfaction show evidence of environmental niche specialization in aquatic, terrestrial, and flying mammals, even after controlling for phylogeny (31, 66, 67). Similar loss of function in short-wave opsin visual genes has been found in bats with advanced echolocation capabilities (30). Bats have undergone the most extensive loss of function of the pheromone-detecting vomeronasal system compared with any other mammalian group (68–70), offering an opportunity to better understand the process of sensory vestigialization and the vomeronasal loss seen throughout mammals, including humans. Therefore, studying the evolution of these genes and genomic regions in bats—the sensory specialists—will elucidate how the mammalian genome has responded to past evolutionary pressures driven by changing environmental conditions. Comparative genomic analyses will also help illuminate the evolution and inheritance of blindness and deafness in humans by identifying regions of these genes that are most likely to cause disease and thus identifying putative targets for downstream gene therapeutics (71–73). This is an important advance, as the World Health Organization has estimated that 285 million people worldwide are visually impaired, 39 million of them are blind (74, 75), and over 360 million people have severe hearing loss. Bats have also been shown to be an excellent model for sensory-driven speciation [e.g., *Rhinolophus philippinensis* (76), *Craseonycteris thonglongyai* (77)]. High-quality bat genomes will enable further elucidation of the molecular basis of sensory adaptation and finally untangle the evolutionary mechanisms driving speciation (77, 78).

Model of Human Communication

Humans are unique in their capacity for language, but a core component of spoken language is the ability to learn new vocalizations, and this is shared with only a few other species, including some bats, birds, cetaceans, pinnipeds, and elephants (79–82). Comparative analyses of humans with other vocal learning species will be key for revealing its biological and evolutionary underpinnings. To date, vocal learning has been studied extensively in birds. For example, comparative analyses facilitated by the release of avian genomes have revealed genes with unique signatures in vocal

learning birds (83, 84). Genome-wide expression analyses have also found that many of these genes are differentially expressed in vocal learning brain regions, pointing to a link between these genes and the distinctive vocal abilities of these birds (85). Mammalian vocal learning is, by comparison, understudied, particularly at a neurogenetic level. This is largely because of the scarcity of the trait and the large size and intractability of most vocal learning mammals (e.g., elephants, whales, dolphins). Their diversity, strong reliance on vocal social communication, and small size make bats an attractive and experimentally tractable model for studying vocal learning. Avian research has highlighted shared neurogenetic mechanisms that may underlie vocal learning across divergent species. Adding bats to this field will bridge the wide evolutionary gap between birds and humans and will bring us closer to understanding how this complex behavioral trait can be genetically encoded in the brain. The availability of high-quality bat genomes will provide a mammalian system in which the biological basis of vocal learning can be explored and shed light on how this language-relevant trait has evolved across Aves, Mammalia, and humans. In addition, understanding the genetic bases of language-relevant traits like vocal learning in bats will help us to understand the genetic causes of disorders involving speech and/or language (e.g., language disorder, autism, and speech apraxia) in humans. These disorders are highly prevalent (up to 25% of children; 86) and have a strong genetic component, and understanding their causes will lead to better genetic diagnoses and new potential therapeutics.

Model for Limb Development

The bat wing is a striking example of morphological adaptation and variation in mammals, characterized by dramatically elongated fingers and retained interdigital webbing. Several genomic studies have recently been carried out on developing bat embryonic limbs that identified numerous candidate wing development–associated genes and regulatory elements (87–89). Importantly, complete genomes are critical to this task, which requires tracking changes in expression encoded by regulatory regions, and not just protein-coding changes. The Bat1K project would enable comparative genomic analyses that can highlight specific genes, regulatory elements, and bat-specific nucleotide changes that are associated with wing development. Characterizing bat wing development will also improve our understanding of how changes in limb developmental building blocks can lead to human limb malformations, such as arachnodactyly (long fingers), brachydactyly (short fingers), and syndactyly (webbed fingers).

Evolution of Mammalian Genome Architecture

Bats exhibit the smallest genome size of all extant mammals, ranging in size from ∼1.6 to 3.54 Gb based on 266 records, with the majority of genomes being ∼2 Gb (90; data from **http://www.genomesize.com**). Birds, the other class of flying vertebrates, show similarly reduced genome sizes, an observation that has been used to suggest that the acquisition of flight requires a loss of genomic redundancy and a streamlining of genomic structure within vertebrates (90–92). Bats represent the lower limit of mammalian genome content and therefore can show the essential genomic information required for being mammalian. In addition, vespertilionid bats are unique among studied mammals in harboring multiple lineages of active transposable elements that are not found in other members of the clade (93). Despite this unique accumulation, they continue to maintain small genomes, suggesting an ability to balance genomic gain from transposable repeats via the loss of genomic mass (90). Chromosome-level, high-coverage bat genomes across the order will drive a unique understanding of the limits of mammalian genome structure in general and the genomic consequences of flight adaptation in particular.

Although many other species have some of these attributes to varying degrees, bats are unique because their genomes harbor secrets related to all of them. Thus, obtaining genome assembly data across the bat clade will drive the advancement of these and numerous other fields given the unique biology and evolutionary history of bats.

BAT GENOMES SEQUENCED TO DATE

The first complex eukaryotic genomes were generated using Sanger (dideoxynucleotide) chemistry, which, given the output limitations, rendered them both complex and expensive endeavors. For example, the first human genome draft took well over a decade to produce and cost over US$1 billion (94). Sanger sequencing was the state of the art for more than two decades, with few eukaryotic genomes being released owing to the cost and time commitments. However, since the introduction of new, high-throughput chemistries in the mid-2000s (95), those costs and time commitments have been substantially reduced.

Currently, two major technologies dominate the field. Illumina sequencing is the highest-throughput option available at present, with some equipment able to generate the equivalent of a human genome in just a few hours, at a cost of only a few hundred US dollars. Briefly, Illumina sequencing relies on the sequential addition of fluorescently labeled nucleotides and their subsequent detection. The technology is very accurate, with error rates of only $\geq$0.1% with variation depending on genomic region, read lengths, and sequencing chemistry (96, 97). The trade-off for accuracy and throughput, however, is read length. Illumina sequencing chemistry has yet to break the 300-bp limit, with most users limiting themselves to reads of 150 bp or less, which can make genome assembly across complex repetitive regions difficult if not impossible (33).

Pacific Biosciences (PacBio) has adopted a different sequencing strategy based on single-molecule real-time (SMRT) sequencing. Briefly, this method relies on a tethered polymerase that incorporates labeled nucleotides using the template to be read. The fluorescent labels are cleaved on addition of the nucleotide, and a detector identifies the base added. Current throughput averages approximately 8 Gb/day (98, 99), with reads averaging 10–15 kb and some ranging up to 100,000 bp. Unfortunately, PacBio reads exhibit a substantially higher error rate of 10–15%, mostly in the form of insertions and deletions. Methods exist to improve this to 99.99% accuracy, but this requires at least 50 $\times$ SMRT-based coverage or the addition of Illumina's shorter reads (100–106), making this option more expensive than Illumina-only approaches.

As of writing, 14 chiropteran genome assemblies are readily available from the National Center for Biotechnology Information (NCBI) database (**Table 2**). The first bat to have its genome released, the little brown bat [*Myotis lucifugus* (107)], was sequenced by the Broad Institute using Sanger chemistry. It is this member of the clade Yangochiroptera (**Figure 1**) (5) that has been the subject of most comparative analyses among mammals and has served as the chiropteran representative.

The remaining assemblies vary in quality and completeness. For example, Parker et al. (65) took a low-coverage approach, which was valid given that they were interested primarily in coding sequences. Unfortunately, such low coverage makes overall contiguity of the assembly very low, as evidenced by the low-contig N50s. In comparison, the Egyptian fruit bat (*Rousettus aegyptiacus*) assembly, made possible by the combination of long- and short-read technologies, is of higher quality (**Table 2**). The search for genomic signatures for complex traits underlying the unique biology of bats, however, requires a common standard for greater coverage.

Of course, other efforts are under way or completed that are not represented in the NCBI database. Members of Bat1K are, for example, currently involved in efforts to sequence and assemble the genomes of the Northern long-eared bat (*Myotis septentrionalis*), the greater mouse-eared

Table 2 Summary statistics of bat genomes currently available via National Center for Biotechnology Information databases[a]

Suborder	Species/family	Common name	Estimated genome size (C)[b]	Assembly size (Gb)	GenBank accession	Publication	Sequencing technology	Coverage	Contigs	Contig N50	Scaffolds	Scaffold N50	Assembly score
Yinpterochiroptera	*Eidolon helvum*/ Pteropodidae	African straw-colored Fruit bat	2.03	1.44	AWHC0+.1	65	IL	18×	288,446	12,668	6,789	27,684	0.1.1Q35
	Hipposideros armiger/ Hipposideridae	Great leaf-nosed bat	ND	2.24	JXIK0+.1	168	IL	218×	128,956	39,863	7,571	2,328,177	0.3.1Q40
	Megaderma lyra/ Megadermatidae	Greater false vampire bat	ND	1.44	AWHB0+.1	65	IL	18×	518,327	7,043	192,872	16,881	0.1.1Q35
	Pteropus alecto/ Pteropodidae	Black flying fox	ND	1.99	ALWS0+.1	47	IL	110×	170,164	18,318	65,598	15,954,802	1.4.1Q40
	Pteropus vampyrus/ Pteropodidae	Large flying fox	2.37	2.14	ABRP0+.2		IL	188×	225,433	21,866	36,094	5,954,017	2.3.1Q40
	Rhinolophus ferrumequinum/ Rhinolophidae	Greater horseshoe bat	1.92–2.68	1.31	AWHA0+.1	65	IL	17×	290,685	11,695	160,500	21,151	1.1.1Q35
	Rhinolophus sinicus/ Rhinolophidae	Chinese rufous horseshoe bat	ND	2.07	LVEH0+.1	168	IL	146×	182,995	37,803	63,439	3,754,400	1.3.1Q40
	Rousettus aegyptiacus/ Pteropodidae	Egyptian rousette	2.11	1.91	LOCP0+.2		IL & PB	169×	3,049	1,488,988	2,490	2,007,187	3.3.1Q40
Yangochiroptera	*Eptesicus fuscus*/ Vespertilionidae	Big brown bat	2.25–2.70	2.02	ALEH0+.1		IL	84×	137,058	21,392	133,538	13,454,942	1.4.1Q40
	Miniopterus natalensis/ Miniopteridae	Natal long-fingered bat	ND	1.80	LDJU0+.1	89	IL	77×	114,632	29,777	1,269	4,315,193	1.3.1Q40
	Myotis brandtii/ Vespertilionidae	Brandt's bat	ND	2.11	ANKR0+.1	169	IL	120×	325,414	23,289	169,750	3,225,832	1.3.1Q40
	Myotis davidii/ Vespertilionidae	David's myotis	ND	2.06	ALWT0+.1	47	IL	110×	325,280	15,182	101,769	3,454,484	1.3.1Q40
	Myotis lucifugus/ Vespertilionidae	Little brown myotis	2.26	1.96	AAPE0+.2	107	SG	7×	72,785	64,330	11,654	4,293,315	1.3.1Q35
	Pteronotus parnellii/ Mormoopidae	Common mustached bat	2.35–2.71	1.56	AWGZ0+.1	65	IL	17×	443,121	9,502	177,401	22,675	0.1.1Q35

[a]Phylogenetic relationships of bat families are depicted in **Figure 1**.
[b]Data taken from **https://genomesize.com**, the animal genome size database.
Abbreviations: 0+, 00000000.; IL, Illumina; PB, Pacbio; SG, Sanger.

bat (*Myotis myotis*), the common vampire bat (*Desmodus rotundus*), and the pale spear-nosed bat (*Phyllostomus discolor*). These assemblies use multiple strategies with results that vary in ways similar to those observed in **Table 2**. Indeed, one primary goal of Bat1K is to standardize assembly strategies to provide assemblies of uniform optimal quality for the bat genomics community through combining multiple sequencing and scaffolding technologies (e.g., PacBio+Illumina+HiC+10X; see below for details).

Given the more than 1,300 species of bat currently recognized, there is still a long way to go to generate genome sequence data covering Chiroptera (108, 109). However, as we outline in the next section, we believe it is important not just to generate genome-level data but to produce high-quality genome sequences that maximize the usefulness and accessibility of the data for all research fields. Below, we outline the specific goals of the Bat1K Consortium and how we aim to achieve these goals.

THE BAT1K PROPOSAL

The ultimate goal of Bat1K is to sequence, assemble to chromosome-level contiguity, and make publicly available the full genome of every living bat species. As one might imagine, given the specialized needs of bats in captivity, determining species boundaries is difficult in chiropterans. Indeed, applying a particular species concept to bats is difficult. The Biological Species concept (110) is commonly applied to mammals, but investigations of gene flow among bat species have been attempted but are not commonly performed and often rely on indirect evidence (76–78, 111–115). The Genetic Species concept was tested for multiple species and suggested hybridization in several cases (116). Some clades, in particular the vesper bats, likely harbor significant numbers of cryptic species (117). Thus, the actual number of species that we could conceivably target is unknown. However, our best estimate is ∼1,300 (108, 109; **http://www.bat1k.com**). The Bat1K genome consortium unites bat biologists (>147 members as of writing; see full list of consortium members in the Supplemental Appendix), computational scientists, conservation organizations, genome technologists, and any interested individuals committed to a better understanding of the genetic and evolutionary mechanisms that underlie the unique adaptations of bats. Our aim is to catalog the unique genetic endowment and diversity present in all living bats to better understand the molecular basis of their unique adaptations; uncover their evolutionary history; link genotype with phenotype; and ultimately better understand, promote, and conserve bats. To achieve this, we must meet the following objectives.

Acquire high–molecular weight DNA from all living bat species. Highly contiguous assemblies require high–molecular weight DNA as starting material. We are coordinating members of Bat1K to obtain such material. Many members have already contributed or pledged samples, and others have committed to acquiring other samples. Our preference is that fresh tissue should be flash frozen in liquid nitrogen immediately after death (see **http://www.bat1k.com** website for methodology) and remain frozen (at <80°C) until DNA extraction and immediate library preparation on site at the sequencing location. We understand this may not always be possible. In cases where it is not possible to lethally sample a particular species given its conservation/protected status, nonlethal wing-punch biopsies should be taken, placed in viable media, and sent to appropriate laboratories for cell culture, as described by Kacprzyk et al. (118). In these cases, high–molecular weight DNA will be harvested after minimal passage of cells and immediately flash frozen or subjected to DNA extraction. All samples will be taken and transported with full capture, licensing, and legal permits required. Additionally, each individual selected for genomic sequencing must be associated with an electronic voucher or, even better, a specimen voucher deposited in a collection.

The minimal requirements for an adequate specimen voucher are (*a*) species identification based on expert assessment; (*b*) standard mammalian measurements, including forearm and body mass; (*c*) geographical coordinates of capture; and (*d*) photographs documenting the individual, its genitalia, and its dentition. Because male mammals are heterogametic, we recommend sampling both males and females where possible; if only one is available, males should be chosen to allow for Y-chromosome sequencing. However, given that many bat species are typically accessible only at maternity roosts, we will sequence a female representative when no male samples are available. Geographic provenance and morphometric data will be recorded, and when possible, additional tissues harvested from the same animal will be frozen and biobanked by consortium members.

Produce reference quality–based genomes. Informally, a chromosome-level assembly refers to a genome reconstruction for which the sequence is in megabase contigs that are ordered and oriented into scaffolds that effectively cover each chromosome arm. Very few vertebrate genomes today approach such a level of contiguity and completeness. However, the new single-molecule, long-read technologies (119, 120) are changing this situation. Our goal is to produce reconstructions with a contig N50 of 1 Mbp or greater, a scaffold N50 of 10 Mbp or greater, at least 90% of the contigs mapped to chromosomes, and a consensus accuracy of Q40 or better, or what we call a 3.4.2Q40 assembly, as we will now rigorously define.

Formally, a *c.s.m.*Q*x* (contig/scaffold/map-chromosome/Qscore) genome reconstruction is one that has a contig N50 of 10^c Kbp, a scaffold N50 of 10^s Kbp, and a consensus accuracy of Q*x*; $m = 1,2,3$, or 4 is a descriptive designator of how well the scaffolds are mapped to chromosomes, as follows:

1. Not assigned to chromosomes, or under 90% assigned.
2. >90% assigned to chromosomes, either directly to species-specific chromosomal maps, or by in silico assignment to closest reference or ancestral reconstructed chromosomes.
3. >90% assigned to chromosomes as for 2, but also requiring that all interchromosomal breakpoints with respect to the reference are confirmed by at least two independent data sources.
4. >90% assigned to species-specific chromosomal maps via direct within-species map data, with all interchromosomal and a representative sample of intrachromosomal breakpoints with respect to the reference confirmed.

This previously unpublished characterization (Richard Durbin & Harris Lewin, private communication) is the standard that has been adopted by the broader Vertebrate Genome Project (**http://www.genomeark.org**) as part of the Genome 10K initiative (**https://genome10k.soe.ucsc.edu**). By way of reference, at the end of 2016, 3 mammals of the 290 sequenced vertebrates met the 3.4.2Q40 standard that we will target, and the human genome is currently 4.4.2.Q40. Estimated scores are given for the bat genomes in **Table 2**.

Our proposed strategy, to generate highly contiguous and complete assemblies, will be to collect sequence data structured as follows: (*a*) 60× coverage in PacBio long reads, (*b*) 50× coverage in Illumina short reads collected as 10X Genomics read clouds, and (*c*) 10× coverage in Illumina short reads collected as long-range Hi-C read pairs. Initial assemblies will be accomplished by using the most accurate current assemblers, such as Falcon and Canu (121, 122), with long reads of an average length of 10–15 Kb. Assemblies of other mammals (101, 123) suggest that we will obtain initial contigs with an N50 length of over 1 Mb and often as high as 5 Mb. Genomes that are highly repetitive and large, such as those of amphibians (e.g., the axolotl at 32 Gb, with transposable elements comprising ∼60%), do not assemble to this level. However, we have no

reason to expect any bat genomes to prove problematic, as the bat genomes sequenced to date are mostly ~2 Gb (90; **http://www.genomesize.com**), and although some bats contain unique transposable element content by type, they are no more repetitive on average than those of other mammals (90).

Scaffolding of the contigs will then be performed using the 10X Genomics read clouds and Hi-C data. The 10X Genomics device and protocol generate short-read templates that lightly cover (i.e., 0.2–.3×) a handful (i.e., 5–15) of 50–200-Kbp molecules in a droplet microchamber (124). Conceptually, sequencing the templates produced in each droplet yields clouds of reads that gather over a handful of regions of the underlying genome. Directly assembling these data with, for example, Supernova (125) has to date delivered results with large scaffolds but unfortunately small contigs. We will instead use the clouds for short-range scaffolding to link the PacBio contigs that are less than 100–200 Kb apart, thereby bridging many of the gaps between contigs in the long-read assembly. We will use the first-generation software available to do this (126; **https://sourceforge.net/projects/phusion2/files/scaff10x**) and use newer, more optimal methods as they develop. Early anecdotal experience shows that this results in scaffolds well over 10 Mbp. Some large gaps will remain unspanned, and therefore we will use the long-range Hi-C paired-end data to link the 10X scaffolds into large super-scaffolds and separate these into chromosomal units (127, 128) with the aid of syntenic mapping to a phylogenetically close relative.

Finally, to achieve a Q40 consensus accuracy, we will use the 50× coverage in Illumina reads provided by data sets *b* and *c* to polish the consensus (see below). This last step is necessary given that the high error rate of the long reads (10–12%) typically results in a Q30 consensus when only the long-read data are used.

In summary, for Bat1K, we propose to collect (*a*) 60× Pacbio long reads, (*b*) 50× Illumina reads in 10X read clouds, and (*c*) 10× Illumina reads in long-range Hi-C read pairs (4-cutter protocol) and follow the computational sequence below:

1. Assemble the 60× PacBio data (*a*) into contigs.
2. Use the 50× read clouds (*b*) to link contigs into scaffolds.
3. Find missed spanning reads and close the spanned gaps.
4. Use the Hi-C pairs (*c*) to order and orient the scaffolds into superscaffolds covering chromosome arms.
5. Use the 60× Illumina read data from *b* and *c* to polish the PacBio consensus contigs to Q40.

A virtue of the proposed strategy is that only three commonly used library preparations and two sequencing technologies are required. Of course, should the protocol need amendment or augmentation, then such will be undertaken. This is especially true given the rapid advancements in sequencing technology observed today. Indeed, we see Bat1K as an opportunity to benchmark new genomic technologies that will enable a fast and cost-effective 3.4.2.Q40 genome.

Project Phases

This large sequencing project will be accomplished in three phylogenetically delimited phases (**Figure 2**). Phase 1 will target representatives of each bat family ($N = 21$). In Phase 2, we will expand to include representatives for every genus of bat ($N = 220$). Phase 3 will expand further to target all remaining species ($N = 1{,}288$). As funding and samples becomes available from other parties, those efforts will be incorporated and lead the sequencing priority. At each phase, the community will publish key synthesis papers describing progress and exploring the unique adaptations within bats that drive this initiative.

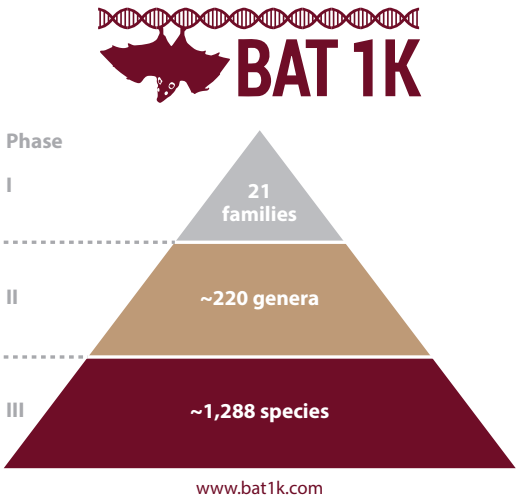

Figure 2

Sequencing phases of Bat1K.

EXPECTED BENEFITS

The resources of Bat1K are of clear value to any researchers interested in linking genes to phenotype and understanding how genomes produce complex organisms, a pursuit that has been identified as one of the Grand Challenges of the Twenty-First Century (129, 130). A set of open-access, high-quality bat genomes that are sequenced, assembled, and annotated using uniform laboratory and bioinformatic pipelines will confer immense benefits to researchers from across the life sciences, ultimately yielding an unparalleled research tool that would form the basis for an uncountable number of research projects. Naturally, many of these researchers will be those whose interests focus principally on bats, and who wish to access the genomes of single, or a few closely related, species. However, as demonstrated in several other recently published phylogenomic-scale projects [for example, those on birds (131, 132), mammals (107), insects (133), and land plants (134)], as both the number and pan-clade distribution of genomes increase, so do the research possibilities. We foresee multiple avenues to pursue but happily acknowledge our inability to anticipate all of the research efforts that will be enabled.

Phylogeny and Population Genomics

In light of the rapidly decreasing costs of resequencing genomes, phylogenetic and population analyses are now increasingly being undertaken at the genome level, as it is widely understood that analyses based upon whole-genome data provide the most accurate reconstructions of species' evolutionary histories. This is particularly true for bats, for which many species are likely to have undergone rapid radiation, diversification, and potentially hybridization and/or incomplete lineage sorting (135–138). In particular, genome-level data sets should at best be able to account for the phylogenetic noise inherent in analyses of single genes or at worst at least clearly indicate the degree of noise that they impart (139). Obviously, the higher the quality of the genomic data, the greater the expected improvement. Given that different parts of the genomes can show strikingly different histories (e.g., 132, 140), it is important that the correct homology is inferred, which requires well-assembled genomes to enable alignment across divergent evolutionary time frames.

The Bat1K genome resource should enable a full reconstruction of the evolutionary history of this phylogenetically controversial clade for the first time and resolve long-standing debates by producing high-quality genomes that should overcome most previously encountered problems.

At the population scale, not only do a suite of genome-scale interpretations of standard tools now exist (e.g., nucleotide diversity, heterozygosity), but this can be complemented by other tools specifically developed to exploit the genome in new ways. Such tools include reconstruction of population size using pairwise sequentially Markovian coalescent (141), multiple sequentially Markovian coalescent (142), and the identification and quantification of admixture between genetically distinct groups using D-statistics (143). Although many of these analyses can be performed through mapping to the genomes of related species, in general the closer the reference genome, the more refined the observations made (144). With such genomes and tools in place, a wide range of questions that are central to current bat population biology and ecology can subsequently be addressed. These include what determines evolutionary significant units (ESUs), what the degree of gene flow is among them, and even the dynamics of the speciation process itself (77, 78, 112), a critical question in bats given their dispersal ability and the high taxonomic diversity of some clades [e.g., genus *Myotis*, with 100+ species (145)]. These are, in turn, critical to more applied efforts, ranging from optimizing conservation strategies, for example, by focusing on ESUs at most risk or most likely to respond to efforts, to defining bat clades that represent possible high risks of cross-species pathogen outbreaks, for example, those exhibiting the greatest degree of inter-ESU, population, or even species admixture, and thus possible cross-host pathogen transmissions.

Uncover Genotype-To-Phenotype

High-quality genomic reference data also lie at the heart of studies aiming to reveal links between genotype and phenotype. Although they have historically been the domain of model organisms, in the era of genomics we are now able to expand our understanding of genotype-to-phenotype links beyond the typical model species. Thus, genomics allows us to choose the most appropriate model for the question, rather than just relying on the usual (and often less–well phenotypically suited) mouse, *Drosophila*, or nematode models. Although it was once acceptable to provide candidate loci identified through genome-wide association studies scans for loci under selection as evidence of genetic mechanisms in nonmodel systems, transcriptional analyses, and even knockout/knock-in experiments, have become feasible and are increasingly required by journals (and the research community) today. Only with such evidence can we develop an understanding of how genes (or other genomic elements) function at a molecular level and thus truly bridge the gap between gene and phenotype. Animal systems with complete genomic information present an ideal opportunity, and indeed are essential to accomplish this. One of the clearest ways to prove gene-to-phenotype links is to create transgenic animals or cell lines in which the candidate gene has been knocked down (e.g., via shRNAs) or knocked out (e.g., via CRISPR/Cas9 genome editing). But these approaches require detailed knowledge of the target organism's genome, as shRNA and CRISPR/Cas9 planning is based on sequence complementarity, and targeting approaches must be designed such that they are complementary only to the gene in question and to no other sequence (146, 147). Such progress has been exemplified by studies in birds in which, for example, genome sequence availability made it possible to knock down a key human language–related gene (*FoxP2*) in zebra finches to determine its role in circuitry underlying vocal learning (148–153).

Genomes also facilitate an understanding of the molecular basis of the gene-phenotype relationship through analysis of gene regulation. Techniques to understand regulatory networks, including small RNAs, transcription factor cascades (e.g., ChIP-Seq, enhancer mapping), epigenetic marks, and chromatin structure (e.g., histone mapping, chromatin conformation capture,

topological domain mapping), rely on a comprehensive understanding of the identity and position of both coding and noncoding DNA sequences (154–156). The perturbation of regulatory networks represents a particularly powerful way to invoke evolutionary change without the need to evolve novel genes (45). Such perturbations have been suggested as influential in bat diversification (157). This represents a potentially rich area of study, especially considering the rapid and massive changes to gene regulation that must have occurred when, over the course of only a few million years, the phyllostomid (Phyllostomidae) bats evolved from an insectivorous ancestor into the clade of carnivores, sanguivores, nectarivores, frugivores, and palynivores we see today (29, 158–160).

Evolution of Genome Architecture

As we move beyond questions of function, we reach those relating to more general questions, such as how genomes evolve. With high-quality reference genomes, this can be studied at levels ranging from the nucleotide up to the chromosome. At the nucleotide level, one series of questions of growing interest relates to constrained sequence evolution, for example, whether specific nucleotides or amino acids are under convergent evolution in phylogenetically disparate taxa, as has been showcased with regard to the evolution of echolocation in bats and marine mammals (e.g., 65, 161). At the larger scale, there is considerable focus today on evolutionary trajectories of autosomal and sex chromosomes (162), and in particular whether major evolutionary transitions accompany significant chromosome-scale rearrangements. The phyllostomids again represent an exciting test case. This clade exhibits a wide range of chromosomal variation, and chromosome-level genome assemblies across the group will allow us, Bat1K members, and other researchers to investigate this phenomenon from nucleotide, syntenic, and phylogenomic perspectives.

A third area that will benefit significantly from high-quality reference genomes is our understanding of the dark matter that large fractions of our genomes are built of—specifically the abundance of seemingly noncoding elements. Estimates from the human-focused ENCODE (163) project predict that between 10% and 80% of these noncoding components are functional (164)—clearly an unsatisfactory answer. Thus, one of the key aims of the ENCODE and other related projects is the combined use of biochemical assays and associated computational analyses to query the underlying DNA sequence, including the application of methods such as Hi-C to refine tertiary structure interactions (127, 165). However, a major challenge is simply the sheer size of genomic data sets—how can one effectively parse the data? In this regard, organisms such as bats, with their naturally reduced genome sizes, represent a unique opportunity to study a naturally filtered subset of this noncoding data. In short, one might hypothesize that elements retained in the reduced genomes of bats and birds (and in particular those with significant cross-species sequence conservation) may play important functions, whereas those purged do not. Indeed, preliminary analyses of bird genomes for both transposable elements and avian-specific highly conserved element content have already been promising in this regard (131, 166), indicating that the secrets within bat genomes may be of considerable use.

Wider Community Benefits

There are potential benefits on an even broader scale. The data set will represent a critical component of wider community efforts to catalog life's diversity, such as the Genome10K (129) and Earth Biogenome (167) projects—the latter of which aims to coordinate the sequencing and eventual public release of a full catalog of life's genomes. As such, these wider efforts will almost certainly contribute to the ongoing developments of not only the technologies underlying DNA

sequencing itself but the associated bioinformatics for both assembling and annotating the genomes and analyzing the data within them. Although many of these efforts will be directly linked to the sequencing industry, we highlight that because the resulting Bat1K data will be accessible by any scientists connected to the internet, it will represent an unparalleled, free opportunity for researchers around the world, including those in tropical countries where bat diversity concentrates, to both be trained in genomics and undertake genomic research. Critically, therefore, the training that this would confer on countless scientists would both strengthen local research across the planet and be taken into other industries, thus radically accelerating developments and discoveries linked to genomics in general—a topic of growing importance in the modern biomedicine and agriscience industries. Bat1K will develop a genomic record of all living bat species, ultimately a genomic ark that can be used to benchmark the genomic health of different bat species to uncover populations in need of immediate conservation efforts. Considering the challenges to well-being facing humanity, from ageing populations to emerging infectious diseases, and from conserving fragmented habitats to maintaining the ecosystems that preserve productivity, undertaking Bat1K is not only opportune but indispensable.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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A full list of Bat1K consortium members is presented in the **Supplemental Appendix**. We think Fiona Reid for her contributed artwork.

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