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# Mass extinctions, biodiversity and mitochondrial function: are bats 'special' as reservoirs for emerging viruses?

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For the past 10–15 years, bats have attracted growing attention as reservoirs of emerging zoonotic viruses. This has been due to a combination of factors including the emergence of highly virulent zoonotic pathogens, such as Hendra, Nipah, SARS and Ebola viruses, and the high rate of detection of a large number of previously unknown viral sequences in bat specimens. As bats have ancient evolutionary origins and are the only flying mammals, it has been hypothesized that some of their unique biological features may have made them especially suitable hosts for different viruses. So the question 'Are bats different, special or exceptional?' has become a focal point in the field of virology, bat biology and virus-host co-evolution. In this brief review, we examine the topic in a relatively unconventional way, that is, our discussion will be based on both scientific discoveries and theoretical predictions. This approach was chosen partially because the data in this field are so limited that it is impossible to conduct a useful review based on published results only and also because we believe it is important to provoke original, speculative or even controversial ideas or theories in this important field of research.

## Addresses

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## Introduction

Bats (order Chiroptera), one of the most abundant, diverse and geographically dispersed vertebrates on earth, have recently been shown to be reservoir hosts of a number of emerging viruses responsible for severe disease outbreaks in humans and livestock [1<sup>•</sup>,2,3]. The first recognition that bats are involved in the ecology of human disease came during the 1920s when rabies virus was identified in bats in South and Central America [4]. However, the discovery of

henipaviruses in the mid-1990s and the subsequent recognition that bats may be a natural host of SARS-like coronaviruses and filoviruses marked a new era of fresh research into the role of bats as an important reservoir host of viruses which have the potential to cause disease in humans and livestock [3,5–9].

The recent surge of interest in bats as a reservoir of viruses was driven by two factors. First, in less than 20 years, several high profile viral pathogens have been proven or hypothesized to have a bat origin. Since Hendra virus was first discovered in 1994, there have been at least 17 known spillover events in Australia with a mortality rate in humans of approximately 60% [10]. The closely related Nipah virus has been responsible for devastating disease outbreaks in Malaysia, Bangladesh and India with mortality rates ranging from 40% to 90%, resulting in the deaths of approximately 200 humans [11]. Filoviruses (Ebola and Marburg viruses) have caused outbreaks in Africa with associated human mortality rates as high as 90%, and have been linked to mass gorilla die-offs, making them both a public health and conservation concern [12–14]. The outbreak of severe acute respiratory syndrome (SARS) in 2002–2003, due to a previously unknown coronavirus, resulted in more than 8000 human infections with a mortality rate close to 10% and an estimated cost of \$50 billion in lost tourism and trade [15–17]. The association of these high profile pathogens and disease outbreaks with bats has led to an increase in public interest, funding and research activities on these and many other bat-borne viruses. However, it should be emphasized that, although closely related SARS-like coronaviruses have been detected in horseshoe bats, the exact natural reservoir of the coronavirus responsible for the SARS outbreaks is still unknown [8,9]. The true natural reservoir of Ebola virus is also still being debated as rodents, insectivores and bats have all been identified as potential sources of infection in primates [18<sup>•</sup>,19].

The second driver for the recent surge in bat virus research has been advances in modern molecular techniques which have presented opportunities for discovery of novel bat viruses, that were considered impossible or nonpractical just a decade ago. Using pan-virus-specific primers and next-generation sequencing, it is now possible to detect and characterize novel viral sequences without the need for virus isolation by cell culture or the identification of virions by electron microscopy. Numerous publications in the past few years have reinforced the observation, first made by Sulkin and Allen in 1974 [20], that bats carry a wide range of

novel RNA and DNA viruses. These results also provide support to the notion, as first observed during the investigations of bat coronaviruses (see below), that bats within a geographic location and/or taxonomic group have an unusual ability to harbor a large number of genetically diverse viruses. More recently, two metagenomic studies on bat fecal samples have revealed a great number of novel bat viruses, some of which have moderate sequence identity to previously known mammalian viruses, including members of the *Parvoviridae*, *Circoviridae*, *Picornaviridae*, *Adenoviridae*, *Poxviridae*, *Astroviridae*, *Herpesviridae* and *Coronaviridae* [21<sup>•</sup>,22<sup>•</sup>]. Further systematic surveillance will be required to determine whether bats are the natural hosts of these novel viruses, but these results clearly indicate that there are many bat viruses yet to be identified. Interestingly, similar to other metagenomic analyses of human or other animal fecal samples, these studies have also identified numerous sequences derived from viruses infecting insects, plants and bacteria. Although these viruses are unlikely to infect bats, one might hypothesize that they could play an important role in facilitating the dispersal of these viruses to different geographical locations and different hosts.

The high detection rate and great genetic diversity of viral sequences from bats have not only propelled further scientific and public interest in this field, but also led to debate on the importance of bats as reservoirs of zoonotic viruses. The question ‘Are bats different?’ has been raised at many international conferences and has been the topic of several previous reviews [1<sup>••</sup>,2,20,23<sup>••</sup>]. While the currently available data are too limited to provide a conclusive answer, this review aims to examine different hypotheses which may eventually allow us to resolve this intriguing and fundamentally important question. It should be noted, however, that some of the discussions presented in this review are largely speculative or even controversial. This has been done intentionally by the authors to invigorate discussion and further research on this topic.

### Special features of bats as reservoirs of viral infection

Bats have several features that might help to explain the seemingly high rate of virus detection. Bats constitute the second largest order of mammals. There are about 1240 bat species worldwide, which represents more than 20% of all mammalian species [24]. Bats are classified in the order Chiroptera in which there are two suborders: the Yinpterochiroptera (also known as Megachiroptera), which contains the megabats, and the Yangochiroptera (Microchiroptera), which includes the majority of microbat families [25]. The wide range of bat species could provide a large ‘breeding ground’ for viruses. The earliest known bat fossil dates to 52.5 million years ago (Mya) [26,27]. Extrapolation of fossil records and genetic data has suggested that the basal split from other placental

mammals in the superorder Laurasiatheria occurred during the late Cretaceous period approximately 80–90 Mya, with extensive diversification of extant bat families commencing approximately 62 Mya [28–30]. Bat viruses may therefore have co-evolved with or adapted to bats over many millions of years. Besides, bats are the only mammalian species that can fly and some bat species can migrate hundreds of miles to their overwintering or hibernation sites [1<sup>••</sup>]. Thus, bats have more opportunities than terrestrial mammals to have direct or indirect contact with other animal species at different geographical locations, thereby enhancing the opportunity for interspecies virus transmission. In addition, some insectivorous bats exhibit exceptionally long life-spans of 25–35 years and live in panmictic populations comprising of millions of individuals. The long life-span of bats may facilitate the transmission of chronic persistent infections, whereas the unusually large and complex structure of bat populations may ensure a sufficient number of immunologically naive juveniles for bat viruses to persist in bat colonies. Some bat species also have a capacity for hibernation over winter or to enter into daily torpor to conserve energy. The reduced body temperature and metabolic rate may suppress robust immune responses and reduce the rate of virus replication, thereby delaying virus clearance from bat populations [20,31<sup>•</sup>].

### Purely a numbers game: more bat species = more viruses?

In one of the most comprehensive reviews on bat viruses, Calisher *et al.* [1<sup>••</sup>] listed 66 different bat viruses that have either been isolated or detected. Since then, many more novel bat viruses, as well as variants of previously known bat viruses, have been reported. In total, 15 virus families — 10 families of RNA viruses and five families of DNA virus — are known to infect 75 bat genera [23<sup>••</sup>]. The detection rate of novel viruses or viral sequences appears to have been higher in bats than that in any other mammalian species for the past two decades or more.

It could be argued that, as bats represent the second largest group of mammals (comprising ~20% of all mammalian species), it is not entirely surprising that there are many bat viruses. However, some of our recent indirect evidence suggests that bats may be atypical hosts of at least some viruses. Firstly, the genetic diversity and prevalence of infection of some RNA viruses in bats is unusually high. We previously reported the detection of genetically highly diverse astroviruses and coronaviruses in bat fecal samples, with the prevalence of infection of these novel bat viruses in the range of 10–50% [32,33]. However, similar surveillance studies for astrovirus and coronavirus in rodents sampled at the same geographic location indicated that none of the samples ( $N = 441$ ) were positive for coronavirus, whereas only 1.6% of the tested Brown rat (*Rattus norvegicus*) samples ( $N = 371$ ) were positive for astroviruses [34<sup>•</sup>]. At least at this

location, bats appear to harbor many more coronaviruses and astroviruses than rodents. Secondly, phylogenetic analysis of viral sequences has revealed that a large number of coronaviruses recognized in other mammalian species share a common ancestor with various other bat coronaviruses (Figure 1). These findings suggest that bats are likely to be the natural reservoir from which all presently known mammalian coronavirus lineages have evolved [35]. The high prevalence of viral infection in bats, together with some of the unusual characteristics of bats discussed above, may have facilitated the transmission of bat viruses to other mammals.

As surveillance data for viruses in wildlife are currently scarce, it may be premature to conclude that bats host a greater diversity of viruses than other animals. For example, more than 45 hantaviruses have been identified in rodents — the largest group of mammals on earth (~40% of all mammalian species) — and each hantavirus appears to have co-evolved with a specific rodent [36,37]. Rodents are also considered to be the natural reservoir of arenaviruses with which they appear to have co-evolved [38] and waterfowls are known to be the natural reservoir of influenza viruses [39]. It is possible that bats, rodents, birds and other wildlife may be ancient reservoirs of different sets of virus taxa. Further systematic surveillance for viruses in different wildlife populations using metagenomics or other molecular approaches is required to determine if the large number of viruses identified in bats is just simply numbers game. Nevertheless, the prevalence of infection of certain bat virus families appears to be much higher than has been reported for the viral families co-evolved with rodent and avian species, suggesting that bats may have some intrinsic properties which make them more suited as a reservoir host.

### Effect of the KT extinction on ancestral bat populations and the virosphere

The five great mass extinctions that have punctuated the history of life on Earth have played a major role in shaping the modern biosphere [40] and it is reasonable to assume

that mass extinctions will also have impacted profoundly on the evolutionary history of viruses. The most recent mass extinction, the K–T extinction, occurred 65 million years ago. It followed the Earth impact of the large bolide that created the 180–300-km-wide Chicxulub crater in northern Yucatan, Mexico [41,42] and resulted in 70–80% reduction in marine diversity at the species level, 50% at the genus level, and the loss of 70% of all species worldwide [43,44]. The K–T extinction will also have impacted on viral diversity. Indeed, as the survival of virus populations is inextricably linked to the survival of their host species, the rate of virus extinction during precipitous mass extinctions is likely to have been far greater than that of their hosts. Virus extinction will have occurred not only as a consequence of host extinction but also through decreases in host population size and host isolation to a level that could not sustain ongoing virus transmission (Table 1). Even temporary host species decline or isolation, followed by recovery and survival, will have had potential for virus extinction. Survival will have favored those viruses that could persist either in the environment or in the host, those that caused no disease or mortalities, those that were transmitted vertically, those with a broad host range, and those for which the host survived with little impact on population size. Surviving host species that were largely unaffected by such a devastating mass extinction event are likely therefore to have been important sources of extant viral biodiversity.

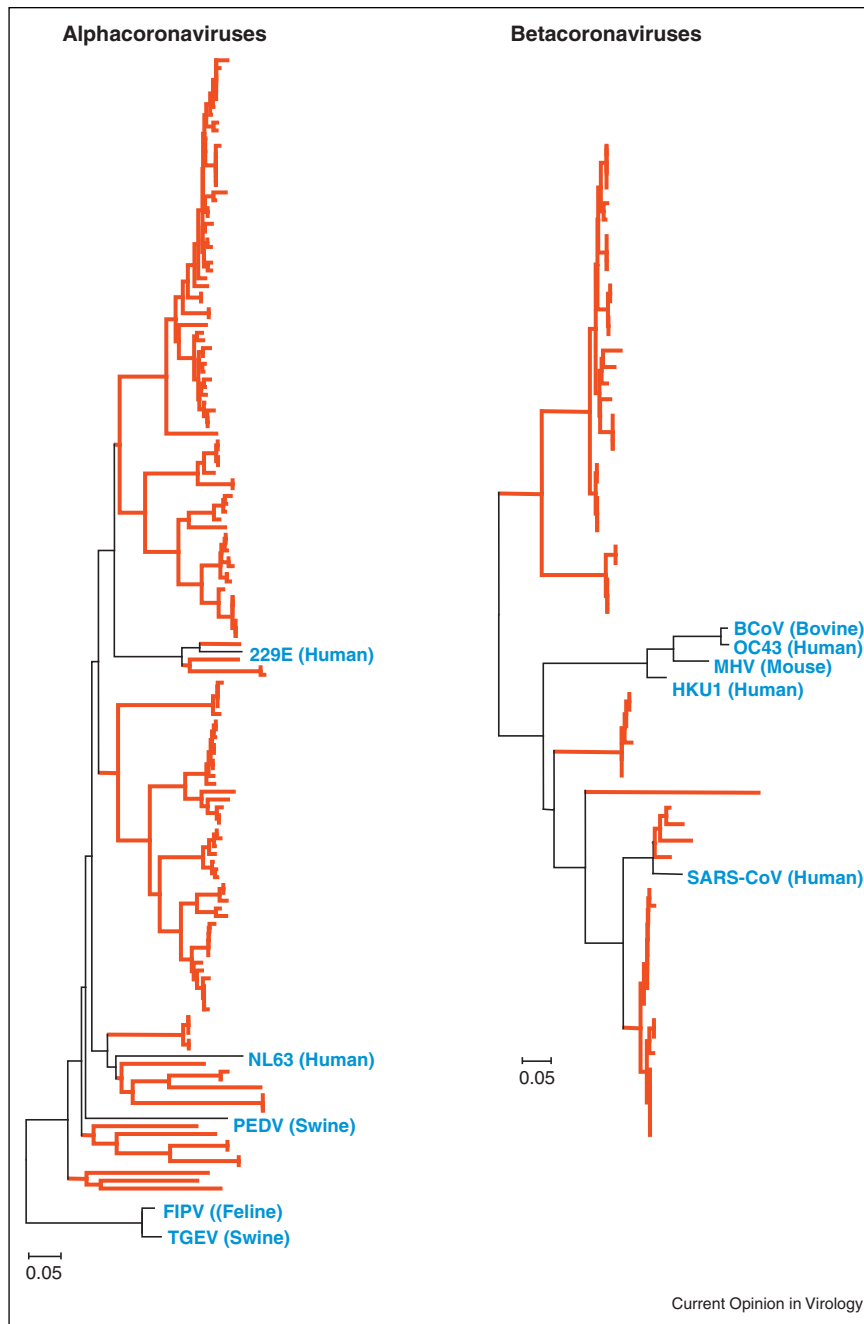
Because of the exceptional paucity of the fossil record, the evolutionary history of bats is not as well documented as many other vertebrate lineages. However, as discussed above, bats are known to have origins in the late Cretaceous Period and appear to have diversified rapidly during the period immediately after the K–T extinction [24,45,46]. It has been argued that the short intense heat pulse caused by the ballistic atmospheric re-entry of ejecta following the bolide impact created a catastrophe that set the stage for later evolutionary events [47]. Indeed, ancestral bats, rodents, insectivores and some birds are likely to have had the characteristics of animals

**Table 1**

#### Effects of the K–T bolide impact on host populations and likely consequences on the contemporary virosphere.

Impact on host/vector population	Consequence for virus population	Favorable virus characteristics
Rapid extinction	Extinction unless alternative surviving host/vector species exist	Broad host range; multiple vector species
Slow progressive extinction	Extinction unless opportunity for adaptation to new host/vector	RNA viruses; mobile hosts
Contraction and isolation	Extinction unless host/vector survives in sufficient numbers to sustain transmission ( $R_0 > 1$ ); reduced genetic diversity; appearance of new genetic lineages	Persistent infection; vertical transmission; nonpathogenic
Migration	Survival unless vector unavailable; potential for exposure to and adaptation to new hosts/vectors	RNA viruses; broad host range; mobile hosts
Contraction and recovery	Survival unless host/vector populations contract to levels that cannot sustain transmission ( $R_0 < 1$ )	Persistent infection; vertical transmission; nonpathogenic
Unaffected	Survival	All viruses

Fig. 1



Phylogenetic analyses of partial RdRp sequences of bat and other representative coronaviruses. Branches representing bat coronavirus sequences are highlighted in red. The hosts of other representative mammalian alphacoronaviruses (Left) and betacoronaviruses (Right) are in blue. All viral sequences were retrieved from Taxonomy Browser of NCBI (<http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Undefined&id=11118&lvl=3&keep=1&srchmode=1&unlock>). The tree was generated by using the Neighbor-joining method in MEGA5 (<http://www.megasoftware.net/>).

that are predicted to have survived the intense heat blast and subsequent global inferno that favored those small enough to shelter in soils, underground, deep in rock piles, or possibly in holes in very large trees (Table 2) [47]. The fossil evidence indicates that bats in the early Eocene

epoch (52.5 Mya) were small winged mammals with morphology very similar to modern species and may already have developed capacity for echolocation [48–50]. Primitive insectivorous bats sheltering in large populations in subterranean habitats may also have had the

Table 2

**Favorable characteristics for survival and proliferation following the K–T mass extinction of bats and other potential sources of extant viral biodiversity.**

Characteristic	Survival advantage	Relevant species	References
Small body size	Shelter, energy conservation	Most or all surviving animals	[47]
Sheltered habitat	Protection from initial heat blast	Bats, rodents, insectivores, some birds	[51]
Flight capability	High mobility to select suitable habitats	Bats, birds	[77**]
Roosting in large colonies	Adequate mating opportunities	Bats, birds	[78]
Hibernation, torpor	Energy conservation, resistance to low temperature, resistance to high ozone	Bats, rodents, insectivores	[79,80]
Echolocation capability	Unaffected by solar occlusion	Bats	[81]
Insectivorous	Food source remained abundant	Bats, insectivores, some birds	[82]

characteristics required for survival of the nuclear winter and food chain collapse that is predicted to have followed the initial impact [51]. Bats are now second only to rodents as the most ecologically and morphologically diverse mammalian clades, adapting to almost every terrestrial environment and accounting for over 20% of extant mammalian diversity [24]. It follows that bats may also be one of the most important sources of extant mammalian virus diversity and supports the view that bat viruses may have ancient origins and a long history of co-evolution with their hosts.

As described above, bats do appear to host a strikingly wide range of viruses and are likely the natural reservoir from which all presently known mammalian coronavirus lineages have evolved [35]. It has also been suggested that the ubiquity, wide genetic diversity and deeply rooted phylogeny of bat lyssaviruses and paramyxoviruses indicate that bats may be their natural ancestral hosts [52,53] and bats, rodents and shrew have been found to contain integrated filovirus-like genome elements that suggest a very ancient relationship [18\*\*]. Although there may not be a direct link between host diversity and virus diversity, the long evolutionary history and the ecological diversity of bats will also have presented a myriad of opportunities for cross-species transmission of viruses to and from many other host species, further enhancing their role as amplifiers of viral biodiversity. The discovery of endogenous viral elements (EVEs) integrated into animal genomes appears to provide the long-sought opportunity to trace the deep evolution of viruses and the role bats may have played in shaping the modern virosphere [54].

### Highly adapted or symbiotic relationship between bats and viruses

As discussed above, persistence in the absence of pathology or disease appears to be a common characteristic of bat viruses in their natural host population and this is also indicative of a highly evolved relationship [1\*\*,18\*\*,32,55\*\*]. The ecological balance that maintains infection and transmission in the absence of disease favors both pathogen and host and it can be argued that each may have contributed to its evolution [1\*\*]. The host aims

to detect and contain or eliminate the pathogen through an effective immune response to avoid disease or mortality. The virus needs only to maintain replication and transmission beyond the extinction threshold ( $R_0 > 1$ ) and the long-term survival of the virus may be improved if this can be achieved in the absence of disease or mortality [56]. However, the high replication rate, mutation frequency and potential for recombination of viruses, particularly RNA viruses, provide a potential for continual adaptation and refinement that far exceeds that of their hosts [57]. It could be argued, therefore, that the most significant characteristic of viral infections in bats may not be the effectiveness of a highly evolved host immune response, but rather the absence of pathology as the result of an ancient and highly evolved viral survival strategy. For many RNA viruses such as those commonly infecting bats, accessory proteins and evolved secondary functions of other viral proteins play a key role in infection by blocking host innate immune defences, modulating cellular signaling pathways and re-directing normal cellular functions [58–60]. The refinement of these functions during a long evolutionary history in bats may well have defined a successful strategy for long-term survival, even through the periods of catastrophic environmental disruption and diminished biodiversity. Conversely, the severe pathology and disease that often occurs as a result of spill-over of bat viruses into other vertebrate hosts may result not from an inherently less effective immune response but from the disturbance of this finely tuned interaction of viral proteins with their targets in host cells.

It can also be argued that there are several ways in which the harboring of well-adapted viruses might also bring a biological advantage to bats. One possibility is through symbiotic enhancement of innate immunity. Although innate immunity has long been considered a broad, non-specific and nonanamnestic first line of host defence, recent studies have demonstrated that persistent infection with one pathogen may prime host innate immunity to provide cross-protection from others. This has been best illustrated by a study in mice demonstrating that herpesvirus latency confers protection from bacterial in-



fection [61]. In a paper by Roossinck, examples of ‘good viruses’ and virus-host symbiosis have also been reviewed for viruses infecting human, wasps, plants, fungi, aphids and bacteria [62\*\*]. Highly adapted viruses persistently infecting bat populations might also serve to protect bats at the species or population level from predators (e.g., tree roosting animals such as raccoons and opossums, owls and hawks, and primates) in a sense acting as defensive ‘biological weapons’. The best defensive weapons are those that do no harm to the host species and are released only when there is an imminent threat of danger and the emerging bat viruses (e.g., henipaviruses and filoviruses) satisfy these requirements. Henipaviruses are believed to persist in bat populations at a very low viral load and are totally harmless to their natural host. However, under stress, the viral load increases, facilitating transmission to other animals [63,64]. They have a very broad range of susceptible hosts and are highly lethal in many different vertebrate species [65]. Such a mechanism might not be able to protect every individual animal in a population, but it would be an effective way to preserve the species.

In principle, such a symbiotic relationship with viruses would benefit any animal species and there is evidence that such relationships do exist in very different hosts including humans, mice to fungi and bacteria [62\*\*]. It is perhaps the long period of co-evolution and some unique selective pressures that have driven its emergence and dominance in bats. Bats have a relatively low reproductive rate (usually one birth with one pup per year) compared to other animals such as rodents and, as discussed above, bats tend to live in very large and dense populations. These biological and behavioral characteristics may demand far more robust mechanisms to fight infection and predation in order to avoid extinction.

### Flight capability, longevity and innate immunity – are they linked?

As discussed above, some of the ‘unique’ biological characteristics of bats are believed to contribute to the observation that they appear to harbor a large number of viruses without clinical signs of disease. While the scientific data are not sufficient to make any conclusive link, it is tempting to speculate on the interplay for some of these factors. In Table 3, three key aspects of the biology of bats

are analyzed in the context of their impact on cellular metabolism and infectious agents.

Flight ability is the most distinguishing feature of bats amongst mammals. Flight consumes a large amount of energy, demanding a much higher rate of metabolism. In general, it is believed that a high metabolic rate, such as that in bats, is likely to generate more metabolic byproducts, which, in turn, will increase the rate of oxidative damage to mitochondrial DNA and other cellular structures [66]. According to the ‘rate of living’ theories, animals with a high metabolic rate are likely to be short-lived [67\*,68]. Although the combination of small body size, high metabolic rate and long lifespan in bats does not seem to be compatible with this view, recent studies on mitochondrial DNA and cellular processes have indicated that multiple mechanisms exist in bats (and other long lifespan animals such as birds) to allow them to be more efficient in resisting oxidative damages than short lifespan animals [67\*].

Oxidative damage to DNA is also an important mechanism of tumorigenesis [69]. It is therefore interesting that unpublished anecdotal observations suggest that bats have a lower rate of tumorigenesis than most other animals. An extensive literature search revealed only a few recent papers describing tumors in Egyptian fruit bats [70–72]. In one case, a sarcomatoid carcinoma was diagnosed in the lung of a 10-year-old male captive bat, and in the other case a gastrointestinal leiomyosarcoma was found in a 10-year-old female bat. During our own study to establish bat cell lines, a wide international collaborative effort examining bats from Australia, Asia and Africa failed to identify any tumors from a large number of individual bats representing more than ten different bat species [73] (G. Cramer, L.-F. Wang, unpublished observations). Although the jury is still out, it is not impossible that efficient mechanisms for countering oxidative damage in bats result in a lower rate of tumorigenesis. On the other hand, it is also possible that the low reporting rate of bat tumors results from a lack of appropriate detection/diagnostic methods for bat tumors or general interest in this area of research.

Mitochondria are key organelles in controlling cellular metabolism. For bats, the efficient function of mitochondria

**Table 3**

#### Potential association of unique bat biological features with a symbiotic relationship with viruses.

Unique biological feature	Impact on metabolism	Impact on infectious agents
True ability to fly	Requiring more energy efficient metabolism	Greater chance of inter-species and long distance transmission
Rapid change of body temperature	Highly efficient sensing and regulation of temperature	Effect on immune system favoring persistence
Long lifespan relative to body size	More efficient mechanism to prevent oxidative damage to DNA	More opportunity for co-evolution and persistency

is likely to be essential for key biological characteristics such as flight, body temperature changes and lifespan, all of which could impact on the ecology of viral infection (Table 3). Until very recently, it was not recognized that mitochondria also act as a center of signaling pathways for apoptosis, inflammation and innate immune responses [74,75,76]. This is a very new and rapidly evolving field of research but it is clear that mitochondria are involved in signaling for antiviral and antibacterial immunity [76]. All published studies to date have been conducted in human or mouse cell lines so it will be extremely interesting and important to conduct parallel studies in bat cells to determine whether mitochondria have similar functions in controlling innate immune responses in bats.

In summary, we speculate that the key unique biological features of bats, that is, ability to fly, high metabolic rate and longevity, are functionally interconnected and mitochondria are the key cellular organelles that link all of these processes. These features, in turn, all have an impact on the bat's ability to control tumors and infection. This fundamental and common innate ability of bats may help explain their seemingly super anti-ageing, anti-tumor and anti-infection characteristics.

### Concluding remarks

Multiple hypotheses are presented in this review in an attempt to address the question as to whether bats are special as reservoir hosts of viruses. While we are not able to provide a definitive answer to the question, we hope that the range of new ideas and angles presented here will stimulate those who work in the field to explore further in the future. It is possible that all of the aspects discussed here, although some of them seem to be mutually exclusive, may play a part in the overall picture of high-rate detection of viruses and infection with no diseases in bats.

If bat's innate ability to counter biological imbalance proves to be different from or more robust than other mammals in whatever way or shape, it will provide a tremendous opportunity for us to 'learn from bats' and apply some of these principles to human and animal health, either via therapeutic intervention in humans or transgenic modification in livestock animals.

However, one must recognize that despite the great interest in bat viruses in recent years, bat biology research is in its infancy compared with existing knowledge of infection in humans and other animals such as rodents. There is a total lack of research tools and reagents to address any of the hypotheses in depth. Thus, there is an urgent need to advance the basic study of bat biology and bat immunology to help remove the road blocks. The following have been identified as priority areas for immediate action: (i) bat genomics and transcriptomics; (ii) establishment of different bat cell lines, both primary and immortalized lines; (iii) reagents for bat immunology

research; (iv) bat breeding colonies to facilitate infection and immunology studies; (v) establishment of genome-wide siRNA library for model bats (both microbat and megabat) to facilitate in-depth virus-host interaction study; (vi) bat physiology and behavior studies; and (vii) system biology to integrate the various aspects of bat biology which are believed to contribute to the overall virus-host interaction process.

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