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Conservation Genomics and Metagenomics of Giant and Red Pandas in the Wild

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Abstract

Giant pandas and red pandas are endangered species with similar specialized bamboo diet and partial sympatric distribution in China. Over the last two decades, the rapid development of genomics and metagenomics research on these species has enriched our knowledge of their biology, ecology, physiology, genetics, and evolution, which is crucial and useful for their conservation. We describe the evolutionary history, endangerment processes, genetic diversity, and population structure of wild giant pandas and two species of red pandas (Chinese and Himalayan red pandas). In addition, we explore how genomics and metagenomics studies have provided insight into the convergent adaptation of pandas to the specialized bamboo diet. Finally, we discuss how these findings are applied to effective conservation management of giant and red pandas in the wild and in captivity to promote the long-term persistence of these species.

INTRODUCTION

When reading the word panda, the first image that comes to people's minds might be the cuddly, adorable, and lazy black-and-white animals munching on bamboos—the star animal, the giant panda. In reality, however, the word panda was first applied to the red panda (also known as the lesser panda), a smaller, lesser-known, bamboo-eating animal with a red coat and a ring tail, in 1825. French zoologist Frédéric Cuvier first described the red panda based on a specimen from the Himalayas; he gave it the scientific name *Ailurus fulgens* (meaning fire-colored cat) and the common name panda. Later, in 1869, a large, bearlike, bamboo-eating animal was spotted by a French missionary in the Sichuan province in China, and its specimen was sent to Paris, where it was studied and given the scientific name *Ailuropoda melanoleuca*. Based on its similarity to the red panda, the larger one was then named the giant panda. As a result, from the moment giant and red pandas became known to the western world, they were regarded as closely related species.

However, as more anatomical and molecular evidence has accumulated, it has finally become clear that despite sharing a name, the two species are not phylogenetically related. Giant pandas belong to family Ursidae, and red pandas belong to their own family, Ailuridae, within the superfamily Musteloidea (1–4) (**Figure 1*a***). Although they diverged more than 40 Mya, they do share some similarities in their bamboo diet, morphology (**Figure 1*b***), and distribution (**Figure 1*c***). The extant giant pandas are endemic to six mountain ranges (Qinling, Minshan, Qionglai, Xiaoxiangling, Daxiangling, and Liangshan Mountains) in China (5), whereas the extant red pandas can be found at the southeastern and southern edges of the Qinghai–Tibetan Plateau in China, Myanmar, India, Bhutan, and Nepal (6). Giant and red pandas coexist in the bamboo forests in the Qionglai, Xiaoxiangling, Daxiangling, and Liangshan Mountains in China (**Figure 1*c***). It was estimated that the number of wild giant pandas has been increasing, reaching approximately 1,864 in 2013 (7). In 2016, it was reclassified from Endangered to Vulnerable under the International Union for Conservation of Nature Red List of Threatened Species (8). In contrast, the latest estimation of the red panda in 2015 suggests its population size has been decreasing, with fewer than 10,000 individuals in the wild, and it is listed as Endangered (9). However, a recent study presented genomic and morphological evidence of two genetically distinct and geographically separated species of the red panda: the Himalayan red panda (*A. fulgens*) in Nepal, India, Bhutan, and southern Tibet in China and the Chinese red panda (*Ailuropoda styani*) in northern Myanmar, southeastern Tibet, Sichuan, and Yunnan provinces in China (10). Particularly, the study suggested that the Yalu Zangbu River, rather than the previously believed Nujiang River, is the species distribution boundary, which was later validated by additional samples (11). Thus, it is now necessary to update some traditional views on red pandas and reevaluate the conservation statuses of the Himalayan and the Chinese red panda.

Since their discovery, pandas have drawn much attention from the general public and the academic community due to their charismatic appeal, specialized characteristics, and conservation needs. The giant panda is considered a flagship species in conservation. It receives top conservation and research priority in China, and its protection has yielded much more value than the conservation input (12). Due to the species' similar characteristics in diet specialization and sympatric distribution in China, red panda conservation and research also largely benefit from those of giant pandas, despite relative lags in conservation efforts and knowledge accumulation. Systematic studies using various techniques in different research fields (biology, ecology, genetics, and evolution) have been conducted on these species in the hope of informing better conservation practices (13–15). In particular, many cutting-edge molecular and omics approaches, such as molecular scatology, whole-genome sequencing, and metagenomic sequencing, were employed in wild giant panda research during their emerging stages. These pioneering studies took advantage of new

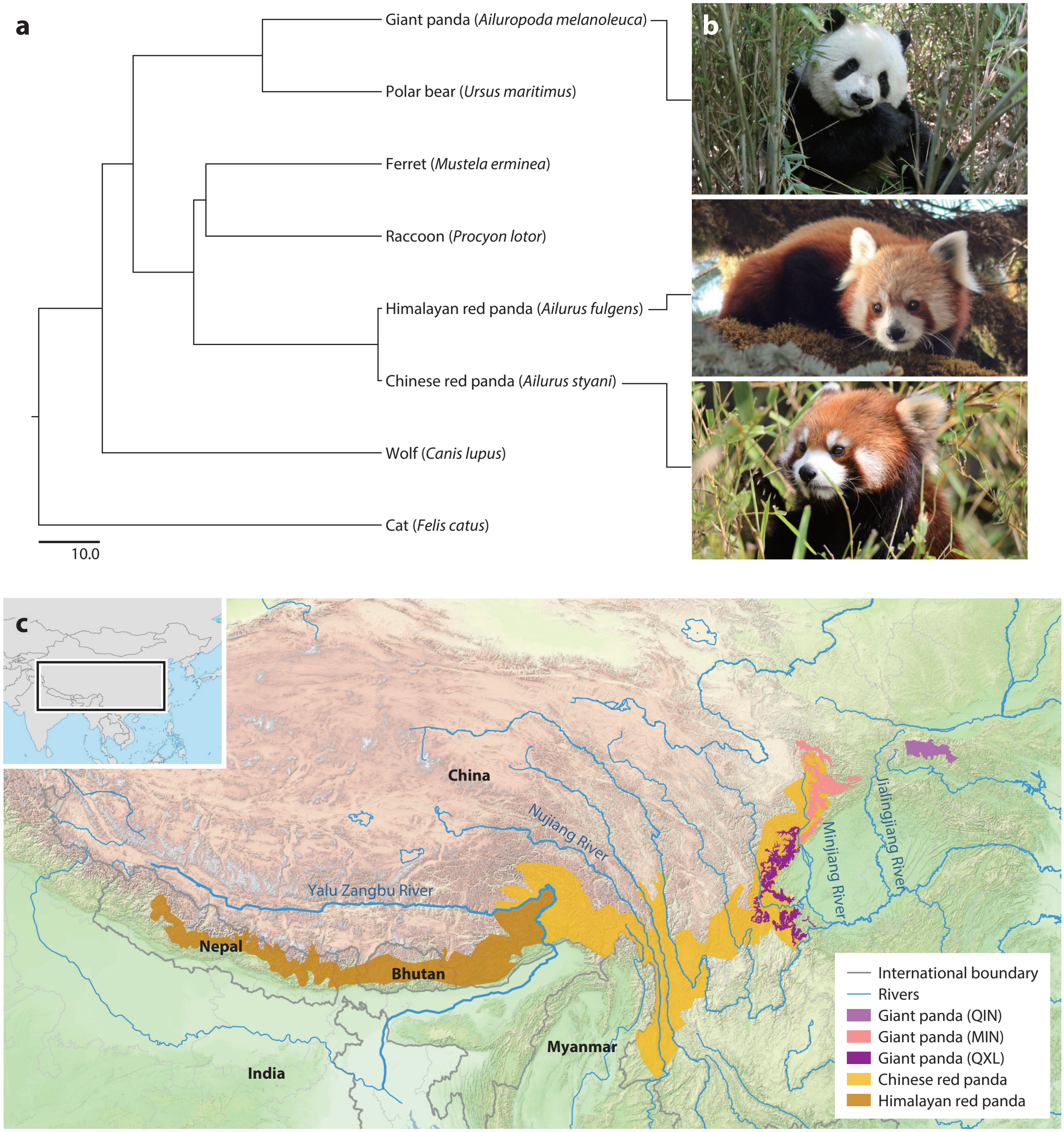

Figure 1

The phylogenetic relationship and distribution of giant and red pandas. (*a*) Giant and red pandas are members of order Carnivora. The giant panda belongs to family Ursidae, and the two red panda species belong to family Ailuridae. The branch lengths are estimated according to Hu et al. (10) and TimeTree (130). (*b*) The giant panda (*top*), Himalayan red panda (*middle*), and Chinese red panda (*bottom*). Photographs courtesy of (*top*) Wenliang Zhou, (*middle*) Arjun Thapa, and (*bottom*) Yisi Hu. (*c*) Distribution map of three wild giant panda populations and two red panda species (8, 9). The QIN giant panda population inhabits the Qinling Mountains; the MIN population inhabits the Minshan Mountains; and the QXL population inhabits the Qionglai, Xiaoxiangling, Daxiangling, and Liangshan Mountains. The giant panda and Chinese red panda coexist in the Qionglai, Xiaoxiangling, Daxiangling, and Liangshan Mountains in China.

DEVELOPMENT AND RESEARCH FOCUS OF CONSERVATION GENETICS/GENOMICS AND CONSERVATION METAGENOMICS

Conservation biology is a field with a long history, established to address the factors that threaten biodiversity and guide efforts to conserve biodiversity in the form of genetic, species, and ecosystem diversity (16, 17). Traditional conservation biology research focuses primarily on wildlife ecology, behavior, and physiology directly associated with survival. As research progresses, the emphasis has broadened to include the mechanisms underlying how species could persist in the face of intensifying anthropogenic and environmental challenges. This is also related to their genetic diversity, adaptive evolution, and capacity to respond to environmental changes.

Conservation Genetics/Genomics

With the development of molecular technologies, various molecular markers have been applied in conservation biology to resolve taxonomic uncertainties and investigate population genetics of potentially endangered species (18). The arrival of next-generation sequencing technologies has also enabled the application of genomics tools for conservation, which largely deepens and broadens the conservation research for non-model species (reviewed in 19). Combined with population genomics data, these techniques can provide information across a range of timescales, from phylogenetic status and demographic history over millions of years to precise estimates of current population parameters, such as effective population size (N_e), population structure, and inbreeding, all of which are relevant to conservation management. In addition, genomic analyses may reveal the genetic basis of adaptive traits, including population-level adaptation to local environments, which underpins predictions of potential expansion in distribution and population size, as well as vulnerability to rapid external changes, thus highlighting conservation needs and gaps (20–24).

Conservation Metagenomics

The microbiome plays a critical role in host health, nutrition, physiology, and even behavior (25–29). In the past two decades, research on symbiotic microbiomes has extended from humans to wildlife to address some novel questions in conservation. These studies apply 16S ribosomal RNA (rRNA) sequencing and metagenomics to examine symbiotic microbiome composition and function to resolve ecological, evolutionary, and conservation problems in wild animals (30–36). Specifically, the gut microbiome engages in various metabolic pathways, including carbohydrate, amino acid, and lipid metabolism, thereby influencing host animal nutrition status and health condition. To understand and emphasize the microbiome's importance to wildlife conservation, conservation metagenomics has become an emerging subdiscipline of conservation biology. This field studies the roles of the microbiota in host ecology, evolution, and physiology to inform conservation assessments, monitoring, and management of wildlife (37).

techniques, broadened their applications from model species to wild animals, and endeavored to solve problems arising from the fact that they were not designed for conservation purposes. They have thus laid a strong foundation for conservation genetics, conservation genomics, and conservation metagenomics (introduced in the sidebar titled Development and Research Focus of Conservation Genetics/Genomics and Conservation Metagenomics), and many of the findings have gone beyond published papers to real-world conservation practices. These studies not only guided panda conservation but also provided examples for the conservation of other endangered species worldwide. Below, we explore the application of genomics and metagenomics to the evolutionary history, population ecology, and adaptive evolution of wild giant and red pandas and discuss how these studies have benefited their conservation.

ORIGINS AND EVOLUTIONARY HISTORY OF PANDAS

Based on the fossil record, the direct ancestors of the giant panda can be traced back to primal pandas (*Ailurarctos lufengensis* and *Ailurarctos yuanmouensis*) that originated in southwestern China approximately 8 Mya, during the Late Miocene (38). Red pandas originated even earlier, during the Late Oligoene to Early Miocene. However, their close relatives have all gone extinct, leaving red pandas the only extant species in the family (39). What has happened over the long evolutionary histories of giant and red pandas, and what has affected their demographic dynamics and shaped their current population structure? Because genomic analyses have substantially complemented the limitations of fossils, we can now explore these questions in detail.

Population History of Giant Pandas

The first giant panda genome was sequenced in 2010 via next-generation sequencing (40), allowing us to unveil the effective population size changes from giant panda ancestors to extant species with the pairwise sequentially Markovian coalescent model (41) and to determine whether their population has been affected by climate change or human-mediated events (42). We detected two population expansions and two bottlenecks in the demographic history of giant pandas (**Figure 2*a***). The first population expansion occurred with the emergence and flourishing of pygmy pandas (*Ailuropoda microta*) 3 Mya, during a warm and wet climate period in China, which was ideal for the spread of forests. This coincides with the switch from a carnivorous or omnivorous diet in primal pandas to a vegetarian diet in pygmy pandas, as indicated by cranial and dental changes of the fossils as well as stable isotope analysis (43). Their population declined approximately 0.7 Mya and encountered its first population bottleneck 0.2 Mya, during a cold and dry glacial period. The second expansion occurred 30 to 40 Kya during the Greatest Lake Period, when baconi pandas (*Ailuropoda melanoleuca baconi*) flourished and spread throughout China to southeast Asia (44). Later, after the return of substantial glaciations, the second population bottleneck occurred around 20 Kya. The extant giant panda (*A. melanoleuca*) is believed to have originated during the Holocene. Ancient DNA and a complete paleogenome of Middle Holocene and Late Pleistocene giant pandas in their historical range also indicated other lineages that diverged from the extant giant pandas but unfortunately became extinct (45–47). The extinct lineage might have contributed to the genetic diversity of the extant species through directional gene flow (47).

Research based on single-nucleotide polymorphism (SNP) data from whole-genome resequencing of 34 wild giant pandas from different mountain ranges reveals three distinct genetic clusters of Qinling (QIN), Minshan (MIN), and Qionglai–Daxiangling–Xiaoxiangling–Liangshan (QXL) wild giant panda populations (42). The recent demographic history modeled by software ∂a∂i (48) showed that the QIN population first diverged from other populations approximately 0.3 Mya, during the onset of the Penultimate Glaciation, whereas the other cluster diverged into the MIN and QXL populations approximately 2.8 Kya. The effective population size of these populations also fluctuated in different ways, which coincides with the influence of anthropogenic disturbances of ancient kingdoms and the resulting habitat loss (42).

Population History of Red Pandas

Early studies on red pandas using mitochondrial or microsatellite DNA yielded contradictory results on their population structure and demographic history and were unable to detect significant lineage divergence to clarify the proposed subspecies differentiation of red pandas (49–51). Recent population-level genome sequencing of red pandas found that they are in fact two distinct phylogenetic species with different demographic histories (10) (**Figure 2*b***). The Chinese red panda experienced two population bottlenecks approximately 0.3 Mya and 20 Kya, presumably owing

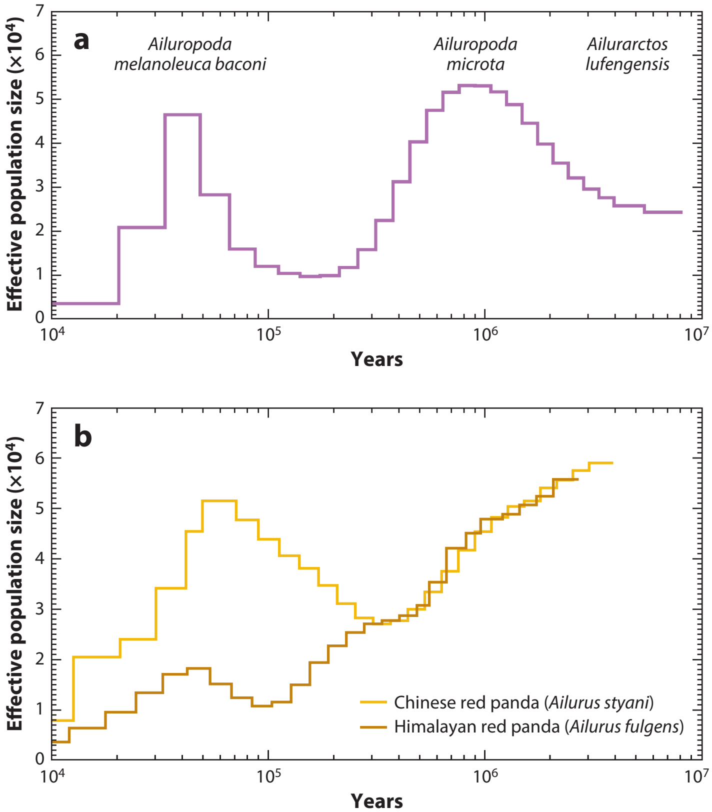

Figure 2

The population history of giant pandas and red pandas, inferred by genomic data. (*a*) Changes in effective population size from giant panda ancestors to extant species based on a pairwise sequentially Markovian coalescent (PSMC) model (adapted from 42). (*b*) The changes in effective population size of the Chinese and Himalayan red panda based on PSMC models (adapted from 10).

to population decline during several glaciations. During the interglacial period, however, it experienced a large population expansion, and the population peaked around 50 Kya. Meanwhile, the Himalayan red panda underwent three population bottlenecks and one small expansion. It diverged from the Chinese red panda 0.22 Mya after the first bottleneck (0.25 Mya) and continued to decrease until the second bottleneck (90 Kya). It then gradually increased and reached a climax at only one-third of the effective population size of the Chinese red panda 50 Kya but soon decreased in the last glaciations (10).

Results using whole-genome SNP data, maximum likelihood phylogenetic tree (52), ADMIXTURE (53), and principal component analysis (54) consistently identified four distinct genetic populations for two red panda species: the middle Himalaya (MH) population of Himalayan red panda and the eastern Himalaya–Gaoligong (EH-GLG), Xiaoxiangling–Liangshan (XXL-LS), and Qionglai (QL) populations of Chinese red panda. The EH-GLG population first diverged with other Chinese red panda clusters 0.1 Mya, and the QL population diverged from the XXL-LS population 26 Kya, during the Last Glacial Maximum. The MH, EH-GLG, and QL populations all declined after population divergence, and only the XXL-LS population increased (10).

The Influence of Paleoclimate Changes and Human Activities on Panda Population Fluctuations

As mountain-dwelling and diet-specialist species, giant and red pandas are susceptible to shifts in suitable habitats caused by paleoclimate changes and suffered range and population fluctuations during glacial–interglacial cycles in the Pleistocene. After Pleistocene glaciation episodes, the interglacial warm climate and vast mountain areas provided suitable habitats for the rapid expansion of giant and Chinese red pandas distributing in the Hengduan Mountains (10, 55). However, Himalayan red pandas from the platform and southern edge of the Qinghai–Tibetan Plateau were more severely affected by glaciers and recovered slowly in limited potential habitats throughout the interglacial period.

In the recent past, habitat fragmentation, along with other human influences, may have arisen as the primary driver underlying population divergence and decline for these animals (10, 42, 51). The habitats of giant and Chinese red pandas largely overlapped with prosperous areas of many dynasties in Chinese history for more than 3,000 years; deforestation and land use change thus may have resulted in the decline and isolation of different populations. For instance, in the Xiaoxiangling Mountains, where giant and Chinese red pandas coexist, the giant panda population is estimated to have decreased by approximately 60-fold over the past 250 years based on microsatellite data (56), whereas the red panda population is estimated to have declined by 98% starting more than 1,000 years ago (51). The historical human population explosion and introduction of non-native crop species (such as potatoes and maize) in the Xiaoxiangling Mountains during the Qing Dynasty led to land use change and forest fragmentation, which may have reduced the giant panda habitat by as much as 31-fold (56). Taken together, the demographic history and population structure of wild pandas help to decipher the complex reasons for their current situation and inform science-based conservation decisions for species or populations with different genetic backgrounds.

POPULATION GENETICS AND GENOMICS OF WILD PANDAS

Genetic diversity is fundamental to species' evolutionary potential to respond to environment change (57, 58). Higher genetic diversity often indicates greater evolutionary potential. In contrast, the loss of genetic diversity has been considered a sign of risk for endangered species. Therefore, conservation of genetic diversity is also deemed an important goal in global conservation plans, such as the Kunming–Montreal Global Biodiversity Framework adopted by the Convention on Biology Diversity (59). Through large-scale sampling of genetic data and application of population genetics and population genomics approaches, the genetic diversity of the wild population could be estimated, along with other key parameters that reflect population connectivity, such as actual population size, gene flow, and inbreeding level. Maintaining sufficient gene flow and avoiding inbreeding are essential to sustaining genetic diversity, which is fundamental to ensure long-term population viability.

Genetic Diversity and Landscape Genetics of Wild Giant Panda Populations

It has long been suspected that the small population size and low genetic diversity of giant pandas were important drivers of their endangerment (60, 61). Since the 1970s, the Chinese government has undertaken four national population censuses on wild giant pandas. Because giant pandas are elusive animals that are difficult to follow in the bamboo forest, accurate individual identification and population surveys have posed a challenge. This situation changed when we successfully established a molecular scatology method for giant pandas, involving extracting DNA from fresh feces, and conducted genotyping using microsatellite techniques (62), which doubled the previous population size estimate in the study areas using conventional methods based on fecal characteristics such as bamboo bite length (63). This method was subsequently introduced into the Fourth National Giant Panda Survey and routine population monitoring. We then collected fecal and tissue samples from wild pandas across the six mountain ranges they inhabit and quantified their genetic diversity on a large scale. Based on mitochondrial and nuclear markers, we found giant pandas still have a medium to high level of genetic diversity compared to other wild animals (55, 56, 64), indicating high evolutionary potential to adapt to environmental changes. Whole-genome SNP data also confirmed this conclusion (40, 42). It is also suspected that massive bamboo flowering and die-off events in the Minshan Mountains in the 1970s and the Qionglai Mountains in the 1980s contributed significantly to the wild population decline. By comparing the effective population size and genetic diversity of wild populations before and after the bamboo flowering events, we found that bamboo flowering did not seriously impact the population viability of the giant panda, indicating its resilience to environmental stochasticity (65). In addition, long-term monitoring of reproductive activities, sampling of mother–cub pairs, and large-scale genetic analysis of wild QIN giant pandas did not find high levels of inbreeding; however, the level of inbreeding is still greater than expected for a solitary mammal (66).

By combining landscape ecology and noninvasive genetics data, researchers can explicitly quantify how landscape features may affect the spatial patterns of population structure and gene flow, as well as identify barriers between subpopulations. A series of studies found that the dispersal of wild giant pandas is related to landscape features such as habitat heterogeneity and bamboo resource availability (67, 68). A fine-scale landscape genetics study indicated that the QIN population is a continuous genetic cluster with gene flow facilitated by an easterly slope aspect (representing preferable microclimatic conditions for pandas and bamboos) and constrained by topographic complexity (representing obstruction for movement) (69). However, natural barriers and human disturbance (such as road construction) have impacted gene flow between populations, resulting in the isolation of small subpopulations, such as the wild pandas in the Daxiangling and Xiaoxiangling Mountains (70). Restoration of landscape connectivity and construction of corridors between habitat patches guided by these findings could facilitate dispersal and gene flow of wild populations.

Taken together, the establishment of noninvasive genetics and advancements in population genomics demonstrate that the population of wild giant pandas is increasing, and their genetic diversity remains high with substantial gene flow, strongly refuting the pessimistic view that population crash and low genetic diversity of wild giant pandas will lead to further decline and extinction (14). However, if habitat fragmentation and population isolation continue, there will be irreversible damage to the genetic diversity and population persistence of wild giant pandas (65).

Genetic Diversity and Gene Flow of Wild Red Panda Populations

Compared to the in-depth research on giant pandas, population genetics and genomics studies on red pandas are substantially inadequate. Most of these studies were undertaken in China, few in India and Nepal, and none in Bhutan and Myanmar, possibly owing to a lack of research resources in these countries (71, 72). Population genetics research on wild red pandas started in China in the early twenty-first century (49, 50). In 2011, a comprehensive study that incorporated tissue and fecal samples from wild red pandas across their distribution range in China and northern Myanmar reported high microsatellite diversity compared to other endangered carnivore species, including the giant panda (51). Other work using microsatellite data on Himalayan red pandas in India reported lower genetic diversity compared to those of the Chinese red panda populations (73). Consistent with the microsatellite data, whole-genome analysis based on SNP information also revealed that the MH population has lower genetic diversity than do all Chinese red panda populations. It also has a higher level of linkage disequilibrium and carries more homozygous loss-of-function and deleterious mutations, which collectively reflect the genetic consequences of long-term population bottlenecks in its demographic history and risk of future population decline (10). In addition, little research has been conducted on the landscape genetics or current gene flow of red pandas. A study found asymmetric gene flow of Himalayan red pandas from west to east in 10 protected areas in India and concluded that landscape heterogeneity and habitat suitability may account for the asymmetric migration (73). Apart from these results, there is an urgent need to assess other red panda populations in the wild across their entire range to update the current knowledge on population size and genetic diversity after the split of the two red panda species.

Female-Biased Gene Flow in Giant and Red Pandas

Wild giant pandas exhibit a female-biased dispersal pattern, according to the genetic structure revealed by microsatellite data (67, 74), which contrasts with the male-biased dispersal seen in most mammals (75). Field observations of subadult female pandas leaving their mothers' home ranges and dispersing long distances to establish their own home ranges also corroborate these findings. Genetic analyses suggest that the female-biased dispersal strategy could facilitate inbreeding avoidance in this solitary animal (66). Interestingly, the female-biased gene flow pattern is also found in the red panda based on population genomic analyses. The haplotypes of Y-chromosome SNPs and mitochondrial genomes presented different phylogeographic structures; the distribution of mitochondrial haplotypes was mixed regardless of geographic origin, whereas the distribution of Y-chromosome SNP haplotypes exhibited a clear phylogeographic structure in accordance with sample origins (10). However, the ecological and reproductive importance of this pattern has yet to be determined in red pandas.

In these genetics and genomics studies, new data and methodologies help bridge the gap between field observations of animal behavior, landscape analyses, and population structure, thus shedding light on animal ecology and genetic outcomes. Understanding wild panda dispersal patterns is beneficial for planning effective conservation strategies to facilitate their movement and reproduction.

ADAPTIVE EVOLUTION OF PANDAS REVEALED BY GENOMIC AND METAGENOMIC RESEARCH

As mentioned above, as members of order Carnivora, giant and red pandas' most peculiar characteristics are their dietary change and specialization on bamboos. Giant and red pandas feed almost exclusively on bamboo (>90%) in the wild. They seasonally forage on the leaves and shoots of different bamboo species, and giant pandas also consume bamboo stems in the winter (76–80). However, bamboo is considered a low-nutrition and low-energy food, because it typically contains only 20–30% protein, fat, and soluble carbohydrate, whereas the other 70–80% consists of cellulose, hemicellulose, and lignin that mammals themselves usually cannot digest. A series of questions arise concerning the diet specialization of giant and red pandas. How do they handle and consume this tough plant? How can they endure its bitter flavor? How can they digest and absorb the nutrients from bamboo, all by themselves or with the assistance of symbiotic gut microbiomes? How can they survive on the limited energy obtained from this low-nutrition diet? And if we comprehend their feeding ecology and evolution, what measures can we take to benefit their conservation?

The bulk of the research has revealed similar phenotypes and convergent evolution in relation to the bamboo diet of giant and red pandas (mostly represented by Chinese red panda) at

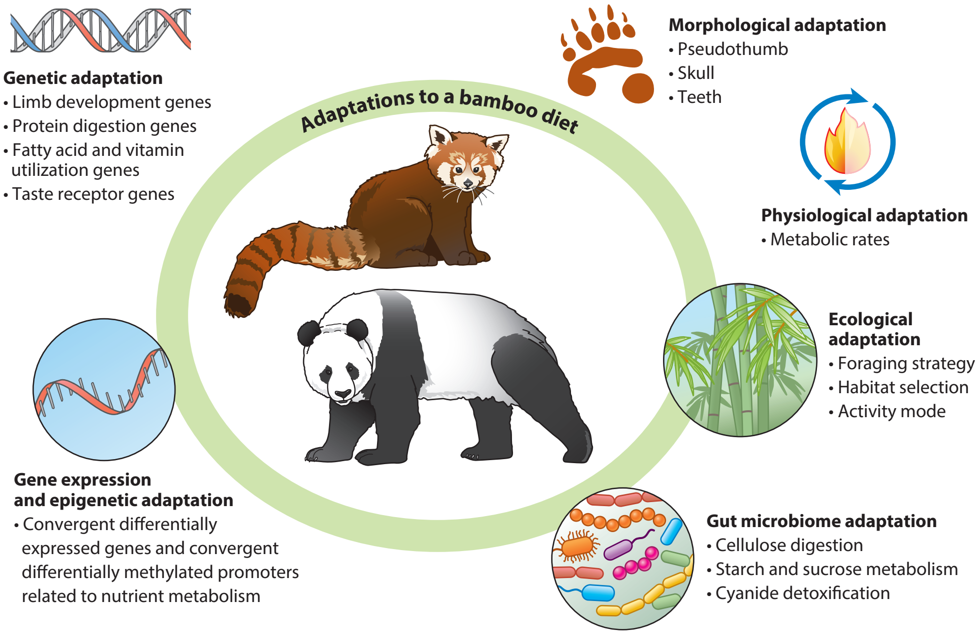

Figure 3

The adaptive evolution of giant and red pandas to diet specialization on bamboo. The giant and red pandas exhibit similar adaptive phenotypes related to diet specialization on bamboo, including morphological, physiological, ecological, genetic, epigenetic, and gut microbiome adaptation.

multiple levels, including morphological, physiological, ecological, genetic, epigenetic, and gut microbiome adaptation (**Figure 3**). For instance, to feed on such a low-nutrition and high-fiber food, pandas need to spend long hours feeding, and both species evolved similar structures of abnormally enlarged wrist bones, known as pseudothumbs, to manipulate bamboo food while feeding (81, 82). Comparative genomics research found that this phenotypic convergence may be driven by genetic convergence of two genes involved in limb development, *DYNC2H1* and *PCNT*, which are positively selected with convergent amino acid substitutions in both pandas but not in other mammals investigated. Mutations in these genes could cause polydactyly and abnormal skeletogenesis phenotypes in mice and humans, and their adaptive changes in both pandas may contribute to pseudothumb development (6). Both pandas also show morphological changes in their skulls and teeth to break down bamboo fibers (83). In addition, bamboo is not an appetizing food due to its low umami flavor and high bitter-tasting components from secondary plant compounds. The umami taste receptor gene *TAS1R1* has been pseudogenized in both pandas (6, 84), preventing them from sensing umami-rich chemicals such as proteins. This may be an evolutionary response to the dietary shift from carnivory and omnivory to herbivory. In contrast, the copy numbers of intact taste 2 receptor genes (*TAS2R*) that mediate bitterness perception have increased in both pandas' genomes compared to those of other carnivores, but the pseudogenized genes, purifying selected genes, and positively selected genes evolved independently in giant and red pandas, indicating different selective pressures on taste receptor genes during their dietary changes (85). Comparative genomic analyses also found convergent evolution in several positively selected genes involved in the digestion and utilization of bamboo nutrients, including serine protease genes (*PRSS1*, *PRSS36*, and *CPB1*) and several genes related to fatty acid and vitamin utilization (*ADH1C*, *CYP3A5*, *CYP4F2*, and *GIF*). Recent comparative transcriptomics studies comparing both pandas and other non-herbivorous mammals identified convergent differentially expressed genes related to carbohydrate metabolism, lipid metabolism, and lysine degradation in the liver and pancreas of giant and red pandas. The differential expression of these genes may be governed by convergent differential DNA methylation in promoter regions (86). Similarly, convergent differentially expressed genes and differentially methylated promoters related to nutrient metabolism are also found in the stomach and small intestine of both pandas (87), together acting as adaptive responses to the high-carbohydrate, low-lipid and -lysine bamboo diet at the gene-expression and gene-regulation levels.

Despite these genomic and epigenomic alterations, both pandas still possess carnivore-like digestive tracts that are not designed for plant digestion. Giant pandas digest only approximately 17% of the dry matter in bamboo leaves and stems and 40% in bamboo shoots (76). Red pandas use bamboos more efficiently than giant pandas, because they digest approximately 30% of the dry matter in bamboo leaves and 46% in bamboo shoots (78). They also have poor digestibility for bamboo hemicelluloses (27% for giant pandas and 28% for red pandas) and celluloses (8% for giant pandas and 3% for red pandas) (78, 88). Because their genomes lack genes encoding the cellulose digestion enzyme, we decided to investigate the role of the symbiotic gut microbiome by applying 16S rRNA gene sequencing and metagenomic sequencing to panda fecal samples. In 2011, Zhu et al. (89) uncovered the dominant presence of *Firmicutes*, a phylum of bacteria with putative genes encoding cellulose- and hemicellulose-digesting enzymes (such as cellulase, β-glucosidase, and xylan 1,4-β-xylosidase) in giant panda feces, suggesting the potential function of the gut microbiome in digesting bamboo. Nonetheless, the gut microbiota composition of both giant and red pandas resembles that of carnivores and not of herbivores such as ruminants (89–92), indicating phylogenetic effects on symbiotic relationships between animal hosts and their gut microbiota. Upon closer examination of the gut microbiota of pandas and their relatives, we found that giant and red pandas share more similarities in gut microbiota with each other than each species shares with its carnivorous relatives (i.e., giant panda versus polar bear, red panda versus ferret), in both microbiota composition and function (93). The 115 operational taxonomic units giant and red pandas share were mostly from *Firmicutes* and *Proteobacteria*, accounting for 91.6% and 79.7% of their gut microbiota, respectively. Interestingly, metagenomic analysis indicated that the gut microbiomes of both pandas possess a high level of starch and sucrose metabolism and vitamin B12 biosynthesis, which enhances the potential for bamboo digestion and nutrient consumption (93). Their gut microbiomes also harbor more putative genes encoding cyanide detoxification enzymes than those of other herbivorous mammals (94). Therefore, despite their anatomical and physiological constraints as carnivores, giant and red pandas have evolved to structure their gut microbiome to digest bamboo. This also represents a case of convergent evolution between giant and red pandas in terms of gut microbiome.

To gain an integrated understanding of the function of symbiotic bacteria in diet adaptation, a series of studies combined field observation, nutritional ecology, metagenomics, metabolomics, and experimental validation to unveil the interplay between the host and symbiotic microbiome (**Figure 3**). Research found that seasonal dietary shifts and nutrient variation possibly shape the seasonal fluctuations in gut microbiome composition and function of both giant and red pandas (95–97). Wu et al. (95) reported that the giant panda gut microbiome during the shoot stage was always richer and more diverse than during the leaf stage. In the leaf stage, bacteria overrepresented genes involved in raw fiber utilization and cell-cycle control, guaranteeing raw fiber use during the nutrient-deficient leaf stage. During the protein-abundant shoot stage, gut microbiome functional capacity expanded to include prokaryotic secretion and signal transduction activity, indicating active gut microbiome–host interactions. Follow-up experiments applying fecal microbiota transplantation from giant pandas into a germ-free mouse model demonstrated that seasonal fluctuations in the gut microbiome could synchronize the host's metabolic activities and were congruent with the significant increase in host body mass during the shoot stage. In particular, transcriptomics and validation experiments showed that the butyrate-producing bacterium *Clostridium butyricum* that is abundant in the shoot stage could extend the upregulation of hepatic circadian gene *Per2* and subsequently increase phospholipid biosynthesis, compensating for the seasonal lack of essential nutraceutical phospholipids to maintain host hepatic health and growth (98). In this manner, the gut microbiome may contribute to phospholipid circulation and fat storage in the giant panda to facilitate its adaptation to the low-fat diet. A metagenomics and metabolomics study also revealed that the seasonal fluctuation of flavonoid (a group of antibacterial plant secondary metabolites) contents in giant panda's diet may influence the seasonal dynamics of their gut microbiota diversity and microbial virulence factors (99).

Another challenge the bamboo diet imposes on the animals is the energy limitation. Giant pandas have evolved in multiple aspects to minimize their energy demands and expenditure, including low physical activity, reduced organ size, reduced metabolic rates, and low thyroid hormone levels (100, 101). In the giant panda genome, dual-oxidase 2 (*DUOX2*), a gene critical for thyroid hormone synthesis, contains a giant panda–unique single-nucleotide mutation that results in a premature stop and possibly a nonfunctional protein (100). Experiments using gene-edited mice confirmed that the same giant panda–unique point mutation could cause metabolic phenotypes in mice in body size, food intake, physical activity, organ size, serum thyroxine level, daily energy expenditure, and even gut microbiota, demonstrating that this mutation identified by genomic analysis may explain the profound adaptive changes in giant panda (**Figure 4**). Therefore, it links the genetic mechanism of metabolic phenotypes and the ecological consequences important in adaptive evolution (102).

Taken together, these studies imply diet-driven gene evolution and coevolution of host and symbiotic microbiome in giant pandas and red pandas, therefore integrating their phenotypes with underlying genetic and evolutionary mechanisms to elucidate the biology and conservation of these endangered species.

Local Adaptation of Giant and Red Pandas

Because the QIN population of giant pandas has evolved independently since it diverged from other clusters 0.3 Mya, it may display distinctive features of local adaptation. Based on analyses of fixation index (F_{ST}) that identified positively selected genes between different populations (103), we found that genes involved in the sensory system were positively selected across QIN and non-QIN populations of the giant panda, including a bitter taste receptor gene (*Tas2r49*) that was positively selected at two nonsynonymous sites in the QIN population (42). Functional expression of this receptor in engineered cells revealed that it is specifically activated by quercitrin, and the receptor variants carried by QIN pandas confer a significantly decreased sensitivity to quercitrin compared to the wild type found in other populations. This is consistent with the observation that QIN pandas consume more bamboo leaves containing more bitter-tasting quercitrin than do non-QIN pandas. The reduced sensitivity to quercitrin may make the bitter bamboo leaves in the Qinling Mountains taste less bitter to pandas, thus explaining their dietary preference toward bamboo leaves (104). Apart from the genetic adaptation of the panda population, comparative metagenomic studies on the gut microbiomes of wild pandas also reveal differences in microbiota structures between different populations, indicating local-scale coadaptation of host and gut microbiota (105, 106).

Similarly, it is not surprising that two red panda species living in different geographic ranges may have adapted to their local environments through genomic mechanisms. F_{ST} and θ_{π} analyses

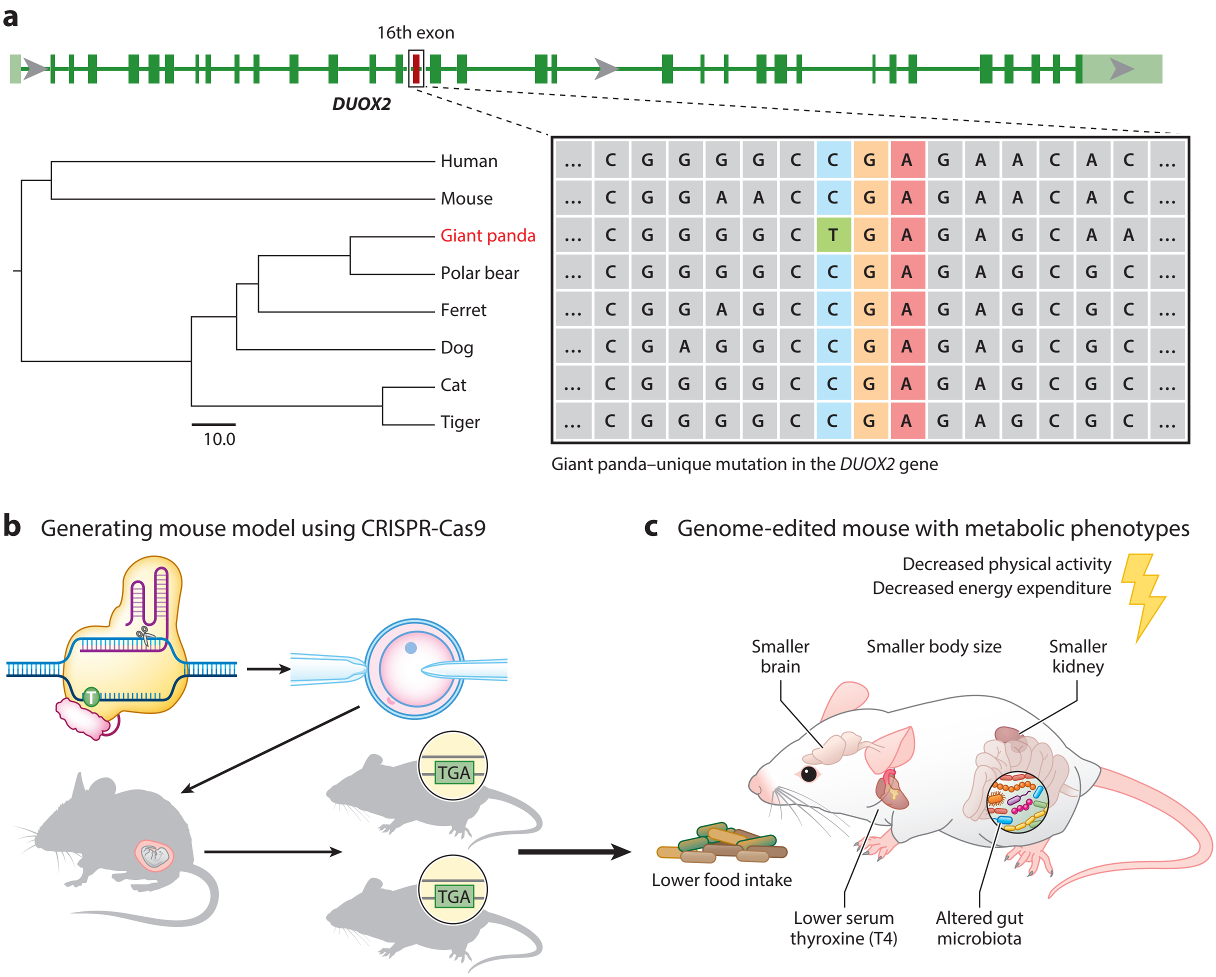

Figure 4

The giant panda–unique point mutation in the *DUOX2* gene and metabolic phenotypes of the genome-edited mouse model carrying the same mutation. (*a*) Gene structure of *DUOX2* and the giant panda–unique single-nucleotide mutation in the sixteenth exon that causes a premature stop codon. The *DUOX2* gene encodes an enzyme participating in thyroid hormone synthesis. (*b*) The main procedures in generating the genome-edited mice using CRISPR-Cas9. (*c*) Metabolic phenotypes of the mutant homozygous mice.

(103, 107) identified genes being selected among the two species. The Himalayan red panda showed genomic signatures of selection in genes associated with coat color and adaptation to hypoxia and a high-elevation environment. In Chinese red pandas, selected genes were found to be related to adaptation to high ultraviolet radiation and hypoxia in the Hengduan Mountains (10). These findings again highlight the importance of considering genetic background in conservation management and captive breeding.

CONSERVATION IMPLICATIONS BASED ON GENOMICS AND METAGENOMICS RESEARCH IN WILD PANDAS

Scientific research on wild giant and red pandas has progressed for nearly 200 years since their discovery by the scientific community. From early field observation to modern GPS collar tracking of animals and biochemical analyses of their food and feces, and to the development of genetics, genomics, and multi-omics, researchers have always attempted to relate their studies to the conservation of these endearing yet endangered species. Specifically, the advances in conservation genomics and metagenomics have provided unprecedented power to look into the biology, ecology, and evolution of wild giant and red pandas. When we finally gather all this information, what can we do to apply this knowledge to their conservation?

Evolutionary Potential of Wild Pandas

Genomic studies have revealed the evolutionary histories of giant and red pandas, allowing us to understand the causes and processes of their endangerment. These studies demonstrate that paleoclimate changes, recent human population expansion, and current natural and anthropogenic barriers are the key drivers of their population decline in different time periods, resulting in the genetic differentiation of current species and populations. For example, compared to the Chinese red pandas, the Himalayan red pandas have experienced long-term population bottlenecks that have severely impacted their genetic diversity and evolutionary potential. The extant wild giant pandas and Chinese red pandas still retain high genetic diversity, indicating high evolutionary potential to respond to future changes. However, populations in the Qinling Mountains have the lowest genetic diversity compared to their conspecific populations, which warrants further conservation concern. In addition, comparison of genomic research based on ancient and historical specimens and extant populations may provide complementary information on changes in genetic diversity and effective population size, which could be incorporated into future conservation policies (108).

Identifying Evolutionarily Significant Units and Management Units for Conservation

Explicitly identifying different species and populations is the prerequisite for effective conservation management of wildlife. The identification of two phylogenetic species of red pandas, as two evolutionarily significant units, urges the need to clearly distinguish between them in assessing their conservation status in a timely manner and developing corresponding conservation plans, taking into account the different environments they inhabit, threats they face, and socioeconomic contexts of their conservation (71). In addition, the historically incorrect distribution boundary between the two red panda species separated the genetically consistent EH-GLG population into two species/subspecies. This may have led to inappropriate management and breeding plans for red pandas in captivity, which should be strictly avoided in the future. Genetic differentiation across Chinese red panda populations suggests that they should be treated as three independent management units (EH-GLG, XXL-LS, and QL) (10, 51), and the knowledge gap in the population genetics or genomics of Himalayan red pandas remains to be filled.

The divergence of wild giant panda populations (QIN, MIN, and QXL) also indicates that they are genetically unique, with specific adaptation to their local environments; hence, they should be regarded as different management units. Anthropogenic gene flow among different genetic populations should be avoided, and the genetic background of individuals involved in conservation interventions such as captive breeding and reintroduction should be considered carefully to prevent hybridization of highly differentiated genetic populations or mismatch between the genetic background and targeted translocation population. In addition, female individuals have inherent advantages in reintroduction projects due to their ability to bear life and the female-biased dispersal pattern that facilitates their settlement in new environments. Under the scientific guidance of genetic rescue, targeted actions have been conducted to aid in population recovery of wild giant pandas (109, 110). Long-term post-release monitoring suggests that several reintroduced giant pandas have survived in the wild and established their own home ranges; one female panda even successfully reproduced an offspring (111). In addition, genetic surveys of giant pandas from four breeding centers in China provide guidance for maintaining the genetic diversity of captive breeding populations and reducing inbreeding and outbreeding based on the genetic backgrounds of individuals (112).

Implications for Habitat Conservation and Protected Area Design

Understanding the population ecology of wild animals, including the pattern of gene flow and dispersal, the impact of habitat loss and fragmentation, and animals' resilience to environmental changes, is another crucial step in devising effective conservation strategies to promote the survival and reproduction of animals in the wild. The Fourth National Giant Panda Survey found wild giant panda populations were divided into 33 local subpopulations of various sizes, including 18 subpopulations with fewer than 10 individuals (7). To facilitate gene flow and population expansion, academics, local governments, and nongovernment organizations have collaborated to construct several habitat corridors within QIN, MIN, and QXL populations, respectively, which have been used successfully by wild giant pandas (65, 113–115). More excitingly, 60 years after the establishment of the first nature reserve in China (116), Giant Panda National Park has been designed and constructed based on existing nature reserves, protected areas of different categories, natural heritage sites, forest parks, scenic areas, and other kinds of natural sites, spanning three provinces, covering an area of 27,000 km^2, and including 70% of giant panda habitats and 88% of known wild populations. The national park–centric protected-area system will hopefully improve connectivity between isolated habitats and help maintain a sustainable population in the wild (117–119). Other sympatric species living under the protection of this umbrella species will also benefit from the construction of Giant Panda National Park.

However, for red pandas, despite the urgent need to expand protected area coverage for both species (120), significant knowledge gaps exist in integrating landscape ecology and population genetics. Research based on landscape genetics of Himalayan red pandas advises enhancing habitat protection and connectivity among isolated populations to promote gene flow in the Kangchenjunga Landscape in India (73). Much remains unknown in other regions and countries, notably in Bhutan and Myanmar, where research on wild red pandas is largely underrepresented. Consequently, more focused research and conservation plans are needed across the distribution ranges of the two red panda species. Due to the transboundary distributions of both red panda species, landscape and transboundary studies on metapopulations are needed to inform regional as well as transboundary conservation planning.

Health and Disease Management Based on Metagenomics Research

Research on the evolutionary adaptation of giant and red pandas indicates that they have become well-suited to the bamboo diet during their long evolutionary history. Apart from connected bamboo forests of suitable bamboo species, they also rely on a wide variety of bacteria to facilitate digestion of and nutrition intake from bamboo, both in the wild and in captivity. Maintaining the indigenous gut microbiota of wild and captive pandas has thus emerged as an important aspect of their conservation and fitness improvement. Abuse of antibiotics in captive breeding and antibiotic pollution in the wild warrant specific attention (121). The transmission of pathogens from humans or livestock to wildlife also poses a threat for the maintenance of their indigenous microbiomes. Monitoring of the compositional and functional changes of the gut microbiome, as well as the presence and transmission of other parasites (122) and viruses (123, 124), using metagenomic techniques on fecal samples provides potential avenues for noninvasive health management of wild distributed and captive breeding pandas, as well as assessment of the adaptation of reintroduced individuals. The patterns of microbial diversity and early indicators of imminent microbial disruption help inform disease prevention and treatment. Metagenomic analysis combined with gas chromatography–mass spectrometry on the anogenital gland secretions of giant pandas also revealed that the microbiome of this scent gland participates in the production of chemical compounds used for olfactory communication in reproduction. The difference in microbial communities between wild and captive giant pandas implies that inappropriate microbiota may interfere with chemical communication and reproduction in captivity (125). Future research that combines metagenomics, metatranscriptomics, metaproteomics, and metabolomics may provide valuable information for monitoring the nutritional status, controlling the pathological microbes, improving the physiological condition, and assisting the reproduction of the animals.

PROSPECT

With increasing attention being paid to global change and loss of biodiversity, it is critical to understand the past, present, and future challenges that wildlife face and to use available tools for wildlife management and conservation. Over the past two decades, the development of sequencing techniques and accompanying analysis tools has aided substantial progress in the field of conservation biology, allowing us to better understand a wide range of questions regarding the evolutionary history, endangerment processes, population structure, adaptive evolution, and current status of endangered wildlife (126–128). These findings should give us more faith as well as a sense of urgency to continue paving the route for the brighter future of their recovery.

In addition, the portable and user-friendly sequencing equipment now available also opens up the window of immediate data generation in field study. The emerging telomere-to-telomere genome sequencing technique also offers the opportunity to build a digital Noah's ark containing the complete genomes of endangered species, which may one day be employed in de-extinction efforts (129). Although more and more studies are applying genomics, metagenomics, and multiomics (including transcriptomics, epigenomics, proteomics, and metabonomics) approaches to endangered species, there is a critical need to better integrate the findings into readily understood information that can inform policy decisions and conservation actions. With all the information in hand, we have a greater chance to halt the population decline of not only wild pandas but other wildlife, and eventually reverse the trend of biodiversity loss.

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