Giant Pandas Are Not an Evolutionary cul-de-sac: Evidence from Multidisciplinary Research

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Associate editor: Bing Su

Abstract

The giant panda (*Ailuropoda melanoleuca*) is one of the world's most endangered mammals and remains threatened by environmental and anthropogenic pressure. It is commonly argued that giant pandas are an evolutionary cul-de-sac because of their specialized bamboo diet, phylogenetic changes in body size, small population, low genetic diversity, and low reproductive rate. This notion is incorrect, arose from a poor understanding or appreciation of giant panda biology, and is in need of correction. In this review, we summarize research across morphology, ecology, and genetics to dispel the idea, once and for all, that giant pandas are evolutionary dead-end. The latest and most advanced research shows that giant pandas are successful animals highly adapted to a specialized bamboo diet via morphological, ecological, and genetic adaptations and coadaptation of gut microbiota. We also debunk misconceptions around population size, population growth rate, and genetic variation. During their evolutionary history spanning 8 My, giant pandas have survived diet specialization, massive bamboo flowering and die off, and rapid climate oscillations. Now, they are suffering from enormous human interference. Fortunately, continued conservation effort is greatly reducing impacts from anthropogenic interference and allowing giant panda populations and habitat to recover. Previous ideas of a giant panda evolutionary cul-de-sac resulted from an unsystematic and unsophisticated understanding of their biology and it is time to shed this baggage and focus on the survival and maintenance of this high-profile species.

Key words: **adaptation, diet specialization, evolutionary dead-end, genetics, giant panda.**

Introduction

The giant panda (*Ailuropoda melanoleuca*) is one of the most endangered mammals on earth, a flagship species for wildlife conservation and an adored icon on every inhabited continent. Its evolutionary history can be traced back 7–8 Ma to the late Miocene and the earliest recorded ancestor *Ailurarctos lufengensis* in Yunnan province, China (Qiu and Qi 1989). Later, *A. microta* appeared in the early Pleistocene and had the smallest body size of all known pandas, but sometime in the mid-Pleistocene it evolved into the largest known panda, *A. melanoleuca baconi* (Pei 1974; Wang 1974; Jin et al. 2007). The current giant panda, *A. melanoleuca*, arose in the Holocene, and because of human-induced habitat loss and fragmentation remains restricted to six isolated mountain ranges across China: The Qinling, Minshan, Qionglai, Liangshan, Daxiangling, and Xiaoxiangling Mountains (Schaller et al. 1985; Hu 2001; Wei et al. 2012). According to the Third National Survey of Giant Pandas completed in 2002, it was estimated there are 1,596 animals in the wild across 23,049 km^2 of habitat (State Forestry Administration of China 2006).

In addition to its conservation plight, the giant panda is well-known for its unique biology. It belongs to the Order Carnivora and has a digestive tract typical of the carnivorous members of the group, but feeds exclusively on low nutrition and low-calorific content bamboo (Dierenfeld et al. 1982; Schaller et al. 1985; Wei et al. 2012). The body weight of a giant panda cub at birth is about 1/900th of its mother, the lowest amongst all Eutherians (Schaller et al. 1985; Gittleman 1994; Zhang and Wei 2006).

It has been repeatedly and regularly argued that the giant panda forms a so-called evolutionary cul-de-sac and is doomed to extinction at some point in the near future (Pei 1965, 1974; Wang 1974; Wei et al. 1990; Feng et al. 1991; Huang 1993; Schaller 1993; Gittleman 1994). This argument follows the general lines that the evolutionary history, population status, and unique biology of the giant panda are all evidence of its doomed fate. Specifically, the arguments are that 1) a specialized bamboo diet is evolutionarily adverse because bamboo provides low nutrition and energy, and is further detrimental because of large-scale bamboo flowering and die off events (Hu 1997, 2001); 2) a small population size and shrinking habitat negatively impact giant pandas (Schaller 1993; Hu 2001); 3) captive giant pandas are characterized by low estrous, low conception, and low cub survival rates (Feng et al. 1991; Zhang and Wei 2006), qualities that have been theoretically applied to wild populations; 4) genetic diversity is low (Su et al. 1994; Fang et al. 1997; Zhang et al. 2002) and represents poor evolutionary potential; 5) an extremely low newborn body weight and slow rate of maturation are evolutionary disadvantages (Gittleman 1994); and 6) a species whose body size evolves from the smallest of its kind to the largest of its kind, and then declines somewhat, has reached an evolutionary dead-end (Pei 1965, 1974). The above concepts permeate scientific, and popular, literature and media. For example, Chris Packham, a wildlife reporter with

Table 1. Summary of Morphological, Ecological, and Genetic Adaptations to a Specialized Bamboo Diet in Giant Pandas.

Adaptive Traits	Morphology	Ecology	Genetics	Coadaptation of Gut Microbiota
1	Pseudothumb	Prefer nutritious bamboo shoots, tender leaves and first-year bamboo to maximum nutrient intakes	Pseudogenization of umami receptor gene *TAS1R1*	Have intestine microbes and genes associated with digestion of cellulose and hemicellulose
2	Skull has extremely expanded zygomatic arches, associated with zygomatico-mandibular muscle attachment	Seasonal shift toward different parts of bamboo and different bamboo species to balance nutrient intakes	Significant positive selection of bitter receptor genes *TAS2R49* and *TAS2R3*	
3	Developed mandible suitable for masticating bamboo food	Prefer old-growth forest with gentle slope and lower density of fallen logs, shrubs, and bamboo culms to save energy expenditure	Pseudogenization of *COMT* gene associated with catecholamine metabolic pathways	
4	Large and flat teeth have elaborate crown patterns	Eat much daily to maximum energy intakes	More AGT targeted to the peroxisomes and positive selection of AGT mitochondrial targeting sequence	
5		Move short distance daily to save energy expenditure		

Note.—COMT, catechol-o-methyltransferase; AGT, alanine:glyoxylate aminotransferase.

the BBC, said, “here’s a species that, of its own accord, has gone down an evolutionary cul-de-sac. It’s not a strong species . . . I reckon we should pull the plug. Let them go, with a degree of dignity.” (http://www.radiotimes.com/news/2009-09-22/chris-packham-let-pandas-die, last accessed October 3, 2014).

The view that giant pandas are somehow an evolutionary dead-end has puzzled many conservation biologists, animal managers, and members of the public, but countering these claims has been difficult because the data needed has been spread across different branches of science and limited by methodological barriers. However, a range of recent and incremental findings spanning the biology, population history, and population status of giant pandas paints a clearer picture of the complexity underpinning this animal and clearly debunks the myth that giant pandas are an evolutionary dead-end, or “cul-de-sac,” or any other analogy implying this species is, and always has been, doomed to fail. Here, we focus most attention on the first four arguments stated above, and do not discuss the last two arguments because these two views were personal and based on supposition.

Adaptation to a Specialized Bamboo Diet

Fossil records suggest that giant pandas probably started to consume bamboo in the late Pliocene or early Pleistocene (Pei 1974; Wang 1974; Jin et al. 2007). Although the modern giant pandas still retains the ability to feed on meat as observed often in captivity and very occasionally in the wild, 99% of their diet now consists of bamboo (Schaller et al. 1985). However, bamboo is a low nutrition/energy food, comprising 70–80% cellulose, hemicellulose, and lignin and 20–30% protein, soluble carbohydrate, and fat (Schaller et al. 1985). Giant pandas digest a small proportion of this: 75–90% of the protein, only 27% of the hemicellulose, and 8% of the cellulose (Dierenfeld et al. 1982). Despite this, they have survived on a bamboo diet probably for more than 2 My, and findings from morphological, ecological, and genetic studies confirm that they are well-adapted to their specialized bamboo diet (table 1).

Morphological Adaptation

The forepaw of giant pandas has evolved a pseudothumb: An enlarged radial sesamoid bone (Endo, Yamagiwa, et al. 1999; Salesa et al. 2006) that plays a critical role in grasping bamboo and facilitating feeding (Endo, Hayashi, et al. 1999) and is generally regarded a perfect example of adaptive evolution (fig. 1*A*). Their skull is composed of dense compact bones and compared with other bears, has extremely expanded zygomatic arches (fig. 1*B*; Sicher 1944; Davis 1964) and well developed mandible structure (fig. 1*C*; Zhang et al. 2007), associated with zygomatic-mandibular muscle attachment. These structures facilitate mastication of bamboo, which is necessary for such a tough and fibrous food. The teeth are large and flat and have elaborate crown patterns, providing efficient crushing surfaces which enable effective mastication of coarse bamboo (fig. 1*D*; Davis 1964).

Ecological Adaptation

The giant panda has evolved a suite of optimal foraging, habitat use, and activity rhythm strategies as adaptations to the

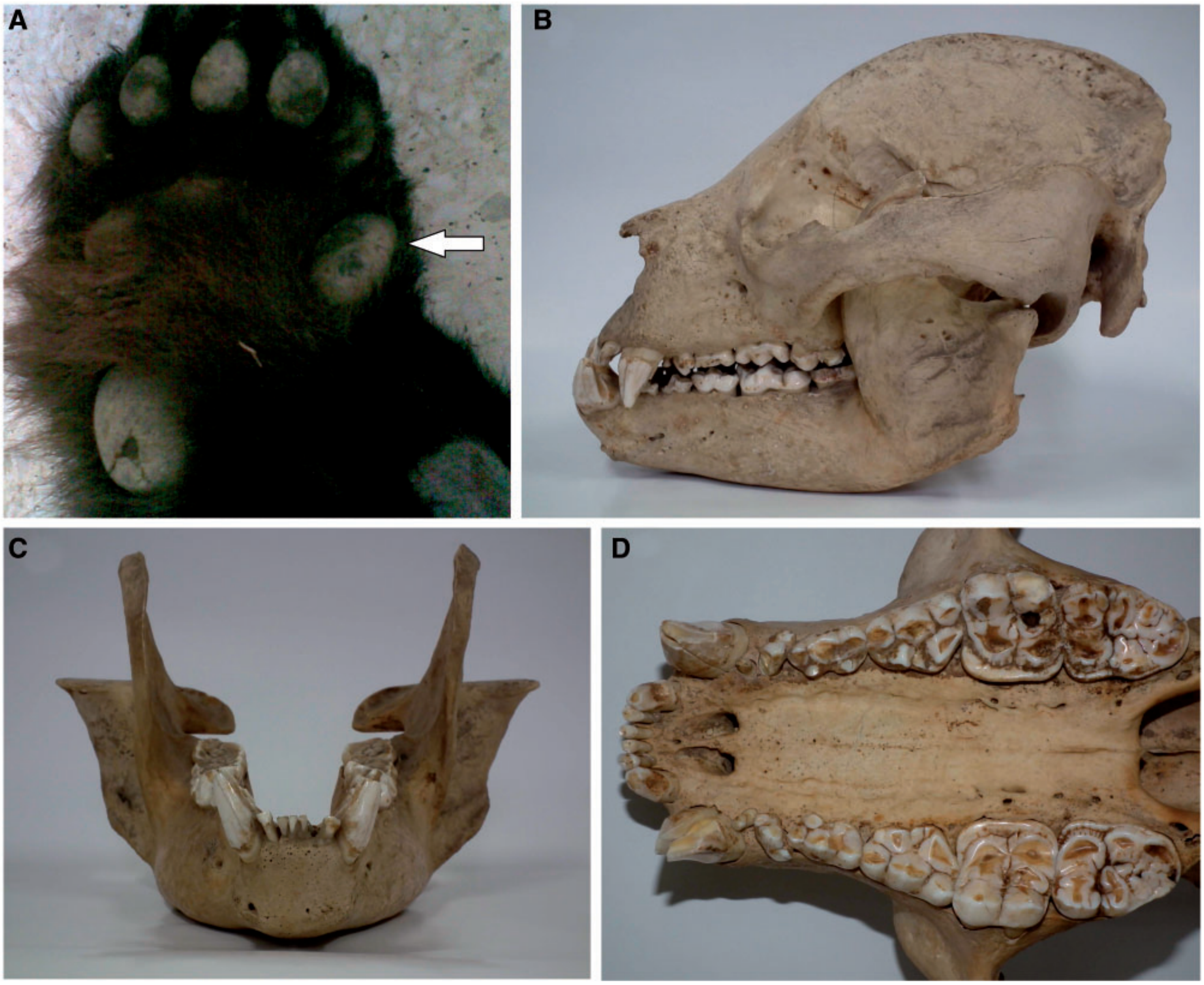

Fig. 1. Adaptive morphological characteristics of giant pandas. (*A*) pseudothumb (denoted by the arrow); (*B*) skull; (*C*) mandible; (*D*) teeth.

low energy diet. Field research shows that it prefers the most nutritious bamboo shoots, tender leaves and first-year bamboo, and chooses different bamboo species and bamboo parts at different times of the year in different mountain ranges (Schaller et al. 1985; Yong et al. 1994; Wei et al. 1999; Pan et al. 2001; Zhang et al. 2014). Nutritional analysis suggests that their foraging habits are tied to changes in the nutritional composition of bamboo (Schaller et al. 1985; Wei et al. 1999) and their dietary shifts are related to balances of nitrogen, phosphorus, and calcium (Nie et al. 2014). Giant pandas have also evolved a strategy of consuming a great deal of food per day (10–18 kg of leaves or stems or about 38 kg of shoots; Schaller et al. 1985) to maximize nutritional and energetic intake. Moreover, the bamboo resource is widely available in their habitat and is sufficient to accommodate the actual population size (Schaller et al. 1985; Wei et al. 1997; Linderman et al. 2005).

Giant pandas have a clever way of utilizing their habitat. They often live in old-growth forest characterized by gentle slopes and a low density of fallen logs, shrubs, and bamboo stands, because feeding and moving in an open microhabitat facilitates easier access to preferred bamboo and reduces energy expenditure (Schaller et al. 1985; Reid and Hu 1991; Wei et al. 2000; Zhang et al. 2006, 2011, 2014). To further minimize energy expenditure, pandas have an optimal activity rhythm whereby they often feed during two activity peaks, one near dawn and one at dusk, and 55% of the daily time is spent in foraging and 41% resting (Schaller et al. 1985). They often move only a short distance daily, between 300 and 500 m (Schaller et al. 1985; Pan et al. 2001; Zhang et al 2014).

Genetic Adaptation

Adaptation to a specialized bamboo diet has also occurred at the molecular level. The giant panda genome reveals that the umami receptor *TAS1R1* gene has become pseudogenized due to a 2-bp insertion in exon 3 and a 6-bp deletion in exon 6 (Li et al. 2010). The umami receptor senses components of meat and other protein-rich foods. Therefore, the loss of function of the *TAS1R1* gene may have contributed to the panda's dietary switch (Li et al. 2010). To test this hypothesis, Zhao et al. (2010) sequenced all six *TAS1R1* exons of another individual and found another 4-bp deletion in exon

6, confirming pseudogenization of this gene. It is estimated that this pseudogenization occurred 4.2 Ma, a timeline that matches the approximate date of the dietary switch in pandas (Zhao et al. 2010). For the bitter receptor gene, Zhao et al. (2013) detected significant positive selection in bitter receptor genes *TAS2R49* and *TAS2R3* in the Qinling Mountain population compared with non-Qinling populations based on whole-genome-wide single nucleotide polymorphism (SNP) analysis, and the difference in selection may be associated with a higher bitter content (e.g., alkaloids) in bamboo leaves (the main food of the Qinling Mountain population). Besides taste receptor genes, Jin et al. (2011) analyzed 166 major genes involved in the "appetite-reward system" of giant pandas and found a 12-bp deletion in the catechol-o-methyltransferase gene, which likely results in loss of function in catecholamine metabolic pathways. This finding suggests that unusual metabolic processes may affect this species' food choices.

The subcellular distribution of the intermediary metabolic enzyme alanine:glyoxylate aminotransferase (AGT) is related with dietary choice, and AGT distribution tends to be peroxisomal in herbivores, mitochondrial in carnivores, and both peroxisomal and mitochondrial in omnivores (Danpure et al. 1994). Birdsey et al. (2004) examined the subcellular distribution of AGT enzyme and the molecular evolution of AGT mitochondrial targeting sequence in the giant panda, and found that more AGT targeted to the peroxisomes and positive selection occurred on the AGT mitochondrial targeting sequence. This finding shows that molecular adaptation has occurred related with the bamboo metabolism. However, the molecular evolution study on the pancreatic ribonuclease gene (*RNASE1*) in carnivores, a digestive enzyme that plays an important role in foregut-fermenting herbivores, found only one *RNASE1* gene copy and no gene duplication in the giant panda (Yu and Zhang 2006), suggesting that the *RNASE1* gene may be not important in bamboo metabolism for giant pandas.

Coadaptation of Gut Microbiota

Research shows that 8% of the cellulose and 27% of the hemicellulose in bamboo is digested by giant pandas (Dierenfeld et al. 1982), but how this species digests cellulose has remained a puzzle for some time. Whole-genome sequencing of giant pandas found no specific genes responsible for the digestion of cellulose and hemicellulose (Li et al. 2010), suggesting that gut microbes may play a role in digesting bamboo fibers. Zhu, Wu, et al. (2011) combined 16S *rRNA* gene sequencing and metagenome analysis, and for the first time identified the microbe group and specific genes associated with the digestion of cellulose and hemicellulose in giant pandas. They found 13 operational taxonomic units closely related to *Clostridium* groups I and XIVa which contain taxa known to digest cellulose, and recovered putative genes coding two cellulose-digesting enzymes (cellulase and beta-glucosidase) and one hemicellulose-digesting enzyme (xylan 1,4-beta-xylosidase) in *Clostridum* group I. Their findings highlight that giant pandas have adapted to a fiber-rich bamboo diet and maximize nutritional intake through symbiosis with specialist gut microbes.

Response to Bamboo Flowering

Bamboo flowering is a natural phenomenon thought to occur every 40–100 years (Campbell and Qin 1984). After bamboo flowers, it dies off, thus reducing food availability for giant pandas. In the 1970s and1980s, two large-scale bamboo flowering events across the main habitat of giant pandas in the Minshan and Qionglai Mountains affected large areas of habitat and resulted in 138 and 141 panda deaths, respectively (Hu 1997, 2001). These bamboo flowering events elicited worldwide concern regarding the fate of giant pandas and are regarded as key evidence for their "bad evolution" despite the fact that the precise genetic effect of bamboo flowering on giant pandas was unknown. Zhu et al. (2013) estimated that over 1,000 pandas were removed from the wild from the 1950s to 1980s, and evaluated the genetic effects of population decline resulting from massive bamboo flowering, hunting, zoo collection, and habitat loss during that period. Comparing genetic diversity and effective population sizes between historical and modern samples, they found no significant genetic signatures for rapid population decline. These findings highlight that the population decline, including that arising from massive bamboo flowering, has not affected current genetic evolutionary potential (Zhu et al. 2013). Giant pandas cope with massive bamboo flowering by switching to alternative nonflowering bamboo species (Reid et al. 1989; Hu et al. 1990; State Forestry Administration of China 2006) or dispersing long distances for foraging (Johnson et al. 1988). Accordingly, giant pandas may have encountered thousands of massive bamboo flowering events, as these are natural ecological phenomena, during more than 2 My of their existence on earth, and under good habitat connectivity they can survive massive bamboo flowering events. Unfortunately, increasing anthropogenic habitat fragmentation is weakening this capacity to respond.

Population History

Based on its rare and endangered status, it has long been inferred that the giant panda is undergoing long-term decline. Quaternary glaciations are thought to have seriously affected the panda population, resulting in continuous population decline (Ma and Hu 1994). Microsatellite data simulation detected recent population declines for giant panda populations dating 300–10,000 years ago (Zhang et al. 2007; Hu, Qi, et al. 2010; Zhu et al. 2010), implying that human activities have in fact played a role in the endangerment process. However, an ancient-to-today detailed demographic trajectory remains unclear until more advanced genomic techniques are developed.

Using giant panda whole-genome data (Li et al. 2010) and the pairwise sequentially Markovian coalescent model (Li and Durbin 2011), Zhao et al. (2013) reconstructed giant panda demographic history from about 8 Ma to the present day and found two population bottlenecks, two expansions, and two divergences. The first bottleneck occurred about 0.2 Ma, which was related with the two largest Pleistocene glaciations

(Naynayxungla and Penultimate Glaciations) in China, and the second bottleneck occurred during the last glacial maximum about 20,000 years ago. Three genetically distinct populations were identified. The Qinling population diverged from non-Qinling populations (Minshan and Qionglai-Xiangling-Liangshan) about 0.3 Ma, corresponding with the onset of the Penultimate Glaciation, whereas the divergence between Minshan and Qionglai-Xiangling-Liangshan occurred about 2,800 years ago, likely resulting from anthropogenic barriers such as regional deforestation. These patterns reveal that historical climate changes were the primary drivers of population fluctuations for millions of years, and then human activities likely drove recent population divergence and decline. This recent study is fundamental to understanding historical demography and reconstructing the endangerment process in giant pandas.

Does the population decline trend of this species reflect the possibility of being doomed to extinction? The demographic histories of other sympatric endangered mammals may provide indirect answers to this question. The red panda (*Ailurus fulgens*) is sympatric with giant pandas in Sichuan, shares the same diet specialization as giant pandas, and is an endangered species according to the IUCN Red List. Based on the simulation of microsatellite data, Hu et al. (2011) found that red panda populations have also experienced recent, rapid population declines, most likely due to increased human activity. Similarly, the Yunnan snub-nosed monkey (*Rhinopithecus bieti*) is an endangered primate and also distributed at the eastern edge of the Tibetan Plateau. Studies show that Yunnan snub-nosed monkeys may have suffered from Quaternary glaciations and experienced ancient population contraction and subsequent expansion (Liu et al. 2007) and that human activity may have resulted in recent population divergence (Liu et al. 2009). The population demographic trajectories of these endangered mammals distributed at the eastern edge of the Tibetan Plateau highlight that similarly, these species have been affected by historical climate fluctuations and then recently suffered increased human activity, and also suggest that the endangerment status of the giant panda is likely attributable more to regional environmental pressures and less to its unique life history.

Population Biology

Based on its endangered status and captive breeding behavior, it has been suggested and widely believed that giant pandas have low reproductive rates and negative population growth. Until the 1990s, giant panda captive-breeding programs faced three main obstacles: Low oestrus rates, low conception rates, and high neonatal mortality (Zhang and Wei 2006). Poor captive-breeding success was attributed to poor reproductive abilities in giant pandas (Feng et al. 1991) which was arbitrarily thought to be the same for the wild population. However, these inferences and conclusions are arbitrary because of a lack of biological knowledge of wild giant pandas and populations.

In the wild, female adult pandas reproduce once every 2 years. After the cub is 1.5 years old and separated from its mother, the female can enter estrous again and start a new cycle of reproduction (Schaller et al. 1985; Pan et al. 2001). Wei and Hu (1994) found that the female adult gives birth to her first cub at 7.5 years old, and the annual reproductive rate is 62.5% in Wolong Reserve, Sichuan. Pan et al. (2001) conducted a field study on a wild giant panda population in Changqing Reserve, Shaanxi, for more than 10 years and found that this species has both high annual reproductive rate (65.4%) and high cub survival rate. For instance, during the study period a collared female "JiaoJiao" gave birth to five offspring which all survived. These findings demonstrate that wild pandas have excellent reproductive ability. In the case of captive breeding, with more understanding of panda reproductive biology and the use of scientific-breeding techniques, giant panda breeders have conquered these main breeding obstacles and cub survival rate has increased to over 90% (Zhang et al. 2006). In 2013, there were 375 individuals living in captive centers and zoos (Xie 2013), and the focus of the captive-breeding program has shifted from the quantity to quality of newborn cubs, such as the avoidance of inbreeding and maintenance of genetic variation. The latest study indicated that the captive panda populations harbor high genetic diversity and low inbreeding level under the effective captive-breeding management (Shan et al. 2014), highlighting the success of captive breeding. These breeding achievements, along with the insights into the reproductive biology of wild populations, have thoroughly corrected the view that this species has poor reproductive capacity.

Field studies have also shown a positive population growth rate (Wei et al. 1989; Pan et al. 2001). Combining the life table of giant pandas with other life history parameters, Wei et al. (1989) estimated a net reproductive rate of >1 ($R_0 = 1.0672$) and an intrinsic growth rate of >0 ($r = 0.0056$) for this species, indicating a trend of potential population growth for the wild population. A positive annual population growth rate (4.1%) of giant pandas was also reported in Changqing Reserve, Shaanxi (Pan et al. 2001).

It is well-known that wild giant pandas comprise small populations; however, accurate population estimates have remained obscure due to a lack of thorough population census methods. With the development of noninvasive genetic sampling, methods based on feces or hair samples and microsatellite DNA genotyping have opened a new door for wild giant panda population censuses. For example, Zhan et al. (2006) performed exhaustive noninvasive sampling of a giant panda population in Wanglang Reserve, and identified 66 individuals based on genotyping nine microsatellite loci for 302 noninvasive samples. The population size was twice the previous population estimate based on traditional methods (27 individuals) using fecal bamboo bite length and home range size (State Forestry Administration of China 2006). This shows that traditional population census methods have probably underestimated the population size of wild giant pandas, and consequently the total population size is more likely to be approximately 2,500 animals (Zhan et al. 2006). This population size is still small compared with other endangered large mammals.

Table 2. Summary of Genetic Diversity in Wild Giant Panda Populations.

Reference	Type and Number of Molecular Markers	Sample Size	Mountain Population	Genetic Diversity
Low genetic diversity				
Su et al. (1994)	40 allozymes or proteins	12	MS, QIO, LS	Hp = 0.008
Fang et al. (1997)	1 DNA fingerprint probe	15	LS, XXL	Ht = 0.64
Zhang et al. (2002)	655–978 bp of mtDNA D-loop region	32	QIN, MS, QIO, LS	13 variable sites, 16 haplotypes
Moderate genetic diversity				
Lu et al. (2001)	mtDNA RFLP	19	QIN,MS, QIO	8 variable sites, 5 haplotypes, $\pi = 0.22$
	268 bp of mtDNA D-loop region	36	QIN,MS, QIO	16 variable sites, 17 haplotypes
	2 DNA fingerprint probes	18	QIN, QIO	MAPD = 0.383 or 0.315
	18 microsatellite loci	36	QIN,MS, QIO	$H_O = 0.44$, MNA = 3.7
Moderate to high genetic diversity				
Zhang et al. (2007)	655 bp of mtDNA D-loop region	159	QIN, MS, QIO, LS, XXL	24 variable sites, 39 haplotypes, Hm = 0.943
	10 microsatellite loci	115	QIN, MS, QIO, LS, XXL	$H_O = 0.565$, $H_E = 0.642$, MNA = 7.1
Zhan et al. (2006)	9 microsatellite loci	66	A part of MS	$H_O = 0.625$, $H_E = 0.609$, MNA = 5.4
He et al. (2008)	13 microsatellite loci	33	A part of MS	$H_O = 0.488$, $H_E = 0.68$, MNA = 6.2
	13 microsatellite loci	30	A part of QIO	$H_O = 0.553$, $H_E = 0.819$, MNA = 7.6
Hu, Qi, et al. (2010)	12 microsatellite loci	52	LS	$H_O = 0.683$, $H_E = 0.592$, MNA = 4
Hu, Zhan, et al. (2010)	655 bp of mtDNA D-loop region	42	LS	9 haplotypes, Hm = 0.7364
Zhu, Zhang, et al. (2011)	655 bp of mtDNA D-loop region	32	XXL	5 haplotypes, Hm = 0.532
	655 bp of mtDNA D-loop region	21	DXL	5 haplotypes, Hm = 0.747
	9 microsatellite loci	32	XXL	$H_O = 0.704$, $H_E = 0.656$, MNA = 4.556
	9 microsatellite loci	21	DXL	$H_O = 0.66$, $H_E = 0.634$, MNA = 4.667
Yang et al. (2011)	10 microsatellite loci	42	A part of MS	$H_O = 0.686$, $H_E = 0.703$, MNA = 5.9
Zhao et al. (2013)	13020055 SNPs	34	QIN, MS, QIO/LS/XXL/DXL	$\theta_w = 1.04–1.3 \times 10^{-3}$, $\theta_\pi = 1.13–1.37 \times 10^{-3}$

NOTE.—mtDNA, mitochondrial DNA; RFLP, restriction fragment length polymorphism; Hp, mean heterozygosity for protein; Ht, mean heterozygous ratio; Hm, haplotype diversity for mtDNA; π, nucleotide diversity; H_O, observed heterozygosity; H_E, expected heterozygosity; MNA, mean number of allele per locus; MAPD, mean average percent difference. QIN, Qinling Mountains; MS, Minshan Mountains; QIO, Qionglai Mountains; LS, Liangshan Mountains; DXL, Daxiangling Mountains; XXL, Xiaoxiangling Mountains.

Genetic Diversity

The giant panda was considered to have low genetic variation based on studies that used a single type of molecular marker and a small sample size (table 2). For example, Su et al. (1994) used protein electrophoresis and showed only one locus to be polymorphic, suggesting very low levels of genetic diversity. Using a DNA fingerprinting probe, Fang et al. (1997) also found low levels of genetic diversity for the Liangshan and Xiaoxiangling populations. Zhang et al. (2002) analyzed variation in mtDNA D-loop sequences and further inferred low genetic variation. However, with the application of giant panda genome sequencing and resequencing (Li et al. 2010; Zhao et al. 2013), multiple types of molecular markers (Zhang et al. 1995, 2009; Lu et al. 2001; Shen et al. 2005; Wu et al. 2009), and larger sample sizes, our understanding of giant panda genetic diversity changed. Lu et al. (2001) applied mtDNA RFLP, D-loop region, DNA fingerprinting and microsatellites to detect moderate levels of genetic variation. Combining microsatellite markers and mtDNA sequence, Zhang et al. (2007) revealed moderate-to-high levels of mtDNA and microsatellite diversity across the five extant mountain populations compared with other endangered carnivores (table 3), and refuted the evolutionary dead-end view from a genetic diversity perspective. Noninvasive genetic studies based on large-scale fecal collection also detected relatively high levels of genetic diversity in giant pandas (e.g., Zhan et al. 2006; Hu, Qi, et al. 2010; Hu, Zhan, et al. 2010; Yang et al. 2011; Zhu, Zhang, et al. 2011) (table 2).

Moderate-to-high genetic variation is also confirmed at the genome-wide scale. Zhao et al. (2013) sequenced the whole genomes of 34 wild pandas, identified over 13 million genome-wide SNPs, and estimated genome-wide genetic diversity. They found that the giant panda genetic diversity indices, Watterson's estimator (θ_w) ($1.04–1.3 \times 10^{-3}$) and average pairwise diversity within populations (θ_π) ($1.13–1.37 \times 10^{-3}$) are similar to those in human, indicating relatively high genetic variation and thus high genetic evolutionary potential in giant pandas.

Hope for the Giant Panda

The giant panda has an evolutionary history of 8 My and during this process it has survived diet specialization, massive bamboo flowering, and rapid climate oscillations. This species' evolutionary history itself highlights that it is a viable animal. Previous determinations that giant pandas are an

Table 3. Comparison of Microsatellite Genetic Diversity of Giant Pandas and Other Endangered Carnivores.

Species	Sample Size	MNA	H_O	H_E	Reference
Giant panda (*Ailuropoda melanoleuca*)	115	7.1	0.565	0.642	Zhang et al. (2007)
Spectacled bear (*Tremarctos ornatus*)	155	5.67	–	0.56	Ruiz-Garcia et al. (2005)
Polar bear (*Ursus maritimus*)	473	6.5	–	0.68	Paetkau et al. (1999)
Brown bear (*U. arctos*)	380	6.8	0.66	0.71	Waits et al. (2000)
Tiger (*Panthera tigris*)	113	7.3	0.537	0.72	Luo et al. (2004)
African cheetah (*Acinonyx jubatus*)	50	4.92	0.447	0.528	Luo et al. (2004)
African lion (*P. leo*)	50	5	0.547	0.61	Luo et al. (2004)
Golden jackal (*Canis aureus*)	120	5.07	0.47	0.51	Fabbri et al. (2014)

NOTE.—MNA, mean number of alleles per locus; H_O, observed heterozygosity; H_E, expected heterozygosity.

evolutionary cul-de-sac resulted from an unsystematic and poor understanding of the species. Everything people used to think about giant pandas needs updating. The panda is well-adapted to its bamboo diet, potential population growth is present, ample genetic variation exists, and we have underestimated its population size. However, this species is suffering from enormous human interference and we hope it could survive modern anthropogenic impacts. So, to decrease the effects of human interference, we have to work hard to conserve this evolutionary “hopeful” species. Fortunately, humans have realized these anthropogenic threats and have made a series of conservation actions to protect this species.

In 1988, the Chinese Government enacted the Wildlife Protection Law to protect endangered animals from human interference and persecution. Since then, poaching has been banned and poachers have been severely punished. To protect giant pandas and their habitat, 63 nature reserves for this species have been established, covering 85% of its entire habitat (Hu et al. 2011). The implementation of natural forest protection and grain-to-green programs has also significantly facilitated the conservation of giant pandas through protecting and restoring habitat (State Forestry of Administration of China 2006). For fragmented or isolated habitat patches, habitat corridors have been planned or constructed to facilitate dispersal and gene flow. For small and isolated populations, translocation or reintroduction programs have been implemented to improve reproduction success and genetic diversity. For instance, in the smallest and most isolated Xiaoxiangling Mountain population, studies have estimated that this population has a very high risk of extinction (Zhu et al. 2010) and so the Chinese Government initiated a translocation program in 2009 that released a rescued wild female panda called Luxin into this population (Schenkman 2010). Then in 2012 and 2013, two captive-born subadults “Taotao” and “Zhangxiang” were also released to this population (http://www.pandasinternational.org/wptemp/program-areas-2/reintroduction-program/, last accessed October 3, 2014). We know these three animals survived through GPS and molecular monitoring.

Achievements in protecting giant pandas abound, but we should not reduce conservation effort because major threats such as habitat loss and fragmentation to the survival and maintenance of this species remain. Although large-scale deforestation has been prohibited, road construction and human settlements along roads are fragmenting and eroding giant panda habitat (Fan et al. 2011). Additionally, new threats are emerging, such as tourism, power stations, large-scale mining, and environmental pollution (Dai et al. 2006). These new threats present serious challenges to the continued conservation and management of giant pandas.

Although we have learned much about the biology and history of giant pandas, much remains a puzzle. Giant pandas have a specialized bamboo diet, but we do not know when and why these animals altered their dietary preferences. The pseudothumb is a typical morphological adaptation to a specialized diet; however, its genetic architecture and evolutionary origins remain obscure. Moreover, what factors drive the persistence of giant panda populations only in isolated mountain range refuges? Only once we understand these issues will we be able to further protect this viable species from historical and emerging threats.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (grant numbers 31230011 and 31270418). The authors are grateful to the Editor-in-chief for inviting this review and commenting on the paper.

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