Genetic evidence of recent population contraction in the southernmost population of giant pandas

Yibo Hu · Dunwu Qi · Hongjia Wang · Fuwen Wei

Abstract Anthropogenic habitat loss and fragmentation have been implicated in the endangerment and extinction of many species. Here we assess genetic variation and demographic history in the southernmost population of giant pandas (*Ailuropoda melanoleuca*) that continues to be threatened by habitat degradation and fragmentation, using noninvasive genetic sampling, mitochondrial control region sequence and 12 microsatellite loci. Compared to other giant panda populations, this population has medium-level genetic diversity based on the measure of both mitochondrial and nuclear markers. Mitochondrial DNA-based demographic analyses revealed that no historical population expansion or contraction has occurred, indicating a relatively stable population size. However, a Bayesian-coalescent method based on the observed allele distribution and allele frequencies of microsatellite clearly did detect, quantify and date a recent decrease in population size. Overall, the results indicate that a population contraction in the order of 95–96% has taken place over the last 910–999 years and is most likely due to anthropogenic habitat loss. These findings highlight the need for a greater focus on habitat protection and restoration for the long-term survival of this giant panda population.

Keywords Conservation genetics · Liangshan Mountains · Microsatellite · Mitochondrial DNA · Noninvasive genetic sampling · Population contraction

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Introduction

Anthropogenic habitat loss and fragmentation have been implicated in the endangerment and extinction of many species (Tilman et al. 1994; Hughes et al. 1997; Sih et al. 2000) and cause fluctuations in population size and structure, inbreeding, decreased genetic diversity and increase the risk of local extinction (Newman and Pilson 1997). This is especially true for rare and endangered species (Frankham et al. 2002; Goossens et al. 2006). The giant panda (*Ailuropoda melanoleuca*) is a focal species of global conservation concern that has been heavily impacted by habitat loss and fragmentation (Hu et al. 1985; Hu 2001). Giant pandas are found in six isolated mountain-ranges at the eastern edge of the Qinghai-Tibetan Plateau. Giant panda habitat is highly fragmented because of large-scale deforestation, road construction and human settlements (Hu et al. 1985; Hu 2001). An assessment of genetic viability and population demography is needed for effective conservation and management (Zhang et al. 2007).

In a paper that shifted public opinion of these animals, Zhang et al. (2007) showed that the genetic viability and population history of giant pandas indicate they are far from a so-called 'evolutionary dead-end'. However, some populations are at a high risk of extinction because of habitat fragmentation and isolation (Hu 2001; Zhu et al. 2010). The Liangshan Mountains are an important biogeographic region and form a transition zone between the Sichuan Basin and Yunnan-Guizhou Plateau (Fig. 1). This area supports the southernmost population of wild giant pandas and has suffered habitat degradation and fragmentation due to human development (SFA 2006). For example, human activities such as deforestation and agriculture have severely eroded giant panda habitat, and as a result,

Fig. 1 Study area and locations of giant pandas in the present study. The *inset* shows the extant geographical distribution of giant pandas in China including the southernmost Liangshan Mountains (in the *black box*)
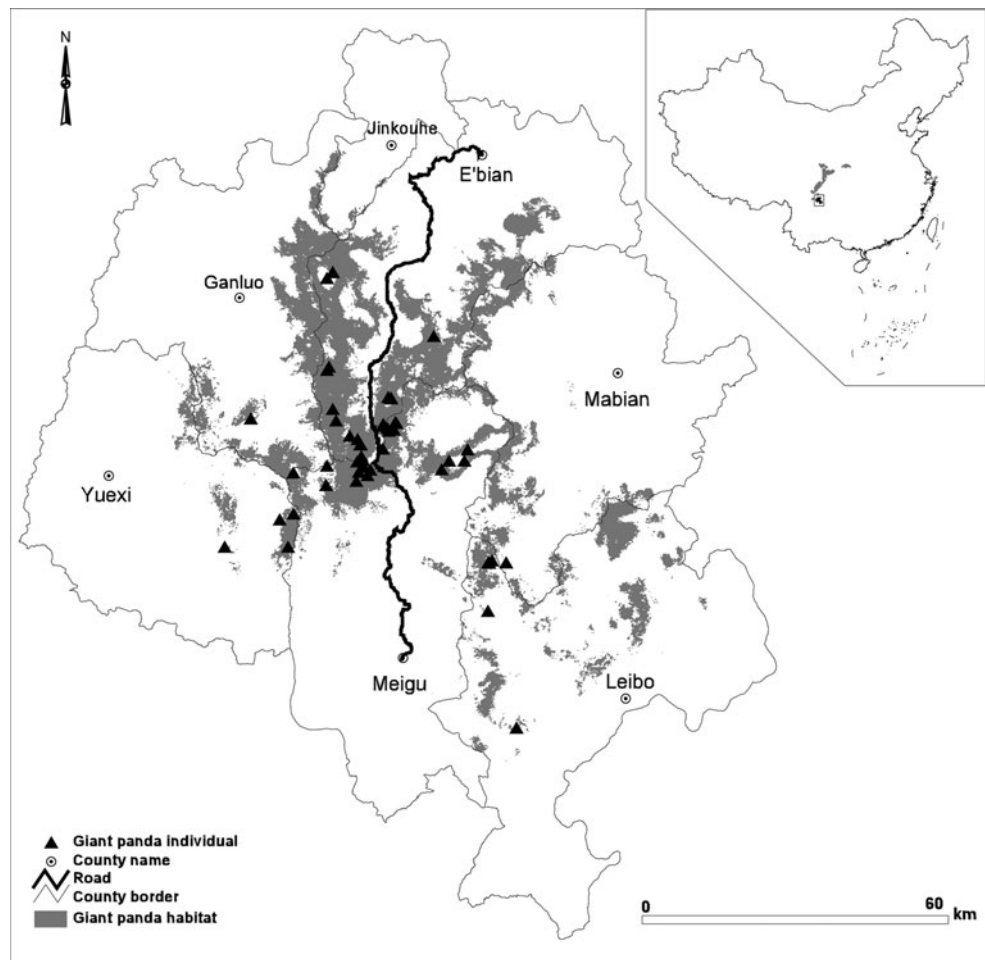
the area of suitable habitat has decreased from 11,607 km^2 in the 1950s to 2,204 km^2 in 2002 (Hu 2001; SFA 2006). Further, giant pandas are confined to middle to high elevations and a trend of population contraction has been reported that 115 giant pandas inhabit the area, down from 230 estimated in the 1970s (Hu 2001; SFA 2006). This mountainous population has been isolated from the adjacent Daxiangling and Xiaoxiangling populations due to human activity (Hu 2001; SFA 2006) and a road currently bisects core habitat causing fragmentation and facilitating further human disturbance. Given historical and current human impacts on this population and its position at the southern extreme of this species' distribution, the Liangshan giant panda population is now a major concern for conservation biologists.

Here we focused on genetic variation and demographic history of the Liangshan population using noninvasive genetic sampling to assess mitochondrial and nuclear genetic diversity. Specifically, we asked: Has the genetic variation in the Liangshan population of giant pandas been lost as a result of a population contraction? If so, has the population contraction produced a genetic imprint? And

what was the magnitude and date? Our findings will contribute to the practical management and conservation of this high-profile species.

Materials and methods

Sample collection and molecular analysis

Giant panda feces and hair were collected along randomly selected transects covering the study region following Qi et al. (2009) (Fig. 1). Most fecal samples were less than 2-week-old, based on the status of the mucosal outer-layer. We collected 156 fecal and 30 hair samples over two periods, March–September 2006 (81 fecal and 18 hair samples) and April–May 2007 (75 fecal and 12 hair samples).

Total DNA was extracted from feces following Zhang et al. (2006) and from hair using the Chelex-100 method (Walsh et al. 1991). Blank controls were also performed in both extractions and downstream amplifications. Twelve microsatellite loci: Ame-μ5, μ10, μ13, μ15, μ22, μ26 (Lu et al. 2001), AY116213 (Shen et al. 2005), Ame-μ11,

μ24, AY79, AY95, AY217 (redesigned loci; Wu et al. 2009) were used to amplify DNA extracts from fecal and hair samples. To obtain reliable genotypes, a stringent multi-tube approach was used (Taberlet et al. 1996). Firstly each extract was amplified three times simultaneously and if the genotype could not be determined four additional amplifications were performed. Detailed polymerase chain reaction (PCR) procedures are described in Hu et al. (2010). PCR products were separated using an ABI 3700 prism automated sequencer and scored using GeneScan 3.7 and Genotyper 2.5 (Applied Biosystems). The mitochondrial control region (mtDNA CR) of 655-bp was PCR-amplified for identified giant panda individuals using a pair of primers: P-tp (5′-CTC CCT AAG ACT CAA GGA AG-3′, Zhang et al. 2007) and BEDH (5′-GGG TGA TCT ATA GTG TTA TGT CC-3′, Zhang et al. 2002). PCR amplifications were performed starting with 95°C for 15 min, followed by a touchdown PCR (a total of 38 cycles of 94°C/30 s, T_a/40 s, 72°C/50 s) and a final step of 72°C for 10 min. T_a was decreased by 2°C every four cycles from 54°C to a touchdown temperature (50°C), which was used for 30 subsequent cycles. PCR fragments were sequenced using an ABI 3700 prism automated sequencer.

Individual identification

Individual identification was performed following Zhan et al. (2006). The probabilities of pairs of individuals and full-sibs bearing an identical multilocus genotype, P(ID) and P(sibs), were estimated using GIMLET 1.3.1 (Valière 2002). The overall genotyping error rate was calculated using the mathematical method described in Zhan et al. (2010). For final individual identification results we utilized MICRO-CHECKER (Van Oosterhout et al. 2004) and DROPOUT (McKelvey and Schwartz 2005) to verify whether genotyping errors were reduced to a non-significant level.

Genetic diversity and phylogeography

Ten mtDNA CR sequences of giant pandas from the Liangshan Mountains, originally reported in Zhang et al. (2007), were analyzed together with the 32 new sequences reported in this study. These CR sequences were aligned using MEGA 4.0 (Tamura et al. 2007). Unique haplotypes were identified, and haplotype diversity (h) and nucleotide diversity (π) (Nei 1987) were calculated using DnaSP 5.0 (Librado and Rozas 2009). Microsatellite variation was summarized as described in Hu et al. (2010) through the number of alleles per locus (A), expected (H_E) and observed heterozygosities (H_O), and inbreeding coefficients (F_{IS}), all estimated using FSTAT 2.9.3.2 (Goudet 2001). Linkage disequilibrium and Hardy–Weinberg equilibriums for each locus and the whole population were also tested

(see Hu et al. 2010). In order to assess the level of genetic diversity of the Liangshan population, we compared our results with studies on genetic diversity in other giant panda populations (Zhan et al. 2006; Zhang et al. 2007; Zhu et al. 2010).

To understand the phylogeographical status of the Liangshan population, we performed phylogeographical analysis under the context of the whole distribution of giant pandas. The haplotypes of the Liangshan population were pooled together with all mtDNA CR haplotypes of giant pandas from Zhang et al. (2007). The median-joining network (Bandelt et al. 1999) based on maximum-parsimony was used to reconstruct the phylogeographical relationship among haplotypes as implemented in NETWORK 4.5.1.6 (http://www.fluxus-engineering.com).

Population demography inference

We performed population demography analyses using all samples because of the absence of population genetic substructure (Hu et al. 2010). To explore the demographic history underlying the observed mtDNA pattern, mismatch distribution analysis, neutrality tests and a Bayesian coalescent-based method were combined to provide the best approximation of population history. First, distribution of pairwise nucleotide differences between sequences (mismatch distribution; Rogers and Harpending 1992) was examined under a model of demographic expansion using ARLEQUIN 3.5 (Excoffier et al. 2005). The validity of the estimated demographic model was tested by the sum of squared differences (Durka et al. 2005) and the Harpending's (1994) raggedness index. Significance was assessed by 10,000 bootstraps and the significant value was taken as evidence for deviation from the estimated demographic expansion model. Second, Fu's (1997) Fs, Ramos-Onsins and Rozas's R_2 (Ramos-Onsins and Rozas 2002) and Fu and Li's (1993) D^* tests were carried out with 10,000 simulations using DnaSP 5.0. Although these tests are commonly used to test the selective neutrality of genetic markers, these estimators are also sensitive to demographic processes such as population expansion or contraction (e.g. Zhao et al. 2008). Finally, a coalescent-based method implemented in FLUCTUATE 1.4 (Kuhner et al. 1998) was used to estimate the population growth parameter (g) of the Liangshan population. The running parameters were as follows: empirical nucleotide frequencies; starting θ-value from Watterson's estimate; initial g value of 0.0; 10 short chains of 4,000 steps and five long chains of 400,000 steps, sampling every 20 steps. Runs were repeated five times to ensure consistency of estimates.

Four different methods based on microsatellite data were used to assess recent demographic history of the

Liangshan population. First, a heterozygosity excess test (Cornuet and Luikart 1996) was performed under three microsatellite mutation models: infinite allele model (IAM), stepwise mutation model (SMM), and two-phase mutation model (TPM). The Wilcoxon signed-rank test was used to evaluate significance based on 10,000 repetitions. Under the TPM model, the proportion of one-step mutation was set as 90 or 95% and variance as 12. Second, a mode-shift test (Luikart et al. 1998) was carried out to detect a distortion of the expected L-shaped distribution of allele frequency. Both heterozygosity excess and mode-shift tests were carried out using BOTTLENECK 1.2.02 (Piry et al. 1999). Third, M-ratios (the ratio of the number of alleles to the size range of alleles) were calculated from microsatellite genotypes using M_P_VAL (Garza and Williamson 2001). The critical value of M (M_c) is set at the lower 5% tail and generated by CRITICAL_M (Garza and Williamson 2001). Three TPM parameters (Δ_g, p_g, θ) can be set to model different mutation processes. We set the proportion of multi-step mutation (p_g) as 5 or 10%, the mean of multi-step mutations $\Delta_g = 3.5$, and θ ($= 4N_e\mu$) = 1, 2, 5 or 10 (μ is the locus mutation rate), as used in most studies (e.g. Busch et al. 2007); 10,000 repetitions were used to test for significance. Fourth, MSVAR, a Bayesian coalescent-based method (Beaumont 1999; Storz and Beaumont 2002), was used to infer population demographic change as implemented in MSVAR 1.3, which is based on the observed allele distribution and allele frequencies (Goossens et al. 2006; Olivieri et al. 2008). It assumes that a stable population of ancestral size N_1 started to decrease (or increase) at time T and finally changed the current population size N_0. The change in population size is assumed to be either linear or exponential, and mutations are assumed to occur under a SMM model at the rate $\theta = 2N_0\mu$. This method allows not only the estimation of N_0 and N_1, but also the estimation of the time T since the population change. The prior distributions for N_0, N_1, T and θ, are assumed to be log normal. The means and standard deviations of these log-normal distributions are themselves drawn from prior (or hyperprior) distributions. Means were drawn from normal distributions and standard deviations from normal distributions, truncated at zero. Wide priors and 3–5 independent runs were used and variances for these prior distributions were large in order to affect posterior distributions as little as possible (Table 1). In order to represent possible demographic histories (stable, decreased or increased), different hyperprior means for the mean of ancestral population size (N_1) were used. The total number of iterations was 1×10^9 and thinning interval was 1×10^4. The first 10% of total iterations were discarded to avoid bias in parameter estimation, and the remaining data were used to obtain the lower (10%), the median (50%), and the upper (90%) quantiles of the posterior distributions. The generation time of the giant panda was set as 5 or 6 years (Hu et al. 1985) for independent simulations, as used in Zhang et al. (2007). In all MSVAR 1.3 simulations, only 11 loci were used as the locus Ame-μ13 did not show evidence for a regular SMM.

Results

Individual identification

Of the analyzed fecal and hair samples, 114 genotypes were obtained and 52 unique genotypes were identified. P(ID) analysis revealed that the set of 12 loci produce an identical genotype with a probability of 1.4×10^{-8}, and a probability of 3.49×10^{-4} for a full-sib. The mean genotyping error rate per locus in our data set was 0.17% and combined genotyping error rate across 12 loci was 2.03%. Accordingly, we were to expect two to three unreliable combined genotypes among 114 genotypes. MICRO-CHECKER and DROPOUT analyses indicated that genotyping errors were reduced to a non-significant level.

Genetic diversity and phylogeographical structure

MtDNA CR sequences of 655-bp were successfully amplified from 32 individuals and five haplotypes were

Table 1 Running parameters for MSVAR 1.3 simulations

Runs	Starting values								Hyperpriors												
	$\log(N_0)$		$\log(N_1)$		$\log(\theta)$		$\log(T)$		$\log(N_0)$				$\log(N_1)$				$\log(\theta)$				$\log(T)$
Run 01	4	1	4	1	−3.5	1	5	1	4	3	0	0.5	4	3	0	0.5	−3.5	0.5	0	2	4	3	0	0.5
Run 02	4	1	4	1	−3.5	1	5	1	4	3	0	0.5	5	3	0	0.5	−3.5	0.5	0	2	4	3	0	0.5
Run 03	4	1	4	1	−3.5	1	5	1	4	3	0	0.5	3	3	0	0.5	−3.5	0.5	0	2	4	3	0	0.5

Columns 2–5: the two figures correspond to the starting values of the mean and variance of the corresponding parameters on a logarithmic scale. These values are updated during the MCMC, using hyperpriors defined by columns 6–9. Columns 6–9: the four figures correspond to the hyperprior mean and variance for the mean and variance for the corresponding parameters on a logarithmic scale. The only differences between Runs 01–03 are that different hyperprior means for the mean of the ancestral population size (N_1) were used to represent different population demographic history, namely stable, decreased and increased

defined including one new haplotype GH40 (GenBank accession number: HQ540590). Four additional haplotypes were defined in 10 individuals of the Liangshan population from Zhang et al. (2007). In total, 9 haplotypes were found from 42 individuals in the Liangshan population (see details in Fig. 2), including 11 variable sites (7 transitions, 4 indels). Haplotype diversity (h) and nucleotide diversity (π) were 0.7364 ± 0.0597 and 0.0038 ± 0.0004, respectively. For microsatellite markers, a mean allele number per locus of 4.0 was detected for 52 individuals in the Liangshan Mountains. Mean H_O and H_E were 0.683 and 0.592, respectively. A Hardy–Weinberg equilibrium test

showed that three loci (Ame-μ22, Ame-μ24 and Ame-μ26) significantly deviated from equilibrium, and no significant linkage disequilibrium was found (see Hu et al. 2010). The comparison of genetic diversity of the Liangshan population with other giant panda populations (Table 2) showed that the Liangshan population has medium-level genetic variation based on the measure of both mtDNA and microsatellite markers.

The median-joining network analysis showed that the nine mtDNA CR haplotypes of the Liangshan population are widely distributed throughout the network (Fig. 2),

Fig. 2 Median-joining network among the mitochondrial DNA control region haplotypes based on the combined data of this study and Zhang et al. (2007), with an emphasis on the Liangshan population. Different colors represent different mountainous populations and empty white circle denotes undetected median haplotypes. The definition of haplotype name follows Zhang et al. (2007). *Circle* areas are proportional to haplotype frequencies and length of *branches* to the number of changes from one haplotype to the following (one or two nucleotide substitutions). The *table* in the *lower left corner* contains detailed information of the haplotypes found in the Liangshan population from this study and Zhang et al. (2007)

Haplotype No.	GeneBank accession No.	Number of individuals	
		This study	Zhang et al. (2007)
GH17	EF100835	18	2
GH30	EF100848	3	1
GH31	EF100849	5	1
GH32	EF100850	4	2
GH33	EF100851	0	1
GH34	EF100852	0	1
GH35	EF100853	0	1
GH36	EF100854	0	1
GH40	HQ540590	2	0
Total		32	10

Liangshan
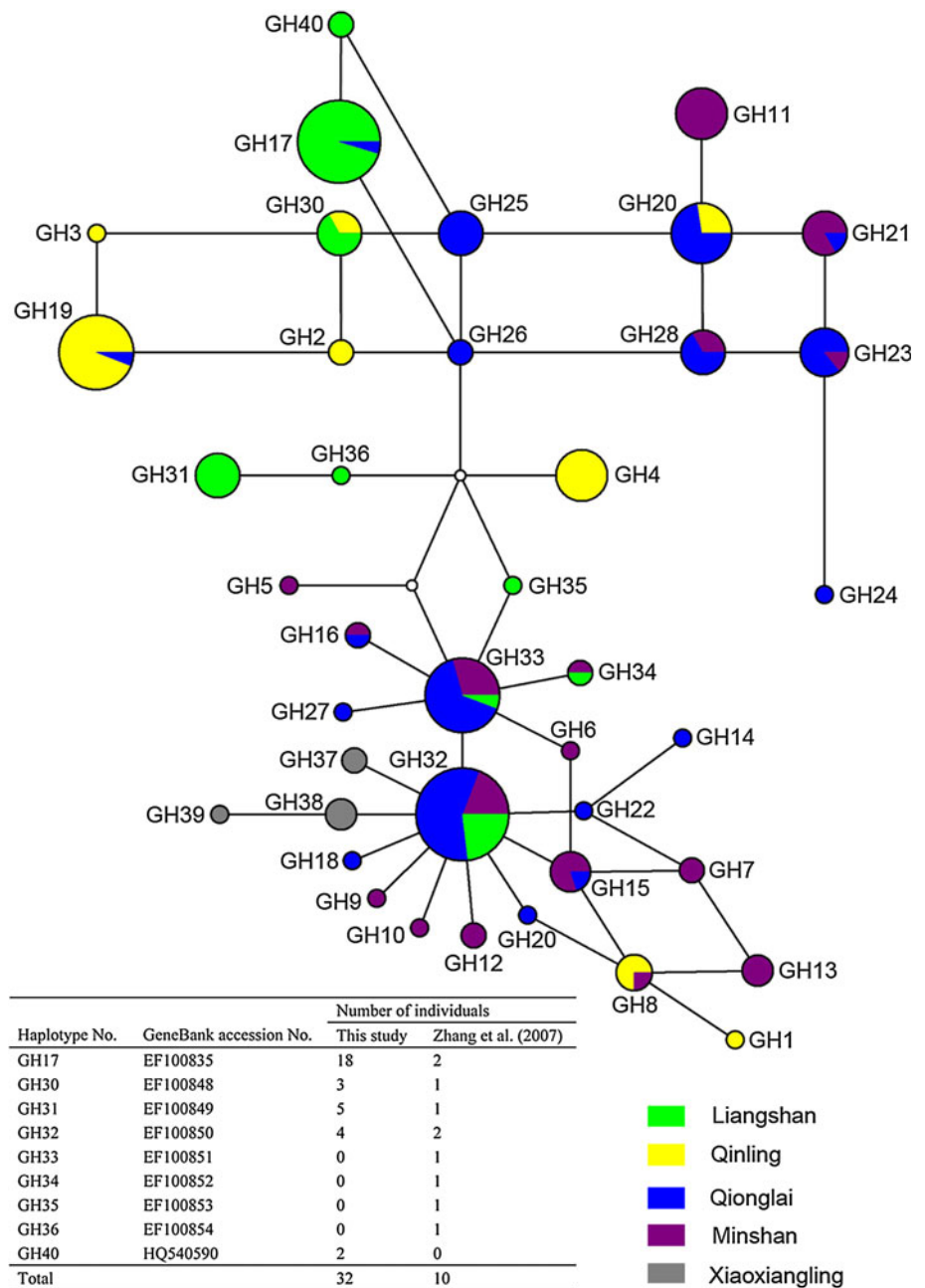Qinling
Qionglai
Minshan
Xiaoxiangling

Table 2 Comparison of genetic diversity of the Liangshan population with other giant panda populations for microsatellite and mitochondrial control region (CR) markers

Population	Microsatellite				Mitochondrial CR			References
	N	MNA	H_E	H_O	N	h	π	
LIS	52	4.0	0.592	0.683	42*	0.7364	0.0038	This study
LIS	4	2.3	0.366	0.425	10	0.956	0.0036	Zhang et al. (2007)
QIN	32	3.5	0.486	0.525	36	0.753	0.0039	Zhang et al. (2007)
MIN	29	4.8	0.559	0.561	44	0.926	0.0051	Zhang et al. (2007)
QIO	40	5.3	0.610	0.595	63	0.885	0.0043	Zhang et al. (2007)
XXL	10	4.0	0.635	0.685	6	0.733	0.0022	Zhang et al. (2007)
XXL	32	–	0.550	0.654	–	–	–	Zhu et al. (2010)
HL	10	4.8	0.670	0.647	–	–	–	Zhan et al. (2006)
WJ	6	2.9	0.633	0.570	–	–	–	Zhan et al. (2006)
WL	74	5.4	0.609	0.625	–	–	–	Zhan et al. (2006)

N the number of analyzed individuals for each marker; MNA mean number of alleles per locus, H_E expected heterozygosity, H_O observed heterozygosity, h haplotype diversity, π nucleotide diversity, * 42 samples include 32 individuals from this study and 10 individuals from Zhang et al. (2007). LIS Liangshan Mountains, QIN Qinling Mountains, MIN Minshan Mountains; QIO Qionglai Mountains, XXL Xiaoxiangling Mountains, HL, WJ and WL are three nature reserves located in the Minshan Mountains

suggesting no obvious phylogeographical structure for the Liangshan population or the whole giant panda population.

Demography

The mtDNA mismatch distribution analysis showed a multimodal distribution which might result from a relatively stable population size during the ancient period (Fig. 3). This violation of the null hypothesis of sudden population expansion was verified by the significant sum of squared differences (0.062, $P = 0.045$), although the
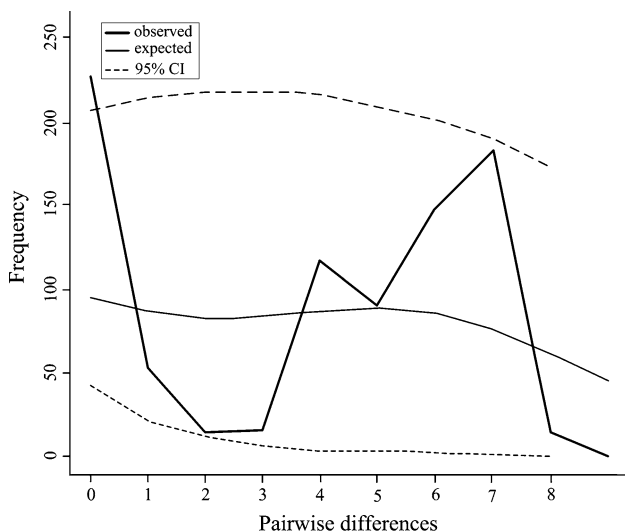

Fig. 3 Observed and expected mismatch distributions showing the frequency of pairwise nucleotide differences and 95% confidence interval of mismatch distribution under a model of population expansion

Harpending's raggedness index was not significant (0.102, $P = 0.083$). Fu's Fs, Ramos-Onsins and Rozas's R_2, and Fu and Li's D^* tests were not statistically significant ($Fs = 0.791$, $P = 0.710$; $R_2 = 0.174$, $P = 0.929$; $D^* = 0.501$, $P = 0.822$), which also rejected population expansion or contraction models. Furthermore, the coalescent-based analysis using FLUCTUATE 1.4 revealed a non-significant population growth parameter ($g \pm SD = 122.209 \pm 323.063$) with the confidence interval of g including zero, indicating a relatively stable population size in this population.

For microsatellite data, the heterozygosity excess test showed that, regardless of the proportion of one-step mutation, no significant heterozygosity excess was found under either TPM or SMM. However, significant heterozygosity excess occurred under the IAM model which is thought to a less appropriate model for microsatellites than the SMM (Shriver et al. 1993). Hence, we considered the detection result based on the IAM as invalid. In addition, after removing three loci that deviated from Hardy–Weinberg equilibrium, the result remained the same. The mode-shift test demonstrated a normal L-shape distribution of microsatellite allele frequencies in this population. M-ratio tests did not give a consistent conclusion that the Liangshan population experienced recent bottlenecks. The average M in this population was 0.803, and only at $\theta = 1$ (or 2) and $p_g = 5\%$, was significantly lower than relevant M_c ($P < 0.05$).

However, MSVAR 1.3 simulations revealed that the Liangshan population has experienced a recent population contraction that commenced 910–999 years before present (Table 3; Fig. 4). Multiple runs produced similar results

Table 3 Posterior distributions of N_0, N_1 and T (year) by MSVAR 1.3 simulations

	N_0 50% ± SD (10–90%)	N_1 50% ± SD (10–90%)	T (years) 50% ± SD (10–90%)
GT = 5	196 ± 73 (4–1936)	3982 ± 393 (721–23470)	910 ± 381 (9–51501)
GT = 6	189 ± 67 (2–2042)	4208 ± 77 (810–24136)	999 ± 372 (5–63556)

The posterior distributions of N_0 (current population size), N_1 (ancestral population size) and T (time since population change) were described by the 10, 50 and 90% quantiles, with one SD value (standard deviation) computed across multiple repeated runs. T was estimated using GT (generation time) as 5 and 6 years, respectively

Fig. 4 Posterior distributions of population demographic parameters from MSVAR simulations based on a generation time of 6 years (on a logarithmic scale). **a** current population size (N_0) and ancestral population size (N_1); **b** time since population decrease (T)
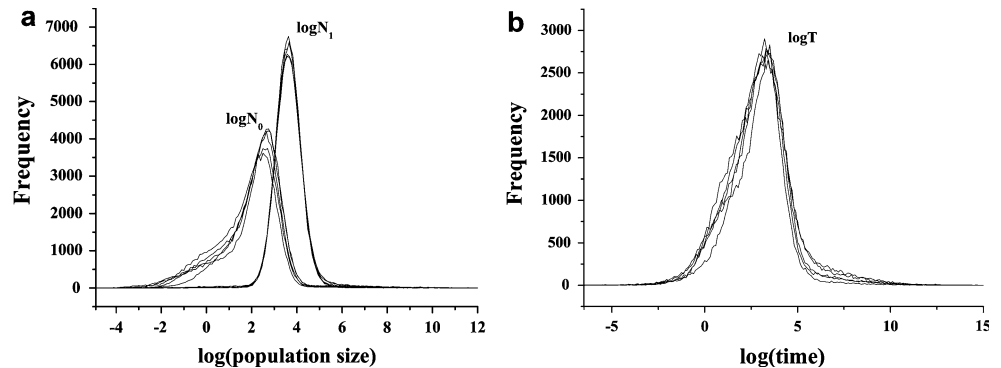
regardless of the demographic models and the generation times used. Different prior distributions (stable, decreased or increased population size) had very little effect on the posterior distributions of population demographic parameters. Because the exponential model is considered more accurate than the linear model for modeling recent population declines (Beaumont 1999), here we only report results from the exponential model. Based on analyses of the generation time of 5 or 6 years, the ratio of median current population size (N_0) to ancestral population size (N_1) was 0.05 (Table 3) and 0.04 (Table 3; Fig. 4a), respectively, equivalent to population contractions of 95–96%. We dated this decline to have commenced between 910 and 999 years before present (Table 3; Fig. 4b). We posit that this population experienced a slow population contraction but not a bottleneck, because the posterior distributions for log (N_0) and log (N_1) had some overlap and population size decreased by only 20-fold in the past approximate 1,000 years.

Discussion

Compared to genetic diversity reported by other studies of giant pandas (Zhan et al. 2006; Zhang et al. 2007; Zhu et al. 2010), the Liangshan population has medium-level genetic diversity and relatively ample genetic variation. Zhang et al. (2007) reported a low microsatellite diversity for the Liangshan population on a limited sample size of four, whereas our findings are based on a comprehensive sample ($N = 52$) covering the entire mountains, and hence should

represent a more accurate level of microsatellite diversity. Additionally, similar-level of mtDNA haplotype diversity and nucleotide diversity were found in the Liangshan population. These results show that the Liangshan population harbors relatively high genetic diversity despite a trend of population decline. Our findings also indicate that, regardless of the measure of mtDNA or nuclear microsatellites, this population has a similar-level of genetic variation to the Qionglai and Minshan populations. Moreover, the median-joining network of all the known CR haplotypes of giant pandas from our study and Zhang et al. (2007) showed that the haplotypes of the Liangshan population are distributed broadly throughout the network, which is also similar to the patterns of the Qionglai and Minshan populations (Fig. 2). Hence, in terms of genetic variation, the Liangshan population may share a similar biogeographic status to the Qionglai and Minshan populations, the two largest populations of giant pandas.

The mtDNA-based demographic history analyses showed that the Liangshan population did not experience an ancient population expansion or contraction, which could be explained by the geographical location of the Liangshan Mountains. Comparative phylogeography in the Qinghai-Tibetan Plateau showed that for five avian species, edge species suffered fewer effects from the Quaternary glaciation and demonstrated relatively stable population demography, different from plateau platform species (Qu et al. 2010). Similarly, the Liangshan Mountains are located at the eastern edge of the Qinghai-Tibetan Plateau and are adjacent to the Sichuan Basin, and species inhabiting this area may have suffered fewer demographic

impacts from the Quaternary glaciation. However, the microsatellite-based MSVAR simulation did detect, quantify and date a recent decrease in population size of 20-fold commencing 910–999 years before present, independent of demographic model (exponential versus linear) or generation time (5 or 6 years). The differences of results between mtDNA and microsatellite markers are not necessarily contradictory because the two types of markers are likely to be affected by demographic events occurring at different time-scales. For instance, Sousa et al. (2008) found evidence for a historical population expansion with mtDNA data and a recent population contraction for microsatellite data for the critically endangered cyprinid (*Chondrostoma lusitanicum*).

Our MSVAR analysis points to a large ancestral population size of 4,000 individuals in this area. Giant pandas were once widely distributed across Sichuan, Yunnan and Guizhou (Hu 2001), and few human settlements would have been present within the Liangshan Mountains (Zhu 2007a). We posit that giant pandas once inhabited the majority of the 19,200 km^2 Liangshan Mountain range and that, assuming a core habitat requirement of 0.33 km^2 (Hu et al. 1985) and a carrying capacity of 1.2 km^2 per giant panda (Qin 1990), this area could have easily supported such a larger number of giant pandas than it currently supports. Even a conservative use of a 5 km^2 home range (Hu et al. 1985) and no overlap still results in an estimate of the former population at around 3,840 giant pandas.

Multiple factors can result in population decline, including climate change, poaching and capturing for zoos or breeding centers, and habitat loss (Goossens et al. 2006; Okello et al. 2008). Historic records show that in the past 10,000 years, the average temperature in the Liangshan Mountains changed 1–2°C only (Lan 1993; Zhu 2007b), and has not experienced rapid climatic change for at least the past 2,000 years. Hu (2001) reviewed poaching and capture records and showed that giant pandas captured or poached were mainly from the Minshan (Pingwu county) and Qionglai Mountains (Baoxing county) and uncommon from the Liangshan Mountains. Records of human activity in the Liangshan Mountains show that anthropogenic habitat loss may be responsible for population decline in this area (Lin 1986; Zhu 2007a). Approximately 1,000 years ago, agricultural activity expanded in this region alongside a growing resident human population, deforestation and wood-related production. With the introduction of high-productive crops such as cotton and potato during the Qing Dynasty, agricultural areas replaced large tracts of forests and accelerated habitat loss and degradation (Zhu 2007a). For the first 90 years of the twentieth century, large-scale commercial logging was the main cause of habitat loss and degradation (Lin 1986). Consequently, habitat in the Liangshan Mountains and adjacent Xiaoxiangling and Daxiangling Mountains is isolated (SFA 2006). During this period of contraction giant pandas may have been forced to reduce their use of space to a small core area, utilize higher elevations, and inhabit more remote areas farther from people.

The demographic history of the Liangshan population is different from that of the Xiaoxiangling population (Zhu et al. 2010), but similar to those of the Qinling, Minshan and Qionglai populations (Zhang et al. 2007). The Xiaoxiangling population is the smallest and most fragmented, and experienced a 60-fold population decline over the last 250 years (Zhu et al. 2010). Isolated and small populations are vulnerable to genetic drift and inbreeding, and rare alleles are lost more quickly. In contrast, the Liangshan population is relatively large with 115 individuals (SFA 2006), and has suffered less from drift and inbreeding.

Although the Liangshan population has medium-level genetic variation and lacks genetic substructure, effective conservation and management are essential for its long-term survival due to the trend of population decline. First, core habitat of the Liangshan population should be protected in order to maintain a relatively large effective population size, high genetic diversity, and strong evolutionary potential. Core habitat is currently connected via Mamize, Mabian-Dafengding, Meigu-Dafengding, Heizugou, Maanshan and Shenguozhuang nature reserves; however, a key road continues to bisect core habitat, increasing the risk of habitat fragmentation and affecting gene flow (Qi et al. 2009; Hu et al. 2010). Hence, corridor construction at critical places along the road should be given a high priority. Second, habitat patches should be reconnected to core habitat to provide a greater area for persistence and expansion. Some small isolated patches exist at the periphery of this region and support a few giant pandas. Reconnecting these patches to core habitat is very important for the survival and persistence of the entire population. These practices, if implemented will not only benefit the giant panda but also a large number of threatened sympatric species found throughout the region, such as red pandas (*Ailurus fulgens*), Sichuan snub-nosed monkeys (*Rhinopithecus roxellanae*) and Sichuan partridges (*Arborophila rufipectus*).

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