Conservation Biology

Contributed Paper

Conservation Implications of Drastic Reductions in the Smallest and Most Isolated Populations of Giant Pandas

LIFENG ZHU,*† XIANGJIANG ZHAN,* HUA WU,* SHANNING ZHANG,‡ TAO MENG,*†
MICHAEL W. BRUFORD,§ AND FUWEN WEI*††

*Key Laboratory of Animal Ecology and Conservation Biology, Institute of Zoology, Chinese Academy of Sciences, 1-5 Beichenxilu, Beijing 100101, People's Republic of China
†Graduate School of Chinese Academy of Sciences, China, Beijing 100039, People's Republic of China
‡China Wildlife Conservation Association, No 18, Hepingli East Street, Beijing, 100714, People's Republic of China
§Biodiversity and Ecological Processes Group, Cardiff School of Biosciences, Cardiff University, Cardiff CF10 3AX, United Kingdom

Abstract: *In conservation biology, understanding the causes of endangerment is a key step to devising effective conservation strategies. We used molecular evidence (coalescent simulations of population changes from microsatellite data) and historical information (habitat and human population changes) to investigate how the most-isolated populations of giant pandas (*Ailuropoda melanoleuca*) in the Xiaoxiangling Mountains became highly endangered. These populations experienced a strong, recent demographic reduction (60-fold), starting approximately 250 years BP. Explosion of the human population and use of non-native crop species at the peak of the Qing Empire resulted in land-use changes, deforestation, and habitat fragmentation, which are likely to have led to the drastic reduction of the most-isolated populations of giant pandas. We predict that demographic, genetic, and environmental factors will lead to extinction of giant pandas in the Xiaoxiangling Mountains in the future if the population remains isolated. Therefore, a targeted conservation action—translocation—has been proposed and is being implemented by the Chinese goverment.*

Keywords: *Ailuropoda melanoleuca*, conservation implication, giant panda, habitat fragmentation, population reduction, Xiaoxiangling Mountains

Implicaciones de las Reducciones en las Poblaciones de Pandas Gigantes más Pequeñas y Aisladas

Resumen: *En biología de la conservación, el entendimiento de las causas de riesgo es un paso clave para el diseño de estrategias de conservación. Utilizamos evidencia molecular (simulaciones coalescentes de cambios poblacionales de datos de microsatélite) e información histórica (cambios de hábitat y de la población humana) para investigar cómo las poblaciones más aisladas de pandas gigantes* (Ailuropoda melanoleuca) *en las Montañas Xiaoxiangling llegaron a estar sumamente en peligro. Estas poblaciones experimentaron una fuerte y reciente reducción demográfica (60 veces) en los últimos 250 años. La explosión de la población humana y el uso de especies agrícolas no nativas en el esplendor del Imperio Qing resultaron en cambios de uso de suelo, deforestación y fragmentación de hábitat, que muy probablemente condujeron a la reducción drástica de las poblaciones más aisladas de pandas gigantes. Pronosticamos que factores demográficos, genéticos y ambientales llevarán a la extinción de pandas gigantes en las Montañas Xiaoxiangling sí las población permanece aislada. Por lo tanto, una acción de translocación se ha propuesto y está siendo implementada por el gobierno chino.*

Palabras Clave: *Ailuropoda melanoleuca*, fragmentación de hábitat, implicación para la conservación, Montañas Xiaoxiangling panda gigante, reducción de la población

---

††*Address correspondence to F. Wei, email weifw@ioz.ac.cn*
Paper submitted April 19, 2009; revised manuscript accepted December 15, 2009.

Conservation Biology, Volume 24, No. 5, 1299–1306

DOI: 10.1111/j.1523-1739.2010.01499.x

Introduction

The processes that lead to extinction are different now than they were in the geological past because anthropogenic factors increasingly influence their suvival (Tilman et al. 1994; Hughes et al. 1997). Small and isolated endangered populations have received considerable attention from conservationists (Schonewald-Cox et al. 1983; Soulé 1987; Loeschcke et al. 1994) because they may experience genetic erosion and be more susceptible to demographic and environmental variation than large populations (Lande 1988; Packer et al. 1991; Pimm & Raven 2000). Nevertheless, data on the trajectory of endangerment and extinction are comparatively rare and controversial (Lande 1988; Caro & Laurenson 1994; Spielman et al. 2004), especially for large mammals (Yang et al. 2006).

The giant panda (*Ailuropoda melanoleuca*) is often cited as one of the most endangered mammals in the world. Historically, this species ranged from southern China into northern Myanmar, northern Vietnam, Laos, and Thailand (Schaller et al. 1985; Hu 2001). Currently, it is confined to six mountain ranges on the edge of the Tibetan Plateau in China: Qinling, Minshan, Qionglai, Liangshan, Daxiangling, and Xiaoxiangling Mountains (Fig. 1, Hu 2001). The Xiaoxiangling population is the most isolated and smallest (State Forestry Administration 2006). The major cause for population declines in this species is still debated. Lack of evolutionary potential, low reproductive rate, anthropogenic effects, and climate change have all been cited (Zhu & Long 1983; Gittleman 1994; Fang et al. 1997; Lu et al. 2001), but evidence to support these hypotheses is lacking (Zhang et al. 2007). We used genetic, demographic, and historical data to identify the main processes that have lead to collapses of panda bear populations in the most isolated mountain region. On the basis of our findings, we devised conservation actions that may help prevent the extinction of panda bears.

Methods

Study Area and Sampling

The Xiaoxiangling Mountain Range includes Mianning, Shimian, Jiulong, Hanyuan, part of Luding, Yuexi, Ganluo, and Xide counties in China. The Xiaoxiangling Mountains are currently the most southwestern range of the giant panda and the area is bisected by the 108 National Road into two patches (A and B) (Fig. 1). We used the sampling strategy of Zhan et al. (2006) to collect 142 fecal samples and one blood sample. Most samples were <2 weeks as determined from the status of the outer mucosa layer on the feces. Locations where samples were taken were recorded with a geographic positioning system and mapped in Arcview (version 3.2a; ESRI, Redlands, California). Up to 5 g of feces were peeled from the outer layer and stored in 95% ethanol.

DNA Extraction and Amplification and Individual Identification

We adopted a widely used protocol for DNA isolation from feces (Zhang et al. 2006) under standard blank controls. We screened feces DNA with 18 giant panda microsatellite loci from Lu et al. (2001) and three redesigned loci from Shen et al. (2005) and selected nine of these (Ame-μ05, Ame-μ10, Ame-μ13, Ame-μ15, Ame-μ16, Ame-μ26, Ame-μ22, AFAY161179, AY161195) for this study on the basis of their efficiency with fecal DNA, polymorphism, and yield. To obtain reliable genotypes, we used a multitube approach (Zhan et al. 2006). Polymerase chain reaction (PCR) amplification of 50 cycles was carried out for up to four loci simultaneously with combinations selected based on the range of fragment sizes, T_m, and fluorescent dye (FAM, TET, or HEX). We used the QIAGEN Multiplex PCR kit according to the manufacturer's protocol to optimize annealing temperatures. Products were resolved with an ABI 377 prism automated sequencer and analyzed with GeneScan (version 3.1.2) and Genotyper (version 2.5) (Applied Biosystems, Foster City, California). We used Zhan et al.'s (2006) methods to determine sex. Genotyping errors have been reported in the use of fecal DNA samples in amplification (Taberlet et al. 1996; Pompanon et al. 2005); thus, preselection of samples and rigorous laboratory procedures need to be undertaken to produce accurate genotypes. As part of this process, we conducted mitochondrial DNA (mtDNA) analysis for species verification, and our microsatellite genotyping protocol followed strict criteria (Taberlet et al. 1996). Moreover, we took a mathematical approach to estimate genotype error rates (Zhan et al. 2009). Additionally we used GIMLET (Valière 2002) to calculate probabilities of identity ($P_{[\mathrm{ID}]}$ and $P_{[\mathrm{ID-sibs}]}$) so that we could examine the discriminatory power of the nine loci in combination.

Estimation of Population Size

We used the DNA-based mark–recapture software (CAPWIRE; Miller et al. 2005) to estimate population size. Unlike traditional mark–recapture approaches, this method deals efficiently with data inferred from multiobservations of individuals within a sampling session. Pandas are mostly solitary and defecate on average 40 times/day (Hu 2001). Therefore, we used the ECM (even capture probability model, which assumes similar deposition rates and even sampling effort among individuals) in CAPWIRE to estimate population size, but compared these results with an alternative model, TIRM (two innate rates model, which assumes the population includes individuals with two kinds of capture probabilities). We

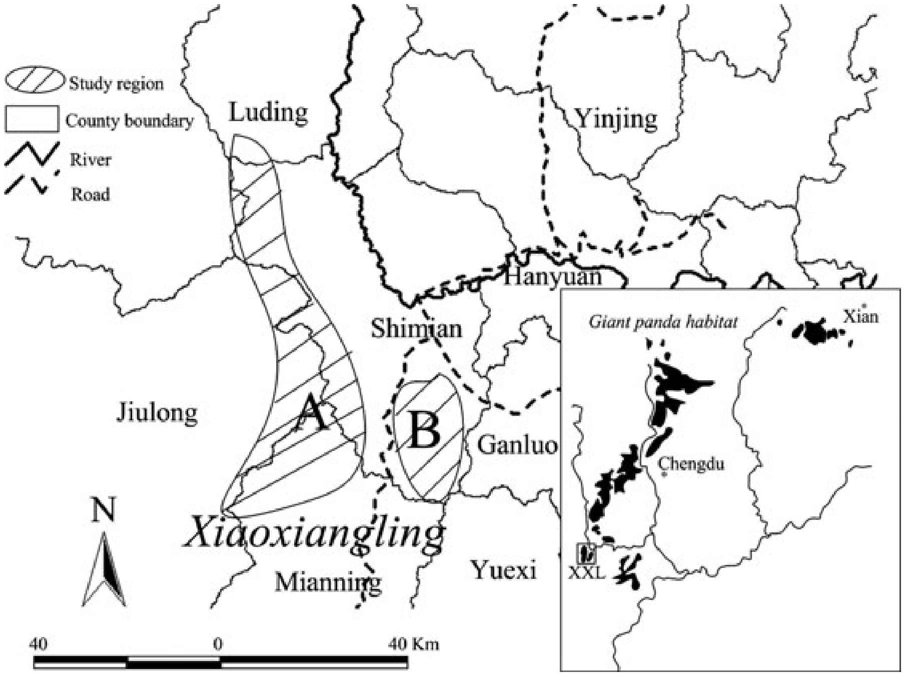

Figure 1. Study areas and major distribution region of giant panda in the Xiaoxiangling Mountains. Inset shows the position of the Xiaoxiangling population (XXL) within the extant distribution of giant pandas (populations in six mountain ranges) in China.

used 1000 bootstrap replicates to produce 95% CIs of the census population size (N_c) under two models.

Simulation of Population Change

Recent population bottlenecks can produce distinctive genetic signatures in the distributions of allele size, expected heterozygosity, and in the genealogy of microsatellite loci (Cornuet & Luikart 1996; Beaumont 1999; Goossens et al. 2006). We used the heterozygosity test in BOTTLENECK (Piry et al. 1999) to detect the signature of a recent demographic bottleneck in the two models, the infinite allele (IAM) and two-phase mutation models (TPM) with various (75–95%) single-step mutations (Di Rienzo et al. 1994).

Using the allelic spectra of present day samples and coalescent simulations (Beaumont 1999), we estimated the likelihood of population increase or decrease. We assumed that the population of effective size (N_1) started to decrease (or increase) t_a generations ago to the current effective population size (N_0). The change in population size can be assumed to be either linear or exponential, and mutations are assumed to occur under a stepwise model, with a rate $\theta = 2N_0\mu$, where μ is the per-locus mutation rate. Thus, θ indicates the rate of production of new neutral alleles. Bayesian coalescent-based approaches were used to estimate the posterior probability distributions of the rate of population size change ($r = N_0/N_1$), the time since the population started changing size ($t_f = t_a/N_0$) and $\theta = 2N_0\mu$. The method uses a Markov Chain Monte Carlo (MCMC) approach to sample the posterior distribution of the parameters. To avoid favoring regions of the parameter space, at least three independent runs were performed for each analysis with different starting values (Supporting Information) so that some of the runs were carried out with positive starting values of log(r). In this method, rectangular prior distributions are assumed for log(r), log(θ), and log(t_f). Wide bounds for these prior distributions were chosen (between 10^{-3} and 10^3 on a natural log scale) so that posterior distributions would not be affected much.

We used the Storz and Beaumont extension of Beaumont's original method to re-estimate N_0, N_1, and T (time since the population change) (Storz & Beaumont 2002). The prior distributions for N_0, N_1, T, and μ were assumed to be log normal. The means and SDs of these log-normal distributions were themselves drawn from prior (or hyperprior) distributions. Variances for these prior distributions were large so they would affect them as little as possible. Different runs were carried out by allowing N_1 to be either greater than or equal to N_0 (Table S2), which did not affect the posterior distribution. Runs assuming that N_1 and N_0 were of the same size were repeated three times as a test and provided exactly the same results. The number of iterations was 2×10^9, and burn-in was 2×10^4.

Human Population Size and Habitat Loss

The human population sizes of Mianning, Shimian, and Hanyuan counties were derived from county annals published in the Qing Dynasty (from National Science Library, Chinese Academy of Sciences; Cao 2001) before 1953 and from the National Human Survey or provincial yearbooks after 1953. Recent habitat loss was estimated through classification of land types seen in three satellite images taken in 1975, 1988, and 1994, respectively, which covered the entire Xiaoxiangling region.

Results

Individual Identification and Population Estimation

A total of 102 genotypes were obtained from 142 fecal samples, among which 32 were unique. The consistency of genotypes was checked according to standard replication criteria (Taberlet et al. 1996). Using the formulae designed by Zhan et al. (2009), we estimated the mean genotype error rate per locus was 0.16% and the whole genotype identification error across nine loci was 1.4%. Therefore, we expected at most two incorrect genotypes identified among the 102 produced, which has the potential to upwardly bias our population estimates slightly. The probability of identity based on these loci and their allele frequencies ($P_{[ID]}$ and $P_{[ID\text{-}sibs]}$) was 1.0×10^{-8} and 7.3×10^{-4}, respectively. Thus, the nine loci combinations would only produce an identical genotype by chance in the case of full sibs with a probability of 0.0007. In the CAPWIRE simulations, the predicted population size under the even capture probability model for patch A was 22 (95% CI 20–26) or 25 (95% CI 20–35, TIRM) and the size in patch B was 12 (95% CI 12–12, ECM; 12–15, TIRM).

Change in Population Size

The bottleneck test indicated that, regardless of the mutation model assumed, the Xiaoxiangling population exhibited a strong and significant signal of a population bottleneck (Table 1).

Table 1. Results of the bottleneck analysis for the panda population in the Xiaoxiangling region.

		TPM	
Locus	H_o	H_e	p
Ame-μ5	0.813	0.678	0.0155
Ame-μ10	0.824	0.730	0.0511
Ame-μ26	0.506	0.229	0.0364
Ame-μ15	0.602	0.532	0.3717
Ame-μ16	0.598	0.621	0.3372
Ame-μ13	0.747	0.537	0.0065
Ame-μ22	0.371	0.409	0.3797
AY161179	0.772	0.679	0.1370
AY161195	0.655	0.532	0.1939

Abbreviations: H_o, *observed heterozygosity;* H_e, *expected heterozygosity; TPM, two-phase mutation models. We used Wilcoxon tests: TPM,* $p = 0.005$; *IAM (the infinite allele),* $p = 0.001$.

Results based on Beaumont's method (1999) and Storz and Beaumont's method (2002) revealed a genetic signature for a recent decline in the giant panda population in the Xiaoxiangling region. The Beaumont method showed that giant panda populations decreased by a factor of approximately 56 (25%, 50%, 75%, and 95% of the posterior distribution are below 154, 56, 23, and 8, respectively) and no support for growing or even stable populations (Fig. 2a). This result was independent of the demographic model we used (exponential versus linear, Supporting Information), and it suggests the giant panda population has decreased by approximately 60-fold (median value).

The Storz and Beaumont approach showed the same pattern of posterior distributions for $\log(N_0)$ and $\log(N_1)$ and a median value of 40 and 2570 for N_0 and N_1, respectively (Supporting Information). This approach allowed us to date the collapse by providing a posterior distribution for T, the time at which the population starts to decrease. The generation time of the giant panda is 5 years (Hu 2001); thus, the posterior distribution for T had a median value of approximately 250 years before present (Fig. 2b; Supporting Information; 10%, 25%, 50%, 75%, and 95% of the distribution masses were below 30, 80, 250, 1290, 3680 years, respectively), despite the fact that, in the prior approach, the median, mean,

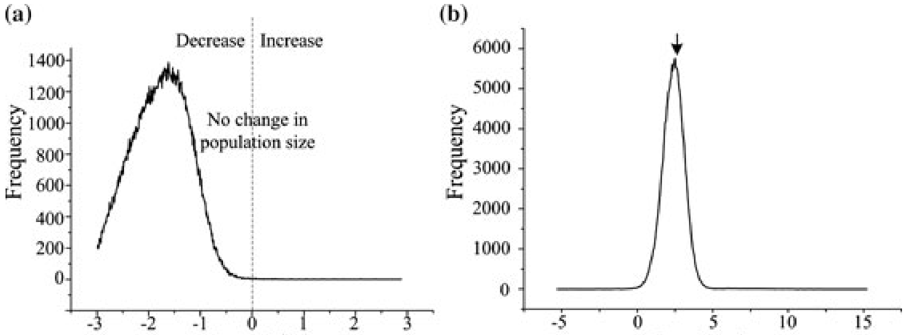

Figure 2. (a) Size of giant panda population in the Xiaoxiangling Mountains relative to the ratio of present (N_0) to past (N_1) population size (solid curve, posterior distribution under a model of exponential change in population size; for linear models see Supporting Information). (b) Time since the panda population collapse relative to population size (posterior distribution for time since population collapse is on a logarithmic scale; median distribution approximately 250 years; black arrow, start of human population explosion).

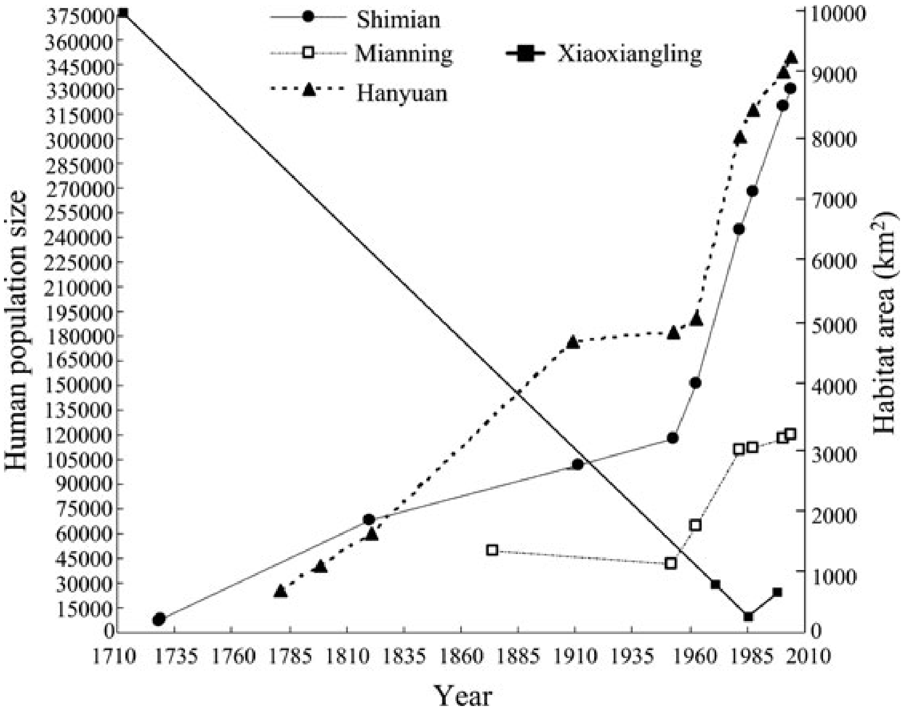

Figure 3. Human population and estimated habitat loss of giant pandas over the past 300 years in the main Xiaoxiangling region. The specific amount of panda habitat is not clear for the 1990s, but it would be far lower than that of 1985 because of deforestation that took place before the 1998 logging ban (solid line, habitat changes in the Xiaoxiangling region; dashed line, filled circle, and open square, human population size changes for each county in the Xiaoxiangling region).

and mode were 20,000 years ago (Supporting Information), that is, 80 times older. Data also show that nearly 50% of the posterior distribution coincides with the last three centuries and the modal value (in natural rather than logarithmic scale). Given that human population explosion and the exploitation of forests began in the 18th century (Ho 1959), this distribution is informative. Moreover, to quantify the posterior distribution, dates older than 10,000 years ago have only a posterior distribution support of 0.41%. These results suggested that prehistoric human activity and Pleistocene climatic events do not reasonably explain the detected genetic signature of a population decrease.

The human population during the past 300 years increased sharply in the Xiaoxiangling region (Fig. 3). For example, the population in Mianning County, one of the main distribution regions of the giant panda in the Xiaoxiangling region, increased 41-fold from 1728 to 2004 and nearly 10-fold from 1728 to 1820. The population size in Hanyuan County increased near eight-fold from 1781 to 1909. According to archaeological evidence from the Paleolithic and Neolithic, human activities were limited to valleys along the Dadu River, and the human population size was very small (Ge 2006). From the Qin Dynasty to the Tang Dynasty (221 BC–AD 907), to ensure national defense major exploitation of lands was still along the river. From the Yuan Dynasty to the Ming Dynasty (1271–1644), humans, including Yi and Zang minorities, began to immigrate to these mountainous areas near the panda habitat (Fang 1984). Even in the early Qing Dynasty, the human population was still small (e.g., only 1754 families lived in Mianning county). In the middle of the Qing Dynasty, however, a sharp population increase was recorded in the Xiaoxiangling region (Fig. 3).

Assuming almost complete coverage of the Xiaoxiangling area with panda habitat 300 years ago, panda habitat may have decreased nearly 31-fold (Fig. 3). Panda habitat decreased about 30% between 1975 and 1994, and land use increased nearly 2-fold (Fig. 4).

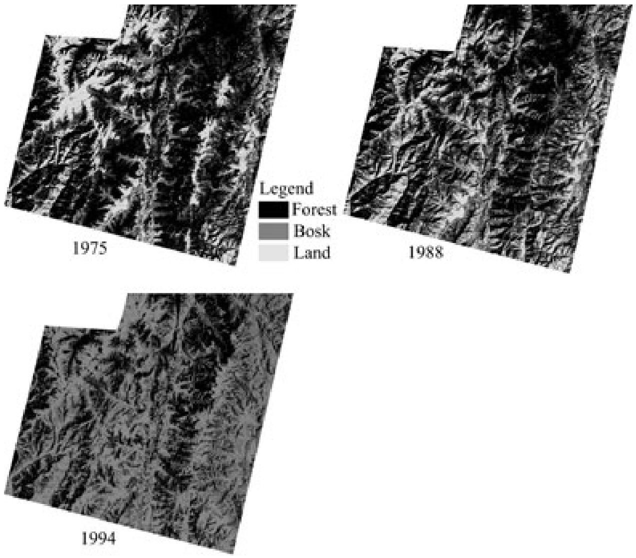

Figure 4. Changes in forest area from 1975 to 1994 in the Xiaoxiangling region derived from the classification analysis of satellite images. Land mainly includes farm and bare land; Bosk is shrub and scrub vegetation.

Polices and Social Development in Recent Local History

In the 17th and 18th centuries people subsisted mostly on agriculture, and slash and burn methods were used to make land arable in the mountainous areas. As the population grew, forests were converted to farm lands and panda habitats were lost and fragmented. About 300 years ago, to increase the local economy, four major polices were enacted in the Qing Dynasty (1661–1911) that resulted in a human population explosion. First, the government recruited many people from adjacent provinces, such as Hunan, Hubei, and Jiangxi provinces, to exploit the mountain area. Second, in 1712 Emperor Kangxi enacted a law that stated newly born children would not be taxed, which alleviated the farmer's burden and accelerated the population explosion. Third, after 1723, in order to further stimulate social development, Emperor Yongzheng decreased the poll and farming taxes. In addition, after 1728 he appointed many officials to help people develop agricultural technology in rural areas, such as the Xiaoxiangling region.

Discussion

Our results provide evidence for a recent demographic reduction (around 60-fold) in the Xiaoxiangling population of giant pandas. We dated this reduction back to approximately 250 years ago. This reduction was independent of the demographic models used and was supported by our analysis in BOTTLENECK. The confidence intervals of the current census population size estimated by genetic capture-recapture analysis (range 32–50) overlapped with the current effective population size (N_e) (40 estimated by the Storz and Beaumont method). Although the N_e:N_c ratio is expected to be substantially less than 1:1 for most animal populations, in certain circumstances it may be higher in small populations (Palstra & Ruzzante 2008) due to allee effects and nonrandom survival during demographic bottlenecks (e.g., Keller et al. 2001). Furthermore, variables commonly known to affect this ratio similarly include fluctuations in population size, unequal sex ratio, and overlapping generations (e.g., Wright 1969; Frankham 1995; Pray et al. 1996). In the Xiaoxiangling region, the giant panda population is small, has overlapping generations, high mortality of juveniles, and a sex ratio that does not deviate from 1:1(Wei et al. 1989), which are the main reasons for the high N_e:N_c ratio. Moreover, we found that the large increases in the human population have led to environmental changes such as deforestation, which have destroyed and fragmented giant panda habitat. It is likely that these habitat changes have led to the giant panda population collapse in this isolated region.

Results from the Storz and Beaumont showed that the most notable feature of the demographic history of the giant panda in the Xiaoxiangling region is the pronounced decline in effective population size starting approximately 250 years ago. This finding was similar in all the Bayesian analyses we conducted. Although the population size of giant pandas is small, we were able to reconstruct their demographic history because sampling has been extensive and the Xiaoxiangling region is the most isolated of giant panda habitats. This region is potentially ideal for an examination of population dynamics and extinction on the basis of coalescent theory (Kingman 1982a, 1982b; Edwards & Beerli 2000; Emerson et al. 2001). Our findings are similar to those from work on a Bornean orangutan (*Pongo pygmaeus*) population, which has also suffered a recent demographic collapse due to human-induced deforestation and habitat fragmentation starting about 210 years ago (Goossens et al. 2006). Using the Storz and Beaumont method, Heller et al. (2008) detected a strong climate-mediated decline (75–98%) in African buffalo (*Syncerus caffer*) populations starting in the mid-Holocene (approximately 3000–7000 years ago). Therefore, on the basis of our results and the results of others (see also Storz & Beaumont 2002; Storz et al. 2002; Lucchini et al. 2004), Bayesian coalescent modeling can successfully restructure demographic history.

The posterior distribution of the collapse of the panda population in China mainly overlaps with human population expansions in recent history. Nevertheless, dates before 10,000 years ago only have a posterior support of 0.41%, and human population size was small before the Qing Dynasty. Thus, the drastic decline of populations of giant panda is concomitant with a sharp increase in the human population in the Xiaoxiangling region during the past 300 years (Ho 1959; Cao 2001). During this period, the panda habitat may have decreased nearly 31-fold. More recently (early 1970s to mid-1980s), panda habitat decreased about three-fold (from 925 km^2 to 330 km^2) due to deforestation in the area (Hu 1993). Our example reinforces the fact that loss and fragmentation of high-quality habitat have been at least partially responsible for the dramatic decrease in the number of pandas in other wild populations, such as Wolong Nature Reserve, (145 pandas in 1974 to 72 in 1986 (Liu et al. 2001)) and even resulted in population extinctions in Hunan, Hubei, Guizhou, Chongqing, and Yunnan provinces during the 18th and 19th centuries (Wen & He 1981).

Anthropogenic harvest, including poaching and capturing for zoos, is another possible reason for population collapse of the giant panda (Hu 1998*a*, 1998*b*; Li et al. 2003). Nevertheless, unlike other distribution ranges of the giant panda, up to now no poaching has been recorded and only three injured individuals have been rescued and removed from in the Xiaoxiangling regions since 1980. Poaching has not occurred (State Forestry Administration 2006) because of social development, improved conservation awareness, and government control (e.g., confiscation of guns). Panda capturing has been prohibited since 1990.

Therefore, human population explosion from 1662 through 1795 that resulted in increases in land use, deforestation, habitat loss, and fragmentation is likely to have led to the collapse of the panda population in this region. A potentially important factor in habitat loss was the introduction into the region of maize, potato, and sweet potato in the late 17th century, which made it possible to cultivate crops at middle to high elevations (Ho 1959). Cultivation of these crops still encroaches on panda habitat today. Until the 1950s the proportion of the human population working in agriculture exceeded 90% in the Xiaoxiangling area. Additionally, the traditional and primary fuel of local people for building and heating was wood, and uncontrolled harvesting must have affected panda habitat and forced populations to higher elevations.

During the past several hundred years, population declines due to human activities in China have also been observed in many species distributed sympatrically with the giant panda (Wen et al. 2006), including the red panda (*Ailurus fulgens*) (Wei et al. 1999), the Sichuan snub-nosed monkey (*Rhinopithecus roxellanae*) (Jin & Xie 2002), the takin (*Budorcas taxicolor*) (Wei & Hu 1993), and the Crested Ibis (*Nipponia nippon*) (Ding 2004). Our findings may serve to enhance the conservation profile of other endangered species in the region. To help the Xiaoxiangling population of the giant pandas recover, targeted action, specifically translocation, was proposed in light of our data, and in April 2009 the first female giant panda was translocated into Lizhiping Reserve (patch B, Fig. 1) by the Chinese government.

Supporting Information

Details of the Markov chain Monte Carlo runs for the model of linear and exponential change in population size (Appendix S1-S3) and for the model of time since population change (Appendix S4-S6) are available as part of the on-line article. The authors are responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

Acknowledgments

This study was supported by projects of the National Basic Research Program of China (973 Program, 2007CB411600), National Natural Science Foundation of China (no. 30830020, 30670329), and the National Key Technology R & D Program in the 11th Five Year Plan of China (2008BADB0B04). We thank the staff of Sichuan Forestry Department, Yele Nature Reserve (NR), Lizhiping NR, and Wawushan NR for their kind help during fieldwork.

Literature Cited

Beaumont, M. A. 1999. Detecting population expansion and decline using microsatellites. Genetics **153:**2013–2029.

Cao, S. J. 2001. The history of Chinese population. Publishing House of Fudan University, Shanghai.

Caro, T. M., and M. K. Laurenson. 1994. Ecological and genetic factors in conservation: a cautionary tale. Science **263:**485–486.

Cornuet, J. M., and G. Luikart. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics **144:** 2001–2014.

Ding, C. Q. 2004. Research on the crested ibis. Shanghai Publishing House of Science and Technology, Shanghai.

Di Rienzo, A., A. C. Peterson, J. C. Garza, A. M. Valdes, M. Slatkin, and N. B. Freimer. 1994. Mutational processes of simple-sequence repeat loci in human populations. Proceedings of the National Academy of Sciences of the United States of America **91:**3166–3170.

Edwards, S. V., and P. Beerli. 2000. Gene divergence, population divergence, and the variance in coalescence time in phylogenographic studies. Evolution **54:**1839–1854.

Emerson, B. C., E. Paradis, and C. Thébaud. 2001. Revealing the demographic histories of species using DNA sequences. Trends in Ecology & Evolution **16:**707–716.

Fang, G. Y. 1984. The history of Yi minority. Sichuan Nationalities Publishing House, Chengdu.

Fang, S. G., et al. 1997. The research on genetic diversity of the giant panda. Pages 141–147 in Chengdu Zoo and Chengdu Research Base for Giant Panda Breeding, editors. Proceeding of the international symposium on the protection of the giant panda (*Ailuropoda melanoleuca*). Sichuan Publishing House of Science and Technology, Chengdu.

Frankham, R. 1995. Effective population size / adult population size ratios in wildlife: a review. Genetics Research **66:**95–107.

Ge, L. 2006. The early history and culture of Zang Minority. The Commercial Press, Beijing.

Gittleman J. L. 1994. Are the pandas successful specialists or evolutionary failures? BioScience **44:**456–464.

Goossens, B., L. Chikhi, M. Ancrenaz, I. Lackman-Ancrenaz, P. Andau, and M. W. Bruford. 2006. Genetic signature of anthropogenic population collapse in orang-utans. Public Library of Science Biology **4:** e25. DOI:10.1371/journal.pbio.0040025.

Heller, R., E. D. Lorenzen, J. B. Okello, C. Masembe, and H. R Siegismund. 2008. Mid-Holocene decline in African buffalos inferred from Bayesian coalescent-based analyses of microsatellites and mitochondrial DNA. Molecular Ecology **17:**4845–4858.

Ho, P. T. 1959. Studies on the population of China 1368–1953. Harvard University Press, Cambridge, Massachusetts.

Hu, J. C. 1993. Preliminary analysis on population decline of giant panda. Pages 40–45 in W. Xia and J. Zhang, editors. The successional changes of mammals in China under the influences of human activities. China Science and Technology Press, Beijing.

Hu, J. C. 1998*a*. *Ailuropoda melanoleuca*. Pages 158–165 in S. Wang, editor. China red data book of endangered animals. China Science Press, Beijing.

Hu, J. C. 1998*b*. Returning to the nature and protection of giant pandas. Pages 3–7 in J. Hu and Y. Wu, editors. Resource and conservation of vertebrate. Sichuan Science and Technology Press, Chengdu.

Hu, J. C. 2001. Research on the giant panda. Shanghai Publishing House of Science and Technology, Shanghai.

Hughes, J. B., G. C. Daily, and P. R. Ehrlich. 1997. Population diversity: its extent and extinction. Science **278:**689–692.

Jin, G. Q., and J. H. Xie. 2002. Research on the golden monkeys. Shanghai Publishing House of Science and Technology, Shanghai.

Keller, L. F., K. J. Jeffery, P. Arcese, M. A. Beaumont, W. M. Hochachka, J. N. M. Smith, and M. W. Bruford. 2001. Immigration and the ephemerality of a natural population bottleneck: evidence from molecular markers. Proceedings of the Royal Society, London, Biological Sciences **268:**1387–1394.

Kingman, J. F. C. 1982*a*. The coalescent. Stochastic Processes and Applications **13:**235–248.

Kingman, J. F. C. 1982*b*. On the genealogy of large populations. Journal of Applied Probability **19:**27–43.

Lande, R. 1988. Genetics and demography in biological conservation. Science **242:**1455–1460.

Li, Y. M., Z. W. Guo, Q. S. Yang, Y. S. Wang, and J. Niemelä. 2003. The implications of poaching for giant panda conservation. Biological Conservation **11:**125–136.

Liu, J. G., M. Linderman, Z. Ouyang, L. An, J. Yang, and H. Zhang. 2001. Ecological degradation in protected areas: the case of Wolong Nature Reserve for giant pandas. Science **292:**98–101.

Loeschcke, V., J. Tomiuk, and S. Jain. 1994. Conservation genetics. BirkhaÈuser Verlag, Basel.

Lu, Z., et al. 2001. Patterns of genetic diversity in remaining giant panda populations. Conservation Biology **15:**1596–1607.

Lucchini, V., A. Galov, and E. Randi. 2004. Evidence of genetic distinction and long-term population decline in wolves (*Canis lupus*) in the Italian Apennines. Molecular Ecology **13:**523–536.

Miller, C. R., P. Joyce, and Waits L. P. 2005. A new method for estimating the size of small populations from genetic mark–recapture data. Molecular Ecology **14:**1991–2005.

Packer, C., A. E. Pusey, H. Rowley, D. A. Gilbert, J. Martenson, and S. J. O'Brien. 1991. A case study of a population bottleneck: lions of the Ngorongoro Crater. Conservation Biology **5:**219–230.

Palstra, F. P., and D. E. Ruzzante. 2008. Genetic estimates of contemporary effective population size: what can they tell us about the importance of genetic stochasticity for wild population persistence? Molecular Ecology **17:**3428–3447.

Pimm, S. L., and P. Raven. 2000. Biodiversity: extinction by numbers. Nature **403:**843–845.

Piry, S., G. Luikart, and J. M. Cornuet. 1999. A computer program for detecting recent reductions in the effective size using allele frequency data. Journal of Heredity **90:**502–503.

Pompanon, F., A. Bonin, E. Bellemain, and P. Taberlet. 2005. Genotyping errors: causes, consequences and solutions. Nature Reviews Genetics **6:**847–859.

Pray, L. A., C. J. Goodnight, L. Stevens, J. M. Schwartz, and G. Y. Yang. 1996. The effect of population size on effective population size: an empirical study in the red flour beetle *Tribolium castaneum*. Genetics Research **68:**151–155.

Schaller, G. B., J. C. Hu, W. S. Pan, and J. Zhu. 1985. The giant pandas of Wolong. The University of Chicago Press, Chicago.

Schonewald-Cox, C. S., S.M. Chambers, B. MacBryde, and L. Thomas. 1983. Genetics and conservation: a reference for managing wild animal and plant populations. Benjamin-Cummings, London.

Shen, F. J., W. Phill, Z. H. Zhang, A. J. Zhang, S. Stephanie, J. K. Steve, and B. S. Yue. 2005. Enrichment of giant panda microsatellite markers using dynal magnet beads. Acta Genetic Sinica **32:**457–462.

Soulé, M. E. 1987. Viable populations for conservation. Cambridge University Press, Cambridge.

Spielman, D., B. W. Brook, and R. Frankham. 2004. Most species are not driven to extinction before genetic factors impact them. Proceedings of the National Academy of Sciences of the United States of American **101:**15261–15264.

State Forestry Administration. 2006. The 3rd national survey report on giant panda in China. Science Press, Beijing.

Storz, J. F., and M. A. Beaumont. 2002. Testing for genetic evidence of population expansion and contraction: an empirical analysis of microsatellite DNA variation using a hierarchical Bayesian model. Evolution **56:**154–166.

Storz, J. F., M. A. Beaumont, and S. C. Alberts. 2002. Genetic evidence for long-term population decline in a savannah-dwelling primate: inferences from a hierarchical Bayesian model. Molecular Biology and Evolution **19:**1981–1990.

Taberlet, P., S. Griffin, B. Goossens, S. Questiau, V. Manceau, N. Escaravage, L. P. Waits, and J. Bouvet. 1996. Reliable genotyping of samples with very low DNA quantities using PCR. Nucleic Acids Research **24:**3189–3194.

Tilman, D., M. M. Robert, L. L. Clarence, and A. N. Martin. 1994. Habitat destruction and the extinction debt. Nature **371:**65–66.

Valière, N. 2002. Gimlet: a computer program for analyzing genetic individual identification data. Molecular Ecology Notes **2:**377–379.

Wei, F. W., and J. C. Hu. 1993. The distribution of the takin (*Budorcas taxicolor*) in Sichuan province. Sichuan Journal of Zoology **12:**32–33.

Wei, F. W., Z. J. Feng, and Z. W. Wang. 1999. Current distribution, status and conservation of wild red pandas (*Ailurus fulgens*) in China. Biological Conservation **89:**285–291.

Wei, F. W., J. C. Hu, G. Z. Xu, Z. M. Zhong, and Q. T. Deng. 1989. A study of the life table of wild giant pandas. Acta Theriologica Sinica **9:**81–86.

Wen, H. R., and Y. H. He. 1981. The distribution of giant panda in Henan, Hubei, Hunan and Sichuan provinces in last five thousands years. Journal of Southwest Technical College **1:**87–93.

Wen, H. R., Y. H. He, and Y. T. Gao. 2006. The change of plant and animal in China during different historical period. Chongqing Publishing House, Chongqing.

Wright, S. 1969. Evolution and the genetics of populations. Vol. 2, the theory of gene frequencies. University of Chicago Press, Chicago.

Yang, G., M. W. Bruford, F. W. Wei, and K. Y. Zhou. 2006. Conservation options for the Baiji: times for realism? Conservation Biology **20:**620–622.

Zhan, X. J., M. Li, Z. J. Zhang, B. Goossens, Y. P. Chen, H. J. Wang, M. W. Bruford, and F. W. Wei. 2006. Molecular censusing doubles giant panda population estimate in a key nature reserve. Current Biology **16:**451–452.

Zhan, X. J., X. D. Zheng, M. W. Bruford, F. W. Wei, and Y. Tao. 2009. A new method for quantifying genotyping errors for noninvasive genetic studies. Conservation Genetics DOI: 10.1007/s10592-009-9950-9.

Zhang, B. W., M. Li, L. C. Ma, and F. W. Wei. 2006. A widely applicable protocol for DNA isolation from fecal samples. Biochemical Genetics **44:**503–512.

Zhang, B. W., M. Li, Z. J. Zhang, B. Goossens, L. F. Zhu, S. N. Zhang, J. C. Hu, M. W. Bruford, and F. W. Wei. 2007. Genetic viability and population history of the giant panda, putting an end to the "evolutionary dead end"? Molecular Biology and Evolution **24:**1801–1810.

Zhu, J., and Z. Long. 1983. The vicissitudes of the giant panda. Acta Zoologica Sinica **29:**93–104.