

Molecular censusing doubles giant panda population estimate in a key nature reserve

Xiangjiang Zhan^{1,2},
Ming Li¹, Zejun Zhang^{1,5},
Benoit Goossens³,
Youping Chen⁴, Hongjia Wang⁶,
Michael W. Bruford³,
and Fuwen Wei^{1*}

The giant panda (*Ailuropoda melanoleuca*), a flagship species for conservation, once inhabited most of China and its neighboring countries in Southeast Asia. Nowadays, giant pandas are confined to fragmented mountain habitats in Western China because of ecological and anthropogenic pressure [1,2]. In order to establish effective conservation strategies, it is critical to know the number and distribution of giant pandas in the wild. However, accurately censusing panda populations remains problematic, because individuals are elusive, wary and very difficult to observe in their complex habitat. Previously, a number of indirect censusing methods have been used [1–3]. These methods were essentially based on transect lines and proved poor at identifying individuals, resulting in a questionable precision of estimates. Considering the keen interest of the conservation community and the millions of dollars already spent on three major national surveys, it is important to find an accurate method for censusing giant pandas. Recently, microsatellite analysis using fecal DNA has proven effective in estimating population size of elusive animals [4,5]. Large numbers of fecal samples can be easily obtained from giant panda habitat without disturbance due to its diet and high deposition rates [2]. Here, we conducted for the first time an exhaustive non-invasive

genetic survey of giant pandas in a key reserve and found that the molecular census was double that previously estimated.

The population, in Wanglang Nature Reserve (Figure 1), a key giant panda reserve, has been intensely studied since the 1960s [6]. Using fecal samples and nine microsatellite loci, we identified a total of 95 unique genotypes in Wanglang and its neighboring areas, including 66 in Wanglang Nature Reserve, eight in Baima, ten in Huanglong, six in Wujiao and five shared between Wanglang and Wujiao. In the Wanglang/Baima population, although three loci departed from Hardy-Weinberg expectations, overall the population was in equilibrium and the mean observed heterozygosity was 0.625. The average number of alleles was 5.4 (Table 1) and mean F_{IS} was -0.033 , not significantly different from zero.

The population size, estimated by DNA-based mark-recapture using CAPWIRE [7], ranged between 67 (95% confidence interval; 66–68) and 72 (66–81) individuals, depending on the deposition model assumed. The lowest range estimate from

both models was 66 individuals, which should therefore serve as a conservative census size for Wanglang Nature Reserve (Figure 1). Molecular sexing detected 35 males and 31 females in Wanglang (Supplemental Data).

The question of how many wild giant pandas remain in nature has been a major debate. Wanglang was the earliest reserve to be surveyed in China. Its giant panda population size was estimated to be 196 in 1968, but only 19 in 1985 by the Second National Survey, and just 27 in 1998 by the Third National Survey. In contrast, using DNA-based mark-recapture, we estimate the 2003/2004 population size to be 66, more than a 100% increase compared to 1998. This figure is incompatible with the 1998 estimate under even the most optimistic recruitment models during the intervening period. The question is, therefore, why our results are so different from those of the Third National Survey.

First, using bamboo bite sizes in feces to differentiate individuals underestimates the number of pandas present, while the molecular approach is more accurate (Supplemental Data). Second, in contrast with partial

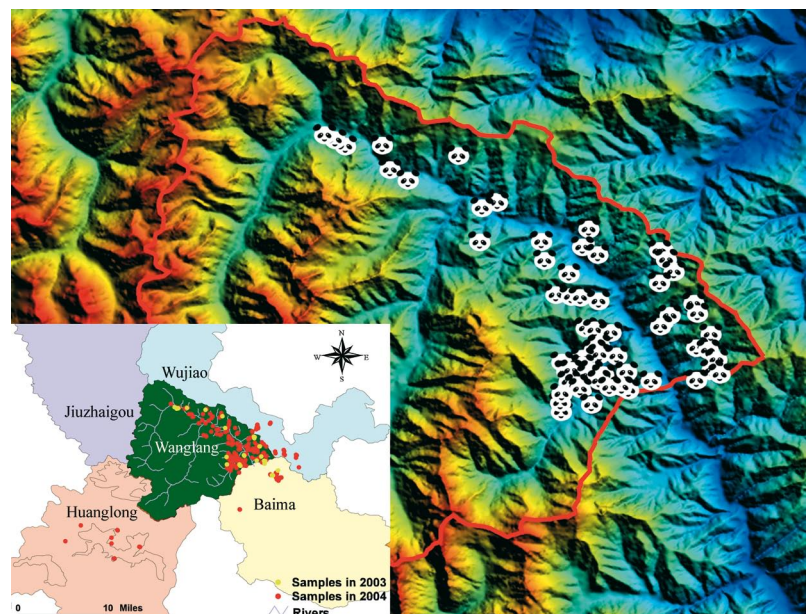


Figure 1. Sampling locations in the study area (lower-left panel) and identified individuals of giant pandas in Wanglang.

The sixty-six individuals are represented by panda icons on the Digital Elevation Model (DEM) map of Wanglang NR. XTools and Center of Mass v.1 in Arcview 3.2a were used to produce polygons and their centers for feces found in multiple locations, which are used to locate individuals on the DEM map.

Table 1. Characteristics of nine microsatellite markers in the study populations.

Locus	A			Ho			He		
	HL	WJ	WL	HL	WJ	WL	HL	WJ	WL
Ame- μ 5	9	4	8	0.778	0.833	0.690*	0.837	0.818	0.698
Ame- μ 10	4	3	8	0.500	0.667	0.761*	0.595	0.621	0.765
Ame- μ 26	5	2	6	0.778	0.500	0.648	0.758	0.530	0.576
Ame- μ 15	4	2	5	0.600	0.667	0.465	0.679	0.485	0.400
Ame- μ 19	4	4	4	0.625	0.800	0.529	0.750	0.756	0.530
Ame- μ 22	3	2	2	0.600	0.500	0.423	0.595	0.530	0.429
Ame- μ 27	3	3	4	0.500	0.667	0.696*	0.500	0.636	0.703
Ame- μ 13	4	2	5	0.444	0.167	0.594	0.484	0.318	0.606
Ame- μ 24	7	4	7	1.000	0.333	0.818	0.833	1.000	0.775
Mean	4.8	2.9	5.4	0.647	0.570	0.625	0.670	0.633	0.609

HL: Huanglong; WJ: Wujiao; WL: Wanglang/Baima; A: the average number of alleles; Ho: observed heterozygosity; He: expected heterozygosity; * significantly different from expected heterozygosity.

sampling strategy using transect lines in the Third Survey, we sampled the whole reserve, a fact borne out by the agreement between the estimated population size and the number of unique genotypes. Third, exhaustive fecal sampling could be more likely to detect smaller pellets deposited by the youngest individuals in the population. Fourth, immigration and emigration can change population estimates (Supplemental Data). Finally, temporary mating movements from outside the reserve could have contributed to our estimates, but their influence is likely to be restricted because of the relatively limited mobility of giant pandas [2,3]. The methods used in the Third Survey are, therefore, probably more suitable for approximate estimation over large-scale areas, but lack the precision of an intensive molecular approach.

Through a combination of anthropogenic disturbance and stochastic events, such as the local large-scale flowering and bamboo die-offs in the 1980s, it seems likely that many pandas in Wanglang Nature Reserve have disappeared since 1969. Fortunately, this decline in population size has not yet resulted in serious genetic consequences, with no evidence of inbreeding, intermediate to high genetic diversity for endangered carnivores [8–10], and no evidence for recent population bottlenecks (data not shown). It seems, therefore, that the giant panda population in Wanglang has the potential to

be restored if habitat protection, local socio-economic measures and population monitoring issues are resolved. However, these data have wider implications: if similar disparities between traditional and molecular census estimates are found for the other key giant panda reserves — and the same factors pertain across the species' range — it seems likely that many more individuals are extant in the wild than estimated in the Third National Survey (1,596 in total), which itself showed a substantial increase compared to the Second Survey. Our molecular census estimate for Wanglang NR is more than double that of the Third Survey, leading to the possibility that there may be as many as 2,500–3,000 giant pandas in the wild. This estimate assumes that the Chinese government now directly protects approximately 71% of wild pandas through its current reserve system, which when taken together with strictly enforced bans on poaching and deforestation in giant panda habitat, augurs well for giant panda conservation in the medium term, provided such measures remain in force.

Supplemental Data

Supplemental Data including experimental procedures are available at <http://www.current-biology.com/cgi/content/full/16/12/R451/DC1/>

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¹Key Lab of Animal Ecology and Conservation Biology, Institute of Zoology, Chinese Academy of Sciences, 25 Beisihuan Xilu, Beijing 100080, China. ²Graduate School of Chinese Academy of Sciences, Beijing 100039, China. ³Biodiversity and Ecological Processes Group, Cardiff School of Biosciences, Cardiff University, Cardiff CF10 3TL, UK. ⁴Wanglang Nature Reserve, Pingwu County, Sichuan 622550, China. ⁵Institute of Rare Animals and Plants, China West Normal University, Nanchong, Sichuan 637002, China. ⁶Wildlife Conservation Division, Sichuan Forestry Department, Chengdu, Sichuan 610081, China.

*E-mail: weifw@ioz.ac.cn

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