



Reintroduction of the giant panda into the wild: A good start suggests a bright future



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ABSTRACT

Reintroduction is an important approach in the conservation of endangered species and in recent decades a number of reintroduction programs have been conducted for conservation purposes. Generally, the success rate of reintroduction is low, but long-term monitoring of most reintroduction programs remains uncommon and this may influence the evaluation of reintroduction. The giant panda is a flagship species of biodiversity conservation. The Chinese government runs a giant panda reintroduction program and here we present reintroduction monitoring data from 2009 to 2015 for three giant pandas released into a small isolated wild population in western Sichuan, China. The results indicate that all three giant pandas display similar activity patterns to wild giant pandas, however, the wild-caught female demonstrated better adaptive ability to the wild environment than the two captive-born individuals in that she established a stable home range in the quickest time and gave birth to a cub. Genetic analysis indicates that new genetic material has been introduced into the local population. Our study provides the latest update on the giant panda introduction program and indicates that it is progressing well.

1. Introduction

Conservation translocation is the deliberate movement of organisms from one site to another to save endangered species from extinction (Kleiman, 1989; Sarrazin and Barbault, 1996; Tenhumberg et al., 2004; Pérez et al., 2012; Germano et al., 2015). From the original terminology outlined by the IUCN, translocation includes introduction, reintroduction and re-stocking (IUCN, 2013). Among these, reintroduction is the most common strategy and in a broad sense it is a translocation that releases animals of any origin into an area within their original geographic range, usually where populations have significantly declined or disappeared due to natural catastrophes or human interference. Many translocation programs of endangered species have been carried out for conservation purposes such as those involving black-footed ferrets (*Mustela nigripes*), black bears (*Ursus americanus*) and Mexican wolves

(*Canis lupus baileyi*) (Dobson and Lyles, 2000; Clark et al., 2002; Oakleaf et al., 2004). Although reviews generally find that the average success rates of these programs are low (Wolf et al., 1996; Fischer and Lindenmayer, 2000), reintroduction or translocation remains an important way to save endangered species. This method is a key conservation tool that prevents some species from disappearing and the number of reintroduction projects continues to grow (Seddon et al., 2012). To improve the success, standards for documenting and monitoring the methods and outcomes associated with reintroduction projects are important (Sutherland et al., 2010).

The giant panda (*Ailuropoda melanoleuca*) is a flagship species for biodiversity conservation. It has a long evolutionary history which can be traced back 7–8 Mya to the late Miocene (Hu, 2001; Zhao et al., 2013). Historically, the giant panda ranged from southern China into northern Myanmar, Vietnam, Laos and Thailand (Schaller et al., 1985;

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Hu, 2001). It is now confined to six mountain ranges on the edge of the Tibetan Plateau in China because of anthropogenic habitat loss and fragmentation. With decades of conservation efforts, the giant panda is no longer Endangered on the International Union for the Conservation of Nature's (IUCN) storied Red List, however it does not mean it has been out of woods (Swaisgood et al., 2017). Habitat loss and fragmentation have seriously impacted this species and resulted in many isolated and small populations. For example, population genetic studies in the Xiaoxiangling Mountains revealed that the smallest fragmented population is at a high risk of extinction if the population remains isolated with low gene flow (Zhu et al., 2010a, 2010b, 2011). To protect and save these small endangered populations, Chinese authorities have ensured that these habitats are protected and begun constructing habitat corridors to connect isolated populations (Wei et al., 2015a, 2015b). Releasing new individuals into these small populations is also an important strategy. Great achievements in captive breeding over the past 30 years has seen the captive population rise to over 400 animals with high genetic diversity (Shan et al., 2014), however the ultimate goal of establishing self-sustaining captive populations using wild animals is to re-establish or reinforce wild populations.

In the last few years the national giant panda reintroduction program has released three individuals into the Xiaoxiangling Mountains. The released animals include two captive-bred giant pandas that underwent two years of pre-release training, and one wild-caught giant panda rescued from the Qionglai Mountains. All animals were fitted with GPS/VHF collars and monitored from the first day of release. Here, we present post-release data spanning behavior and genetics for these three individuals in order to update the conservation community about the progress of the giant panda reintroduction project. The lessons being learned from this high-profile program also have implications for the continued conservation management of this and other endangered species.

2. Material and methods

2.1. Release area and animals

Giant pandas were released into Liziping National Nature Reserve (LNNR) in the Xiaoxiangling Mountains. Giant panda habitat in the Xiaoxiangling Mountains is located in southwest corner of the giant panda distribution region. This area contains the most isolated and smallest giant panda population remaining in the wild. Total panda habitat in these mountains spans 119.36 km² but is bisected by the 108th National Road into two patches which causes severe suppression of gene exchange (Zhu et al., 2011; Fig. 1).

Three giant pandas, Luxin (LX), Taotao (TT) and Zhangxiang (ZX), were released into habitat patch-A in LNNR (Fig. 1). LX is a 3.5 years old wild female rescued from the Qionglai Mountains on 27th March 2009 and released on 29th April 2009 after one month of care. TT is a male and ZX is a female, both are captive-born animals whose parents were captured from the Qionglai Mountains. TT and ZX were released into the wild on 11th October 2012 and 6th November 2013, respectively, after two years of pre-release training. TT and ZX both released when they were 2 years and 2 months old.

2.2. Activity rhythm and home range

Giant pandas were fitted with global positioning system telemetry collars (GPS7000MU, Lotek, Canada) programed to collect locations every 2 h. Activity was measured every 5 min by a dual-axis acceleration sensor mounted on the collar.

To look for changes in the activity rhythms of the released animals we summed the counts of the two acceleration sensors every 5 min, and defined the giant panda as active when the sums were > 32, and as resting when below this value (He et al., 2016). The difference in activity between released individuals was analyzed using ANOVA (if

variables followed a normal distribution) or Mann-Whitney *U* test (if variables did not follow a normal distribution) in the R environment (R Development Core Team, 2014).

We employed Brownian Bridge Movement Models (BBMM, Horne et al., 2007) to compute the utilization distribution (UD), which represents the probability distribution defining the animal's use of space. Home range sizes of giant pandas were calculated as the minimum areas encompassing the 95% of the UD estimate volumes. We used the BBMM package (Nielsen et al., 2013) in the R environment (R Development Core Team, 2014) to generate all BBMMs.

2.3. Genetic analysis

2.3.1. Sample collection

We collected fresh fecal samples in the giant panda reintroduction area for noninvasive genetic study. From April 2012 to December 2013, we laid a total of 46 transect lines, and the density of transect lines was one line every 2 km² in the study area. We also positioned and recorded all collected samples using GPS. Totally, we collected 92 samples including 90 fecal samples and two blood samples.

2.3.2. Extraction and analysis of fecal genomic DNA

We used the QIAamp DNA Stool Mini kit (QIAGEN) to extract fecal DNA. The PCR amplification of sex identification and microsatellite genotyping referred to the methods used by Hu et al. (2010) and Zhan et al. (2006). We selected 12 microsatellite loci (Ame- μ 10, Ame- μ 11, Ame- μ 22, Ame- μ 13, Ame- μ 15, Ame- μ 24, Ame- μ 26, Ame- μ 27, AY161179, AY161195, AY161213, AY161217) to genotype fecal DNA of giant pandas.

We used MICRO-CHECKER (Van et al., 2004) to detect genotyping errors such as null alleles, large allele drop out and stutter scoring. In order to confirm whether the number of selected microsatellite loci could reduce the shadow effect (Mills et al., 2008) to a less significant level, we used GIMLET 1.3.3 (Valiere, 2002) to calculate the combined *P* (ID) values of 12 microsatellite loci.

2.3.3. Individual identification and population size estimation

We identified matching genotypes in our genotyping data using the MStools plug-in (Park, 2001). Genotypes from different samples were considered to represent the same individual when all alleles in all loci were identical or if only one mismatch for one allele was found (Zhan et al., 2006). Because our fecal collection efforts focused on the main activity area of the reintroduced giant pandas, we inferred that recapture probability of individuals outside this area or rarely appearing in this area was lower than those living in the main activity area of the reintroduced animals. We chose the Two Innate Rates Model (TIRM) in CAPWIRE to estimate local population size (Miller et al., 2005).

2.3.4. Genetic diversity and relatedness

We used ARLEQUIN3.5 (Excoffier et al., 2007) to calculate expected heterozygosity (*H_e*), observed heterozygosity (*H_o*), and mean number of alleles per locus (MNA). Deviations from the Hardy-Weinberg equilibrium for each locus and the whole population were assessed using an exact probability test implemented in GENEPOP 3.4 (Raymond and Rousset, 1995). FSTAT2.9.3.2 (Goudet, 1995) was used to test linkage disequilibrium across loci. To estimate genetic relationships among individuals, Kingroup v2 (Konovalov et al., 2004) was used to estimate pairwise relatedness, with a maximum likelihood estimate based relatedness estimate used (Konovalov and Heg, 2008).

2.4. Population viability analysis (PVA)

To find the optimal release strategy combinations to ensure long-term viability of giant panda populations in the Xiaoxiangling Mountains, we used population viability analysis (PVA) to test the effects of release number and sex on population viability. The main

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