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Evaluating the reintroduction project of Przewalski's horse in China using genetic and pedigree data



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Gang Liu^a, Aaron B.A. Shafer^b, Waltraut Zimmermann^c, Defu Hu^{a,*}, Wenting Wang^a, Hongjun Chu^d, Jie Cao^e, Chongxue Zhao^f

^a Laboratory of Non-invasive Research Technology for Endangered Species, College of Nature Conservation, Beijing Forestry University, Beijing, China

^b Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden

^c Cologne Zoological Garden, Cologne, Germany

^d Forestry Bureau of Altay Prefecture, Altay, China

^e Wild Horse Breeding Center, Urumqi, China

^fGansu Endangered Species Research Center, Wuwei, China

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ABSTRACT

Przewalski's horse went extinct in the wild in the mid 1960s. Starting in 1985, individuals were brought from western zoos to two centers in China and breeding programs were initiated. With the increasing size of captive populations, two reintroduction projects were launched in the northwestern China in 2001 and 2010. Knowledge on genetic diversity in China's horse populations is limited, but would help improve the genetic management and assess the success of the reintroduction. Accordingly, one reintroduced and two captive populations were examined with 10 microsatellite loci together with pedigree data. The results showed higher level of diversity within the captive populations than the reintroduced population, indicating some alleles may have been lost during reintroduction. Genetic differentiation was detected among populations (F_{ST} = 0.09 ± 0.05, Rho_{ST} = 0.05 ± 0.02) and Bayesian clustering supported the presence of three subpopulations. The highest genetic differentiation was observed between the captive and reintroduced populations, and inbreeding coefficients were generally higher in the reintroduced population. Temporal estimates of both pedigree and microsatellite data showed a high, but decreasing level inbreeding. Through simulations, we estimated that the reintroduced population needs more than 100 individuals to retain approximately 90% of its current, already depauperate, genetic diversity. We have provided recommendations for the management program concerning introgressed genes from domestic horse and the number and origin of individuals for future reintroductions.

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1. Introduction

Captive breeding has helped recover numerous endangered species from the brink of extinction. Despite the obvious importance of captive breeding programs, limitations do exist. Apparent adaptation to captive environments has resulted in the poor success of reintroductions (Snyder et al., 1996), and rapid inbreeding depression has been documented (Araki et al., 2007; Armbruster and Reed, 2005). While species appear differentially vulnerable to the effects of inbreeding, it is generally viewed that inbreeding depression depends largely on the amount of standing genetic diversity present within a population (Charlesworth, 2003). Further, the reduction of genetic diversity and subsequent inbreeding can be attributed to multiple factors, including bottlenecks, subdivided populations and reduced gene flow (Lacy, 1987). The loss of diversity and the importance of these aforementioned demographic processes should be considered by managers when deciding to translocate individuals for conservation purposes (Weeks et al., 2011).

With the widespread use of molecular tools and diagnostics in endangered species, a greater emphasis has been placed on understanding the loss of genetic diversity (its causes and effects), which has led to rather integrated conservation management strategies (Robert, 2009). Microsatellite genotyping, combined with pedigree data, have become effective tools for conservation and reintroduction programs in a number of endangered species (e.g. Alpine ibex – Maudet et al., 2002; gazelles – Ruiz-López et al., 2009; Parma wallabies – Ivy et al., 2009; Arabian oryx – Alqamy et al., 2012; Brown bear – Barba et al., 2010; Mississippi sandhill



^{*} Corresponding author. Tel.: +86 010 62337051. *E-mail address:* hudf@bjfu.edu.cn (D. Hu).

crane – Henkel et al., 2012; and Stewart Island robin – Townsend and Jamieson, 2013). Specifically, more tailored management actions can be implemented (i.e. source population selection, breeding group selection) and the potential loss of genetic diversity mitigated by monitoring the genetic diversity and the level of inbreeding in reintroduced populations.

The Przewalski's horse (Equus ferus przewalskii) is a flagship species for conservation that once inhabited the Eurasian steppes. It is considered the only extant wild horse species (Goto et al., 2011; Orlando et al., 2013), but was extirpated in the wild in the mid 1960s due to hunting, military activities, climatic change, competition with livestock, and increasing land use pressure (Wakefield et al., 2002). It is believed that only 12 individuals (9, 7; 3, 5), captured in the wild between 1899 and 1947 and a maximum of 4 domestic horses (Bowling et al., 2003), have contributed all the genetic material present in living individuals. In 2012, more than 5000 individuals, of which almost 2000 are currently living, have been documented in the international studbook of the Przewalski's horse (founded in 1959): this corresponds to ~ 11 generations of captive breeding based on a generation time of 12 years (Sokolov and Orlov, 1986). Around twenty-five years ago, reintroduction efforts started in Mongolia, Ukraine, China, and Kazakhstan (Dierendonck and Vries, 1996; Zimmermann, 2005). These conservation efforts have increased the number of individuals in the wild to 350 in Mongolia (Walzer et al., 2012) and 70 in the Ukraine (Chernobyl), but the reintroduction attempt in Kazakhstan failed (Zimmermann, 2005). As result of these efforts the species was downlisted from Critically Endangered (as recent as 2008) to Endangered in 2011 because of the growing population numbers (Boyd and King, 2011). The Chinese population was established in 1985 with two breeding centers situated in the Xinjiang and Gansu provinces in China and is now on its fourth generation. Since 2001, small groups (totaling 27 individuals) have been released into the Kalamaili Nature Reserve (KNR) in Xinjiang province that is now estimated to have 99 individuals (ca. 2012 census). In addition, two groups consisting of 28 individuals were released into Dunhuang Xihu National Nature Reserve, Gansu, in 2010 and 2012 (Fig. 1).

Previous genetic studies on Przewalski's horse have focused on systematic relationships and genetic diversity using an array of molecular markers, including protein polymorphisms (Bowling and Ryder, 1987), chromosomal variation (Benirschke et al., 1965; Lau et al., 2009; Wallner et al., 2003), mitochondrial DNA (Oakenfull and Ryder, 1998), microsatellites (Bowling et al., 2003; Breen et al., 1994), immune genes (Hedrick et al., 1999) and genome-wide markers (Goto et al., 2011; Wade et al., 2009; Orlando et al., 2013). Historically, wild Przewalski's horses went through a major bottleneck during the last glacial maximum (see Supplemental material in Orlando et al., 2013), and even in captivity bottlenecks occurred during and after World War II (Wakefield et al., 2002). One major challenge associated with the management of the Przewalski's horse population is that some founders are overrepresented (Miller, 1995) and studies have shown inbreeding depression in the form of high mortality, abnormal reproduction, and shorter lifespans (Bouman and Bos, 1979; Collins et al., 2011; Hedrick et al., 1999). To date, the Chinese Przewalski's horse populations have only been studied with mitochondrial DNA (Liu et al., 2013), and an assessment of nuclear and genome-wide variability (through pedigree data) is urgently needed to gauge the population genetic health and evaluate the success of the reintroduction program.

This aim of this study was to evaluate the genetic diversity and level of inbreeding in the three Przewalski horse populations in China (two captive and one reintroduced to the wild) using microsatellite and pedigree data. We simulated the trajectory of genetic diversity in the reintroduced population and provided population

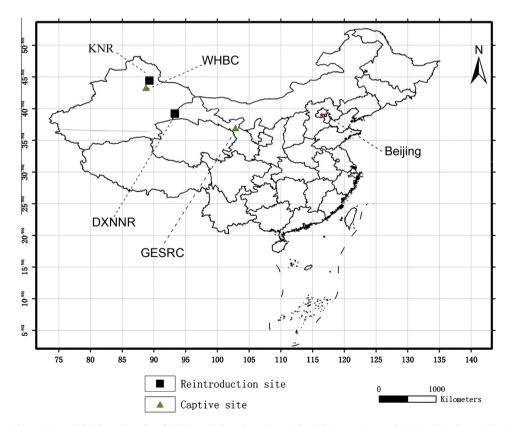


Fig. 1. Locations of two Chinese Przewalski's horse breeding facilities and the release sites, Kalamaili Nature Reserve (KNR) and Dunhuang Xihu National Nature Reserve (DXNNR). WHBC is the Wild Horse Breeding Center and GESRC is the Gansu Endangered Species Research Center.

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