



## Original Investigation

## Genetic structure in Mongolian gazelles based on mitochondrial and microsatellite markers

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## ABSTRACT

Mongolian gazelles (*Procapra gutturosa*) are among the last remaining abundant and wide-ranging grassland ungulates, although they have experienced a 75% decline in habitat historically. The effect of their mobility on their genetic structure has not been fully investigated, especially given recent anthropological disturbances. We carried out a molecular study of Mongolian gazelles along the international railroad in the eastern Gobi-steppe of Mongolia using mitochondrial control region sequences and microsatellite markers. Both markers had high genetic diversity with no evidence of a population bottleneck. Genealogies using control region sequences revealed two distinct genetic lineages; however, they were unrelated to geographic location. No significant population genetic structure was found with mtDNA or microsatellites; no isolation-by-distance was detected in our study. Our results suggested that the large population and high mobility of the animals has allowed sufficient gene flow to maintain a homogenous population, and detectable genetic differentiation has not been caused in spite of the anthropological disturbances including the railroad.

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## Introduction

Genetic structure in a wild population is mainly determined by gene flow, which is a consequence of both of dispersal from the natal site and subsequent reproduction (Freeland et al., 2011). The amount of gene flow can be influenced by factors such as a species' mobility, the extent of its fidelity to the natal site, and landscape factors, such as rivers, topographic relief, habitat discontinuities, or environmental gradients, as well as human-made barriers (Storfer et al., 2010; Freeland et al., 2011). Among them, railroads have strong effects on wildlife. These barriers increase mortality, modify behavior, and alter the environment (Trombulak and Frissell, 2000). They can cause habitat fragmentation but also create corridors (Coffin, 2007). Wildlife gene flow patterns change as well. Railroads often cause decreased genetic diversity and increased population differentiation by fragmenting the habitat, and increased gene flow

along the road verges are also reported (Holderegger and Wagner, 2008; Holderegger and Giulio, 2010).

Mongolian gazelles, *Procapra gutturosa*, represent one of the largest remaining wildlife populations in Asia. During the 1950s, they were distributed across 780,000 km<sup>2</sup> of steppe and semi-desert ecosystems throughout Mongolia, parts of Kazakhstan, the Russian Federation, and in China (Bannikov, 1951; Heptner et al., 1988; Lhagvasuren and Milner-Gulland, 1997). In the past 50 years, their range has decreased by 75% to approximately 190,000 km<sup>2</sup> (Lhagvasuren and Milner-Gulland, 1997; Milner-Gulland and Lhagvasuren, 1998). The present range is mainly confined to the Eastern Steppe of Mongolia and adjacent areas of Russia and north-eastern China, except for a small population in central and western Mongolia (Lhagvasuren and Milner-Gulland, 1997; IUCN, 2013).

High mobility is characteristic of the species, and they move long distances searching for resources and shelter (Olson et al., 2011). Recent studies with a global positioning system (GPS) or the Argos system reported that the animals occasionally travelled up to 160 km a week (Ito et al., 2013b), and their ranges exceeded 30,000 km<sup>2</sup> in a year (Ito et al., 2006; Olson et al., 2010; Ito et al., 2013b; Fleming et al., 2014). The movement patterns of Mongolian gazelle are characteristic in that the animals do not necessarily

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return to the same places seasonally; instead, they move in a more nomadic pattern (Olson et al., 2010; Ito et al., 2013a). Furthermore, adult female gazelles did not use the same calving ground each year, and calves did not return to the natal site (Olson et al., 2010), suggesting that they lack philopatry. They aggregate throughout the year (Lhagvasuren and Milner-Gulland, 1997; Olson et al., 2009), but the groups vary in size seasonally, so the animals associate in loose herds. The formation of herds is related to mating in winter and to calving in summer (Lushchekina et al., 1985; Lhagvasuren and Milner-Gulland, 1997; Wang et al., 1997; Jiang et al., 1998; Leimgruber et al., 2001), although the natal sites and genetic bases of memberships in a herd have not been investigated yet.

Even if nomadism is an intrinsic character of Mongolian gazelles, the behavior might be prevented locally by several factors, including recent artificial alterations of the environment. The species has experienced severe shrinking of its range. The loss of historical habitat suggests this species is susceptible to human activity. Human settlement or artificial constructions, such as industrial plants and transportation pathways, may have fragmented the habitat of Mongolian gazelles (Milner-Gulland and Lhagvasuren, 1998; Olson, 2010; Ito et al., 2013a). The related Przewalski's gazelle (*Procapra przewalskii*) shows distinct genetic differentiation related to human settlement (Yang et al., 2011). For Mongolian gazelles, the international railroad between Russia and China, which was established in 1955, could be regarded as the most serious threat to population continuity. It bisects the gazelle habitat, appearing to prevent their crossing and driving them along the construction and may even affect gazelle survival (Ito et al., 2005; Ito et al., 2008; Ito et al., 2013a). Additionally, along the railroad are cities that disrupt the movements of wild animals. The combination of the railroad and these anthropologic landscapes may be barriers to gene flow for gazelles, even in small areas relative to their migrating abilities.

When individuals move and mate randomly throughout the habitat, as expected based on field observations, a population should be genetically homogeneous. Conversely, when movement is prevented by barriers or limited by fidelity to the natal site, the population will show discontinuous genetic structure or isolation by distance (Wright, 1969; Rousset, 2004; Holderegger and Wagner, 2008). Several molecular studies on Mongolian gazelles have been carried out with mitochondrial sequences. All results suggested the species contains quite high genetic diversity despite the historical habitat loss (Sorokin et al., 2005; Sorokin and Kholodova, 2006; Chen et al., 2012; Okada et al., 2012). Analyses of mitochondrial cytochrome b gene and control region sequences in samples from Russian and Mongolia suggested there are two genetic lineages within the population that are unrelated to geographical location (Sorokin et al., 2005; Sorokin and Kholodova, 2006). Okada et al. (2012) obtained similar results by analyzing samples from a wide range of Mongolia and additionally suggested a past population expansion based on molecular indices. Although these studies hinted at nomadism, the population genetic structure must be investigated with biparental hypervariable markers such as microsatellites, as results from different types of markers are not always consistent (Toews and Brelsford, 2012). The spatial distribution of autosomal microsatellite alleles reflects gene flow in both sexes, while matrilineal mitochondrial markers show that of maternal lineages. Gene flow is caused by dispersal from the natal site and consequent reproduction, so the genetic structure in markers with different modes of inheritance is used to investigate sex-biased dispersal (Prugnolle and de Meeus, 2002). Additionally, microsatellite markers evolve faster than mitochondrial sequences and should allow the examination of recent reductions in gene flow caused by anthropologic barriers, such as rails or roads (e.g., Novembre et al., 2008; Balkenhol and Waits, 2009; Edwards and Bensch, 2009).

In this study, we obtained carcass samples of Mongolian gazelles along the railroad in the eastern steppe. We successfully analyzed

mitochondrial control region sequences for 103 individuals and microsatellite markers for 138 individuals. To clarify the effects of human-induced past population decline, we calculated molecular indices, including effective population size, and tested for a past population bottleneck using microsatellite markers. We also constructed a maximum likelihood genealogy using the mitochondrial DNA (mtDNA) data we previously obtained (Okada et al., 2012), performed a Bayesian clustering analysis with the microsatellite markers, and tested the isolation-by-distance effect with both types of markers to examine the extent of geographic differentiation caused by the construction of the international railroad bisecting their habitat combined with sex-biased philopatry. Our study elucidated the present genetic status of a presumably nomadic species that maintains a large population but suffers from past and present anthropologic activities.

## Material and methods

### Study area

We carried out this study along the international railroad running through the largest habitat of Mongolian gazelles in central to eastern Mongolia. The climate in the area is continental, and annual precipitation is low (<100 mm) in the southern part of the area. The vegetation is generally steppe in the north, dry steppe in the middle, and Gobi Desert in the south of the region. The railroad bisects the habitat latitudinally from Russia to China, and large cities exist along the railroad, including Ulaanbaatar (>1.2 million residents), Choir (>10,000), and Sainshand (>20,000).

### Samples

We conducted a carcass survey of Mongolian gazelles in June/July 2005 along the international railroad from Ulaanbaatar to the Chinese border by driving along the tracks (for details see Ito et al., 2008) (Fig. 1). Excluding two samples near Ulaanbaatar, all samples were obtained along the railroad, with 95% of samples typed with microsatellite markers were obtained within a limit of 200 m off the railroad. When a gazelle carcass was spotted, skin tissues from the ear were collected for molecular analysis and preserved in 99.5% ethanol. The phenol–chloroform method was used to extract DNA according to Sambrook et al. (1989). Approximate age and location (GPS coordinates) of each carcass were determined using GPSMAP 76CSx (Garmin International, Inc., Olathe, KS, USA). Because the sampling locations were widespread from north to south (>500 km), the samples were divided into three latitudinal groups (N, north; M, middle; S, south) at the cities of Choir and Sainshand, then additionally into three longitudinal groups (E, east; I, within the fences; W, west) based on which side of the railroad the carcass was found. Thus, there were a total of nine location groups: NW, NI, NE, MW, MI, ME, SW, SI, and SE.

### Laboratory methods and statistical analysis

A fragment of the mitochondrial control region was amplified from each sample, as previously reported (Okada et al., 2012). The first polymerase chain reaction (PCR) used the primers forward PGLo (5'-CTTCAAGGAAGAAGCTATGGCT-3') and reverse PGHo (5'-GGTGATGCTCAAGATGCAGT-3') designed to avoid amplification of mitochondrial copies in the nuclear genome. For nested PCR, forward L15376 (5'-CACTATCAACACCCAAAGCTGAAG-3'), designed at the San Diego Zoo Center for Reproduction of Endangered Wildlife (USA) (Sorokin et al., 2005), and reverse PGHr (5'-GCCCTGAAGAAAGAACCAGATG-3') were used. The first and nested PCRs were both performed in 20- $\mu$ L reaction volumes containing 0.4  $\mu$ M of each primer, 0.2 mM of each dNTP, and 0.5 units of Takara

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