

Genetic structure and population size of Marco Polo sheep (argali) in the Pamir region of Afghanistan, China, Pakistan, and Tajikistan

by Gordon Luikart and Richard Harris

Introduction

This portion of the Afghanistan Biodiversity Project is addressing issues of connectivity, corridors, isolation, loss of genetic diversity, and possible barriers to movement among sub-populations of Marco Polo argali throughout the 4 country transboundary region. The genetics team is using non-invasive sampling (primarily of feces) to provide data on the genetic structure of this meta-population because: the region is extremely remote and difficult to access. DNA information is also forming the basis of a mark-recapture estimate of population size for the Big Pamir subpopulation.

Progress during the fourth quarter of 2008

Sampling

During the 4th quarter, the genetics team received and initiated analyses on 99 argali samples from the Little Pamirs and 5 samples from just north of the Big Pamir on the Tajikistan side. These samples added to the existing collection of 391 samples from the Big Pamir, 33 from the Wakhjir Valley, 30 from the Murghab district of adjacent Tajikistan, and 48 from adjacent Taxkorgan County in Xinjiang, China.

Microsatellite identification

For the study to estimate population size in the Big Pamir study area, the genetics team has genotyped 232 samples at 6-10 loci. Over 300 more samples were genotyped with 5 loci but were excluded from the study because they did not yield consistently useful genotypes. The 232 samples were from four different sampling periods (times) including 42, 81, 61, and 48 for sampling sessions 1, 2, 3 and 4, respectively. Importantly, approximately 40 'recaptures' (samples with identical genotypes) were identified, which will be the basis of the mark-recapture analysis to estimate population size for the Big Pamir study area, currently ongoing. Gender identification is currently being finalized: when this is done, statistical analysis can commence on population size estimates for the Big Pamir. This will also be compared with visual estimates of population size from the field teams.

To assess gene flow and population connectivity, the genetics team is genotyping 18 loci on 20-40 samples from each of 5 study areas. The five study areas are Atabek in Tajikistan, Taxkorgan in China and the Big Pamir, Little Pamir, and Wakhjir in Afghanistan. Approximately 120 samples are being genotyped at 18 loci to assess population connectivity.

The genotyping for the connectivity study has been delayed because most efforts have

been directed toward re-genotyping difficult samples from Big Pamir for the population size estimation study. In addition, samples from sampling session 4 from Big Pamir arrived relatively late after being held up by custom officials. Thus, final information on genetic structure of argali is not yet available from these. Preliminary results based on 8 loci are below.

Table 1. Preliminary results from a suite of 6-18 microsatellite loci used for individual identification and for connectivity assessment. Six-to-ten loci are used for individual identification and population size estimation. Eighteen loci are being used for the assessment of population connectivity and gene flow.

Samples	18 loci genotyped for gene flow study	6-10 loci genotyped for individual identification	Poor msats amplification	Not yet examined	Total
Big Pamir Summer 2007 (BP1)	0	42*	19	0	61
Wakhjir Valley Summer 2007	7	22	11	0	33
Big Pamir November/December 2007 (BP2)	14	81*	53	0	134
Atabek, Tajikistan October, November 2007	14	26	4	0	30
Taxkorgan, China, October 2007	28	39	9	0	48
Big Pamir January 2008 (BP3)	0	61*	1	0	62
Shimhal Valley, Pakistan May 2008	0**	**	30	0	30
Big Pamir June/July 2008 (BP4)	0	48*	12	75	135
Little Pamir June 2008	0	42***	18	39	99
Ishkashim area Tajikistan	0		0	6	6
Total	63	361	157	120	638

*From BP, 4 samples had only 6 loci (all from BP1), 11 samples had 7 loci, 20 had 8 loci, 42 had 9 loci and the remaining 154 sample had all 10 loci successfully genotyped.

**All samples were extracted genotyped twice but all yielded poor DNA and unusable genotypes

***Only 60 were extracted due to poor quality fecal pellets and limited time and funding.

Preliminary results

It appears that the genetics team has identified no fewer than 192 individual Marco Polo

sheep in the Big Pamir region. However, pending further analysis, it is unclear if all of these are truly resident in the Big Pamir area, or if alternatively, some spend only part of the year there, traveling to other parts of the Pamirs during other seasons. The sample size of 232 genotypes should be sufficient to shed light on this, although confidence limits will probably be wide. If further funding permits extraction of DNA from the remaining 75 samples from the Big Pamir (collected during summer 2008), a more accurate and precise mark-recapture population estimate will be possible.

As reported during the 3rd quarter, it appears that heterozygosity and gene flow is relatively high among the sub-populations examined.

ACRONYMS

AFIR	AFIR, a Kabul-based architectural/design company
AKDN	Aga Khan Development Network
BAPAC	Band-i-Amir Protected Area Committee
BEDP	AKDN's Bamiyan Ecotourism Development Program
BSP	Ecodit implemented Biodiversity Support Program
MoAIL	Ministry of Agriculture, Irrigation and Livestock
NEPA	National Environmental Protection Agency
PRT	Provincial Reconstruction Team
Q3	Third Quarter, 11 July - 11 October
Q4	Fourth Quarter 12 October- 11 January
USAID	United States Agency for International Development
WCS	Wildlife Conservation Society