Project Update: July 2018

Field survey: Fecal and hair samples were searched with the support of citizen scientists following 12 transects in Ghandruk and Mustang management units of Annapurna Conservation Area by considering available forest habitat for Himalayan black bears and previous records of bear distribution.

Bear population and sex ration in Annapurna Conservation Area: For microsatellite analysis, 27 loci were initially tested with 16 fecal samples for genotyping determination of which 11 loci from *U. americanus* (Paetkau et al. 1995; Paetkau et al. 1998; Meredith et al. 2009), 10 loci from *U. arctos* (Taberlet et al. 1997), and 6 loci from *U. t. japonicus* (Kitahara et al. 2000). The eight best loci were selected (**Table 1**) for further experiments based on ease of use in multiplex PCR, amplification efficiency, readability and success, relatively small PID values (<0.25) and having high PIC (>0.5) value (Uno et al. 2012). We used *Amelogenin* gene with the primers SE47/SE48 for molecular sexing of individuals (Ennis and Gallagher 1994), which was applied to the Asiatic black bears (Yamamoto et al. 2002; Mukesh et al. 2013). Of 147 non-invasive sample collected from Annapurna Conservation Area, 24 samples were unsuccessful for DNA extraction and 26 additional samples that amplified inconsistently for several loci or did not yield complete genotypes even after the third round of PCR replication were discarded from further analyses.

Table 1. Characteristics of microsatellite markers tested in the present study. Analysis were carried out based on 16 fecal samples which were later identified 8 unique genotypes.

Multiplex set	Locus	Labelling dye	Ta	Allele size	N_A	Amplification efficiency*	Amplification readability*	Amplification success (%)	H_O	H_E	PIC	P_{ID}	P_{ID}Sibs	P
MP1	**MU50**	FAM	55	212-224	7	good	good	100%	0.875	0.817	0.735	0.086	0.389	0.867
	G10B	VIC	55	154-166	5	good	good	100%	0.500	0.800	0.711	0.102	0.400	0.037
	MU23	NED	55	110-124	6	good	good	100%	0.875	0.858	0.776	0.067	0.364	0.424
MP2	G1A	FAM	55	192-196	3	Good	good	44%	0.000	0.714	0.555	0.211	0.490	0.031
	MU05	VIC	55	136-146	6	good	good	100%	0.875	0.817	0.730	0.090	0.390	0.842
	MU51	NED	55	108-122	4	fair	good	63%	0.625	0.617	0.510	0.246	0.523	0.610
MP3	G10X	FAM	55	186-188	2	fair	good	100%	0.375	0.325	0.258	0.530	0.730	1.000
	G10P	VIC	55	152-160	4	good	good	100%	0.250	0.758	0.658	0.136	0.429	0.003
	G10C	NED	55	102-118	6	good	good	100%	1.000	0.850	0.766	0.072	0.370	1.000
MP4	G10M	FAM	55	204-210	4	good	good	100%	0.625	0.592	0.510	0.243	0.533	0.778
	MU09	VIC	55	130-148	5	good	good	81%	1.000	0.817	0.727	0.094	0.391	1.000
	MU59	NED	55	106-130	6	fair	good	88%	1.000	0.842	0.759	0.075	0.374	1.000
MP5	**MU61**	FAM	55	201-219	6	good	good	100%	0.875	0.850	0.766	0.072	0.370	0.742
	G1D	VIC	55	180-186	3	good	good	100%	0.500	0.633	0.511	0.248	0.515	0.270
	MU10	NED	55	122-128	4	good	fair	100%	0.500	0.725	0.618	0.164	0.451	0.097
MP6	**UamD2**	VIC	55	206-226	5	good	good	100%	0.750	0.792	0.701	0.108	0.406	0.456
	UamB5	NED	55	144-160	4	good	good	100%	1.000	0.692	0.592	0.180	0.471	1.000
MP7	MU26	VIC	55	190-210	4	good	good	100%	0.750	0.700	0.605	0.169	0.464	0.796
	G10L	NED	55	124-142	6	good	good	100%	0.625	0.775	0.691	0.110	0.414	0.181
MP8	MU64	FAM	55	191-203	5	good	good	100%	0.750	0.700	0.595	0.180	0.467	0.731
	G10J	VIC	55	98-112	6	good	good	100%	0.625	0.733	0.654	0.131	0.439	0.350
MP9N	MSUT8	FAM	55	114-124	4	good	good	100%	1.000	0.758	0.658	0.136	0.429	1.000
	MSUT2	PET	55	77-99	6	good	good	100%	0.750	0.833	0.748	0.081	0.380	0.131
MP10N	MSUT7	NED	50	118-138	4	good	good	100%	0.625	0.692	0.582	0.191	0.473	0.488
	MSUT4	VIC	50	92-102	5	good	good	100%	0.500	0.533	0.474	0.276	0.569	0.608
Mean/combined					4.8					0.729	0.636	$3.53x10^{-22}$	$1.45x10^{-09}$	

Ta, annealing temperature °C; N_A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; PIC, polymorphic information content; P_{ID}, probability of identity; P_{ID}Sibs, probability of identity of siblings; P, P values for exact test of Hardy-Weinberg equilibrium (level of significance, $\alpha = 0.05$).

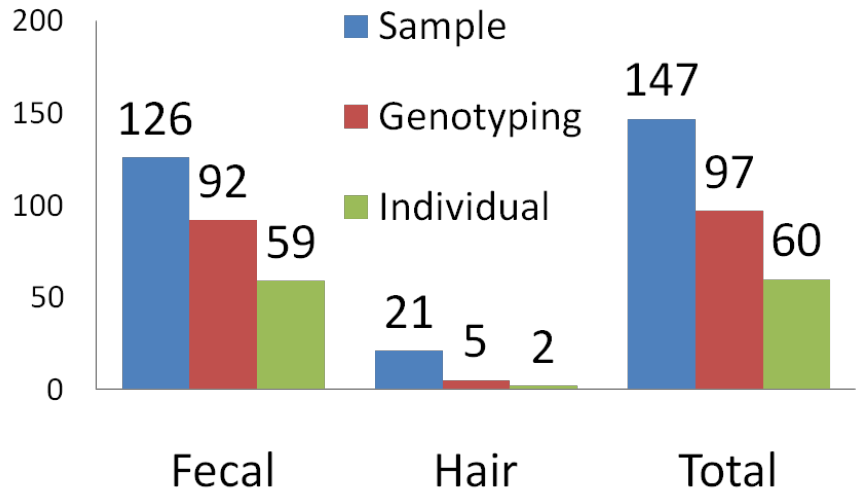
*Amplification efficiency and readability were estimated following Uno et al. 2012.

The highlighted loci were selected for amplification of all samples.

We were able to successfully genotype these eight microsatellites in both round from 73% (n = 92) of the fecal samples and 24% (n = 5) of the hair samples. The low rate of success of genotype from hair samples was possibly due to poor quality of DNA extracts from old samples. We detected an overall genotyping success of 66% (n = 97) including both hair and fecal samples with 0-4% allelic dropout errors whereas the presence of false alleles was negligible. The micro-checker program did not detect any errors due to stutter bands, large allele dropout or deficit of heterozygotes. The average number of alleles (NA) of the eight microsatellite loci was 7.6, ranged from 5 to 10, which was higher than the effective numbers of alleles (4.4). Combined across all loci, the average values of observed heterozygosity (HO) and expected heterozygosity (HE) were 0.798 and 0.760, respectively.

From the total of 97 samples that were successfully genotyped, we identified 60 individuals, of which 20 were found multiple times (**Figure 1**). Two individuals were detected a maximum of seven times, whereas 40 individuals (67%) were recorded only once during the sampling period. Sex identification resulted in 32 (54%) males and 28 (46%) females. Most of the bears (n=48) were recorded in the southern forest of three management units during multiple surveys. Similarly, 10 and two individuals were recorded in the two management units of high mountain region located in Jomsom and Manang unit, respectively. In total, 60 individuals were found in about 525 km^2 (11.5 individuals/100km^2) of ACA that contained both forests and surrounding farmlands. One quarter of the bears visited in agricultural land mostly during the rainy season of which, 62% were males and 38% were females. A female bear was sampled seven times from maize land of Jomsom which suffered frequent damage of crops by bears. We did not detect any evidence of bear in millet crops during the autumn season.

Figure 1. Status of Genotyping success and individual identification of Himalayan black bear population from Annapurna Conservation Area.

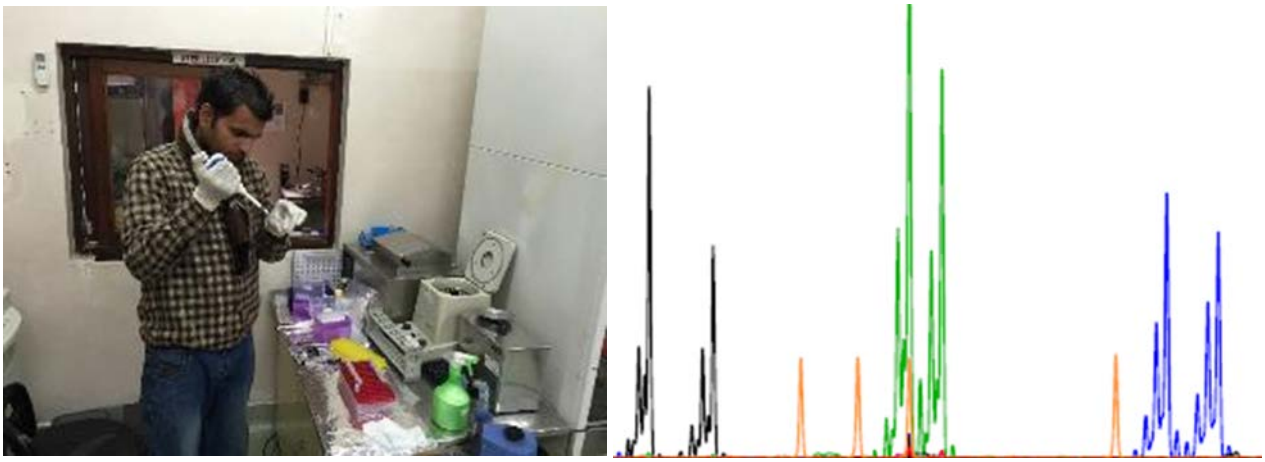


Community based crop damage compensation scheme: Kunjo village, located in northern part of Annapurna conservation area was selected for the development of community based crop damage compensation scheme. This is a small village of around 150 households situated in the middle of the mountain forest. Maize is the major crops and maximum no of maize were raided by bears in last 5 years.

The forest around the village has less fruiting during the cropping season. We organised an orientation workshop with the village leaders for sharing the scheme development mechanism and seeking of their support during the process. The participants decided for the provision of 70% financial compensation of crop damage made by bear. The payment will be made by counting the maize cob damaged by bears at end of harvesting time. Another meeting will be organised in August 2018 for the filling of damage assessment form and the assessment form will be distributed to all farmers who involved in maize cultivation. It gives the no of farmers loose maize, no of times bear visit in land, amount of maize damage and the action taken for protection of crops. Each farmer will be requested to collect residue of maize cob left after bear damage.

Field assistants in Rhododendron forest of Ghandruk

Left: Bear hairs searched by field assistant in broken branched of beard raided tree. Right: The exterior surface of each putative fresh fecal samples was slightly rubbed using a cotton swab and stored in 15 ml vial containing 10 ml 100% ethanol.



Left: DNA extraction and PCR of noninvasive samples. Right: Electrophoretogram display of PCR products of three microsatellite loci.

Himalayan black bears in Annapurna conservation area (Photo: ACAP/Jack)

Orientation workshop for the development of community based crop damage compensation scheme in Kunjo village of Mustang district. The workshop was facilitated by secretary of Conservation Area Management Committee, Forest guard and Ranger of ACAP. (Photo: Suresh Thapa/ACAP)