



## Y chromosome haplotype diversity of domestic sheep (*Ovis aries*) in northern Eurasia

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### Summary

Variation in two SNPs and one microsatellite on the Y chromosome was analyzed in a total of 663 rams representing 59 breeds from a large geographic range in northern Eurasia. SNP *A-oY1* showed the highest allele frequency (91.55%) across the breeds, whereas SNP *G-oY1* was present in only 56 samples. Combined genotypes established seven haplotypes (*H4*, *H5*, *H6*, *H7*, *H8*, *H12* and *H19*). *H6* dominated in northern Eurasia, and *H8* showed the second-highest frequency. *H4*, which had been earlier reported to be absent in European breeds, was detected in one European breed (Swiniarka), whereas *H7*, which had been previously identified to be unique to European breeds, was present in two Chinese breeds (Ninglang Black and Large-tailed Han), one Buryatian (Transbaikial Finewool) and two Russian breeds (North Caucasus Mutton-Wool and Kuibyshev). *H12*, which had been detected only in Turkish breeds, was also found in Chinese breeds in this work. An overall low level of haplotype diversity (median  $h = 0.1288$ ) was observed across the breeds with relatively higher median values in breeds from the regions neighboring the Near Eastern domestication center of sheep. *H6* is the dominant haplotype in northwestern and eastern China, in which the haplotype distribution could be explained by the historical translocations of the *H4* and *H8* Y chromosomes to China via the Mongol invasions followed by expansions to northwestern and eastern China. Our findings extend previous results of sheep Y chromosomal genetic variability and indicate probably recent paternal gene flows between sheep breeds from distinct major geographic regions.

**Keywords** microsatellite, paternal lineage, SNP, Y chromosome

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Y chromosomal genetic markers provide important information about genetic origins and the diversity of livestock from the paternal perspective, which is complementary to those revealed by autosomal and mitochondrial variations (e.g. Meadows *et al.* 2006; Perez-Pardal *et al.* 2010). In sheep, so far only very few Y chromosomal genetic markers, including two SNPs (*oY1* at g.88A>G, Meadows *et al.* 2004; *oY2* at g.460G>T, Meadows *et al.* 2004; Zhang *et al.* 2012)

in the 5'-promoter region of the *sex-determining region Y* (SRY) gene and one compound microsatellite (SRYM18; Meadows *et al.* 2006), have been available for paternal analyses. Earlier paternal analyses in sheep identified a total of 18 haplotypes and two main lineages with specific geographic patterns of frequency distribution (Meadows *et al.* 2006; Meadows & Kijas 2009; Ferencakovic *et al.* 2013). Following domestication in the Fertile Crescent region c. 11 000 years ago (Ryder 1984), sheep have colonized a wide geographic range across Eurasia. In this study, we extend previous studies on Y chromosomal genetic diversity of sheep by including breeds from northern Eurasia that remain largely uninvestigated.

A total of 663 samples from rams representing 59 Eurasian breeds were included in the study (Table 1). Most of the breeds originate from northern Eurasia (Fig. 1). In all cases, particular efforts were made, using both pedigree information and the knowledge of local herdsman, to ensure that animals were unrelated and typical of the breed. Genomic DNA was extracted from ear tissue using the standard phenol/chloroform protocol (Sambrook & Russell 2001) or from blood using the General AllGen Kit (ComWin Biotech), following the manufacturer's instructions.

Three male-specific genetic markers, microsatellite SRYM18 (Meadows *et al.* 2006) and two SNPs (*oY1* and *oY2*), were genotyped in all the samples. The primers SRY3F and SRY3R (Meadows *et al.* 2004) were used to amplify a 472-bp fragment containing the SNP variations. The microsatellite SRYM18 was amplified with the primers SRYM18F and SRYM18R (Meadows *et al.* 2006), and the forward primer was fluorescently labeled. PCR was carried out in a total volume of 20  $\mu$ l containing 2 $\times$  Taq MasterMix (ComWin Biotech), 0.4  $\mu$ M of each primer and 2 ng of template genomic DNA. Amplifications were carried out in a thermal cycler (Applied Biosystems) with the following cycling program: pre-denaturation at 95 °C for 5 min, followed by 30 cycles at 95 °C for 30 s, 58 °C (for primers SRY3F and SRY3R) or 62 °C (for primers SRYM18F and SRYM18R) for 30 s, 72 °C for 1 min and a final extension at 72 °C for 10 min. The PCR products were sequenced directly from both orientations on an ABI 3730 capillary sequencer (Applied Biosystems, Life Technologies) using a BigDye Terminator v3.1 Cycle Sequencing Kit (Life Technologies).

Sequences were aligned with BIOEDIT software v.7.1.8 (Hall 1999). By combining the repeat structure of SRYM18 with the two SNPs, haplotypes were constructed, and haplotype diversity was calculated using ARLEQUIN suite v.3.5 software (Excoffier & Lischer 2010, available at <http://cmpg.unibe.ch/software/arlequin35/>).

## Results and discussion

We observed the two SNPs (i.e. *oY1* and *oY2*) identified earlier. Similar to the observations reported previously

(Zhang *et al.* 2012), the T-*oY2* allele was very rare and was detected in only six samples of two Chinese breeds (two in Hetian sheep and four in Tan sheep). The A-*oY1* allele had the highest frequency (91.55%) in the samples, whereas G-*oY1* was detected in only 56 samples (8.45%) from different breeds.

We obtained seven haplotypes (*H4*, *H5*, *H6*, *H7*, *H8*, *H12* and *H19*; Table 2; see Table S1 for the haplotype nomenclature) by combining the genotypes from the SNPs and the microsatellite. Haplotype distribution within each population is presented in Table 1. The most frequent haplotype was *H6* (65.01%), which was present across all the breeds with only a few exceptions such as Ninglang Black, North Caucasus Mutton-Wool, Russian Karakul, Kuibyshev and Russian Romney Marsh sheep breeds. *H8* was the second most common haplotype (20.81%), although it was rare (0.9%) in the breeds of southwestern China (Tengchong, Ninglang Black, Lanping Black-bone, Zhaotong, Shiping Gray, Diqing and Weining). Haplotype frequency distribution for the breeds from distinct major geographic regions is shown in Fig. S1. A previous study showed that *H12* was present only in Turkish breeds (Ferencakovic *et al.* 2013), but in our work, *H12* was also found in three breeds (Sishui Fur, Taihang Fur and Luzhong Mountain sheep; Fig. 1) of eastern China. *H4* was completely absent from European breeds in one previous study (Meadows *et al.* 2006), but it was detected once in one European breed (Swiniarka; Fig. 1) here. We also found *H7*, which had been identified to be unique in European breeds, in two Chinese native breeds (Ninglang Black and Large-tailed Han), one Buryatia breed (Transbaikal Finewool) and two Russian breeds (North Caucasus Mutton-Wool and Kuibyshev; Fig. 1). This observation may be a result of the use of exotic breeds, such as Suffolk, Merino, Lincoln and Romney, to improve the meat or wool production of local breeds. For example, Kuibyshev originates from Romney  $\times$  Cherkassy backcrossed to Romney, and both North Caucasus Mutton-Wool and Akasaraisk type of Soviet Mutton-Wool were improved through the use of Lincoln and Romney (Mason 1988). Our results reveal a hidden breed history of foreign introgression in two Chinese breeds (Tengchong and Ninglang Black), although the breed development information for them has not been readily available.

In general, a low level of paternal genetic diversity was observed in northern Eurasian sheep breeds. Haplotype diversity (*h*) varied from 0 in several breeds, such as Altay, Baerchuke, Bashbay, Duolang, Kazakh, Tashkurgan, Hulun Buir, Tong, Guide Black Fur, Zhaotong, Russian Karakul and Russian Romney Marsh, to 0.3849 in Transbaikal Finewool with a median value of 0.1288 (Table 1). The reduced genetic variability in Y chromosomal markers might be due to the use of only a few rams in breeding practices. Nevertheless, the breeds in the Eastern Europe, West Russia and Caucasus regions show a relatively higher median *h* value (Caucasus: *h* = 0.1705; Eastern Europe and

**Table 1** Geographic origin, breed code, sample size (*n*), haplotypes distribution and haplotype diversity (*h*) of 59 Eurasian native sheep breeds.

Region	Sampling regions	Breeds	Code	<i>n</i>	Haplotype							<i>h</i>		
					H4	H5	H6	H7	H8	H12	H19			
Central Asia	Xinjiang, China	Altay sheep	AL	8			8						0.0000	
		Baerchuke sheep	BRK	8			8						0.0000	
		Bashbay sheep	BSB	16			16						0.0000	
		Bayinbuluke sheep	BYK	14			12		2				0.0705	
		Dulang sheep	DL	24			24						0.0000	
		Kazakh sheep	HS	18			18						0.0000	
		Hetian sheep	HT	34	3		28		1		2		0.0547	
		Kirghiz sheep	KR	24			22		2				0.0325	
		Kunlun sheep	KL	22			19		3				0.0526	
		Lop sheep	LB	24			11			13			0.1058	
		Turfan Black sheep	TLF	10	2		8						0.1125	
		Tashkurgan sheep	TSK	16			16						0.0000	
		Yecheng sheep	YH	6	1		5						0.1361	
		Celei black sheep	CLS	14			12		2				0.0705	
		Inner Mongolia and Buryatia	East of Caspian Sea, Kazakhstan	Kazakh Edilbai	EDIL	5			2		3			0.2683
Inner Mongolia, China	Hulun Buir sheep		HLS	10			10						0.0000	
	Sunite sheep		SNS	12	2		8		2				0.1575	
	Wuranke sheep		WRS	12			4		8				0.1399	
Buryatia	Ujimqin sheep		WZS	12	1		6		5				0.1793	
	Baidarak		BAJ	9			4		5				0.1852	
	Transbaikal Finewool		ZAB	11	1	5	3	1	1				0.2302	
Northwest and East China	Gansu, China		Lanzhou Large-tailed sheep	LAN	11	1		10						0.0548
			Minxian Black Fur sheep	MIX	1					1				1.0000
	Ningxia, China		Tan sheep	TAN	20			15		1		4		0.0930
			Shaanxi, China	Hanzhong sheep	HZS	10	5		5					0.1757
	Shanxi, China		Tong sheep	TS	9			9						0.0000
			Jingzhong sheep	JZS	14	1		7		6				0.1615
			Guangling fat-tail sheep	GLS	3			2		1				0.3849
	Qinghai, China	Guide Black Fur sheep	GDS	4			4						0.0000	
		Qinhai Tibetan sheep	OLS	9			7		2				0.1296	
		Shandong, China	Sishui Fur sheep	SSS	10			3		2	5		0.2178	
	Southwest China	Henan, China	Wadi sheep	WDS	13			1		12				0.0427
			Luzhong Mountain sheep	LZS	14		1	11			2			0.1028
			Large-tailed Han sheep	HDW	19			4	1	14				0.0993
		Henan, China	Small-tailed Han sheep	SXW	18			15		3				0.0693
			Yuxi Fat-tailed sheep	YXZ	6			4		2				0.2177
Taihang Fur sheep			THQ	6			3		1	2			0.2994	
Jiangsu, China		Hu sheep	HU	14			11		3				0.0969	
		Tengchong sheep	TCS	14		12	2						0.0705	
		Yunnan, China	Ninglang Black sheep	NLS	10		5		5				0.1757	
Caucasus		Lanping Black-bone sheep	WGS	19	3		12		4				0.1288	
			Zhaotong sheep	ZTS	15			15						0.0000
			Shiping Gray sheep	SPS	12			11		1				0.0481
		Guizhou, China	Diqing sheep	DQS	5			4		1				0.1789
			Weining sheep	WNS	7	2	1	4						0.2520
			South Caucasus, Azerbaijan	Mazekh	MAZ	12			3		9			0.1181
	South Caucasus, Azerbaijan	Bozakh	BOZ	7			4		3				0.2160	
		Gala	GALA	8			5		3				0.1894	
		Karabakh	KRB	8			6		2				0.1515	
	Stavropol, Russia	North Caucasus	NCMW	6		1		4	1				0.2449	
		Mutton-Wool												
		Caspian depression, Russia	Akasaraik tp. of Soviet Mutton-Wool	ASKT	6		5	1					0.1361	
	West Russia	Caspian depression, Russia	Grozny	GRO	5			2		3			0.2683	
			Russian Karakul	KARA	6					6			0.0000	
		Middle Volga region, Russian	Kuibyshev	KUIB	10		7		3				0.1476	
Volga-Kama region, Russian		Udmurtian local	UD	3			2		1			0.3849		
West Russia, Russia		Russian Romney Marsh	ROMA	6					6			0.0000		

Table 1 (Continued)

Region	Sampling regions	Breeds	Code	n	Haplotype						h	
					H4	H5	H6	H7	H8	H12		H19
Central Europe	Poland	Olkuska	OLK	4			3		1			0.2500
	Poland	Swiniarka	SW	7	1		1	5				0.1980
	Poland	Wrzosowka	WR	3			1		2			0.3849

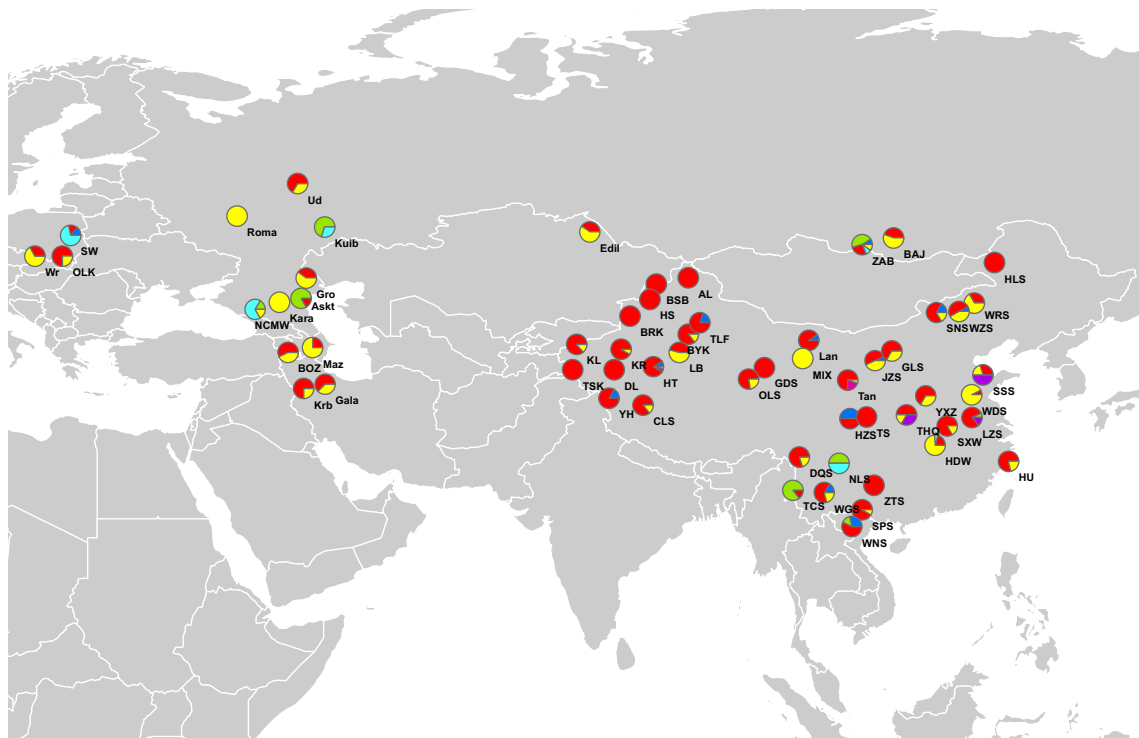


Figure 1 Haplotype frequency distribution (H4 in blue, H5 in green, H6 in red, H7 in aqua blue, H8 in yellow, H12 in purple and H19 in pink) in 59 northern Eurasian native sheep breeds. Size of the circles is not proportional to the population sample size.

Table 2 Y chromosome haplotypes of 59 Eurasian native sheep breeds.

Haplotype <sup>1</sup>	SRYM18 <sup>2</sup>				SRY SNPs	
	[TTTTG] <sub>m</sub>	Indel G/–	[TG] <sub>n</sub>	Allele (bp)	g.88 SNP	g.460SNP
H4	3	G	16	145	A	G
H5	3	G	16	145	G	G
H6	3	G	15	143	A	G
H7	3	G	15	143	G	G
H8	3	G	14	141	A	G
H12	3	G	13	139	A	G
H19	3	G	15	143	A	T

<sup>1</sup>The nomenclature of haplotypes (H4, H5, H6, H7, H8, H12 and H19) is according to Meadows & Kijas (2009).

<sup>2</sup>SRYM18 comprises a pentanucleotide [TTTTG]<sub>m</sub> and dinucleotide [TG]<sub>n</sub> repeat separated by an insertion/deletion (indel G/–).

West Russia:  $h = 0.2240$ ) than that of breeds in other regions (Table 1), which could be due to the fact that the two regions are neighboring the presumed Near Eastern domestication center of sheep. However, we observe a low level of  $h$  for breeds in northwestern China ( $h = 0.0426$ ),

and most of the breeds consist of exclusively H6 (Table 1). This observation is surprising and deserves further studies because northwestern China is situated on the important overland trade route linking middle Asia and China and, thus, frequent animal exchanges in the past should have

promoted gene flows among sheep breeds in the regions. One possible explanation could be that the sheep exchange had involved mainly ewes instead of rams. Alternatively, the scenario of haplotype distribution in northwestern and eastern China could be explained by the historical translocation of the *H4* and *H8* Y chromosomes to Mongolia, via the Mongol invasions during the Mongol Empire, followed by expansions to northwestern and eastern China. Nevertheless, *H6* would have been fixed before these events. The low level of *h* in northwestern Chinese breeds also could be due to the small number of available paternal markers used in this study.

In conclusion, this study represents one of the most comprehensive studies of sheep Y chromosomal genetic variability in northern Eurasia. We observed an overall low level of intrapopulation haplotype diversity and region-specific SNPs and haplotypes. Our findings indicated a small number of rams used in the breeding practice and restricted historical paternal gene flows among breeds in distinct major Eurasian regions. However, differences in geographic distribution of haplotypes between this and earlier studies could probably be attributed to recent ram exchange between European and Asian breeds.

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### Supporting information

Additional supporting information may be found in the online version of this article.

**Table S1.** Previous haplotype nomenclature (Meadows & Kijas 2009; Zhang *et al.* 2012; Ferencakovic *et al.* 2013).

**Figure S1.** Haplotype distribution and relationship. The relationships of haplotypes *H4* (blue), *H5* (green), *H6* (red), *H7* (aqua blue), *H8* (yellow), *H12* (purple) and *H19* (pink) in seven distinct major geographic regions (see Table 1). Sizes of the circles are proportional to the sample sizes.