THE TRANSFERRINS AND HEMOGLOBINS OF WILD IRANIAN SHEEP (*OVIS LINNAEUS*)

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Abstract—1. Serum transferrins and hemoglobins were analyzed by starch–gel electrophoresis from ninety-seven wild sheep collected from eight localities across northern Iran and from two Iranian domestic sheep.

2. Comparison of transferrins from the wild sheep with domestic sheep reference sera demonstrated Tf alleles A, B, C, D and E in the former; new transferrin alleles designated Tf's A^+, B^+ and E^- were also observed.

3. Transferrin allele frequencies provided a means for differentiating wild sheep populations of northwestern Iran from those of northeastern Iran; natural hybrids displayed transferrin frequencies of an intermediate nature. Applications of the transferrin data to some taxonomic problems and to the origin of domestic sheep are discussed.

4. Iranian wild sheep were found to possess Hbg B only.

INTRODUCTION

DOMESTIC sheep possess serum transferrin polymorphisms that result from the expression of at least nine codominant alleles (Ashton, 1958; Ashton & Ferguson, 1963; Stormont *et al.*, 1968). Each allele controls two electrophoretically separable transferrin fractions called a "zone pair" and therefore homozygous phenotypes are characterized by a single "zone pair" of fractions whereas heterozygote individuals display four fractions or two "zone pairs" (Ashton & Ferguson, 1963). Populations from numerous breeds of domestic sheep have been studied. Despite differences in geographic sampling, each breed generally exhibited greater similarities in transferrin gene frequencies than was observed between breeds (Stormont *et al.*, 1968). Although the selective advantages of transferrin polymorphism in sheep remain unknown, the value of transferrins as genetic markers for population analysis is clearly established (Stormont *et al.*, 1968).

Hemoglobin, another polymorphic protein useful in population studies of domestic sheep, occurs in the electrophoretic forms Hbg A, B and the heterozygote AB. These alleles vary among different breeds (Harris & Warren, 1955; Evans *et al.*, 1958; Stormont *et al.*, 1968). Hbg C, with slower mobility than Hbg B, appears in anemic sheep (van Vliet & Huisman, 1964). A fourth allele, Hbg D

with electrophoretic mobility faster than Hbg A, B and C, has been found to characterize some Yugoslavian sheep (Vaskov & Efremov, 1967). Evans *et al.* (1958) have suggested that Hbg A has a selective advantage at higher altitudes because it constitutes the most common allele in highland breeds of English and Scottish sheep. More recently, Efremov & Braend (1965) demonstrated different and perhaps characteristic gene frequencies for Hbg A and B among several breeds of Norwegian sheep, but failed to substantiate the correlation between Hbg A and life at higher altitudes.

Transferrin and hemoglobin gene frequencies have not been studied in wild sheep populations. This study describes such data from ninety-seven wild specimens collected from eight localities within virtually continuous range across northern Iran from Turkey in the west to Turkmen SSR in the east. Transferrins from this study are correlated with chromosomal data from these same specimens which were collected from cytologically homogeneous herds. Diploid numbers ($2n$) of 54 characterize populations in northwestern Iran, $2n = 58$ in northeastern Iran and hybrid herds with $2n = 54$, 55 and $2n = 56$, 57, 58 are found in north-central Iran (Nadler *et al.*, 1971).

MATERIALS AND METHODS

Specimens were examined from the following localities (Fig. 1):

(i) Marakan Protected Region (38°53′ N; 45°11′ E), four females and three males.

(ii) Goyoon Daghi Island, Lake Rezaiyeh (37°28′ N; 45°37′ E), six females and five males.

(iii) 20–40 km N and NNE of Bijar (36°06′ N; 47°40′ E), five females and two males.

(iv) Imperial Reserve (35°41′ N; 51°34′ E), six females and twelve males.

(v) Parvar Protected Region (36°06′ N; 53°35′ E), seven females and six males.

(vi) Kosh Yeilagh Protected Region (36°52′ N; 53°35′ E), seven females and fifteen males.

(vii) Mohammad Reza Shah Wildlife Park (37°20′ N; 56°07′ E), six females and six males.

(viii) Mooteh Protected Region (33°38′ N; 50°07′ E), four females and three males.

Two domestic sheep, a male and female, obtained in Tehran were also studied.

Animals were killed by shooting and blood was collected from the heart and allowed to clot, although in some instances coagulation did not occur.

Serum or plasma was obtained by centrifugation. These samples, and red cells suspended in plasma, were stored in a liquid nitrogen freezer ($-320°F$) until electrophoretic examination.

Horizontal starch–gel electrophoresis was utilized for transferrin analysis (Kristjansson, 1963). Eight sera were run simultaneously in each gel and transferrins were identified by means of radioautography with ^{59}Fe; one-half of the gel was used for radioautography, and the other was stained for protein with buffalo black. Transferrins from Iranian sheep were classified by comparing them with reference sera exhibiting Tf's AE, BD, BE and CE phenotypes that were provided through the kindness of Dr. C. Stormont and Miss Y. Suzuki, School of Veterinary Medicine, University of California, Davis, California.

The frozen red cell samples were thawed and an equal volume of water and a volume of chloroform equal to one-fifth of the total red cell volume were added and mixed. Centrifugation for 15 min resulted in sedimentation of the stromal material and the clear supernatant was removed for electrophoresis. A Beckman microzone apparatus with Tris–EDTA borate buffer (Beckman Methods Manual RM-TB-01D, 1967) was used for hemoglobin

electrophoresis. Samples from Iranian sheep were compared with Hbg AB and Hbg B reference sera from domestic sheep supplied by Stormont and Suzuki. Additional samples obtained from domestic sheep displayed Hbg A, AB and BC and they too were compared with the Iranian specimens.
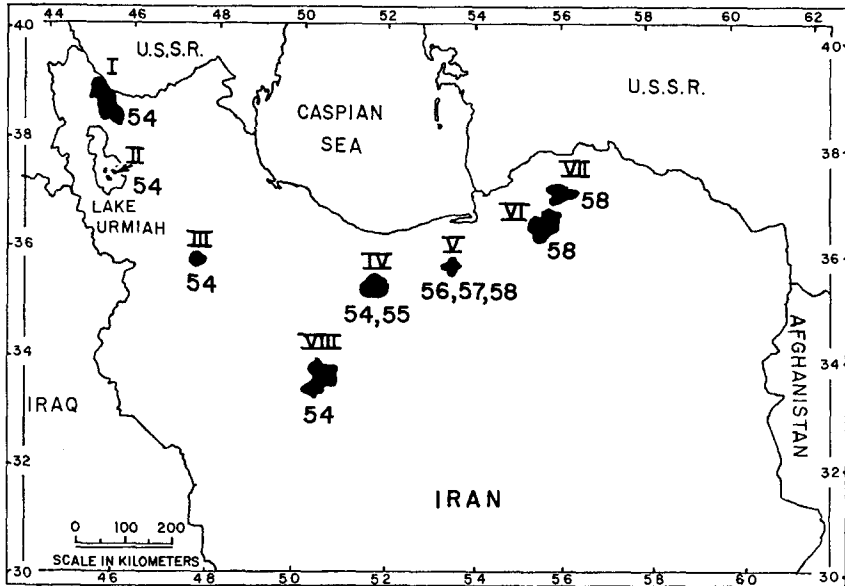

FIG. 1. Map illustrating the collecting localities of wild sheep in northern Iran (i–viii). The diploid chromosome numbers ($2n$ = 54–58) of these sheep are listed for each locality (Nadler *et al.*, 1971).

RESULTS

Transferrins appeared as either two ("zone pair") or four (two "zone pairs") fractions after separation on starch–gel and thus conformed to the hypothesis, based on domestic sheep sera, that each allele controls a pair of fractions consisting of a faintly staining fraction followed by a slower, darker staining fraction, the latter being the main diagnostic band (Ashton, 1958; Stormont *et al.*, 1968). "Zone pairs" with mobilities identical to domestic sheep reference transferrins Tf's A, B, C, D and E were observed. Additional transferrins with mobilities faster than reference Tf's A and B, and a third with mobility slower than Tf E were recognized and designated Tf's A^+, B^+ and E^- respectively (Fig. 2); E^- migrates more slowly than Tf E_2 of Stormont *et al.* (1968) and is probably a different transferrin. Table 1 lists the transferrin phenotypes identified at each locality and includes the results of two Iranian fat-tailed sheep (domestic) that possessed Tf's CC and DD respectively. Gene frequencies derived from the above data are tabulated in Table 2.

Among the Tf's found in all populations, Tf D was most prevalent in the two northeastern populations (localities VI, VII, Fig.1), frequencies were intermediate in the central population (localities IV, V, VIII in Fig. 1), whereas the lowest

TABLE 1—TRANSFERRIN PHENOTYPES OF IRANIAN SHEEP

Locality (specimens)	A+A	A+D	AA	AC	AD	B+D	BC	CC	CD	CE-	DD	DE
Marakan P.R. (7)*		1		1	1	1		1	2			
Goyoon Daghi Is. (11)*			1	4				4	1		1	
Bijar P.R. (7)*		1		1	2	1		1				1
Mooteh P.R. (7)*	1				1				3		2	
Imperial Reserve (18)†					1		1		7	1	8	
Parvar P.R. (13)†		1	1		2				3		5	1
Khosh Yeilagh P.R. (22)‡					2			1	8		11	
M.R.S.W.P. (12)‡					1			1	3		7	
Domestic sheep (2)								1			1	

*2n = 54.   † Hybrids 58 × 54.   ‡2n = 58.

TABLE 2—TRANSFERRIN GENE FREQUENCIES IN IRANIAN SHEEP

Locality	Tf A+	Tf A	Tf B+	Tf B	Tf C	Tf D	Tf E	Tf E-
Marakan P.R. *	0·070	0·140	0·070		0·360	0·360		
Goyoon Daghi Is. *		0·270			0·590	0·140		
Bijar P.R. *	0·070	0·215	0·070		0·215	0·360	0·070	
Mooteh P.R. *	0·070	0·140			0·220	0·570		
Imperial Reserve †		0·030		0·030	0·250	0·660		0·030
Parvar P.R. †	0·040	0·150			0·120	0·650	0·040	
Khosh Yeilagh P.R. ‡		0·040			0·230	0·730		
M.R.S.W.P. ‡	0·040				0·210	0·750		
Domestic sheep					0·500	0·500		

*$2n = 54$.   †Hybrids 58×54.   ‡$2n = 58$.

frequencies of Tf D were found in northwestern Iran (localities I, II, III, Fig. 1). The decreasing frequencies of Tf D progressing from northeastern to northwestern Iran were gradual and not stepwise. In contrast, the frequency of Tf A was low in sheep from northeastern Iran and a gradual increase in Tf A occurred proceeding from central to northwestern Iran. Finally, Tf C appeared in nearly equal frequencies in populations from the northeast and north-central regions while animals from northwestern localities I and II displayed higher frequencies of Tf C.

Sheep from Goyoon Daghi Island (locality II) exhibit differences in the frequencies of Tf's A, C and D when compared to neighboring populations from Bijar (locality III) and Marakan (locality I); this variation may reflect either small sample size or genetic drift in an originally small insular transplant population. Despite these differences the Island sheep clearly belong to the northwestern Iran group of sheep because of their high frequency of Tf A and low frequency of Tf D.

The sporadic appearances of Tf's A+, B+, B, E and E− with their concomitant low gene frequencies cannot be correlated with any geographic section of the sheep range analyzed in this study.

Hemoglobin electrophoresis of wild Iranian sheep demonstrated a single Hbg B fraction in ninety-six specimens; one sheep displayed two fractions with mobility corresponding to Hbg B and Hbg C of domestic sheep (Fig. 2).

DISCUSSION

Comparison of the transferrin polymorphisms known for domestic (Ashton, 1958; Ashton and Ferguson, 1963; Stormont et al., 1968) and wild Iranian sheep indicates a close similarity in the alleles characterizing both groups. The five alleles most commonly observed in domestic sheep, Tf's A, B, C, D and E (Stormont et al., 1968) were also found in wild sheep and Tf's C and D predominate in both kinds of sheep. The additional alleles (Tf's A+, B+ and E−) seen in wild sheep may represent alleles of low frequency not yet described in domestic sheep, or they may be peculiar only to wild populations. Although alleles of Tf's C and D predominate either alone or in combination in all sheep, the wild sheep are unique in their consistently low frequency of Tf B, an allele shown by Stormont et al. (1968) to vary from low to moderately high frequency in domestic breeds. The two domestic fat-tailed sheep obtained from a flock in Tehran have Tf's C and D only and thus conform to the major frequencies described in domestic sheep.

Among the wild sheep, those from northwestern Iran (localities I, II, III) resemble the domestic sheep most closely with respect to their Tf gene frequencies while those from the northeast (localities VI, VII) are most divergent due to their high frequency of Tf D and low frequency of A. These differences may reflect either phylogenetic differences between sheep from northwestern and northeastern Iran, or indicate that the former group of wild sheep and domestic sheep may share a common ancestry.

Support for the existence of phylogenetic differentiation among sheep of northern Iran is derived from studies of gross morphology and chromosomes. Sheep
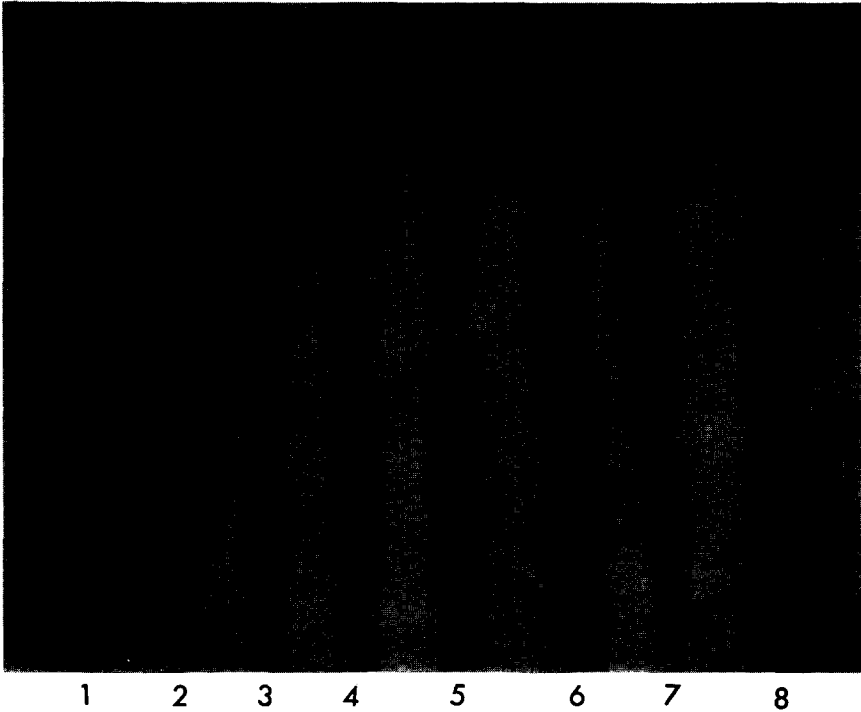
FIG. 2. Starch–gel electrophoresis of serum proteins from wild Iranian sheep. The major transferrin bands of each zone pair, designated by arrows, were identified by ^{59}Fe radioautography. The following phenotypes are illustrated (Tf's): (1), DE; (2), CE$^-$; (3), CD; (4), CE (domestic sheep reference serum); (5), BD; (6), B$^+$D; (7), A$^+$D; (8), AD.

of northeastern Iran are characterized by horns having a "normal" downward, forward curl with everted tips and white throat ruffs (Nadler *et al.*, 1971) and constitute one group that is referable to *vignei* Blyth, 1841. These sheep (VI, VII, Fig. 1) are characterized by $2n = 58$ (Nadler *et al.*, 1971). In contrast, sheep from northwestern Iran have horns that curve downward and either into the neck (inverted tips) or behind the neck (supracervical horns) and the throat ruffs are smaller and black (Nadler *et al.*, 1971). This group (I–III, Fig. 1) uniformly exhibits $2n = 54$ (Nadler *et al.*, 1971), and is referable to *orientalis* Gmelin, 1774. Between these two groups are sheep with intermediate horns and throat ruffs comprised of both black and white hair; chromosome analyses from sheep collected at localities IV and V (Fig. 1) reveal $2n$'s of 54 and 55, and $2n = 56, 57, 58$ respectively and they constitute *orientalis* × *vignei* hybrids.

Correlation of transferrin gene frequencies with gross morphological and chromosomal features suggests that the *vignei* ($2n = 58$) populations are identifiable by a high Tf D frequency (0·73–0·75) and a low Tf A frequency (0·04) in contrast to the *orientalis* ($2n = 54$) group which has a lower frequency of Tf D (0·36–0·57) and much higher frequency of Tf A (0·14–0·27). Sera from the hybrid zone have intermediate Tf frequencies. The gradual changes in Tf's A and D frequencies could be interpreted as a cline within a single taxon but this view is not supported by the chromosomal or morphological data; the gradual rather than stepwise changes across the hybrid zone may reflect allelic introgression extending beyond the zone of chromosomally detected hybridization. In addition to supporting the concept of two sheep taxa, the transferrins also suggest that the insular population from Goyoon Daghi Island and the sheep from Mooteh (VIII, Fig. 1), both with $2n = 54$, are referrable to the *orientalis* group; they are clearly separable by transferrins, horns and chromosomes from the *vignei* group. The similarities in Tf C frequencies observed in all localities (except II) may reflect evolution from a common ancestral stock.

It is next pertinent to evaluate the apparent similarity between the transferrins of the *orientalis* group and domestic sheep. The existing knowledge concerning the origins of domestic sheep bears on this question and prevailing opinion recognizes the Aralo–Caspian basin as a major center of origin where Urial (*Ovis ammon vignei*) were domesticated and later these sheep spread throughout the Middle East and to Europe; a second domestic line presumably arose from mouflon stock (*Ovis ammon musimon*) in the Mediterranean–Middle East region and later became incorporated into European stock (Zeuner, 1963). Based on the knowledge that all breeds of domestic sheep that have been analyzed chromosomally have $2n = 54$ (reviewed by Nadler *et al.*, 1971), it was postulated that domestic breeds of European origin probably arose from ancestral wild stock occupying a range west of central Iran where sheep display $2n = 54$ and not from the Aralo–Caspian basin where wild sheep are characterized by $2n = 58$ (Nadler *et al.*, 1971). It therefore seems likely that the transferrin similarities observed in domestic sheep (Stormont *et al.*, 1968) and the *orientalis* group of Iranian wild sheep reflect the probable derivation of domestic sheep from wild stock more like *orientalis* than *vignei*.

Unlike domestic sheep, which are polymorphic for two hemoglobin alleles (Hbg A and B), wild Iranian sheep are characterized by only one allele, Hbg B. As a consequence, hemoglobins are of no value as population markers. The selective advantage of Hbg A to life at higher altitudes that was suggested by population (Evans *et al.*, 1958) and oxygen affinity (Huisman *et al.*, 1958) studies could not be evaluated in the wild sheep because only Hbg B was observed. It is noteworthy that our wild sheep were collected at altitudes ranging from 1100–2650 m and are known to range as high as 3800 m, elevations at which Hbg A might be expected, and the allele was not identified nor was it observed in nine *Ovis canadensis canadensis* Shaw and one *Ovis dalli dalli* Nelson from North America (Nadler, unpublished data). Further studies may confirm the absence of Hbg A in wild sheep and thus indicate that Hbg A evolved following the domestication of sheep.

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Key Word Index—Transferrins; hemoglobins; *Ovis linnaeus*; sheep; alleles; hybrids.