COGITATIONES

Ammotragus lervia: Progenitor of the Domesticated Sheep or Specialized Offshoot of Caprine Evolution?

In a recent article in this journal, NADLER, HOFFMANN and WOOLF[1] postulate on the basis of chromosomal characters that the 'true sheep' lineage (genus *Ovis*) and the 'true goat' lineage (genus *Capra*) diverged first with the 'true sheep' lineage then evolving 'through an intermediate, aoudad-like form'. Behavioural studies have also resulted in considering that the aoudad, *Ammotragus lervia*, also variously known as the Barbary sheep and the Barbary goat, is close to the ancestor of the modern sheep[2]. CURTAIN[3] cites studies on the primary structure of haemoglobin C from *Ammotragus lervia* as suggesting 'a close relationship between *Ammotragus* and the direct ancestors of the domestic sheep, the β-chains of the A and B haemoglobins of the latter having arisen by duplication and mutation of the β^C-gene of the former'.

In the course of reviewing literature on the biochemical polymorphisms of domesticated species[4] we have noted that the biochemical data at present available do not support the idea that *A. lervia* is particularly close to the domestic sheep *Ovis aries*. Data available on the amino acid sequence of various haemoglobin chains from representatives of the genera *Ovis*, *Capra* and *Ammotragus* are summarized:

The α-chains. The α-chain locus is duplicated (I_α and II_α) in both *A. lervia* and the domestic goat *Capra hircus*, but not in the domestic sheep *O. aries*[5,6], although many breeds of domestic sheep have been surveyed for haemoglobin heterogeneity and genetic polymorphism[7]. The domestic goat I_α- and II_α-chains differ by 4 amino acids. *Ammotragus* I_α- and II_α-chains differ by 3 amino acids. Yet, domestic goat I_α and *Ammotragus* I_α differ by only 1 amino acid, a conservative replacement of threonine for serine. Similarly, goat II_α and *Ammotragus* II_α differ by no more than 1 amino acid substitution. The unduplicated α-chain of domestic sheep haemoglobin differs by 2 amino acids from I_α of *Ammotragus*, whereas I_α of the domestic goat is exactly intermediate between the 2, differing by 1 amino acid from each. A difference of this small magnitude is not significant (and, as mentioned later, *A. lervia* β^C differs by 2 amino acids from *C. hircus* β^C and by 1 amino acid from *O. aries* β^C) but the similarity in sequence between duplicated α-chains in domestic goat and *Ammotragus lervia* is significant[5].

The β-chains. There are sufficient differences in the primary structure of various species-specific and genetically polymorphic β-chains to allow useful comparison, best done by the square matrix pattern of differences[8], though the original papers and a more recent source[6] has been used for the actual number of amino acid differences (Table).

The total number of differences from other β-chains is greatest for *Ovis aries* β^B variant and for *Ammotragus lervia* β^B, 39 differences each, and least for *Ovis musimon* β^B, only 18. Are the comparison of total differences between the various species statistically significant? Using the χ^2 goodness-of-fit one-sample test[9], and correcting for continuity, the $\chi^2 = 11.95$ (6 degrees of freedom) in testing the null hypothesis that the total differences do not differ significantly from the expected average difference 28.57; this fails to be statistically significant (p approximately equals 0.07). However, SIEGEL[9] recommends using the more powerful Kolmogorov-Smirnov one-sample test. For the order of β-chains given in the above comparison the maximal difference $|F_0(X)-S_{200}(X)| = 0.0543$, which is statistically significant at the 0.01 level where the maximal difference must be greater than $1.63/(200)^{1/2} = 0.0408$. The χ^2 test can be made more powerful by testing against specific alternative hypotheses, e.g., the alternative hypothesis that the total divergence of *Ammotragus* β^B, 39, is significantly greater than the total divergence of *O. musimon* β^B, 18, for which against the average of 28.57 and correcting for continuity $\chi^2 = 7.00$ (1 degree of freedom), significant at $p < 0.01$. Therefore, as it has the least number of total differences in comparison with other β-chains, mouflon β^B is much more likely to be closer to a hypothetical ancestral ovine-caprine β-chain than is aoudad β^B, which is along with sheep β^B the most divergent.

The β^C-chains. *Ammotragus lervia* is unique among all sufficiently studied ovines and caprines in that some individuals of *A. lervia* synthesize a β^C-like chain continuously as adults, without the requirement of severe

[1] C. F. NADLER, R. S. HOFFMANN and A. WOOLF, Experientia *30*, 744 (1974).
[2] V. GEIST, *Mountain Sheep: A Study in Behaviour and Evolution* (University of Chicago Press, Chicago, Illinois 1971).
[3] C. C. CURTAIN, Antiq. Survival *45*, 303 (1971).
[4] C. MANWELL and C. M. A. BAKER, Proc. Int. Conference on Population Genetics and Ecology, Israel 12–23 March 1975 (Eds. S. KARLIN and E. NEVO, Academic Press, New York), in press.
[5] J. B. WILSON, R. N. WRIGHTSTONE and T. H. J. HUISMAN, Nature, Lond. *226*, 354 (1970).
[6] T. H. J. HUISMAN, Ann. N. Y. Acad. Sci. *241*, 392 (1974).
[7] N. S. AGAR, J. V. EVANS and J. ROBERTS, Anim. Breed. Abstr. *40*, 407 (1972).
[8] M. O. DAYHOFF, *Atlas of Protein Sequence and Structure* (National Biomedical Research Foundation, Silver Spring, Maryland 1972), vol. 5.
[9] S. SIEGEL, *Nonparametric Statistics for the Behavioral Sciences* (McGraw-Hill, New York, N. Y. 1956).

	β^B Sheep	β^A Sheep	β^B Mouflon	β^E Goat	β^A Goat	β^D Goat	β^B Aoudad
Sheep (*Ovis aries*) β^B	0	7	5	6	7	8	6
Sheep (*Ovis aries*) β^A	7	0	2	3	4	5	7
Mouflon (*Ovis musimon*) β^B	5	2	0	1	2	3	5
Goat (*Capra hircus*) β^E	6	3	1	0	3	4	6
Goat (*Capra hircus*) β^A	7	4	2	3	0	1	7
Goat (*Capra hircus*) β^D	8	5	3	4	1	0	8
Aoudad (*Ammotragus lervia*) β^B	6	7	5	6	7	8	0
Total differences	39	28	18	23	24	29	39

anaemia or other hypoxic stress, the unusual β^{C}-like chain called $\beta^{C(na)}$. Other individuals of *A. lervia* synthesize a typical ovine-caprine β^{C} chain which differs by eight amino acids from *A. lervia* $\beta^{C(na)}$[10]. Otherwise, the evolution of C-type chains is relatively conservative, for *A. lervia* β^{C} differs by only 2 amino acids from *C. hircus* β^{C} and by only 1 amino acid from *O. aries* and *O. musimon* β^{C}[11]. Another remarkable feature of *A. lervia* $\beta^{C(na)}$ is that the middle of the chain, tryptic peptides T-9 and T-10, contain 4 amino acid substitutions not found in other β^{C} chains but shared in the sequence of the foetal chain, γ, of sheep and goats.

Accordingly, the haemoglobin data suggest that *A. lervia* is no closer to *Ovis* than to *Capra*; indeed, the nature of the α-chain duplication indicates that there is more in common between *A. lervia* and *C. hircus* than between *A. lervia* and *O. aries*. However, especially with the unusual $\beta^{C(na)}$ chain and the total of 39 differences between *A. lervia* β^{B} and other β-chains, it might be best to regard the aoudad as distinct from both *Ovis* and *Capra*, perhaps representing an early offshoot from the ovine-caprine stock which has retained some primitive characters but has also evolved some uniquely specialized ones.

Such a position would not be incompatible with the zoogeographic information, for *Ammotragus lervia* occurs in a restricted part of northern Africa, distinct from 'the great arc' of *Ovis* in Eurasia and North America[12]. The distinctness of *Ammotragus* is shown by its failure to produce viable hybrids with either domestic goats or domestic sheep, although development of *A. lervia* × *C. hircus* foetuses proceeds to term, whereas *A. lervia* × *O. aries* fails to be conceived[13].

In contrast to suggestions that *Ammotragus lervia* is close to progenitors of the domestic sheep, discussions of data for both biochemical[4] and chromosomal[14] characters suggest the mouflon, *Ovis musimon*, as a better candidate. As CLARK[15] says, 'the Barbary sheep (*Ammotragus*)..., for reasons it would be interesting to have restated, is excluded from any part in the ancestry of African domestic sheep'.

Summary. Data on haemoglobin do not support suggestions that the aoudad *Ammotragus lervia* is close to a hypothetical ancestor to the genus *Ovis* in general or to the domesticated sheep *Ovis aries* in particular. *Ammotragus* haemoglobin is more like that from the domestic goat *Capra hircus* than that from the domestic sheep *Ovis aries*, but also shows some unique characteristics, perhaps more specialized than primitive.

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[10] T. H. J. HUISMAN and A. MILLER, Proc. Soc. exp. Biol. Med. *140*, 815 (1972).
[11] T. H. J. HUISMAN, Ann. N. Y. Acad. Sci. *241*, 549 (1974).
[12] J. L. CLARK, *The Great Arc of the Wild Sheep* (University of Oklahoma Press, Norman, Oklahoma 1964).
[13] A. P. GRAY, *Mammalian Hybrids* (Commonwealth Agricultural Bureaux, Farnham Royal, Bucks., England 1954).
[14] K. V. KOROBITSYNA, C. F. NADLER, N. N. VORONTSOV and R. S. HOFFMANN, Quaternary Res. *4*, 235 (1974).
[15] J. D. CLARK, Proc. prehist. Soc. *37*, 34 (1971).

PRO EXPERIMENTIS

A Method for Distinction Between RNA and DNA in Aldehyde and Osmiumtetroxide-fixed Electron Microscopic Autoradiographs[1]

Histochemical procedures in electron microscopy usually require a special fixation or other treatment of the tissue prior to embedding. If an investigation with tissue fixed and embedded according to a routine procedure is in progress and one wishes to perform a histochemical reaction, the fixation, embedding, and preceeding experiments have to be repeated. This is especially time-consuming with electron microscopic autoradiography, as the exposition time of the autoradiographs ranges from weeks to several months.

The present paper shows that the so-called 'regressive staining method' described by BERNARD[2] for the distinction of RNA and DNA on aldehyde-fixed tissue can also

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[2] W. BERNHARD, J. Ultrastruct. Res. *27*, 250 (1969).

Processing and staining of autoradiographs for conventional contrast and RNA-DNA differentiation

Solution	Time of treatment for	
	Conventional contrast	RNA-DNA differentiation
Microdol-X- (Kodak)	5 min	5 min
Dist. water	a few sec	a few sec
Na-thiosulfate (3%)	no longer than 3 min	no longer than 3 min
Dist. water (room temp.)	3 × 10 min	3 × 10 min
Dist. water 37 °C	30 min	30 min
Acetic acid 37 °C	15 min 4%	up to 20 min, up to 8%
Dist. water (room temp.)	3 × 5 min	3 × 5 min
Uraniumacetate 2,5% in dist. water	6 min	none
Lead citrate[8]	3 min	3 min