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Genetic evidence for the origin of the agrimi goat (*Capra aegagrus cretica*)

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Abstract

The agrimi goat *Capra aegagrus cretica* is unique to Crete and its offshore islands. It has been identified as a sub-species of the wild bezoar goat *Capra aegagrus aegagrus* Erxleben, 1777, which it closely resembles in horn shape, body form and coloration. This classification has been disputed by some researchers who claim that the agrimi are feral goats, derived from early domestic stock brought to the island by the first Neolithic settlers. In order to clarify this issue, DNA analyses (cytochrome *b* and D loop sequences) were carried out on tissue of live and skeletonized agrimi and compared to sequences of wild and domestic caprines. Results conclusively show the agrimi to be a feral animal, that clades with domestic goats (*Capra hircus*) rather than with wild Asiatic bezoar. This study demonstrates that morphometric criteria do not necessarily reflect genetic affinities, and that the taxonomic classification of agrimi should be revised.

Key words: agrimi, *Capra aegagrus cretica*, bezoar goat, *Capra aegagrus aegagrus*, Crete, aDNA, cytochrome *b*

INTRODUCTION

The agrimi goat *Capra aegagrus cretica* is unique to Crete and its offshore islands. It has been identified by numerous researchers (Zimmerman *et al.*, 1942–49; Harris, 1960–61; Schultze-Westrum, 1963; Epstein, 1971; Walker, 1983; Mason, 1984; Valdez, 1990; Harrison & Bates, 1991) as a subspecies of the wild Asiatic bezoar goat (*Capra aegagrus aegagrus* Erxleben, 1777) which it resembles in horn shape and body form (Fig. 1), but it differs from the bezoar goat in being lighter coloured with broader markings and in having a shorter withers height (Mason, 1984; Porter, 1996).

Other researchers (Papageorgiou, 1974; Corbet, 1978; Groves, 1989; Ciani & Masseti, 1991) have argued that the agrimi is probably feral, derived from domestic goats brought to the island by the first Cretan settlers in the Aceramic Neolithic period (*c.* 7000 uncal. BC). This is assumed because agrimi are absent from the Pleistocene fauna of Crete (Lax & Strasser, 1992;

Jarman, 1996). The earliest identification of skeletal remains of agrimi, from the sites of Phaestos and Knossos (Jarman, 1996; Porter, 1996; Wilkens, 1996), date to the Late Neolithic period (*c.* 6000 uncal. BC) such that the timing of their first appearance clearly post-dates the arrival of domestic caprines on the island. Archaeozoological data (Jarman, 1996; Wilkens, 1996; Nobis, 1998), and literary, historical and pictorial evidence (Porter, 1996; Vanschoonwinkel, 1996), document that agrimi have been hunted on Crete from the Late Neolithic period through to the twentieth century.

Today agrimi herds are restricted to Samaria Gorge in the White Mountains, Western Crete (numbering some 500 animals in 1996), and three off-shore islands: Theodorou (*c.* 70 animals in 1996), Dia (*c.* 300 animals) and Agli Pandes (*c.* 90 animals) (Papageorgiou, 1974; Husband, 1977; Porter, 1996) (Fig. 2).

As standard archaeological and zoological methods have failed to resolve the question of the taxonomic status of agrimi, we have applied DNA analysis (cytochrome *b* and D loop sequences) to establish the genetic affinity of the agrimi.

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Fig. 1. Captive agrimi *Capra aegagrus agrimi* at the Hai-Bar Nature Reserve, Mount Carmel, northern Israel. Note the resemblance to the bezoar goat *Capra aegagrus aegagrus* in coat type, horn form and body proportions.

MATERIALS AND METHODS

DNA analyses were carried out on a total of 17 animals derived from 3 recently separated agrimi populations; a population living in Samaria Gorge, western Crete; a herd kept on Theodorou Island off Crete; and a captive herd held in Israel (Fig. 2a, b).

The population in Samaria Gorge, the last refuge for agrimi on the mainland of Crete, is the parent population of all the agrimi studied here. It is a free-living population and represents the remnants of the original agrimi population that was probably more widespread on the island. In 1928, 1937 and 1945, 1 breeding pair of agrimi were taken from the Samaria population and introduced into a reserve on Theodorou Island (Fig. 2b) (Schultze-Westrum, 1963; Papageorgiou, 1974; Husband & Davis, 1984; Nicholson & Husband, 1992). In 1975, as part of a programme to save endangered species and to re-introduce wildlife that had once inhabited the region, 6 agrimi were brought to Israel from Theodorou Island and held in captivity in the Hai-Bar Reserve on Mount Carmel (Fig. 2a) (Yoffe, 1980). In 1993, this herd numbered *c.* 55 goats.

For this study, blood samples were taken from 5 Hai-Bar reserve agrimi (while tranquilized) using a 3-ml tube containing 7.5% EDTA to prevent clotting. DNA was extracted from the blood using a method of isolation of DNA from peripheral blood (Filon *et al.*, 1992). The DNA was amplified in a volume of 50 μ l through 25 cycles. For all PCR positive reactions, DNA was purified using the Boringer DNA purification kit. All the samples, purified and cored bands, were sequenced by the direct sequencing reaction using the Termo Sequenase kit (Amersham, U.K.).

In 1996, DNA samples were obtained from 5 skeletons

of agrimi from Samaria Gorge, Western Crete and 7 skeletons from Theodorou Island. This enabled comparison of the Hai-Bar sample with its parent stock. In all instances, the Cretan agrimi skeletons were collected in the field and represent goats that had lived in the wild and are assumed to have died of natural causes. The samples for DNA included teeth, bones and dry skin adhering to the skeletons.

DNA was extracted from these skeletons using ancient DNA techniques (aDNA). These samples were treated according to protocols established for aDNA analysis as this is defined as any DNA which has suffered autolytic or diagenetic processes (Herrmann & Hummel, 1994). For this purpose guanidine thiocyanate and silica bead extraction methods were used (Boom *et al.*, 1990) followed by a PCR reaction (Saiki *et al.*, 1988). PCR amplification was performed with 6 sets of primers (Table 1), and under conditions set up separately for each reaction. The DNA extracted from bone, teeth and skin was amplified in a volume of 25 μ l through 40 cycles. The double-stranded PCR products were run on 1.5% low melting agarose gels to determine whether there was a successful amplification in the PCR reaction. The positive bands of these skeletal samples were cored directly from the gel using Pasteur pipettes followed by direct sequencing reaction using the Termo Sequenase kit (Amersham).

For the aDNA analyses, stringent precautions were taken to eliminate contamination. These included working under hoods that were sterilized between analyses with ultraviolet light, using different rooms for each stage of the procedure and disposable tips and sterile reagents. For each reaction, 2 sterile negative controls were used to check for contamination. In order to confirm the sequences, the complete analysis from

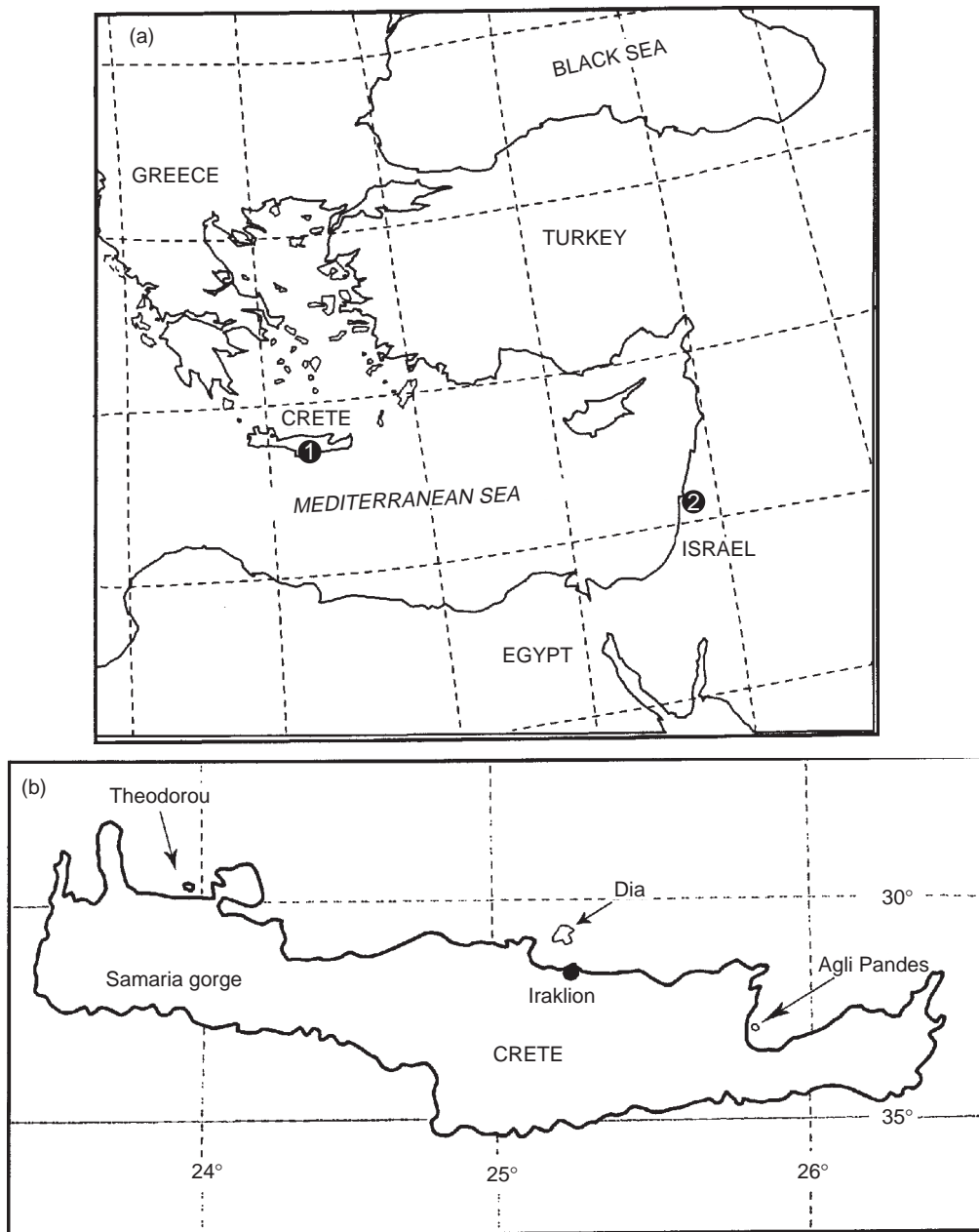


Fig. 2. (a) Locations from which samples of agrimi *Capra aegagrus agrimi* were taken: 1, Crete; 2, Hai-Bar Reserve, Mount Carmel, northern Israel; (b) locations on Crete and adjacent islands where agrimi are found today.

extraction to sequencing was carried out at least twice independently for each sample. Thus, every amplified region has a minimum of 4 sequences, 2 with each primer. In order to analyse the data a consensus sequence was obtained for each set of primers using the ClustalX multiple sequence alignment software from the Molecular Biology Shortcuts website (Rodrigues & Thompson, 1999).

Cytochrome *b* sequences from the skeletal samples from Crete were first compared by geographic location (Fig. 3). They were then compared to the consensus sequence of the Hai-Bar Reserve agrimi, and finally to additional haplotypes for the same region of wild and domestic caprines from GenBank as given in Table 2,

as well as the Baladi breed of goat (also known as the Syrian mountain goat; Hirsch, 1933; Epstein, 1971: 264), sequenced by us (Table 2). Phylogenetic analysis was carried out using the PAUP* version 4.0 (Swofford, 1993). The data were analysed using 3 methods: distance, maximum parsimony and maximum likelihood.

RESULTS

All five cytochrome *b* (1050 bp) and D loop sequences (239 bp) of the living, Hai-Bar agrimi sample were found to be identical (Fig. 3). A consensus sequence was

Table 1. Six sets of primers were used to amplify the cytochrome *b* and D loop sequences. For the skeletal samples, three primers proved to be the most efficient: primers 86–92, G11–G12 and Gd1–Gd2. For agrimi no.21 from Theodorou Island, DNA was also amplified using another set of primers (117–116) while for agrimi no.14 from Samaria Gorge, the cytochrome *b* had a positive reaction using the G9–G10 set of primers

Primers	Position in gene	Primer name	Primer location	Primer sequence	Product size (bp)	Annealing temperature (°C)
1	70	86	Upper	Cytochrome <i>b</i> 5' CCATCCAACATATCAACATGA 3'	165	46–48
	214	92	Lower	Cytochrome <i>b</i> 5' AATRCAGCGTAGTTTACATC 3'		
2	1009	116	Lower	Cytochrome <i>b</i> 5' ACTGGCTGTCTCCAATTCA 3'	176	46–48
	853	117	Upper	Cytochrome <i>b</i> 5' CCTAACAAACTAGGAGGAGTC 3'		
5	68	G9	Upper	Cytochrome <i>b</i> 5' CCCCATCAAACATCTCATCA 3'	196	50–52
	244	G10	Lower	Cytochrome <i>b</i> 5' ATACACGCAAACGGAGCA 3'		
6	615	G11	Upper	Cytochrome <i>b</i> 5' CAACAACCCACAGGAAT 3'	236	50–52
	831	G12	Lower	Cytochrome <i>b</i> 5' ATACGCAATCTTACGA 3'		
7	895	247	Upper	Control region 5' GGGGATGCTTGGACTCAGC 3'	156	46–48
	1051	248	Lower	Control region 5' TTAAAAATGGTGATAAATATTTTAGG 3'		
8	907	Gd1	Upper	Control region 5' GACTCAGCTATGGCCGTCT 3'	216	50–52
	1102	Gd2	Lower	Control region 5' TATCTAGGAGGGAGCGTGTTT 3'		

Table 2. List of specimens, GenBank Numbers and their sources that were used in this study

Code Used	GenBank no.	No. of specimens	Gene	Reference	Goat breed and geographical location
<i>C. hircus</i> 1	AB004070	1	Cytochrome <i>b</i>	Takada <i>et al.</i> , 1997	Japanese native, Saanen, small meat type goat, National Livestock Breeding Center, Nagano Station, Japan
<i>C. hircus</i> 2	AB004071	1	Cytochrome <i>b</i>	Takada <i>et al.</i> , 1997	West African Dwarf, small meat type goat, National Livestock Breeding Center, Nagano Station, Japan
<i>C. hircus</i> 3	AB004072	1	Cytochrome <i>b</i>	Takada <i>et al.</i> , 1997	Korean native, small meat goat, introduced from Korea
<i>C. hircus</i> 4	AB004073	1	Cytochrome <i>b</i>	Takada <i>et al.</i> , 1997	Pakistan lop-eared goats, Miyazaki Phoenix Zoo, Miyazaki, Japan
<i>C. hircus</i> 5	AB004074	1	Cytochrome <i>b</i>	Takada <i>et al.</i> , 1997	Japanese native, Shiba, small meat goat, National Livestock Breeding Center, Nagano Station, Japan
<i>C. hircus</i> 6	AB004075	1	Cytochrome <i>b</i>	Takada <i>et al.</i> , 1997	Japanese native, Tokara, small meat goat National Livestock Breeding Center, Nagano Station, Japan
<i>C. hircus</i> 7	D84201	1	Cytochrome <i>b</i>	Arai <i>et al.</i> , 1997	Unknown
<i>C. hircus</i> 8	X56289	1	Cytochrome <i>b</i>	Irwin, Kocher & Wilson, 1991	DNA from D. E. Dobson, Boston University School of Medicine, USA
Baladi	AF217254	5	Cytochrome <i>b</i>	Kahila Bar-Gal <i>et al.</i> , 1999 ^a	Blood, Baladi breed from a herd kept at Matat, Galilee, North Israel
<i>C. aegagrus</i> 1	AB004069	1	Cytochrome <i>b</i>	Takada <i>et al.</i> , 1997	Gumma Safari Park, Tomiok, Japan
<i>C. aegagrus</i> 2	D84204	1	Cytochrome <i>b</i>	Arai <i>et al.</i> , 1997	Unknown Captive
<i>C. aegagrus</i> 3	AF034739	1	Cytochrome <i>b</i>	Hassanin, Pasquet & Vigne, 1998	Blood, from J. L. Berthier, Menagerie du Jardin des Plantes, Paris, France
<i>C. aegagrus</i> 4	AJ231408	1	Cytochrome <i>b</i>	Manceau <i>et al.</i> , 1999	Bone, from St Petersburg Museum; specimen origin Turkmenistan
<i>C. aegagrus</i> 5	AJ231409	1	Cytochrome <i>b</i>	Manceau <i>et al.</i> , 1999	Bone, from St Petersburg Museum; specimen origin Iran
<i>C. falconeri</i> 1	AF034739	1	Cytochrome <i>b</i>	Hassanin <i>et al.</i> , 1998	Bone, Laboratoire de Mammiferes and Oiseaux, MNHN, Paris, France
<i>C. falconeri</i> 2	D84202	1	Cytochrome <i>b</i>	Arai <i>et al.</i> , 1997	Unknown
<i>C. falconeri</i> 3	AJ231415	3	Cytochrome <i>b</i>	Manceau <i>et al.</i> , 1999	Bone, from the field
<i>C. falconeri</i> 4	AJ231414	2	Cytochrome <i>b</i>	Manceau <i>et al.</i> , 1999	Bone, from the field
<i>C. falconeri</i> 5	AJ231413	1	Cytochrome <i>b</i>	Manceau <i>et al.</i> , 1999	Bone, from St Petersburg Museum
Agrimi	AF217255	5	Cytochrome <i>b</i>	Kahila Bar-Gal <i>et al.</i> , 1999 ^a	Blood, captive herd kept in Hai-Bar Reserve, Mt Carmel, Israel

^a Date of GenBank submission.

Primer 86–92 (Cytochrome *b*)

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70
Agrimi Hi-Bar CCATCAACATCTCATGATGAACTTGGATCCCTCTAGGAATTTGCCTAATCTTACAAATCCTGACAGGCCTATTCTAGCAATACACTATACATCCGACACAATAACAG
Agrimi 7 Th .....
Agrimi 8 Th .....
Agrimi 9 Th .....T..T..G..A..A.....T..Y...
Agrimi 10 Th .....
Agrimi 13 S .....
Agrimi 16 S .....
Agrimi 17 Th .....
Agrimi 19 S .....
Agrimi 20 Th .....
Agrimi 21 Th .....

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223
Agrimi Hi-Bar CATTTCCTCTGTAACCTCACATTGTGCAGATGTAATTTATGGCTGAATCA
Agrimi 7 Th .....C.....
Agrimi 8 Th .....C.....
Agrimi 9 Th .....C.....C.....
Agrimi 10 Th .....
Agrimi 13 S .....
Agrimi 16 S .....Y.....
Agrimi 17 Th .....
Agrimi 19 S .....X....
Agrimi 20 Th .....C.....
Agrimi 21 Th .....T.....

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Primer G11–G12 (Cytochrome *b*)

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615
Agrimi Hi-Bar AACAAACCCACAGGAATCCATCAGACACAGATAAAATCCCAATTCACCCCTTACTACACCATTAAAGATATCTTAGGGCCATGCTACTAATCTTGTCTAATATTACTAGTACTAT
Agrimi 7 Th .....
Agrimi 8 Th .....
Agrimi 9 Th .....
Agrimi 10 Th .....
Agrimi 13 S .....
Agrimi 16 S .....
Agrimi 17 Th .....
Agrimi 21 Th .....

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845
Agrimi Hi-Bar TCACACCCGACCTACTCGGAGACCCAGACAACCTATATCCAGCAATCCCACTCAATACACCCCTCAGATTAAACCTGAGTGGTATTTCCTATTGTCATAGCCAATCCTACGATCAAT
Agrimi 7 Th .....T..T.....
Agrimi 8 Th .....A..A.....
Agrimi 9 Th .....T..T.....
Agrimi 10 Th .....A..A.....
Agrimi 13 S .....T..T.....
Agrimi 16 S .....T..T.....
Agrimi 17 Th .....A..A.....
Agrimi 21 Th .....A..A.....

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Primer Gd1–Gd2 (D loop)

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906
Agrimi Hi-Bar GGACTCAGCTATGGCCCTCTGAGGCCCCGACCCGGAGCATAAATTTAGCTGGACTTAACTGCATCTTGAGCATCCCCATAATGGTAGGCATGGGCATTGCAGTTAATGGTCACAGGAC
Agrimi 7 Th .....
Agrimi 8 Th .....
Agrimi 9 Th .....
Agrimi 10 Th .....
Agrimi 13 S .....
Agrimi 16 S .....
Agrimi 17 Th .....
Agrimi 19 S .....
Agrimi 20 Th .....
Agrimi 21 Th .....

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1122
Agrimi Hi-Bar ATATTATTATGTTGCATTTTCATCATGCATCCGCTCCACCTTTCCCCCTCCTTCTTAGATATATACCACCGTTTAAACACGCTCCCTCCTAGATA
Agrimi 7 Th .....
Agrimi 8 Th .....
Agrimi 9 Th .....
Agrimi 10 Th .....
Agrimi 13 S .....
Agrimi 16 S .....
Agrimi 17 Th .....
Agrimi 19 S .....
Agrimi 20 Th .....A.....G.....
Agrimi 21 Th .....

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Fig. 3. Comparison of agrimi *Capra aegagrus cretica* cytochrome *b* and D loop sequences. First three columns, identity number of the individual specimen and its location of origin; Hi-Bar, Hai-Bar Reserve, Israel; Th, Theodorou Island, Crete; S, Samaria Gorge, Crete. The Hai-Bar sample represents a consensus sequence comprising 10 identical haplotypes. Following columns: dots, identical sequence; X, unknown nucleotide; – , deletion; C, cytosine; T, thymine; A, adenine; G, guanine; Y, cytosine or thymine.

determined for each region for this population (cytochrome *b*, GenBank number AF217255; D loop, GenBank number AF217259).

For 11 of the 12 (92%) agrimi skeletal samples, DNA could be amplified for two regions of the cytochrome *b*

and one region of the D loop; in eight samples (66.7%) DNA was amplified from three different areas; and for four samples only two fragments could be amplified. For the two sets of cytochrome *b* primers that were amplified (Table 1), a maximum of 362 bp were

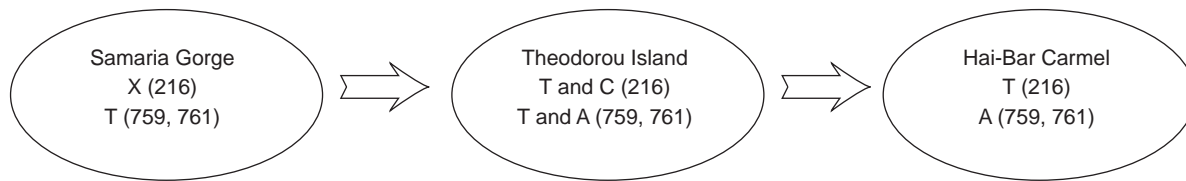


Fig. 4. Diagram showing the relationship between populations of agrimi *Capra aegagrus cretica* and polymorphic sites on two regions of the cytochrome *b* gene. Samaria is the parent population from which the Theodorou Island population is derived. The Hai-Bar agrimi are derived in turn from the Theodorou Island herd. All Hai-Bar agrimi have an identical sequence; two sequences from Samaria Gorge differ from the Hai-Bar agrimi in having thymine in position 759 and 761. The sequences from Theodorou Island are variable with three polymorphic sites at positions 216, 759,761. At position 216, four sequences have cytosine and one sequence has thymine; at position 759, 761 four sequences have adenine and two sequences have thymine.

sequenced, which is about one-third of the gene (which in total comprises 1100 bp).

Comparison of the two main regions of the cytochrome *b* that were amplified showed the presence of a polymorphism in the three agrimi populations; Hai-Bar, Samaria and Theodorou Island (Fig. 4). The sequences obtained using primer G86-92, indicate that nucleotide sequences in four specimens from Theodorou (specimens nos 7, 8, 20 and 21) varied at position 216 (Fig. 3). Three Theodorou specimens (nos 7, 8 and 20) exhibited a transition of thymine to cytosine, which did not change the amino acids, and is indicative of a polymorphism on the island. This polymorphism indicates that a separation of 54 years, this being the date when the last pair was introduced to the island from Samaria, may have been long enough to establish minor genetic differences between populations. However, larger samples from both locations are needed to verify this. Unfortunately, for this region of the cytochrome *b*, the sequences of the Samaria agrimi are missing, and as such cannot be compared. However, comparison of this cytochrome *b* region with other *Capra* species indicates that all the published domestic goats, wild goats and *C. falconeri* have a thymine in position 216, like the agrimi from the Hai-Bar reserve. For this region of the cytochrome *b*, the sequence obtained for agrimi no. 9 was aberrant and so suspected of being an artefact, and was not included in the phylogenetic analysis using this region.

Two groups of agrimi were recognized based on sequences at the second region using primer G11-12 (Fig. 3). The first group has nucleotide thymine in positions 759 and 761 and comprises specimens from Theodorou Island (nos 7 and 9) and Samaria Gorge (nos 13 and 16). The second group, comprising animals from the Hai-Bar reserve and Theodorou Island, has adenine in positions 759 and 761 (specimen nos 8, 10, 17 and 21). The substitutions are two transversions in the second and third codon, which caused one amino acid change (Asp-Val).

The results illustrated in Figs 3 & 4, demonstrate that the Hai-Bar reserve consensus sequence is identical to that of one goat from Theodorou (no. 21). This specimen represents a variant of the sequence occurring on the island that was present in the six founder animals

that established the Hai-Bar herd. Based on these data, the Theodorou Island population seems to be more polymorphic than their parent population in western Crete and their daughter population in Israel.

For the D loop (primer Gd1-Gd2), 16 of the 17 agrimi specimens had identical sequences (Fig. 3). The one exception, specimen no. 20 from Theodorou, differs in two substitutions (a transition and a transversion) and one deletion. The high degree of homology of the D loop sequences (a non-coding region), suggests that we are dealing with a relatively conserved region of the D loop rather than a hypervariable one.

The three agrimi populations studied here closely resemble each other. In the cytochrome *b* sequence there is a similarity of 99.3% among all the sampled individuals with additions of three polymorphic sites at positions 216, 759 and 761 (Fig. 4). This divergence rate is similar to that found among breeds of domestic goats (0.6–0.84%).

In order to determine the genetic affinity of the agrimi to domestic and wild goats, three methods of phylogenetic analysis were used. In these analyses the sequences were compared to published sequences of wild and domestic goats from GenBank (Table 2). All three methods of analysis (distance, maximum parsimony and maximum likelihood) and all three sequenced regions, showed the same pattern of relationship with the agrimi sequences closely related to the different breeds of domestic goats, but differing from the wild goats, *C. falconeri* and two *C. aegagrus* specimens.

As many of the goat cytochrome *b* sequences in GenBank are limited to short sequences, the region that incorporates the greatest number of sequences (i.e. the region amplified with primers 86–92) was used. The resulting phylogeny (Fig. 5), shows that the cytochrome *b* sequences of the agrimi (agrimi nos 1, 2, 3 and 4) all clade with the domestic goats and lie on a different branch to two of the bezoar goats (*C. aegagrus* nos 3 and 4) and the five samples of *C. falconeri*. Moreover, the consensus sequence of the agrimi goats from the Hai-Bar and agrimi no. 21 from Theodorou island, are identical to the Baladi goat; an indigenous breed of domestic goat from the southern Levant (Hirsch, 1933; Epstein, 1971).

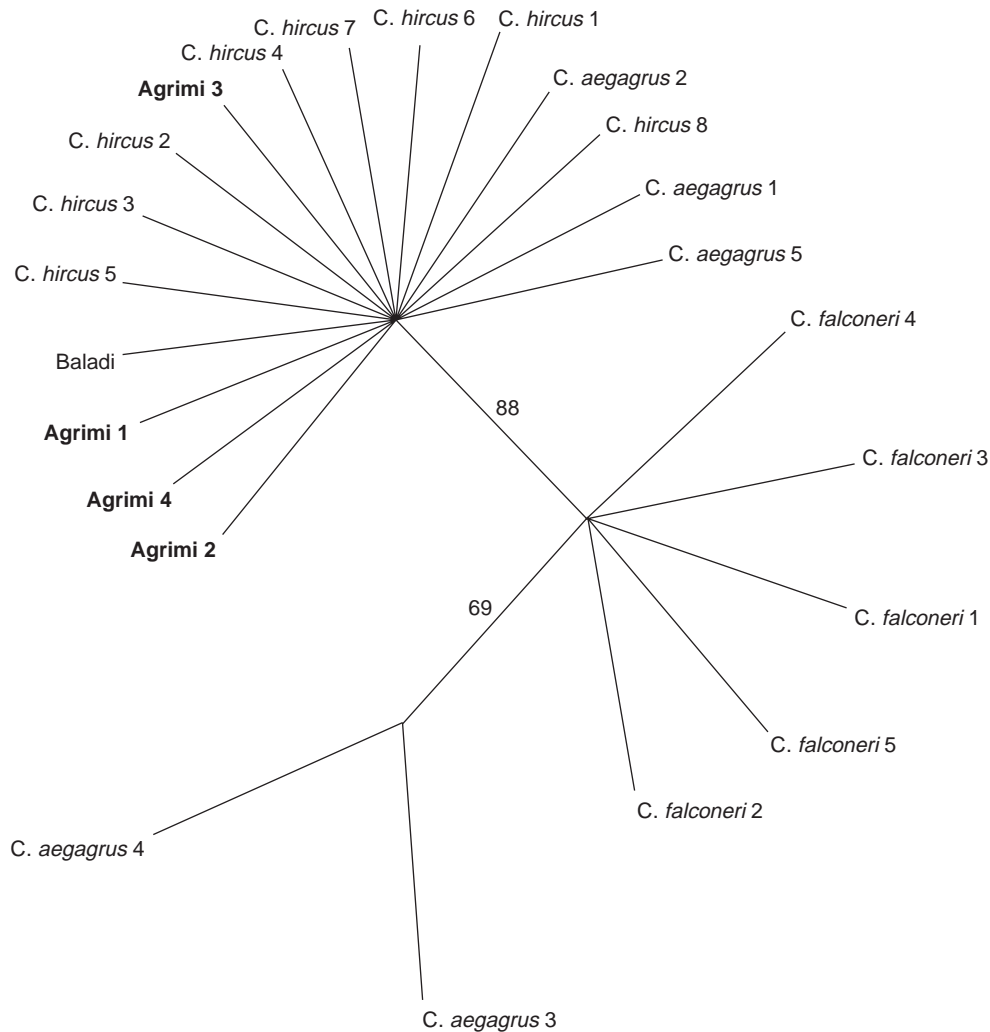


Fig. 5. Phylogenetic analysis of agrimi sequences using primer G86–92 of cytochrome *b* (based on maximum parsimony analysis heuristic search). The bootstrap values (obtained after 100 replicates) are indicated on the internal branches. Data on wild and domestic goats are taken from samples in GenBank as described in Table 2. The agrimi sequences shown in this clade are grouped according to the length of their cytochrome *b* sequences (as given in Fig 3): agrimi 1, Hai-Bar consensus sequence and agrimi no. 21 from Theodorou Island; agrimi 2, agrimi nos 10, 13 & 17; agrimi 3, agrimi nos 7 & 19; agrimi 4, agrimi nos 8, 16 & 20.

DISCUSSION

As shown in Fig. 5, three of the published bezoar sequences (*C. aegagrus* nos 1, 2 and 5), clade with the domestic goats. The first two sequences are derived from zoo specimens and it is not inconceivable that they represent hybrids or even agrimi. The third specimen (*C. aegagrus* no. 5) is derived from a museum sample originating in Iran (Table 2). Recent studies on the systematics of *Capra* (Manceau *et al.*, 1999: fig. 2) have shown a similar dichotomy for sequences of the bezoar goat, with the same *C. aegagrus* specimen from Iran grouping with domestic goats and agrimi, while another two *C. aegagrus* specimens from Turkmenistan (our *C. aegagrus* nos 3 and 4) cluster on a different clade.

Thus, the two sequences of *C. aegagrus* (nos 3 and 4)

that cluster together, are perhaps the only specimens that represent true *C. aegagrus aegagrus*. These sequences group together on a distinct branch and lie furthest from the domestic goats and closest to the other wild goat specimens of *C. falconeri*. As shown in Fig. 5, the bootstrap values, after 100 replicates based on maximum parsimony analysis heuristic search, provide strong support for this separation.

However, the genetic variation exhibited by the *C. aegagrus* specimens is problematic and raises an important issue concerning sampling procedures used during DNA research. Two of the *C. aegagrus* specimens (nos 1 and 2) that clade with the domestic goats, are derived from zoo specimens and not from free-living wild animals (Table 2). However similarly, the two bezoar specimens that are outliers (*C. aegagrus* nos 3

and 4) are derived from a museum collection and the Paris zoo, respectively (Table 2). The results obtained from the genetic analyses, emphasize the problems of using samples taken from captive animals and museum collections for genetic analyses. The source of these specimens is often unknown, frequently no sub-specific determination is given and their identification is based only on morphological characteristics so that there is always a possibility that they may be hybrids, or even agrimi. Indeed, it cannot be discounted that some hybridization with domestic breeds has taken place in the bezoar goat, especially as in most parts of the world *C. aegagrus* survives only in relict populations (Uerpmann, 1987). Alternatively, Manceau *et al.* (1999) may be correct in suggesting that the ancestral populations of wild goats displayed broad heterogeneity and that specifically, those from the Fertile Crescent gave rise to domestic goats. This may account for the resemblance between the *C. aegagrus* specimen from Iran and *C. hircus* specimens.

Thus in the absence of samples from free-living animals of known origin, museum and zoo specimens have to suffice, but as noted here, are problematic. Although we have obtained sequences of goats from ancient DNA samples (Kahila Bar-Gal *et al.*, in press), these data are of limited comparative use with modern samples because of specific problems relating to aDNA (e.g. short sequences as well as the limited number of sequences that can be obtained). Results obtained to date do, however, distinguish clearly between wild and domestic goats (Kahila Bar-Gal *et al.*, in press).

On the one hand, the close relationship of all 17 agrimi sequences studied here to those obtained from a variety of domestic goat breeds (*Capra hircus*) and the separation between them and the sequences of *C. aegagrus* nos 3 and 4 and *C. falconeri* on the other hand, supports the hypothesis that the agrimi (*C. aegagrus cretica*) is not a sub-species of the wild bezoar goat but rather a feral animal. Even though data are based on short sequences, Manceau *et al.* (1999) obtained the same results, and like us concluded that the agrimi is a feral goat.

Note that the agrimi sequences are identical to those of the Baladi goat, a breed that is indigenous to the Levant. This close relationship may be a reflection of the geographic origin of early domestic goats including those initially colonizing Crete. Indeed, the genetic findings corroborate recent archaeozoological hypotheses on the origins of the domestic goat, which suggest a Levantine origin such as the Jordan Valley and its periphery (Horwitz *et al.*, 1999; Peters *et al.*, 1999) or the region adjacent to the mountains of Lebanon or the Anti-Lebanon (Wasse, 2001). If these hypotheses are correct, then the indigenous Baladi goat breed may represent a direct descendent of these early domestic forms. Our genetic data support such a conjecture, as the timing of the initial importation of goats to Crete (c. 7000 uncal. BC) would have taken place relatively close to the hypothesized onset of their domestication in the Levant, c. 8000 uncal. BC (Peters *et al.*, 1999; Wasse,

2001) or even slightly later, c. 7500 uncal. BC (Horwitz *et al.*, 1999).

Despite the physical and behavioural resemblance of the agrimi to the wild bezoar goat (Fig. 1), the molecular data do not support the hypothesis that it is a true subspecies or even a geographic variant of *Capra aegagrus aegagrus*. The agrimi is a feral animal derived from a primitive domestic goat which, following its importation to Crete in the Aceramic Neolithic period, returned to the wild. The apparent contradiction between the morphology and the genetic composition of the agrimi illustrates the limitations of classical taxonomy. As demonstrated by Randi *et al.* (1990) for goats on the island of Montecristo off Italy, when domestic animals become feral, selective pressures may result in a reversion to a 'wild' phenotype.

In the light of these findings, it is suggested that the taxonomic classification of agrimi should be revised. Moreover, this study has illustrated the importance of applying DNA analysis to taxonomic disputes. Finally, the high correlation found between results obtained using conventional analyses of live animals and those using aDNA techniques on skeletonized remains, demonstrates the validity of the latter approach.

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