Pattern of ancient goat migration revealed by AFLP molecular markers

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RIASSUNTO – I marcatori AFLP ricostruiscono gli spostamenti di *Capra hircus* al seguito delle antiche migrazioni umane. Capra hircus *è adattabile a condizioni ambientali molto differenti e possiede un areale di distribuzione estremamente ampio. Studi recenti suggeriscono che la capra abbia giocato un ruolo chiave nel sostentamento delle popolazioni umane durante le migrazioni demiche verso ovest, successive alla rivoluzione agricola del Neolitico. Lo studio della variabilità genetica di questa specie può quindi essere un utile strumento sia per ricostruire le antiche migrazioni umane, sia per preservare la diversità biologica di razze storiche e localmente adattate. L'analisi statistica dei marcatori AFLP effettuata su 44 razze autoctone (campionate in Europa, Medio Oriente, Anatolia) e 2 cosmopolite, affiancata da metodi di interpolazione geografica, ha permesso di evidenziare: - la peculiare composizione genetica delle razze Orobica e Tauernshecken (entrambe di origine incerta) rispetto ai popolamenti europeo e medio-orientale; - un significativo gradiente di distribuzione delle distanze genetiche tra razze che da sud-est procede verso nord-ovest, sovrapponibile al modello di variazione genetica umana, probabile traccia di almeno due differenti eventi di migrazioni umane lungo la via di espansione neolitica dell'agricoltura.*

KEY WORDS: genetic diversity, *C. hircus*, AFLP, spatial analysis

INTRODUCTION – Domestic goat (*Capra hircus*) is a very adaptable and geographically spread livestock species. Recent studies on mitochondrial DNA diversity suggest that goats have been the most widely transported and traded livestock species. For this reason it is thought to have played a central role in the demic spread of agriculture during the Neolithic agricultural revolution (Luikart *et al.* 2001). The investigation of the evolutionary and demographic history of *C. hircus* may indeed shed light on human past history and migrations and contribute to explain human populations biological diversity as we observe it nowadays. Knowledge on the level and distribution of diversity within a species has also immediate importance for the management and conservation of locally adapted populations. The sustainable conservation and management of small ruminant genetic resources are addressed in the EU ECONOGENE project, that combines molecular genetics, socio-economics and geostatistics to accomplish these tasks. One of the major objectives of the project is to investigate geographic patterns of genetic variation at the individual, farm and population level.

Here we present preliminary results of the analysis of the AFLP goat dataset comprising 44 autochthonous and 2 cosmopolite breeds sampled across Europe and Middle East.
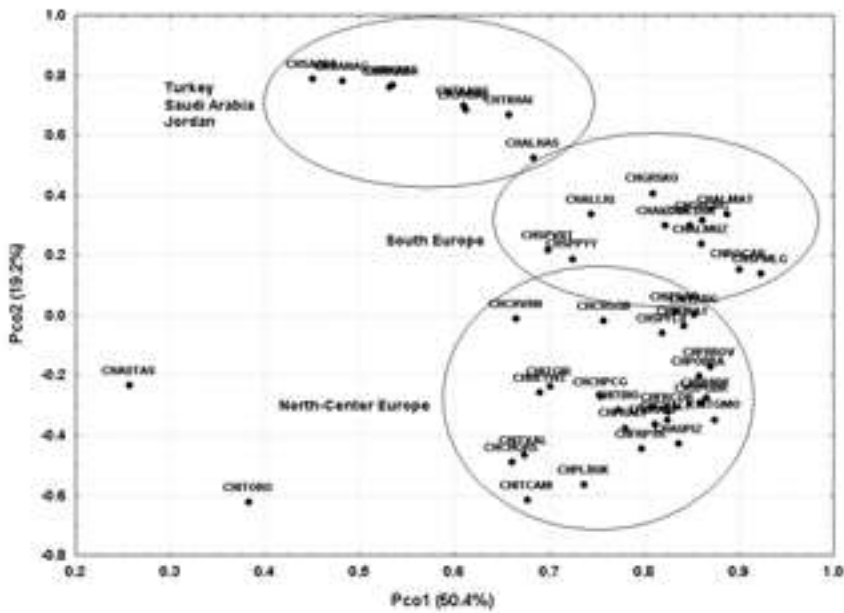
MATERIAL AND METHODS – *Sampling and DNA extraction*: Biological material (blood, hair and tissue) and photo documentation were collected from 33 unrelated animals per breed from 11 different farms located in traditional rearing areas. More than 1300 individuals were sampled from 44 breeds. For each farm sampling site, position and altitude were recorded by Geographical Positioning System (GPS). DNA was extracted with a standard phenol-clorophorm protocol or using commercial kits.

AFLP production and analysis: Animals were typed with three highly polymorphic primer combinations following the protocol published by Ajmone Marsan *et al.* (1997). AFLP polymorphisms were visually scored as dominant markers, coding with 1 the presence and with 0 the absence of the band. Only clearly readable bands were retained for the analysis. Allele frequencies of each marker in each population were estimated using a Bayesian approach with uniform distribution of allele frequencies using the appropriate routine of the program AFLP-surv 1.0 (Vekemans *et al.* 2002). Relationships between populations were inferred computing Reynolds genetic distances. The matrix of genetic distance was then transformed using the principal component analysis (PCOA) and visualised by a 2D scatterplot. The natural neighbor interpolation method was used to plot the first two principal components on a geographic map.

RESULTS AND CONCLUSIONS – In this papers we present some preliminary results obtained analysing with AFLP markers a large number of autochthonous goat breeds sampled in Europe and Middle East, where this species was domesticated about 10,000 years B.C. The integration of GPS technology with genetic data allowed to investigate the pattern of diversity in this species from a geographic perspective. AFLP technology (Vos *et al.* 1995) is based on ligation of linkers to restriction fragments followed by a selective amplification. Polymorphisms are displayed in the form of the presence/absence of bands and correspond to single-nucleotide polymorphisms, insertions or deletions. The markers are as distributed over the genome as the restriction sites and allow estimations of relative distances between genomes of individual animals. AFLP profiles are quite informative and are therefore increasingly used in plant and animal population genetics studies. The three primer combinations assayed generated a total of 279 bands in the interval 50 - 550 bp, of which 102 (36.6%) were polymorphic. The percentage of polymorphic loci having band frequency lying within 0.05 to 0.95 (95% criterion) ranges from 49 in Italian Girgentana to 74 in the Baladie sampled from Jordan. On the basis of allele frequencies estimated by a Bayesian approach we calculated Reynolds distances between populations, graphically visualized by PCOA. The first principal component accounts for the 50% of the total variance and separates the Italian Orobica and the Austrian Tauernshecken breeds from the other populations analysed (Figure 1).

These breeds show the lower genetic distance values with the Eastern European breeds investigated within Econogene and have probably been introduced in the Alps in historical time. To further investigate their origin, the sampling area should be extended towards North-Eastern regions. The second principal component, that accounts for the 19.2% of the total variance, distributes breeds according to geography. Breeds sampled around the traditional centre of domestication (Fertile Crescent) cluster together and the other populations show a South-to-North distribution pattern. Principal Component Analysis of genetic distances and the geographic distribution of *C. hircus* diversity, highest in the Near and Middle East and declining in agreement with the routes of post-domestication human migrations, support the demic diffusion model of pastoralism. The first and the second principal components of Reynolds genetic distances were plotted on the geographic map and interpolated using the natural neighbour interpolation method (data not shown). The geographic centroids of breed sampling sites were used as geographic reference points for locating breeds. The original genetic make up of Orobica and Tauernshecken is evident in the first principal component map. Although independent, both principal components display similar South-East to North-West gradients. These patterns agree with the model of genetic variation of human populations, as described by Cavalli-Sforza *et al.* in 1994, and with the route of agriculture expansion. The two principal components display the signature of two independent events of goat migration, likely together with human population, or alternatively the combined effects of a probably ancient human migration and of more recent large-scale trades.

Figure 1.   PCOA based on Reynolds distances between populations, using 102 AFLP polymorphisms

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