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LIVESTOCK BIODIVERSITY

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LIVESTOCK BIODIVERSITY: PREVIEW

- 1. Livestock biodiversity and human culture
- 2. Local livestock breeds
- 3. Breed characterisation
- 4. In situ conservation strategies
- 5. Ex situ conservation strategies





Origin and domestication of livestock species

Domestic species	Wild Ancestor	MtDNA	Domestication	Time	Location	
		clades	events*	B.P.		
Cattle	Aurochs (3 subspecies) (extinct)					
Bos taurus taurus	B. primigenius primigenius	4	1	~ 8000	Near & Middle East (west Asia)	
	B. p. opisthonomous	2	1	~ <mark>9500</mark>	northeast Africa	
Bos taurus indicus	B. p. nomadicus	2	1	~ 7000	northern Indian subcontinent	
Horse	Extinct					
Equus caballus		17	multiple	~ 6500	Eurasian steppe	
Goat	Bezoar					
Capra ferus	Capra aegragus (3 subspecies)	5	2	~ 10000	Near and Middle East, northern Indian subcontinent	
Sheep	Asian mouflon					
Ovis aries	Ovis orientalis	4	2	~ 8500	Near and Middle East/Turkey (Central Anatolia)	
Pig	Wild boar					
Sus scrofa domesticus	Sus scrofa (16 subspecies)	6	6	~ 9000	Europe, Near and Middle East, China	
					Indian subcontinent, Southeast Asia	

Source: FAO. 2007. The State of the World's Animal Genetic Resources for Food and Agriculture, edited by Barbara Rischkowsky & Dafydd Pilling. Rome.



Fig. 1. The origin and dispersal of domestic livestock species in the Fertile Crescent. Shaded areas show the general region and the approximate dates in calibrated years B.P. in which initial domestication is thought to take place. Dates outside of the shaded areas show the approximate date when the domesticate first appears in a region. Orange, goats (*Capra hircus*); blue, sheep (*Ovis aries*); green, cattle (*Bos taurus*); fuscia, pigs (*Sus scrofa*).

Source: Zeder MA 2008. Domestication and early agriculture in the Mediterranean Basin: Origins, diffusion, and impact. PNAS 105 11597–11604



Cattle diffusion in Europe

Source: Beja-Pereira et al. 2006. The origin of European cattle: evidence from modern and ancient DNA. PNAS 23 103:8113-8.

- Livestock biodiversity is integral to human culture, history, environment, and economy.
- Example: dairy cattle will be considered.
- Cheese was made 9000 years ago.
- Milk from domestic cows has been an important food source for over 8000 years, especially in lactose-tolerant human societies.

• Some human populations have the genetically determined ability to digest lactose by persistent lactase enzyme in adulthood, benefiting from the rich food resources occurring in cows' milk.

Source: Beja-Pereira et al. 2003. Nature Genetics 35:311–313.



Map of allele frequencies at the cattle milk protein genes

Map of lactase persistence allele in European human populations 7

 Thousands of livestock breeds have evolved to fit particular environments and farming systems

• Biodiversity is a prerequisite for genetic improvement and environmental adaptation



http://www.agraria.org/

Giant Italian **Chianina** reared from Roman times



http://www.fao.org/ Trypanosome tolerant

African N'Dama

- However, diversity of Animal Genetic Resources (AnGR) has been in a continual state of decline
- Main causes:
 - trends in the livestock sector
 - disasters and emergencies
 - animal diseases, control measures
- Need for safeguard strategies



Apulian livestock (by Elisa Pieragostini)

• AnGR are managed by Global Plan of Action (Food and Agriculture Organisation, 2007)

- Strategic Priority Area 1: Characterization, inventory and monitoring of trends and associated risks
- World Watch List for Domestic Animal Diversity (WWL-DAD): information system (IS) to identify and monitor livestock breeds



Apulian livestock: Murgese horse (by Elisa Pieragostini)

6379 livestock breeds belonging to 30 different species reported in WWL-DAD 3rd edition

DAD-IS: http://dad.fao.org

Source: FAO 2000 World Watch List for Domestic Animal Diversity. Scherf, B. (ed.)



Year of analysis	Mammalian s	pecies	Avian spec	Countries covered	
-	Number of national breed populations	% with population data	Number of national breed populations	% with population data	
1993	2 719	53	_		131
1995	3 019	73	863	85	172
1999	5 330	63	1 049	77	172
2006	10 512	43	3 505	39	181
2008	10 550	52	3 450	47	181

Table 3. Status of information recorded in the Global Databank for Animal Genetic Reso	ources.
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Source: FAO (2009b).

In 2008, 48% of mammalian breeds and 53% of avian breeds recorded in DAD-IS had no population data recorded (Martyniuk et al., 2010)

Different actions in defense of AnGR are developed.

Animal resources



012: Cattle http://www.regionalcattlebreeds.eu



066: Livestock breeds http://www.elbarn.net



020: Farm animals http://efabis.tzv.fal.de/cgi-bin/ index.cgi



067: Livestock globalview http://www.globaldiv.eu



040: Sheep http://www.heritagesheep.eu

Five projects funded by the European Community Program 2006-2011 for genetic resources

Conservation of AnGR relies on demographic characterisation of breeds and definition of correct breeding schemes.





Trends in level of inbreeding (F), average relatedness (AR) and number of individuals by year of birth in the period 1929-2006.

Martina Franca donkey Apulian livestock (by Elisa Pieragostini)

R. Rizzi (2011): Monitoring of genetic diversity in the endangered Martina Franca donkey population. J. Anim. Sci. (in press)

Zootechnical characterisation is also a prerequisite for safeguard.

Garfagnina goat (Tuscan breed at risk of extinction)

Data on:

- size of the breed
- zoometric measures
- breeding system
- milk quality



Source: Martini et al. (2010). The Garfagnina goat: a zootechnical overview of a dairy local population. J. Dairy Sci. 93, 4659-4667.

Molecular genetic studies: allow to identify and monitor the genetic diversity within and across breeds and to reconstruct their evolution history.



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CLUSTERING OF CATTLE BREEDS FOR $\alpha_{s2}\text{-}\mathsf{CASEIN}$



Erhardt et al. (2009). *60th EAAP Annual Meeting*, Barcelona, Spain. Caroli et al. (2010). J. Dairy Sci. 93, 1260–1265.

Database/project name	URL	Pigs	Cattle	Goats	Sheep	Chicken	Other sp.	Type of data included
AVIANDIV	http://aviandiv.tzv.fal.de/	n	n	n	n	у	n	Microsatellite genotypes, SNPs
CaDBase	http://www.projects.roslin.ac.uk/cdiv/	n	У	n	n	n	n	Microsatellite genotypes
PigDBase	http://www.projects.roslin.ac.uk/ pigbiodiv/index.html	у	n	n	n	n	n	Microsatellite and AFLPgenotypes
PEDE: Pig Expression Data Explorer	http://pede.dna.affrc.go.jp/	у	n	n	n	n	n	Full-length cDNA clones and ESTs
AnimalQTLdb	http://www.animalgenome.org/QTLdb/	У	У	n	У	У	n	Quantitative Trait Loci data
ARKdb	http://www.thearkdb.org/arkdb/index.jsp	У	у	n	у	у	у	Genome mapping data
ChickVD: Chicken Variation Database	http://chicken.genomics.org.cn/index.jsp	n	n	n	n	У	n	Sequence variation map, primarily SNPs
Bovine Genome Project	http://www.hgsc.bcm.tmc.edu/ projects/bovine/	n	У	n	n	n	У	BAC and Whole Genome Shotgun (WGS) libraries
Cattle Genome Database	http://www.cgd.csiro.au/	n	у	n	n	n	n	Comparative mapping, QTL
LivestockGenomics	http://www.livestockgenomics.csiro.au/	n	у	n	у	n	n	Genome Browsers (maps) and SNPs
Pig Genomic Informatics System	http://pig.genomics.org.cn/	у	n	n	n	n	n	Gene annotations, maps, SNPs
Bovine Genome Database	http://genomes.arc.georgetown.edu/ drupal/bovine/	n	У	n	n	n	n	Genome analysis and annotation, QTL
US Poultry Genome Project	http://poultry.mph.msu.edu/	n	n	n	n	У	У	SNPs, BACs linked to genes primers
COMRAD – comparative radiation hybrid mapping	http://www.projects.roslin.ac.uk/ comrad/introduction.html	n	у	n	n	n	n	Whole genome radiation hybrid map
Gallus Genome Gbrowse	http://birdbase.net/cgi-bin/ gbrowse/gallus08/	n	n	n	n	У	у	Wide array of chicken genome data
Geisha – gallus expression in situ hybridization analysis	http://geisha.arizona.edu/geisha/	n	n	n	n	У	n	In situ hybridization information
AgBase	http://www.agbase.msstate.edu/	У	У	n	у	У	У	Genome, functional annotations
BBSRC ChickEST Database	http://www.chick.manchester.ac.uk/	n	n	n	n	У	n	ESTs
Chicken full-length cDNA Database	http://bioinfo.hku.hk/chicken/	n	n	n	n	у	n	cDNA

Source: Groeneveld et al. Genetic diversity in farm animals- a review. Anim Genet. 2010 41:6-31.

Particular nutritional properties of animal products can be characterised for local breed valorisation

Example: null alleles at goat caseins



- Lower milk allergenic reaction observed for two milk samples (α_{s1} *CN 00* and *0F*) from local breeds
- Results confirmed by skin prick test on 6 allergic children



Source: Ballabio et al. (2011). Goat milk allergenicity as a function of alpha_{s1}-casein genetic polymorphism. J. Dairy Sci., 94, 998-1004.

• In situ conservation strategy is very important to conserve a breed within its natural habitat



Apulian local breeds (Courtesy of dott. Silvio Schito)

• In-situ conditions: conditions where genetic resources exist within ecosystems and natural habitats, and, in the case of domesticated or cultivated species, in the surroundings where they have developed their distinctive properties (Convention on Biological Diversity, 1992)



Apulian livestock (by Elisa Pieragostinii)

• The presence of local breeds on a farm can be linked to a precise strategy, e.g. cultural affection of farmers to breeds of their parents.

• Example: Italian Reggiana and Modenese cattle kept by some successful Holstein farmers

Gandini et al. 2010. Motives and values in farming local cattle breeds in Europe: a survey on 15 breeds. Animal Genetic Resources, 47:45–58.





Efficient *in situ* conservation strategies: based on the self-sustainability of the breed



Gandini G., Maltecca C., Pizzi F., Bagnato A., Rizzi R. 2007. Comparing local and commercial breeds on functional traits and profitability: the case of Reggiana Dairy Cattle. J. Dairy Sci., 90: 2004–2011.

Efficient *in situ* conservation strategy Production of "Parmigiano Reggiano of Reggiana"









Figura 4. Forma di Parmigiano Reggiano prodotta con solo latte di vacche Modenesi.



RAZ	ZE ANIMALI DA REDDITO	1 m
Specie	Caprina	AL
Razza	Verzaschese o Nera di Verzasca	

VERZASCHESE O NERA DI VERZASCA



Origine e zona di diffusione

E' una razza originaria del Canton Ticino e in particolare nella Valle Verzasca che si estende dall'estremità settentrionale del Lago Maggiore verso Nord, dove viene allevata in forma semibrada da tempo imprecisabile.

Le scambio di animali ha da sempre interessato le aree prealpine al confine con la Svizzera, per la comunanza di alcune aree di pascolo; il prosso incentivo all'allevamento di questa capra si è verificato però negli anni '70 quando sono stati importati dallSvizzera riproduttori allo scopo di sostituire la locale popolazione caprina, molto eterogenea.

E' una razza rustica e resistente sia alle alte che alle basse temperature. Viene allevata in greggi mediograndi e l'allevamento è di tipo semi-estensivo.





In Lombardia è diffusa soprattutto nella provincia di /arese (Val Veddasca) e nelle valli dell'Alto Lario Como), ed anche in Val Chiavenna (Sondrio) la consistenza registrata nel Registro Anagrafico è di .791 capi in 92 allevamenti. resente anche nella provincia di Bergamo.





E' un animale di taglia medio-grande. Testa: leggera, profilo fronto-nasale retblineo, orecchie sviluppate ed erette, collo robusto, ben unito alla spalla e al garrese. Le corna sono sempre presenti in ambo i sessi, lievemente arcuste all'indietro con una leggera torsione verso l'esterno, Tronco: spalla larga ben legata al torace, garrese di unghezza media, torace profondo e largo, con coste ben arcuate: cosce muscolose.

Mammella: ben sviluppata, larga alla base. Il mantello è completamente nero, lucido, con pelo corto e fine, più fitto nella stagione invernale; i becchi hanno la barba e peli più lunghi sul garrese: la pelle è morbida, fine ed elastica. Altezza media al garrese: Maschi cm.80 - 90, femmine cm. 75 - 85 Peso minimo: Maschi Kg 90, femmine Kg. 70.

Caratteristiche

una razza a duplice attitudine ha una produttività di latte e carne molto buona. Prolificità pari al 121% Capretto alla nascita: 5.00-5.50 kg Produzioni medie latte (AIA 2003): 364 litri con 3,27% di grasso e 3,06 di proteine. Il latte viene destinato alla trasformazione per ottenere prodotti tipici a "latte crudo": formaggi freschi e stagionati a partire dalle lavorazioni lattica o presamica, yogurt, ricotta. A questa razza è legata la produzione di un formaggio tipico "Formaggella del Luinese" per il quale è in corso il riconoscimento di prodotto DOP.



Per saperne di più

Il Registro Anagrafico per guesta razza è stato attivato in Italia nel 1997 ed è tenuto dall' Associazione Nazionale Pastorizia (ASSONAPA) Viale P. Togliatti1587, 00155 ROMA Tel. 1 + 06 409001 9 - fax 06-40900130 nfo@assonapa.com - www.assonapa.com



Erisa

Valtellinese o

Frontalasca Lariana o Capra di Livio Orobica o di Val Gerola Verzaschese o Nera di Verzasca

RegioneLombardia

Cultural value of a local breed (safeguard of landscape, preservation of ancient local traditions)

(Gandini and Villa, 2003, J. Anim. Breed. Genet. 120, 1–11)

	Historical value ^b in the areas of:						
Breed	Landscape	Gastronomy	Folklore	Handicrafts			
Bianca Val Padana	+	+ +	<u> 1979</u>				
Chianina	+	+	+				
Garfagnina	+	355	1	325			
Maremmana	+ +	300	+ +	+ +			
Modicana	+ +	+ +	-				
Reggiana	+	+ +		100			
Valdostana Castana	+ +	+ +	+ +	+ +			
Valdostana Pezzata Nera	+ +	+ +	+ +	+ +			
Valdostana Pezzata Rossa	+ +	+ +	+ +	+ +			

Table 1. Analysis of cattle breeds as historical witnesses^a

^aFor breed antiquity, agricultural systems associated with each breed and their presence in higher artistic expression, see text.

^b-, negligible value; +, limited value; ++, noticeable value.



Grazing Bionde dell'Adamello Source: http://www.associazionerare.it/



Battle of the Queens Source: http://www.naturaosta.it/cultura.htm

When in situ conservation is not feasible, or as a support to *in situ* conservation:

EX SITU CONSERVATION STRATEGIES

EX SITU in vitro refers to conservation external to living animal in an artificial environment, under cryogenic condition including, in alia, the cryoconservation of embryos, semen, oocytes, somatic cells or tissue having the potential to reconstitute live animal (including animal for gene introgression and synthetic breeds) at a later date (FAO, 2007)



National Cryobanks



Table 2 Summary of annual reports provided by 25 European countries reporting on their activities implementing the Global Plan of Action from September 2009 to August 2010

Country	No. of kreeds reported to DAD-IS ³	Strategic Priority 1 Characterization, inventory and monitoring of trends and associated risk	Strategic Priority 2 Sustainable use and development	Strategic Priority 3 Conservation	Strategic Priority 1 Policies, institutions and capacity building	National strategy or/and action plan planned for 2010/11/under development or adopted	National advisory committee to guide national implementation of GPA established	National law planned to review/harmonize or adopted in view of GPA	Cryokank for national AnGR planned for 2010/11/ under development or operational
Albania	42	+	+	++	++	1			1
Belgium	71	+	+	++	++			4	1
Croatia	33	**	+	++	++			~	1
Cyprus	18	**	+	++	+				1
Czech Republic	100	+	+	+++	++	~		~	\checkmark
Finland	23	++	++	+++	++	~			1
Germany	185	++++	++	++++	+++	\checkmark	1	~	~
Greece	37	++	++	+++	++	~	1		1
Hungary	91	++	++	+++	+++		N N	~	
Iceland	6	++	+++	+++	+++	~	~	730/	~
Ireland	34	++	++	++	+++		1		~
Italy	263	++	+	+++	not reported			~	1
Latvia	10	+	++	++	not reported				
Montenegro	6	++	+	++	+	~	1	~	
Poland	114	+++	++	++++	+++	~	1		
Romania	114	+	+	+	+	~			
Serbia	41	+++	++	+	+			~	
Slovakia	39	++	not reported	++	+++			1	
Slovenia	63	+++	+	+++	+			~	\checkmark
Spain	203	++++	++++	++++	++++	\checkmark	1	~	~
Sweden	50	+	+	++	+	~			
Switzerland	38	++++	++	++++	++			*	√ V
Turkey	92	++	+	++++	++	~	√ √		
Ukraine	163	+	+	+	+			N.	~
United Kinadom	264***	+++	++	++++	++	~	√	1	

Actions described as (+) initiated to (++++) if all actions are fully implemented and regular monitoring is udertaken.

National Cryobank planned for 2010-2011

http://www.fao.org/ag/againfo/programmes/en/genetics/documents/ITWG_AnGR_6/CGRFA_WG_AnGR_6_10_Inf10.pdf

Type of genetic material stored

Semen and embryos form the most common material for the cryopreservation of farm AnGR

	Possibilities	Limitations
Embryos	 Carry the entire genome including extra- nuclear genetic material Allow the complete and immediate recovery of the breed in case of extinction 	 Freezing not available for all species High cost In small population not enough embryos donors
Semen	 Freezing technique routinely available in all species Low cost 	•To reconstruct extinct breed several backcrossing and high number of doses are needed •<100% genome recovered, cytoplasmatic effect lost or altered

The costs of breed reconstruction from cryopreserved material in mammalian livestock species

Three strategies:

- •Embryos,
- •Embryos in combination with semen,
- •Semen.

Three cost measures:

- •time required for population reconstruction,
- cost for creation of the gene bank,
- •number of years-keeping-female to reach reconstruction.



Boettcher P.J., Stella A., Pizzi F., Gandini G. (2005). The Combined Use Of Embryos And Semen For Cryogenic Conservation Of Farm Mammal. Genetic Resources. Genetics, Selection, Evolution 37, 657-675

Gandini G., Pizzi F., Stella A., Boettcher P.J. (2007). The costs of breed reconstruction from cryopreserved material in mammalian livestock species .Genetic, Selection, Evolution 39, 465-479



This project is aimed at assisting conservation, development and sustainable use of autochthon, local or regional cattle breeds. These breeds are recognized as **important elements of agrobiodiversity, of agro-ecosystems and of our cultural heritage** in Europe. The most secure **conservation strategy** for those breeds is to **promote measures which contribute to selfsustainability** of the breed.

http://www.regionalcattlebreeds.eu/publications/document s/9789086866977cattlebreeds.pdf





Towards self-sustainable European Regional Cattle breeds

A detailed survey was carried out:

- to compare cryopreservation activities and policies in Finland, France, Italy and the Netherlands.
- to detect similarities and differences between these 4 countries,
- to compare the countries' strategies with the international Guidelines,
- to formulate recommendations for initiating or strengthening cryopreservation programmes.

For a total of **52 local breeds** from Finland, the Netherlands, France and Italy detailed information on **2,536 bulls** was collected.

Breed classification according to FAO criteria						
Country	Critical	Endangered	Critical maintained	Endangered maintained	Not at risk	Total
Finland	0	2	0	0	1	3
France	1	0	1	12	4	18
Italy	0	1	1	9	12	23
Netherlands	1	3	0	0	4	8
Total	2	9	4	17	19	52



Number of bulls and number of doses in storage

	N° of bulls	N° of doses
Finland	223	351 981
France	563	1 307 166
Italy	1091	264 208
The Netherlands	659	1 095 162



Western Finncattle breed









Meuse-Rhine-Yssel cattle (MRY)



Birth date of bulls





Sampling strategy Distribution of semen doses per bull





Semen sample storage – sites /breed



Network of the Italian Animal Genetic Resources Cryobanks – CRIONET-IT

Created in 2010 in collaboration with the Department of Veterinary Science and Technology for Food Safety (UNIMI) in order to:

• Share, through a virtual bank, the information concerning cryo-preserved genetic material of farm animals breeds

• Create a network of institutions involved in cryopreservation of local breeds.



Network of the Italian Animal Genetic Resources Cryobanks CRIONET-IT



Main features

- Single database
- Partners feeds the database with the information of their collection
- Partners are responsible for the information of the collection not necessary the owners of the materials
- Only dose stored as genetic reserve are recordered in the network

Homepage Network



CRIONET-IT è composto da Partners che condividono le informazioni relative a materiale genetico di razze di interesse zoo-economico crioconservato a scopo di riserva genetica. *

In **CRIONET-IT** sono archiviate informazioni relative a materiale genetico (materiale seminale, embrioni, oociti, cellule somatiche) conservato come back-up da utilizzare in caso di comparsa di problemi genetici nelle razze, quali perdita di linee genetiche, eccessiva consanguineità, o per la ricostruzione della razza in caso di estinzione. CRIONET-IT inoltre archivia informazioni relative a materiale stoccato (es. sangue, bulbo pilifero) come possibile fonte di DNA.

ELENCO PARTNERS

- 1. Associazione Nazionale Allevatori Bovini di Razza Reggiana Banca Genetica;
- 2. Banca delle Risorse Genetiche Animali Lombarde LABank;
- 3. Comunità Montana Valli del Verbano;
- 4. Criobanca del Germoplasma Animale "Giuseppe Rognoni" IBBA-CNR
- 5. Riserva genetica della razza Burlina Associazione Provinciale Allevatori di Treviso;
- 6. Riserva genetica della razza Cabannina Associazione Provinciale Allevatori di Genova;

* I Partner sono responsabili dell'informazione archiviata in CRIONET-IT.

http://www.genrescryonet.unimi.it

EX SITU CONSERVATION STRATEGIES Network of the Italian Animal Genetic Resources Cryobanks CRIONET-IT



Material stored: n of breeds for species



EX SITU CONSERVATION STRATEGIES Network of the Italian Animal Genetic Resources Cryobanks CRIONET-IT



Material stored: n of donors for species

Grazie per l'attenzione!



In ricordo di un maestro di signorilita'

L'attivita' del prof. Giuseppe Rognoni nella Pezzata Rossa Italiana

Friuli V.G. si fece conoscere ed sporezzare già dalla fine degli '40 per aver sperimentato il vaccino contro la Brucellosi (BUCK 19) Ha presieduto la CTC ANAPRI dal germaio 1979 fino al 2007, in qualità di esperto zootecnico di nomina ministeriale. Durante questo lungo periodo si è distinto per la capacità di saper ascoltare tutte le istanze, anché se contrastanti tra loro, stando sopra le parti, con l'unico scopo di poter prendere le decisioni migliori a benefició della maggior parte degli allevatori di Pezzata Rossa. Questa sua virtù gli ha consentito di guidare l'ANAPRI per un così lungo periodo, durante la scalte selettive più importanti, in nome del progresso scientifico ma al tempo stesso rispettando la dignità delle tradizioni. Non si possono dimenticare alcune delle decisioni tecniche più importanti prese

L'ANAPRI

dalla CTC ANAPHI durante la sua presidenza: • Ublizzo del Performance test nel programma

di selezione (1980) Prima valutazione genetica in Italia con

il metodo BLUP (1984) • Cambio del nome della razza da FRULANA

 Cambio del nome della razza da Procovi ad ITALIANA (1985)

 Adozione del programma di selezione GROVANI TORI (1990)

 Utilizzo del metodo BLUP ANIMAL MODEL nella valutazione genetica (1991)

 Adozione del sistema lineare di valutazione mortologica amorizzato a livello europeo (1997).

Definizione dell'Indice di selezione IDA (1998)
 Test Day Model (2005)

Un ringraziamento a nome di tutti i componenti della Commissione Tecnica, del Comitato Direttivo, del Direttore e personale Anapri, nonche degli alevatori di Pezzata Rossa Italiana.

PEZZATA BOISA 5



3 Prof. Ragnoni interviene durante l'Assembles Anepri

Al mio professore

Rivervato ed accogliente, coraggioso eppur prudente, campagnolo e gran signore, genetista e allevatore.

Lui, maastro da imitare,pronto sempre ad imparare! sguardo attento a tutto il mondo, per andare fino in fondo della vera Consistenza che fa lieta l'esistenza.

"Siate sempre generosi" "State attenti a figli e sposi: la famiglia innanzi tutto!" "Se volete portar frutto, fate tutto con bentà e cristiana carità!"

Ci riecheggian nella mente, ed ancor ciascuno cente, quelle semplici parole che diceva alla sua "prole" di discepoli ... maldestri nel cercar d'esser maestri!

Ci rimane dentro il cuore il cuo iguardo indagatore, la cua innata cimpatia, la cua timida allegria.