

# 51° Convegno Nazionale della WPSA

## LA RETE DELLA BIODIVERSITA' AVICOLA ITALIANA:

### La Caratterizzazione Genetica



**Martino Cassandro**



UNIVERSITÀ  
DEGLI STUDI  
DI PADOVA

51° CONVEGNO NAZIONALE  
Associazione Scientifica di Avicoltura  
Sezione Italiana della  
World's Poultry Science Association



**Le razze avicole  
italiane, patrimonio  
della biodiversità  
nazionale:  
un'opportunità  
o un hobby?**

organizzato da  
Associazione Scientifica di Avicoltura  
e  
Camera di Commercio, Industria, Artigianato e  
Agricoltura di Forlì-Cesena  
in collaborazione con Fiera di Forlì

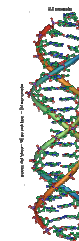
**Forlì – 16 aprile 2015**  
Sala Europa  
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16 APRILE 2015 – Sala EUROPA - Fiera di FORLÌ



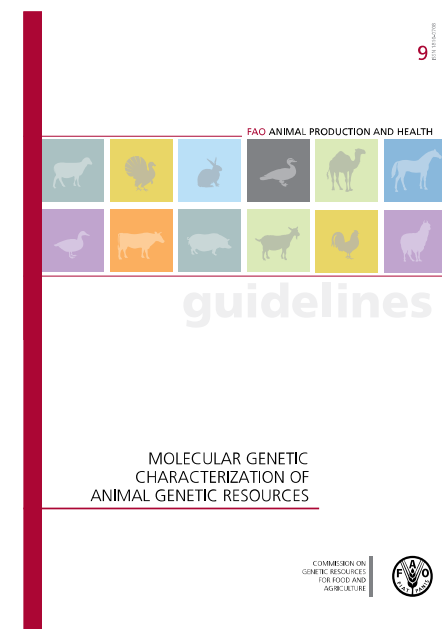
- L'importanza della caratterizzazione genetica (CG)
- Quanti e quali studi di CG
- Conclusioni e Prospettive

# Caratterizzazione Genetica Animale (CGA): *Studio del genoma animale, analisi del DNA*



## Alcuni finalità della CGA:

- *Preservare la variabilità genetica*
- *Stimare distanze genetiche*
- *Studi Filogenetici*
- *Autenticità genetica*
- *Miglioramento genetico*
- *Tracciabilità genetica*
- *Verifica dei Pedigree*
- *Certificazione linee parentali (GGP e GP, P)*
- *Adattabilità ambientale/sistemi di allevamento*
- *Valorizzazione prodotti tradizionali*





## La scoperta del DNA 1953



The Nobel Prize in Physiology or Medicine 1962

"for their discoveries concerning the molecular structure of nucleic acids and its significance for information transfer in living material"



**Francis Harry Compton Crick**

🕒 1/3 of the prize

United Kingdom

MRC Laboratory of Molecular Biology  
Cambridge, United Kingdom

b. 1916  
d. 2004



**James Dewey Watson**

🕒 1/3 of the prize

USA

Harvard University  
Cambridge, MA, USA

b. 1928



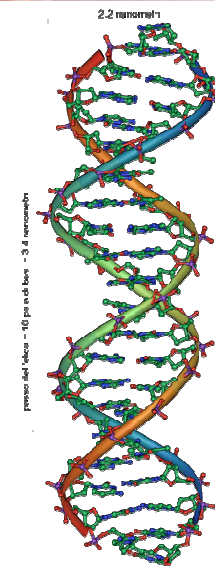
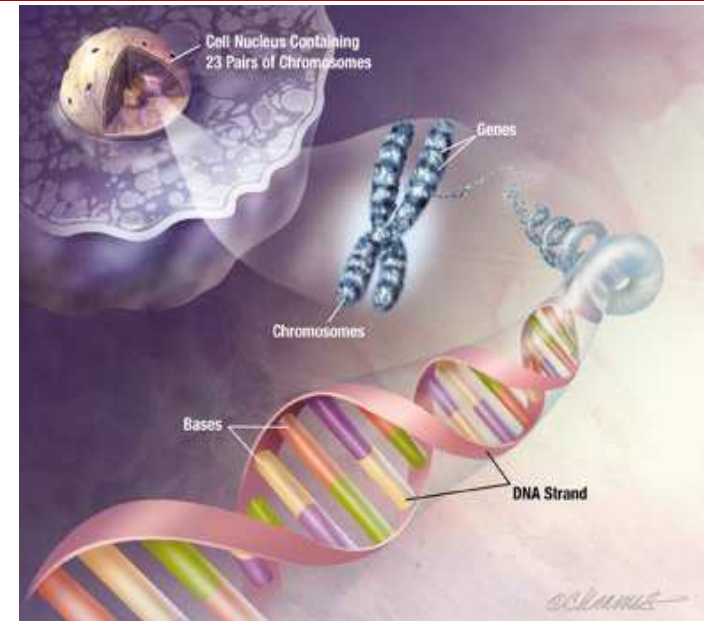
**Maurice Hugh Frederick Wilkins**

🕒 1/3 of the prize

United Kingdom and New Zealand

London University  
London, United Kingdom

b. 1916  
(in Pongaroa, New Zealand)  
d. 2004



# PCR - 1984



## PCR : Polymerase Chain Reaction

30 - 40 cycles of 3 steps :



**Step 1 : denaturation**

1 minut 94 °C



**Step 2 : annealing**

45 seconds 54 °C

forward and reverse primers !!!



**Step 3 : extension**

2 minutes 72 °C  
only dNTP's

(Andy Vietmann 1999)



## The Nobel Prize in Chemistry 1993

"for contributions to the developments of methods within DNA-based chemistry"

"for his invention of the polymerase chain reaction (PCR) method"

"for his fundamental contributions to the establishment of oligonucleotide-based, site-directed mutagenesis and its development for protein studies"



**Kary B. Mullis**

⊕ 1/2 of the prize

USA

La Jolla, CA, USA

b. 1944



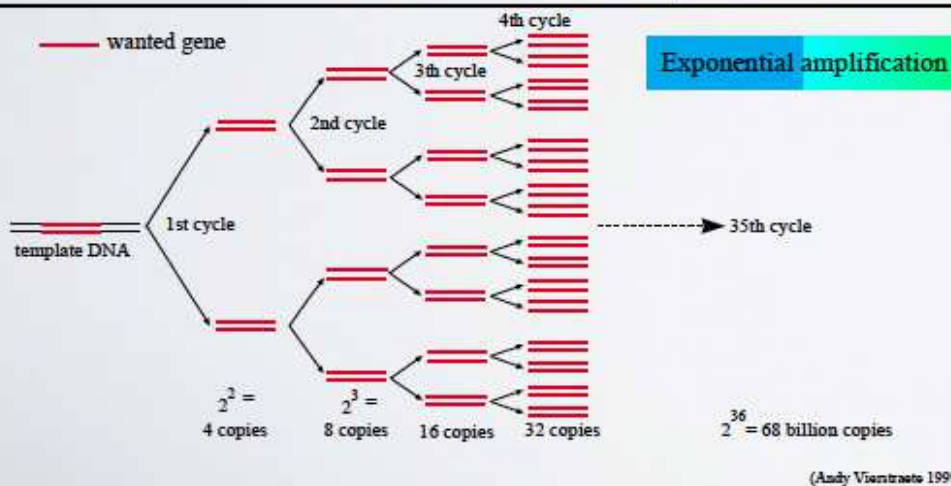
**Michael Smith**

⊕ 1/2 of the prize

Canada

University of British Columbia  
Vancouver, Canada

b. 1932  
(in Blackpool, United Kingdom)  
d. 2000



16 APRILE 2015 – Sala EUROPA - Fiera di FORLI'



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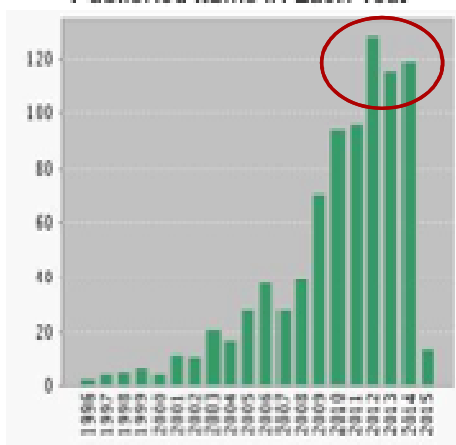
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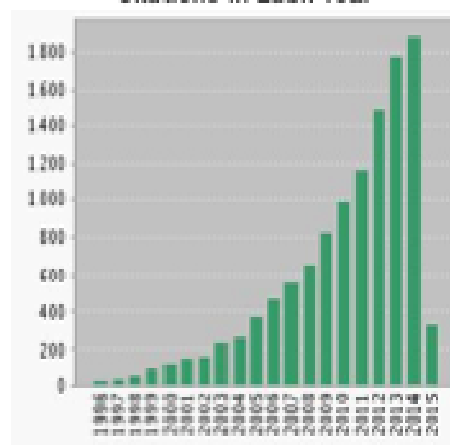
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The latest 20 years are displayed.  
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Results found: 866

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Sum of Times Cited without self-citations [?]: 11252

Citing Articles [?]: 9480

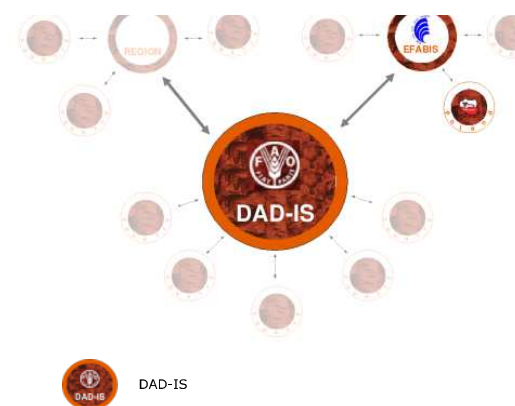
Citing Articles without self-citations [?]: 9115

Average Citations per Item [?]: 13.75

h-index [?]: 50



- FAO creato il DAD-IS
- Sistema Informativo sulla Biodiversità degli Animali Domestici
- Sito web [dad.fao.org](http://dad.fao.org)
- E' uno strumento informativo e di comunicazione per implementare strategie per la gestione dell'(AnGR)





**14.872 RAZZE ANIMALI CENSITE DAD-IS** ([dad.fao.org](http://dad.fao.org), 2015), delle quali avicole:

<b>Nel MONDO</b>	
2.633	CHICKEN
395	DUCK (domestic)
15	DUCK (domestic)/MUSCOVY DUCK
48	MUSCOVY DUCK
262	GOOSE (domestic)
182	TURKEY
76	PIGEON
65	GUINEA FOWL
60	QUAIL
22	OSTRICH
18	PHEASANT
12	PARTRIDGE
5	EMU
3	NADU
1	PEACOCK
<b>3.797</b> (25% del Tot AnGR)	<b>TOTAL</b>





**14.872 RAZZE ANIMALI CENSITE DAD-IS** ([dad.fao.org](http://dad.fao.org), 2015), delle quali avicole:

Nei MONDO		In EUROPA	In ITALIA
2.633 (69%)	CHICKEN	1355	16
395	DUCK (domestic)	182	2
15	DUCK (domestic)/MUSCOVY DUCK	6	
48	MUSCOVY DUCK	9	
262	GOOSE (domestic)	170	3
182	TURKEY	94	3
76	PIGEON	35	
65	GUINEA FOWL	11	3
60	QUAIL	16	
22	OSTRICH	5	
18	PHEASANT	5	
12	PARTRIDGE	2	
5	EMU	1	
3	NADU	1	
1	PEACOCK		
<b>3.797</b> (25% del Tot AnGR)	<b>TOTAL</b>	<b>1.892</b> (50% del Mondo)	<b>27</b> (1,4% dell'EU)

**DNA standard samples for microsatellite typing.**

**Browse the AVIANDIV database.**

---

**50 diversi genotipi** (tra i quali la PADOVA, SICILIANA e diverse linee commerciali)

25 microsatelliti e DNA pools di 50 individui per razza

- Valutare la Biodiversità avicola
- Proporre un metodo di valutazione
- Creare un database/archivio di DNA di razze di pollo



## 8 Partners of the AVIANDIV project

Dr. Steffen Weigend  
The Federal Agricultural Research Centre Braunschweig-  
Völkenrode Institute for Animal Science and Animal Behaviour  
Germany [Steffen.Weigend@fli.bund.de](mailto:Steffen.Weigend@fli.bund.de)

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Institute of Animal Sciences . Wageningen  
The Netherlands [martien.groenen@alg.vf.wau.nl](mailto:martien.groenen@alg.vf.wau.nl)

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Institut National de la Recherche Agronomique  
Centre de Recherches de Jouy-en-Josas France  
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The Hebrew University of Jerusalem  
Faculty of Agricultural, Food & Environmental Quality Sciences  
Israel [hillel@agri.huji.ac.il](mailto:hillel@agri.huji.ac.il)

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Rheinische Friedrich-Wilhelms-Universität Bonn  
Institute of Animal Breeding Science  
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## Partners of the AVIANDIV project

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Prof. Asko Mäki-Tanila  
Agricultural Research Centre  
Institute of Animal Production  
FIN-31600 Jokioinen Finland [Asko.Mäki-Tanila@mtt.fi](mailto:Asko.Mäki-Tanila@mtt.fi) or

## 6 Subcontractants

European Poultry Breeders

- 1.Lohmann Tierzucht GmbH, Germany
- 2.Lohmann Indian River GmbH, Germany
- 3.ISA-HUBBARD, France
- 4.HENDRIX Poultry Breeders, The Netherlands
- 5.ROSS Breeders Ltd, UK
- 6.COBB Breeding Company Ltd, UK

## 8 Others

SPAIN: Dr. Jose Luis Campo Chavarri, MADRID

ITALY: Dr Giuseppe Pidone, CALATAFIMI

HUNGARY: Dr. Gyorgy Virag, Dept. Rabbit and Fur Animal Breeding, Institute for Small Animal Research, GODOLLO

POLAND: Dr. K. Cywa-Benko, Institute of Animal Production, Poultry Department, Balice n. Cracow

RUSSIA: Dr. I. Moiseyeva, Vavilov Institute of General Genetics, MOSCOW

SLOVAKIA: Dr. J. Baumgartner, Research Institute of Animal Production, Poultry Breeding Station, Ivanka pri Dunaj

UKRAINE: Dr. Bondarenko, Poultry Research Institute, Genetics and Selection Department, BORKY

CZECH REPUBLIC : Dr. Pavel Trefil, Research Institute of Animal Production



## Quantitative Trait Loci and Single Nucleotide Polymorphism

Cytogenet Genome Res 117:319–326 (2007)  
DOI: [10.1159/000103194](https://doi.org/10.1159/000103194)

Cytogenetic and  
Genome Research

### Biodiversity of 20 chicken breeds assessed by SNPs located in gene regions

T. Twito<sup>a, b, e</sup> S. Weigend<sup>c</sup> S. Blum<sup>a</sup> Z. Granevitze<sup>a</sup> M.W. Feldman<sup>d</sup>  
R. Perl-Treves<sup>e</sup> U. Lavi<sup>b</sup> J. Hillel<sup>a</sup>

<sup>a</sup>Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot, <sup>b</sup>ARO – Volcani Center, Bet Dagan (Israel)

<sup>c</sup>Institute for Animal Breeding, Federal Agricultural Research Centre, Mariensee (Germany)

<sup>d</sup>Department of Biological Sciences, Stanford University, Stanford, CA (USA)

<sup>e</sup>Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan (Israel)

Manuscript received 4 July 2006; accepted in revised form for publication by I. Nanda, 27 September 2006.

Breed name	Breed No.
<i>Gallus gallus gallus</i>	102
Bedouin	5
Icelandic landrace	16
Green-legged Partridge	27
Orlov	28
Transsylv. Naked Neck	26
Old Scand. Ref. Pop.	18
Marans	13
Jaerhoens	19
Fayoumi	4
Padovana	21
Line Sarcoma-Resistant	34
Gödöllő Nhx	33
Ab line, high	51
White-egg layer A	37
Brown-egg layer line D	45
Brown-egg layer line C	44
Broiler dam line D	50
Broiler sire line B	42
C line	32

We conclude that it is important to use a large number of SNPs, and much less important to genotype a large number of individuals per population. Similar results were previously reported for constructing the phylogenies of closely related populations (Shriver et al., 1995; Chu et al., 1998; Romanov and Weigend, 2001).



Cluster analisi di 60 individui; 5 individui per ciascuna delle 12 popolazioni di pollo usando 25 g-SNP markers, usando STRUCTURE software



Centri di Ricerca e Università attive in Italia per la CG  
avicola:

### UNIVERSITA' di PADOVA

- Caratterizzazione genetica di popolazioni avicole venete e genotipi commerciali

### UNIVERSITA' di PERUGIA

- Studi di DNA nucleare e DNA mitocondriale di popolazioni centro Italia

### UNIVERSITA' di MILANO

- Caratterizzazione genetica di popolazioni di polli lombarde e tacchini autoctoni

### UNIVERSITA' di TORINO

- Caratterizzazione genomica di popolazioni di polli piemontesi e altre

*e UNIVERSITA' di PARMA, PISA, FIRENZE e altre sedi*

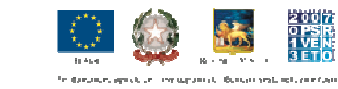
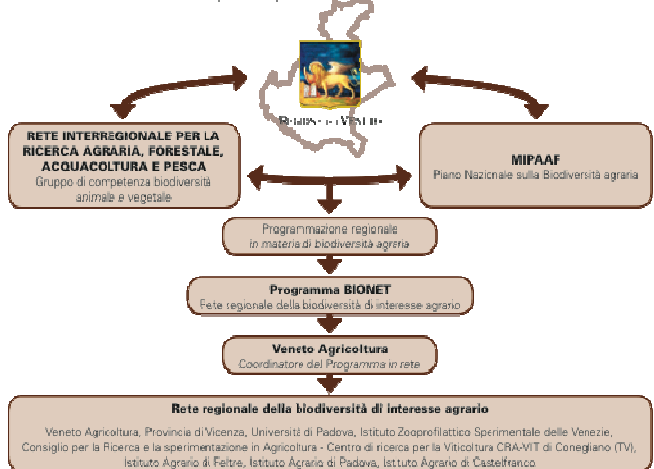


## PROGRAMMA BIONET

Rete regionale per la conservazione e caratterizzazione della biodiversità di interesse agrario

Il Programma, creato dalla Provincia di Padova nel 1998, è stato successivamente coordinato dalla Regione Agricoltura, che ha fornito la struttura organizzativa e finanziaria di fondo al fine di garantire attività mirate a conservare, caratterizzare e valorizzare la biodiversità di interesse agrario, promuovendo la formazione, dimostrando il modello al ricettore e alla conservazione e realizzando il monitoraggio e di valore vegetali e animali di interesse agrario. Le attività di interesse agrario sono: la gestione delle risorse della biodiversità di interesse agrario e l'attività di conservazione e valorizzazione della biodiversità di interesse agrario. Il Programma è coordinato dalla Regione Agricoltura, che ha fornito la struttura organizzativa e finanziaria di fondo al fine di garantire attività mirate a conservare, caratterizzare e valorizzare la biodiversità di interesse agrario, promuovendo la formazione, dimostrando il modello al ricettore e alla conservazione e realizzando il monitoraggio e di valore vegetali e animali di interesse agrario.

- obiettivi del Programma BIONET sono:
  - il recupero, l'identificazione e la valorizzazione della biodiversità genetica di razze e di varietà di specie vegetali, animali e microrganismi di interesse agrario;
  - il mantenimento e l'aumento del numero di varietà di specie vegetali, animali e microrganismi di interesse agrario;
  - la tutela e la valorizzazione delle risorse genetiche di specie di interesse agrario;
  - la promozione e la valorizzazione delle risorse genetiche di specie di interesse agrario;
  - la promozione e la valorizzazione delle risorse genetiche di specie di interesse agrario;
  - la promozione e la valorizzazione delle risorse genetiche di specie di interesse agrario;



## PROGRAMMA BIONET

Rete regionale per la conservazione e caratterizzazione della biodiversità di interesse agrario



### Gruppo di lavoro avicoli





## PROGRAMMA BIONET

Rete regionale per la conservazione e caratterizzazione della biodiversità di interesse agrario

Gruppi di lavoro	Partner operativi	Razze e varietà/secessioni oggetto di conservazione e caratterizzazione	
 BOVINI	<ul style="list-style-type: none"> <li>Veneto Agricoltura</li> <li>Provincia di Vicenza</li> <li>Università di Padova</li> <li>Istituto Zooprofilattico Sperimentale delle Venezie</li> </ul>	Burlina	
 OVINI	<ul style="list-style-type: none"> <li>Veneto Agricoltura</li> <li>Provincia di Vicenza</li> <li>Università di Padova</li> <li>Istituto Zooprofilattico Sperimentale delle Venezie</li> <li>Istituto Agrario di Feltre</li> </ul>	Alpagota Brogna Foza o Vicentina Lamon	
 AVICOLA	<ul style="list-style-type: none"> <li>Veneto Agricoltura</li> <li>Provincia di Vicenza</li> <li>Università di Padova</li> <li>Istituto Zooprofilattico Sperimentale delle Venezie</li> <li>Istituto Agrario di Feltre</li> <li>Istituto Agrario di Padova</li> <li>Istituto Agrario di Castelfranco</li> </ul>	<b>Anatre:</b> Germanata veneta, Mignon <b>Faraona:</b> Camosciata <b>Oche:</b> Padovana e Veneta <b>Polli:</b> Emerlinata di Rovigo, Millefiori di Lonigo, Padovana Argenta, bianca, camosciata e dorata, Polverara bianca e nera, Robusta Linonata, Robusta Maculata <b>Tacchini:</b> Comune bronzato, Ermellinato di Rovigo	<b>Specie Razze-Popolazioni</b> 5 20 (13)
 CEREALICOLO	<ul style="list-style-type: none"> <li>Veneto Agricoltura</li> <li>Provincia di Vicenza</li> <li>Università di Padova</li> <li>Istituto Agrario di Feltre</li> <li>Istituto Agrario di Padova</li> <li>Istituto Agrario di Castelfranco</li> </ul>	<b>Fumenti:</b> Canove, Monococco, Piave <b>Maie:</b> Biancopepa, Marano, Sponcio <b>Orzo:</b> Agordino	
 ORTICOLA	<ul style="list-style-type: none"> <li>Veneto Agricoltura</li> <li>Provincia di Vicenza</li> <li>Università di Padova</li> <li>Istituto Agrario di Feltre</li> </ul>	Asparago Montino, Broccolo di Bassano, Broccolo fiolano di Cressano, Fagiolo Giolet e Fighetta d'oro di Posina, Pomodoro Nasone	
 VITICOLO	<ul style="list-style-type: none"> <li>Veneto Agricoltura</li> <li>Provincia di Vicenza</li> <li>Università di Padova</li> <li>Consiglio per la Ricerca e la sperimentazione in Agricoltura - Centro di ricerca per la Viticoltura (CRA-VIT) di Conegliano (TV)</li> <li>Istituto Agrario di Feltre</li> </ul>	Bianchetta trevigiana b., Boschera b., Cabrusina n., Cavrara, Corbine n., Dall'Oochio b., Dinderella n., Forsellina n., Graperiol, Gropello di Breganze, Grujia n., Marzemina bianca b., Marzemina grossa n., Negrare n., Oseleta n., Pattaresca, Pedevenda b., Perera b., Pinella b., Prosecco lungo b., Recantine, Trevisana nera n., Turchetta n.	
 FORAGGERE	<ul style="list-style-type: none"> <li>Veneto Agricoltura</li> <li>Provincia di Vicenza</li> <li>Università di Padova</li> </ul>	Praterie seminaturali di pianura	

# BIONET

*(Rete regionale per la conservazione e la caratterizzazione della biodiversità di interesse agrario)*



FEASR



REGIONE DEL VENETO



Fondo europeo agricolo per lo sviluppo rurale: l'Europa investe nelle zone rurali

**Tabella 1.** Numero di campioni di sangue prelevati presso i centri di conservazione (anno 2013-2014).

Razza	Sigla	Totale	Castelfranco, Montebelluna	Ceregnano	Feltre	Padova	Montecchio Precalcino
Anatra Germanata Veneta	AGV	71		36	35		
Anatra Mignon	AMG	78		40	38		
Faraona Camosciata	FCM	70	35	35			
Oca Padovana	OPD	11				11	
Ermellinata di Rovigo	PER	137	35	34	34	34	
Millefiori di Lonigo	PML	36					36
Polverara Bianca	PPB	89		17	36	36	
Padovana Camosciata	PPC	66		24		42	
Padovana Dorata	PPD	75		36		39	
Polverara Nera	PPN	88		18	35	35	
Pepoi	PPP	124	35	19	35	35	
Robusta Lionata	PRL	104	16	16	36	36	
Robusta Maculata	PRM	126	35	19	36	36	
Tacchino Comune Bronzato	TCB	79	45	34			
Tacchino Ermellinato di Rovigo	TER	75	36	39			

5 Specie Avicole analizzate (Anatra, Oca, Faraona, Pollo, Tacchino), 15 razze/popolazioni considerate per un totale di **1229 campioni raccolti di singoli individui**





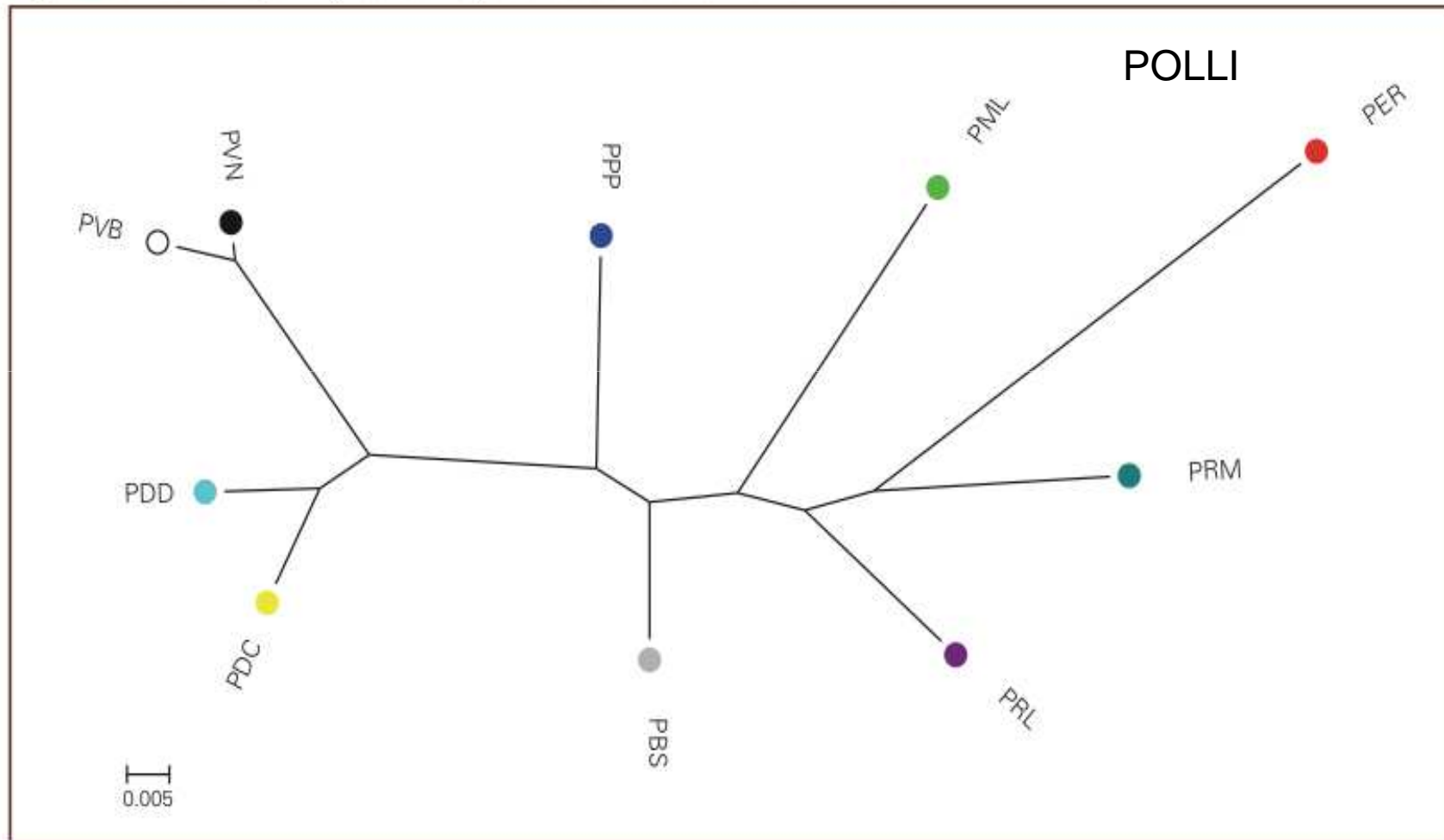
# BIONET

*(Rete regionale per la conservazione e la caratterizzazione della biodiversità di interesse agrario)*



Fondo europeo agricolo per lo sviluppo rurale: l'Europa investe nelle zone rurali

Figura 28. Albero di tipo Neighbor-Joining tracciato con le distanze Nei Minimum.



Martino Cassandro et al. (Università degli Studi di Padova)



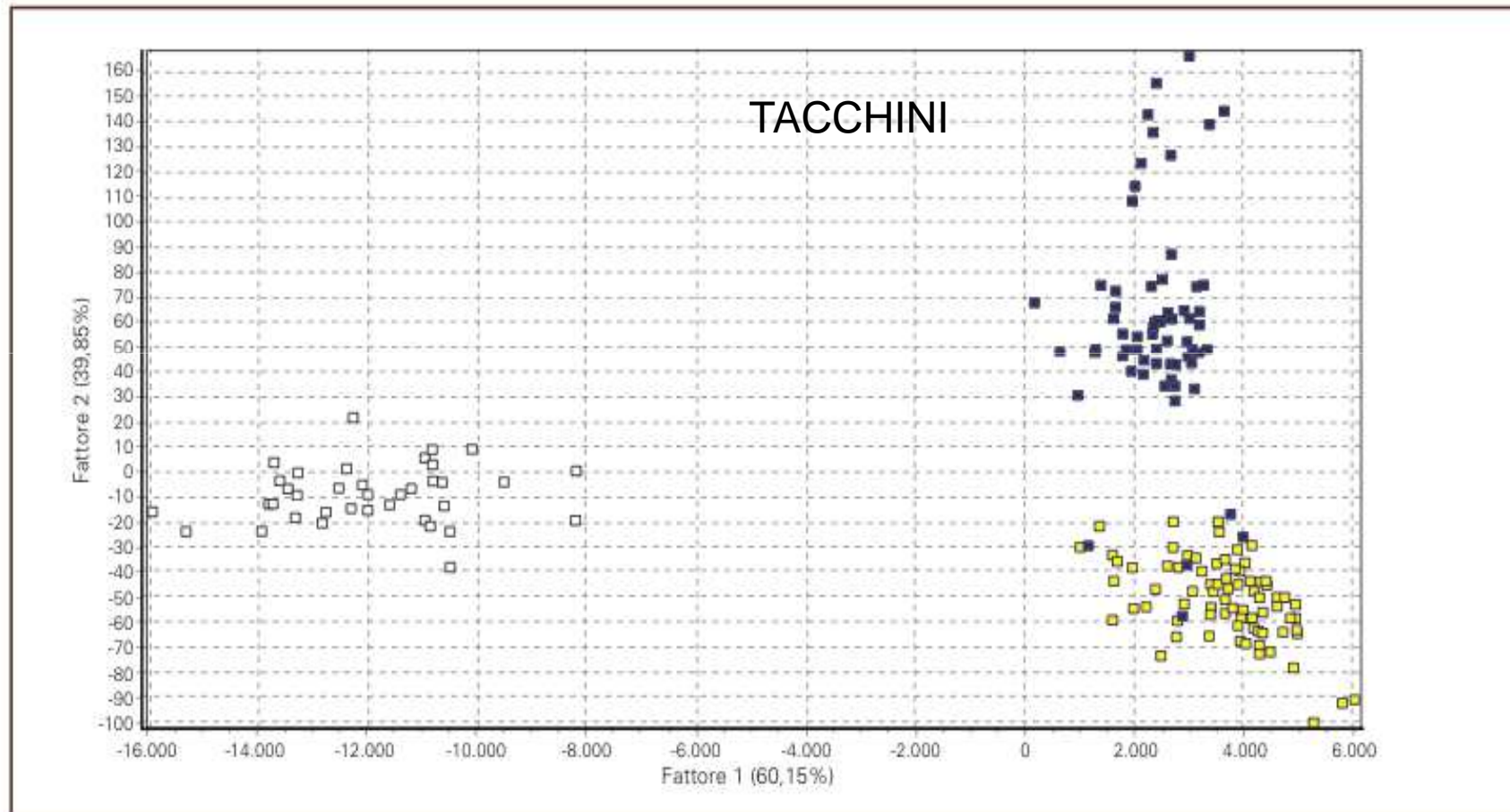
# BIONET

*(Rete regionale per la conservazione e la caratterizzazione della biodiversità di interesse agrario)*



Fondo europeo agricolo per lo sviluppo rurale: l'Europa investe nelle zone rurali

**Figura 33.** Analisi fattoriale delle componenti. Plot 2D dei due fattori principali (bianco: THY, giallo: TER, blu: TCB).



# BIONET

*(Rete regionale per la conservazione e la caratterizzazione della biodiversità di interesse agrario)*



Fondo europeo agricolo per lo sviluppo rurale: l'Europa investe nelle zone rurali

Figura 31. Analisi fattoriale delle componenti per i soggetti di Faraona Camosciata (giallo: Ceregnaio; blu: Castelfranco).

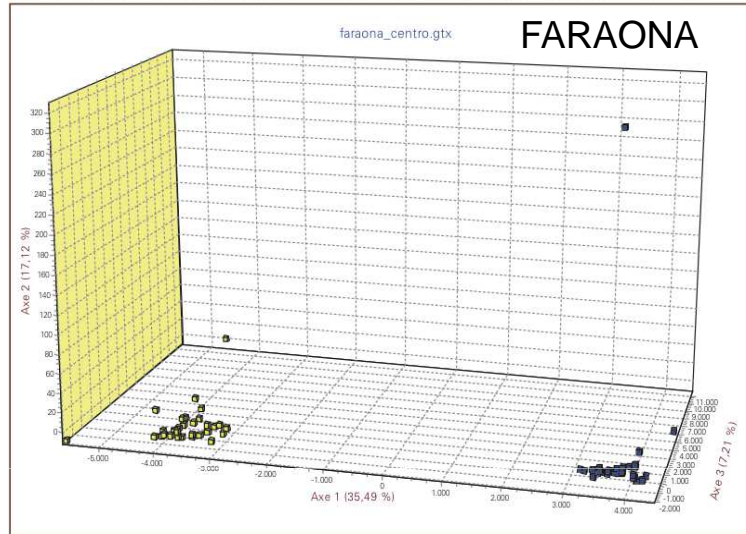


Grafico 3. Analisi fattoriale delle componenti. Giallo: AGV, Blu: AMG.

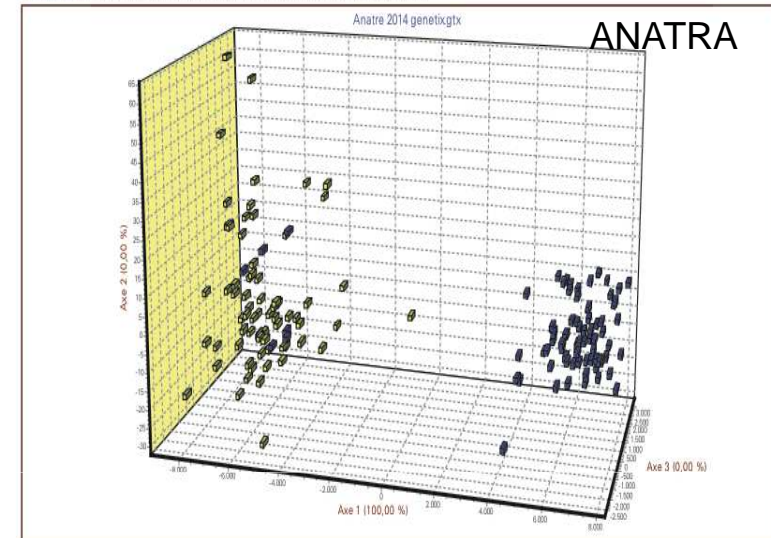
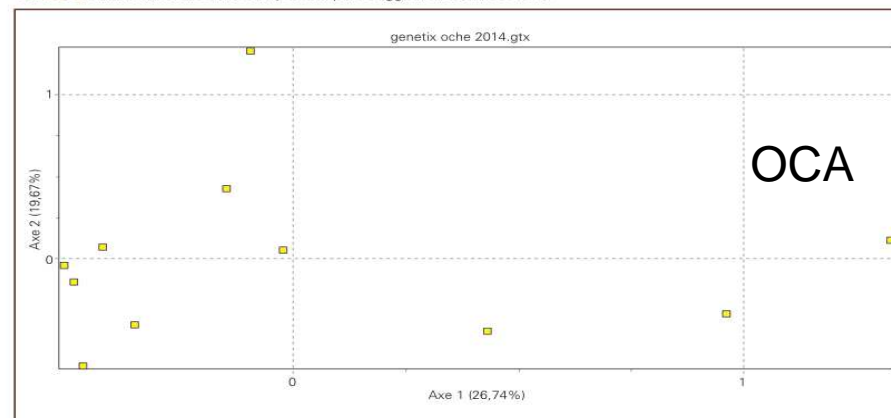


Grafico 5. Analisi fattoriale delle componenti per i soggetti di Oca Padovana.





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Italian Journal of Animal Science

Volume 12, Issue 2, 2013, Pages 313-319

## Conservation of local Turkish and Italian chicken breeds: A case study (Review)

Özdemir, D.<sup>a</sup>, Özdemir, E.D.<sup>b</sup>, De Marchi, M.<sup>c</sup>, Cassandro, M.<sup>c</sup>

<sup>a</sup> Teknik Bilimler Meslek Yüksekokulu, Akdeniz Üniversitesi, Antalya, Turkey

<sup>b</sup> İşletme Bölümü, Akdeniz Üniversitesi, Antalya,, Turkey

<sup>c</sup> Dipartimento di Agronomia Animali Alimenti Risorse Naturali e Ambiente, Università di Padova, Legnaro (PD), Italy

### Abstract

[View references \(59\)](#)

Increased global use of highly productive breeds of farm animals has been associated with a loss of genetic diversity in most species, especially in local poultry species. Since 2000, in Italy, and most of all in Veneto region, various governmental, non-governmental and private organizations have tried to preserve the genetic diversity of poultry resources. This successful conservation effort has included various activities: improving knowledge on biological functions, conserving typical morphological characteristics, developing a selection of strategies, controlling inbreeding and, valorizing strategies to diffuse breeds in local productive systems. These activities represent a good example for developing countries such as Turkey. With the aim of helping to conserve Turkish poultry resources, this study describes the current status of local **chicken** breeds in Turkey and Italy, and makes some recommendations for developing countries such as Turkey. © D. Özdemir et al., 2013 Licensee PAGEPress, Italy.

### Author keywords

Biodiversity; Chicken; Denizli breed; Local breeds; Sustainable conservation

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The first aim of this study was to investigate the maternal genetic origin of five Italian local chicken breeds (*Ancona*, *Livorno*, *Modenese*, *Romagnola* and *Valdarnese bianca*) based on mitochondrial DNA (mtDNA) information.

Italian Journal of Animal Science 2013; volume 12:e66

PAPER

## Phylogeny, genetic relationships and population structure of five Italian local chicken breeds

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### Abstract

Number and population size of local chicken breeds in Italy is considered to be critical. Molecular data can be used to provide reliable

higher degree of inbreeding ( $F_{IS}=0.08$ ) than the commercial populations that served as reference. Structure analysis showed a separation of the Italian breeds from the reference populations. A further sub-clustering allowed discriminating among the five different Italian breeds. This research provides insight into population structure, relatedness and variability of the five studied breeds.

### Introduction

Attention and awareness to genetic conservation has significantly increased in recent years (Allendorf and Luikart, 2007). Preservation of genetic variability plays a crucial role in animal science because its decline may reduce populations' ability to adapt to environmental changes (Lande, 1988). Moreover, autochthonous breeds might be an important resource for research purposes and future breeding programmes.

In Italy, the number of native chicken breeds has suffered a dramatic decline leading to the current critical situation. Zanon and Sabbioni (2001) reported the presence in Italy, in the last fifty years, of 90 rural poultry breeds (9 ducks, 11 guinea fowls, 53 chickens, 5 geese and 12 turkeys): 61.0% of these breeds are extinct, 13.3% are endangered, and only 6.7% are involved in conservation programmes. On the other hand, hybrids based on only few specialized chicken lines provided by globally acting breeding companies are used for industrial

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Key words: Chickens, Genetic diversity,  
Mitochondrial DNA, Microsatellites.

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bianca* breeds and Prof. Francesco Panella for  
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## Abstract

The **genetic diversities and relationships among 16 local breeds of chicken originating from five countries (Italy, Spain, Serbia, Albania and Republic of Malta)** within the Mediterranean basin were assessed by sequencing part of the mitochondrial DNA (mtDNA) D-loop region and by genotyping individuals at 27 autosomal microsatellite loci. The **aim was to study the microevolution of chicken** on the northern shores of the Mediterranean and to determine their present genetic status. A 506bp fragment of the mtDNA control region was sequenced in 160 individual DNA samples. The mtDNA sequence polymorphisms nomenclature that is normally used in these studies suggests that the Mediterranean chicken breeds under investigation are related to haplogroup E. A total of 465 blood samples were collected and utilised for microsatellite analysis. Six breeds (**Ancona, Livornese Bianca-Italy; Pita Pinta Asturiana, Gallina de Sobrarbe-Spain; Albanian population-Albania; and the Maltese Black-Malta**) **showed significant high levels of inbreeding**. About 22% of the total genetic variation observed was due to variability between populations. STRUCTURE analysis confirmed the breed variability result ( $F_{ST}=0.22$ ) also observed in the Neighbor-Net dendrogram. **These results strongly allude that the 16 Mediterranean chicken breeds studied originated from three distinct maternal lineages and retain moderate levels of autosomal genetic diversity.**

## Genetic diversity and phylogeographic structure of sixteen Mediterranean chicken breeds assessed with microsatellites and mitochondrial DNA

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## ABSTRACT

The genetic diversities and relationships among 16 local breeds of chicken originating from five countries (Italy, Spain, Serbia, Albania and Republic of Malta) within the Mediterranean basin were assessed by sequencing part of the mitochondrial DNA (mtDNA) D-loop region and by genotyping individuals at 27 autosomal microsatellite loci. The aim was to study the microevolution of chicken on the northern shores of the Mediterranean and to determine their present genetic status. A 506 bp fragment of the mtDNA control region was sequenced in 160 individual DNA samples. The mtDNA sequence polymorphisms nomenclature that is normally used in these studies suggests that the Mediterranean chicken breeds under investigation are related to haplogroup E. A total of 465 blood samples were collected and utilised for microsatellite analysis. Six breeds (Ancona, Livornese Bianca-Italy; Pita Pinta Asturiana, Gallina de Sobrarbe-Spain; Albanian population-Albania; and the Maltese Black-Malta) showed significant high levels of inbreeding. About 22% of the total genetic variation observed was due to variability between populations. STRUCTURE analysis confirmed the breed variability result ( $F_{ST}=0.22$ ) also observed in the Neighbor-Net dendrogram.

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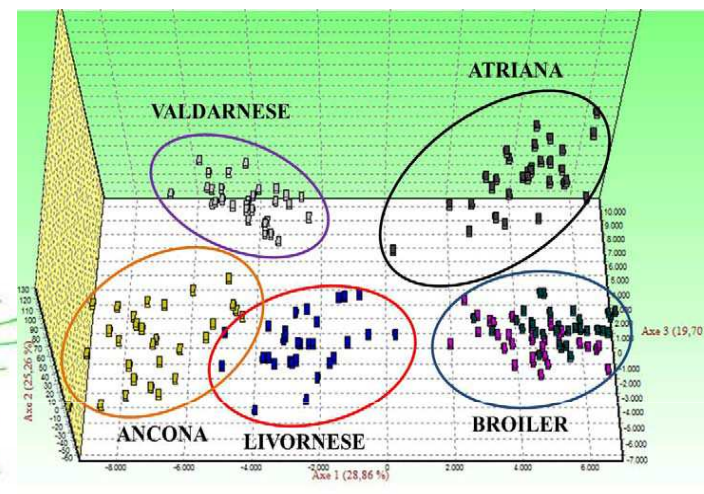
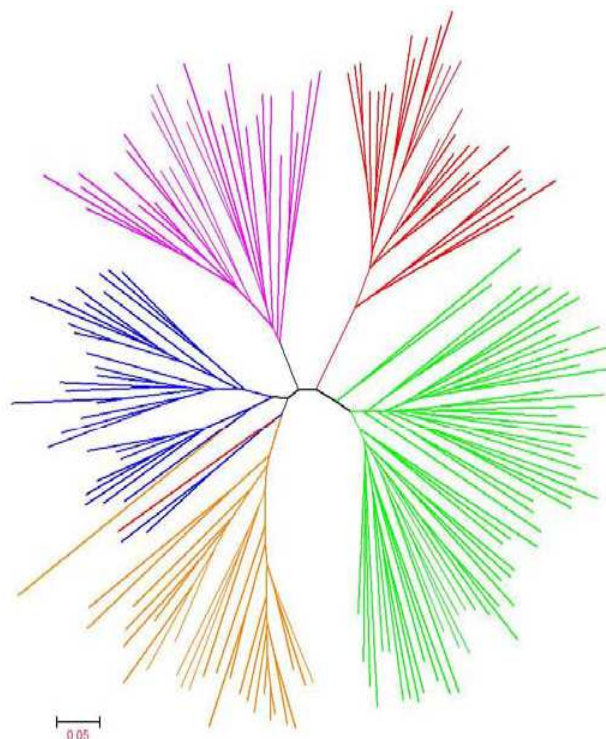
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## RELAZIONE SCIENTIFICA FINALE RELATIVA ALL'ACCORDO DI COLLABORAZIONE PER LA CARATTERIZZAZIONE GENETICA DELLA POPOLAZIONE AVICOLA "ATRIANA"

La razza Atriana è un pollo raffigurato in alcune monete pre-romane rinvenute in territorio Atriano, da cui prende il nome. Si pensa essere progenitrice della razza Ancona e Siciliana, tutt'ora presenti nell'Italia centro-meridionale.

La razza è ufficialmente estinta o diluita in una miriade di incroci succedutesi nel corso dei secoli, ma in alcuni piccoli allevamenti è possibile ritrovare un pollo dalle caratteristiche molto simili.



- Atriana
- Broiler
- Livornese
- Ancona
- Valdarnese

	ANCONA	LIVORNESE	VALDARNESE	BROILER LINEA A	BROILER LINEA B
ATRIANA	0,305	0,282	0,268	0,244	0,270

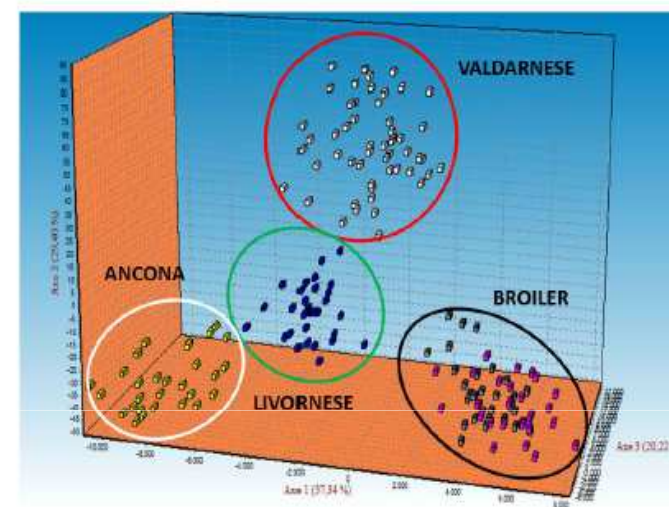
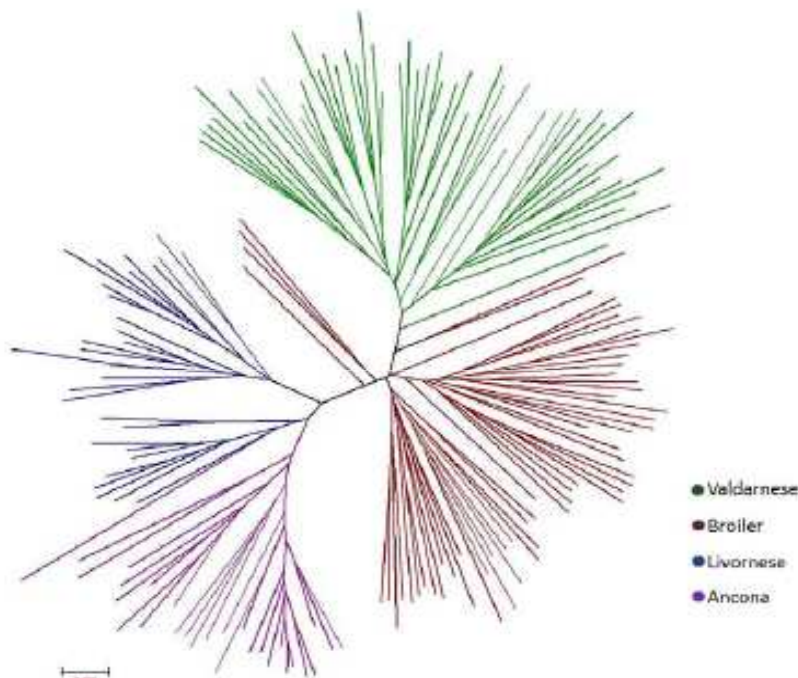
Sulla base di questi valori, l'Ancona risulta essere la razza più distante dalla popolazione considerata in questo studio, mentre una linea di broiler (linea A) quella che presumibilmente dal punto di vista genetico le si avvicina di più.

## Quanti e quali studi di CG



La razza Valdarnese è utilizzata fundamentalmente per la produzione di carne, anche se la sua attitudine alla deposizione di uova non va trascurata (140-170 uova/anno).

A partire dal 2008, è stato attivato un registro anagrafico sotto la responsabilità dell'Associazione Regionale Allevatori Toscana (ARA Toscana), al fine di garantire la sopravvivenza e l'eventuale certificazione di prodotti da essa derivati e identificati da un marchio.



	ANCONA	LIVORNESE	BROILER LINEA A	BROILER LINEA B
VALDARNESE	0,193	0,196	0,142	0,179

In conformità a questi valori, l'Ancona e la Livornese sono le razze più distanti dalla popolazione considerata in questo studio, mentre le due linee di broiler sono quelle che le si avvicinano di più. Questo è confermato dal fatto che anche nell'albero delle distanze individuali (DAS) il cluster del nucleo oggetto di studio si avvicina quantomeno a quello appartenente alle linee broiler.





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Italian Journal of Animal Science

Volume 13, Issue 4, 23 December 2014, Pages 887-890

## Feasibility study on the FAO chicken microsatellite panel to assess genetic variability in the Turkey (*Meleagris gallopavo*) (Article)

Colombo, E., Strillacci, M.G., Cozzi, M.C., Madeddu, M., Mangiagalli, M.G., Mosca, F., Zaniboni, L., Bagnato, A., Cerolini, S. Dipartimento di Scienze Veterinarie per la Salute, La Produzione Animale e la Sicurezza Alimentare, Università di Milano, Italy

[View references \(11\)](#)

### Abstract

The aim of this work was to study the feasibility of the Food and Agriculture Organization (FAO) microsatellite panel developed for chickens to assess genetic variability in turkeys. Genomic DNA was extracted from a total of 37 blood samples collected from turkey of different breeds [15 Brianzolo (BR); 12 Colli Euganei (EU); 10 Nero d'Italia (NI)], and all 31 chicken microsatellite markers recommended by the FAO were tested. The results show that 22 chicken markers out of 31 suggested by FAO guidelines can be applied to turkey populations. In particular, the multiplex groups confirmed in the turkey were the Multiplex Master Mix 1 (ADL0268, ADL0278, LEI0094, MCW0216, MCW0248) and the Master Mix 2 (MCW0034, MCW0069, MCW0081, MCW0222, MCW0295), whereas 13 microsatellites were amplified only under single polymerase chain reaction (PCR) conditions. No PCR products were obtained for 9 markers (LEI0166, MCW0020, MCW0078, MCW0080, MCW0104, MCW0123, MCW0248, MCW0284 and MCW0330), which is 29% of the total markers used. A panel of 22 markers was used to assess genetic diversity in three turkey breeds and a total number of 63 alleles were found. Observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosity and polymorphism information content (PIC) values for each microsatellite and the relative mean values were also calculated. The mean values were 0.210, 0.250, 0.203 for  $H_o$ ; 0.301, 0.348, 0.228 for  $H_e$ ; and 0.265, 0.313, 0.199 for PIC in NI, BR and EU, respectively. © E. Colombo et al., 2014.

### Author keywords

Biodiversity; Breed conservation; Genetic variability; Microsatellite; Turkey

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Theriogenology

Volume 75, Issue 9, June 2011, Pages 1613-1622

## DNA fragmentation in chicken spermatozoa during cryopreservation (Article)

Gliozzi, T.M.<sup>a</sup>, Zaniboni, L.<sup>b</sup>, Cerolini, S.<sup>b</sup>

<sup>a</sup> Istituto di Biologia e Biotecnologia Agraria, Consiglio Nazionale delle Ricerche, IBBA-CNR, via Bassini, 15, 20133 Milan, Italy

<sup>b</sup> Dipartimento di Scienze e Tecnologie Veterinarie per la Sicurezza Alimentare, Università di Milano, via Trentacoste 2, 20134 Milan, Italy

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### Abstract

Semen cryopreservation is fundamental both for the practice of artificial insemination, and for the conservation of genetic resources in cryobanks; nevertheless, there is still not an efficient standard freezing procedure assuring a steady and suitable level of fertility in fowl, and consequently there is no systematic use of frozen semen in the poultry industry. This study examined changes in motility (CASA), cell membrane integrity (Ethidium Bromide (EtBr) exclusion procedure and stress test) and DNA fragmentation (neutral comet assay) in fowl spermatozoa before, during and after cryopreservation and storage at -196 °C. An optimized comet assay for **chicken** semen was studied and applied to the analyses. Semen collected from 18 Mericanel della Brianza (local Italian breed) male **chicken** breeders was frozen in pellets and thawed in a water bath at 60 °C. Measurements were performed on fresh semen soon after dilution, after equilibration with 6% dimethylacetamide at 4 °C (processed semen) and after thawing. Sperm DNA damage occurred during cryopreservation of **chicken** semen and the proportion of spermatozoa with damaged DNA significantly increased from 6.2% in fresh and 6.4% in processed semen to 19.8% in frozen-thawed semen. The proportion of DNA in the comet tail of damaged spermatozoa was also significantly affected by cryopreservation, with an increase found from fresh (26.3%) to frozen-thawed (30.9%) sperm, whereas processed semen (30.1%) didn't show significant differences. The proportion of total membrane damaged spermatozoa (EtBr exclusion procedure) did not increase by 4 °C equilibration time, and greatly and significantly increased by cryopreservation; the values recorded in fresh, processed and frozen semen were 2.9, 5.6, and 66.7% respectively. As regards the proportion of damaged cells in the stress test, all values differed significantly (7.1% fresh semen, 11.7% processed semen, 63.7% frozen semen). Total motility was not affected by equilibration (52.1% fresh semen, 51.9% processed semen), whereas it decreased significantly after cryopreservation (19.8%). These results suggest a low sensitivity of frozen-thawed **chicken** spermatozoa to DNA fragmentation, therefore it should not be considered as a major cause of sperm injuries during cryopreservation. © 2011 Elsevier Inc.

### Author keywords

Chicken; Comet assay; Pellet cryopreservation; Sperm integrity; Sperm motility

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Italian Journal of Animal Science

Volume 9, Issue 4, 2009, Pages 382-385

## Breeding performance in the italian chicken breed mericanel della brianza (Article)

Cerolini, S.<sup>a</sup>, Madeddu, M.<sup>a</sup>, Zaniboni, L.<sup>a</sup>, Cassinelli, C.<sup>a</sup>, Mangiagalli, M.G.<sup>b</sup>, Marelli, S.P.<sup>b</sup>

<sup>a</sup> Dipartimento di scienze, Tecnologie Veterinarie per la Sicurezza Alimentare, Università di Milano, Italy

<sup>b</sup> Dipartimento di Scienze Animali, Università di Milano, Italy

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### Abstract

In Italy, 90 local avian breeds were described; the majority (61%) were classified as extinct and only 8.9% as still widely spread. Therefore, efforts for conservation of Italian avian breeds are urgently required. The aim of this study was to record the breeding performance of the Italian breed Mericanel della Brianza to multiply a small population, in order to develop a conservation programme. Fourteen females and eight males were available at the beginning of the reproductive season in 2009 and organised into eight families (1 male/1-2 females) kept in floor pens. Birds received a photoperiod of 14L:10D and were fed ad libitum. Breeding performance was recorded from March to June. Egg production and egg weight were recorded daily; eggs were set every two weeks and fertility, embryo mortality and hatchability were recorded. Mean egg production was 37% and mean egg weight was 34±3.49 g. High fertility values from 94% to 87% were recorded in the first three settings and the overall mean fertility value was 81.6%. Overall hatchability was only 49.6% owing to a high proportion of dead embryos. Embryo mortality occurred mainly between days 2 and 7 of incubation and during hatching. The highest hatchability values were recorded in settings 1 and 2, 69% and 60% respectively, and a large decrease was found in the subsequent settings. Marked variations in egg production, fertility, hatchability and embryo mortality were found among families. The present results represent the basic knowledge of reproductive parameters necessary to improve the reproductive efficiency of the breed within a conservation plan © S. Cerolini et al.

### Author keywords

Breed conservation; Chicken; Egg production; Fertility; Hatchability

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Italian Journal of Animal Science

Volume 6, Issue SUPPL. 1, 2007, Pages 63-65

## Heterozygosity analysis of Bionda Piemontese and Bianca di Saluzzo chicken breeds by microsatellites markers: A preliminary study (Conference Paper)

Guidobono Cavalchini, L.<sup>a</sup>, Marelli, S.P.<sup>ab</sup>, Strillacci, M.G.<sup>a</sup>, Cozzi, M.C.<sup>a</sup>, Polli, M.<sup>a</sup>, Longeri, M.<sup>a</sup>

<sup>a</sup> Istituto di Zootecnica, Università di Milano, Italy

<sup>b</sup> Istituto di Zootecnica, Facoltà di Medicina Veterinaria, Università degli Studi di Milano, Via Celoria 10, 20133 Milano, Italy

### Abstract

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Conservation of genetic variability is one of the main goals in animal production science and the analysis of breeds genetic asset can supply objective basis for effective conservation programs and selection strategies. Bionda Piemontese (PIB) and Bianca di Saluzzo (SAB) chicken breeds originated in Piemonte region. Breeds conservation programmes started in 1999 in Verzuolo (CN) aiming to preserve the breeds and to improve their diffusion being particularly adapted to free range rearing systems thanks to their resistance. PIB and SAB are both suggested for traditional recipes and production (e.g. Morozzo capon) and are Slow Food presidia. A total of 76 birds were analysed: PIB (n=36), SAB (n=40). Genomic DNA was extracted from blood samples. All birds were genotyped at eight microsatellite loci. Each marker was subjected to PCR and the products were separated by electrophoresis in 4.2% denaturing polyacrylamide gels on ABI Prism 377 DNA Sequencer equipped with Genescan and Genotyper software. The results of this preliminary study highlight the genetic differences occurring between PIB and SAB populations.

### Author keywords

Bianca di Saluzzo; Bionda piemontese; Chicken microsatellites; Heterozygosity

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**Effect of genotype and transport on tonic immobility and heterophil/lymphocyte ratio in two local Italian breeds and Isa Brown hens kept under free-range conditions**

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## Genetic traceability of two local chicken populations, *Bianca di Saluzzo* and *Bionda Piemontese*, versus some current commercial lines

Stefano Sartore, Dominga Soglia, Sandra Maione, Paola Sacchi, Michele De Marco, Achille Schiavone, Simone Sponza, Alessandra Dalmasso, Maria Teresa Bottero, Daniele Pattono, Ivo Zoccarato, Laura Gasco, Alberto Brugiapaglia, Martina Tarantola, Mario Giacobini, Luigi Bertolotti, Roberto Rasero

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### Abstract

The aims of this investigation were to analyse the genetic variation of two Piemonte chicken local breeds, *Bionda Piemontese* and *Bianca di Saluzzo*, and to set them against some commercial lines. A panel of 19 microsatellite markers was used. On the overall, the results of different analyses highlight the genetic uniqueness of the two breeds; therefore they should be considered genetic resources worthy of preservation. The panel of microsatellites used in this investigation turns out to be a consistent and reliable tool for traceability. In fact, these markers are able to distinguish the two local populations from the commercial lines and they are able to confirm the existence of two genetically different clusters within the *Bionda Piemontese*, namely the ecotypes *standard* and *Cuneo*. Mating policies implemented to avoid inbreeding and, if necessary, a marker assisted conservation scheme would be sufficient to solve the problem of inbreeding.

### Introduction

The quality, rather than the quantity, of food of animal origin met recently the attention of the consumers, together with the growing perception that regional and traditional products could be healthier and tastier. This novel tendency is based on a reduced confidence towards food of animal origin, as a consequence of the outbreak of diseases like bovine spongiform encephalopathy, followed by avian influenza crisis (Ciampolini *et al.*, 2000; Goffaux *et al.*, 2005). Moreover, Opara and Mazaud (2001) reported a rising incidence of foodborne disease caused by microbial contamination of processed food, leading to additional mistrust in consumers.

Therefore an increasing need to check food processing aimed to prevent frauds and adulterations is arisen. The idea of traceability, intended to be a method able to identify animal or animal products through different steps of the food chain (McKean, 2001), became familiar to consumers and the development of a reliable traceability

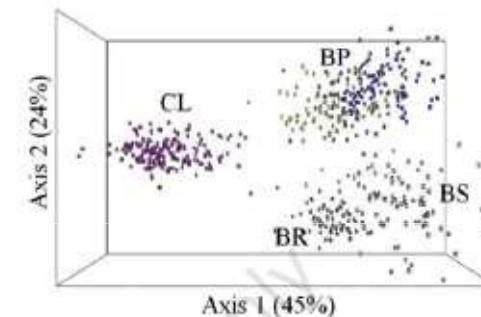


Figure 1. Distribution of individuals based on the factorial correspondence analysis, axes 1 and 2. BP, *Bionda Piemontese*; BS, *Bianca di Saluzzo*; BR, broiler chickens; CL, egg-laying commercial lines.

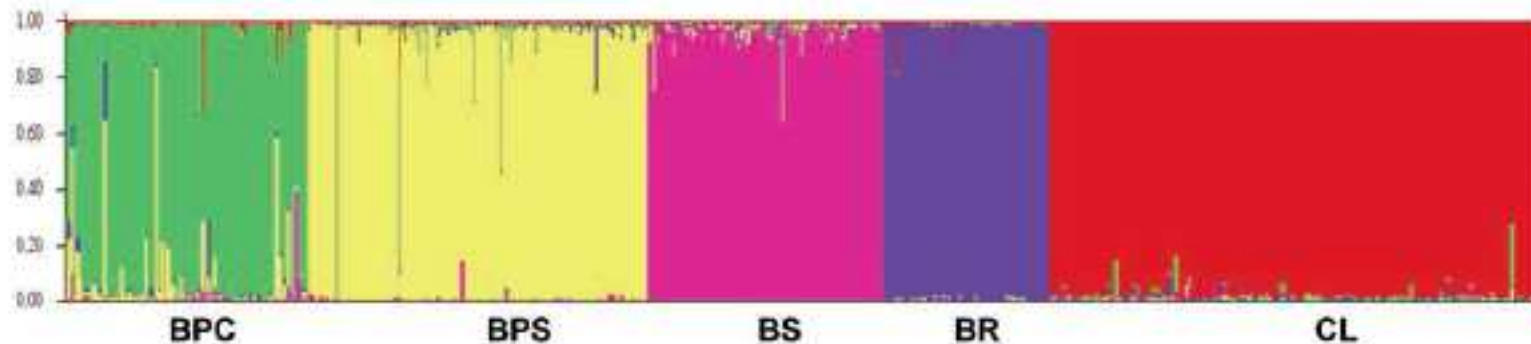


Figure 4. Bayesian cluster analysis at K=5. BPC, *Bionda Piemontese Cuneo*; BPS, *Bionda Piemontese standard*; BS, *Bianca di Saluzzo*; BR, broiler chickens; CL, egg-laying commercial lines.



## **Attività UNITO su razze avicole locali: Gallina Bionda Piemontese e Gallina Bianca di Saluzzo**

Il Dipartimento di Scienze Veterinarie (ex Facoltà di Medicina Veterinaria) si occupa da anni di attività di studio delle due razze avicole piemontesi (Bionda Piemontese e Bianca di Saluzzo) in particolare:

1. in collaborazione con l'Istituto Zooprofilattico di Piemonte Liguria e Valle d'Aosta, ha svolto studi relativi ai parametri di benessere (ematici e comportamentali) delle due razze e le caratteristiche macroscopiche delle uova.

**2. con finanziamento Compagnia di San Paolo 2012-2014 ha condotto studi circa: la consistenza delle due popolazioni in Piemonte, un censimento circa le modalità di allevamento, le caratteristiche qualitative delle carcasse presenti sul mercato, la caratterizzazione genetica (microsatelliti e SNPs)**

3. in collaborazione con il Dipartimento di Scienze Agrarie, Forestali e Alimentari nel 2015 è stato realizzato un centro avicolo per la conservazione e studio delle razze. Il centro avicolo è costituito da 24 parchetti coperti (2x4m) con annesso parchetto esterno (2x4m). Inoltre è presente un impianto di incubazione e schiusa, 24 parchetti (1x2 m) in ambiente controllato per lo svezzamento dei pulcini, e 32 gabbie metaboliche per lo studio della digeribilità.

4. Nel 2015 è in avvio una prova di accrescimento volta a studiare la curva di crescita di femmine, maschi e capponi delle due razze. verranno altresì realizzate macellazioni a diverse età per definire la migliore età di macellazione e verranno studiate le variazioni ormonali e comportamentali durante la crescita.



Caratterizzazione Genetica delle popolazioni Avicole (CGA) è oggi una realtà ed uno strumento a disposizione e a servizio del settore avicolo privato, pubblico, amatoriale, industriale e scientifico

Esiste una rete scientifica nazionale molto qualificata e diversificata che trova nella WPSA Italia la sede naturale per crescere e qualificarsi a tutti i livelli.

La multi-utilità della CGA si ritiene rappresenti una interessante opportunità per l'intero settore avicolo nazionale