ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

Whole genome resequencing detects signature of selection in 23 European pig breeds and wild boars

Luca Fontanesi
Department of Agricultural and Food Sciences
University of Bologna
Bologna, Italy

S. Bovo, G. Schiavo, A. Ribani, M. Muñoz, V.J. Utzeri, M. Gallo, J. Riquet, G. Usai, R. Charneca, J.P. Araujo, R. Quintanilla, V. Razmaite, M. Candek-Potokar, A. Fernandez, C. Ovilo, L. Fontanesi

TREASURE

Diversity of local pig breeds and production systems for
high quality traditional products and sustainable pork chains

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

Global description of genetic variability/diversity in European pig populations

Global description of genetic variability/diversity in European pig populations

Extract interesting information:
Potential application

9 European countries
20 autochthonous breeds


Schwäbisch-Hällisches


Senojo tipo Lietuvos baltosios


Lietuvos vietins


Krškopoljski


Gascon


Basque


Mangulica


Moravka

Bisaro



Alentejano


Crna slavonska


Mora Romagnola


Casertana


Nero Siciliano


Turopoljska


Iberico


Negre Mallorquí


Cinta Senese


Apulo Calabrese


Sarda

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

Italian Wild Boars

Italian Large White

Italian Landrace

Italian Duroc

A N A S
associazione nazionale allevatori suini

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

Materials

Genotyping

- At least 48 animals x breed/population: genotyped with the GGP 70K SNP/60k Illumina chips.
- For some breeds – a larger number of pigs was genotyped

Sequencing

- 30-35 animals x breed/population: DNA-pools
- Whole genome sequencing of all DNA pools (40X – Illumina paired end)

Sequencing data

Breed	No. of reads (in pairs)	No. of animals in pool
Cinta Senese	422120850	35
Alentejano	419690476	35
Apulo Calabrese	418529727	35
Mangalitsa	416663891	35
Black Slavonian	405316112	35
Casertana	435598516	35
Mora Romagnola	411095541	35
Nero Siciliano	405812223	35
Krskopolje	404204144	35
Duroc	420384723	35
Negre Mallorqui	414314159	35
Gascon	408764207	30
Basque	407698128	30
Bisaro	415284437	35
Lietuvos Baltosios	405822217	35
Lietuvos Vietines	409935460	35
Turopolje	416663891	35
Scwabisch Hallisches	428982876	35
Moravka	413100992	35
Sarda	442035147	35
Large White	450673024	35
Landrace	442780637	35
Wild boars	184076376	35

Databases/ items	**Features**	**No. of SNPs**	**No. of INDELs**
dbSNP			
	Common*	19,004,673	961,368
	Rare$^{\#}$	3,315,987	95,287
	Fixed$^{\$}$	5,295	9
Novel	**Common**	3,475,057	2,184,363
	Rare	1,423,002	445,019
	Fixed	4,871	358
Total		27,228,885	3,686,404

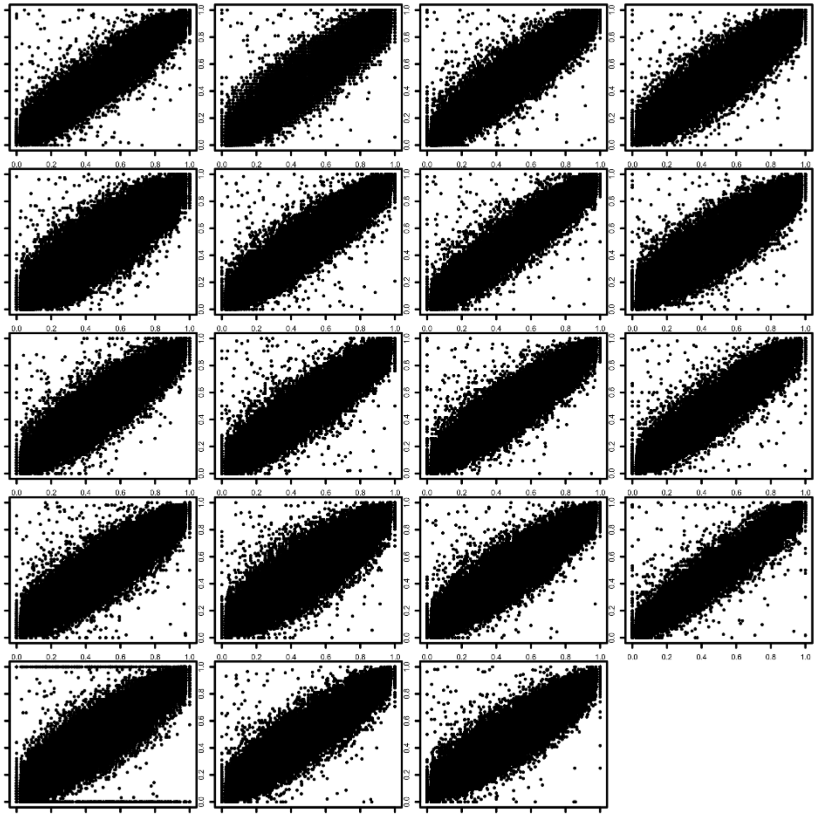
*AFmean ≥ 0.0143; #AF$_{mean}$ < 0.0143; \$Fixed: only the ALT allele is present.

Consequence	**No. of SNPs**
Intron variant	27,723,666
Intergenic variant	15,604,676
3’-UTR variant	469,853
Non-coding transcript variant	396,126
Synonymous variant	274,870
Missense variant	149,180
5’-UTR variant	93,507
Splice region variant	52,788
Non-coding transcript exon variant	15,757
Stop gained	1,357
Splice donor variant	806
Splice acceptor variant	567
Start lost	376
Stop lost	201
Stop retained variant	144
Coding sequence variant	107
Mature miRNA Variant	48
Total*	44,784,029

- **SNPs impacting the gene at the protein level (i.e. start/stop gained/lost and missense SNPs) accounted only for 0.34%.**

Estimation of allele frequency: SNP-CHIP vs DNA Pool-Seq

Breed	PCC	SNPs
Cinta Senese	0.97	46,674
Alentejano	0.96	40,614
Apulo Calabrese	0.96	45,354
Mangulica	0.96	40,278
Black Slavonian	0.92	46,730
Casertana	0.96	44,813
Mora Romagnola	0.97	35,238
Nero Siciliano	0.93	47,283
Krskopolje	0.94	46,625
Negre Mallorquì	0.96	44,895
Gascon	0.96	42,319
Basque	0.97	37,531
Bisaro	0.93	46,948
Lietuvos Baltosios	0.93	45,163
Lietuvos Vietines	0.95	44,376
Turopolje	**0.98**	39,194
Schwäbisch Hällisches	**0.91**	46,946
Moravka	0.95	47,063
Sarda	0.92	47,540



MAX correlation

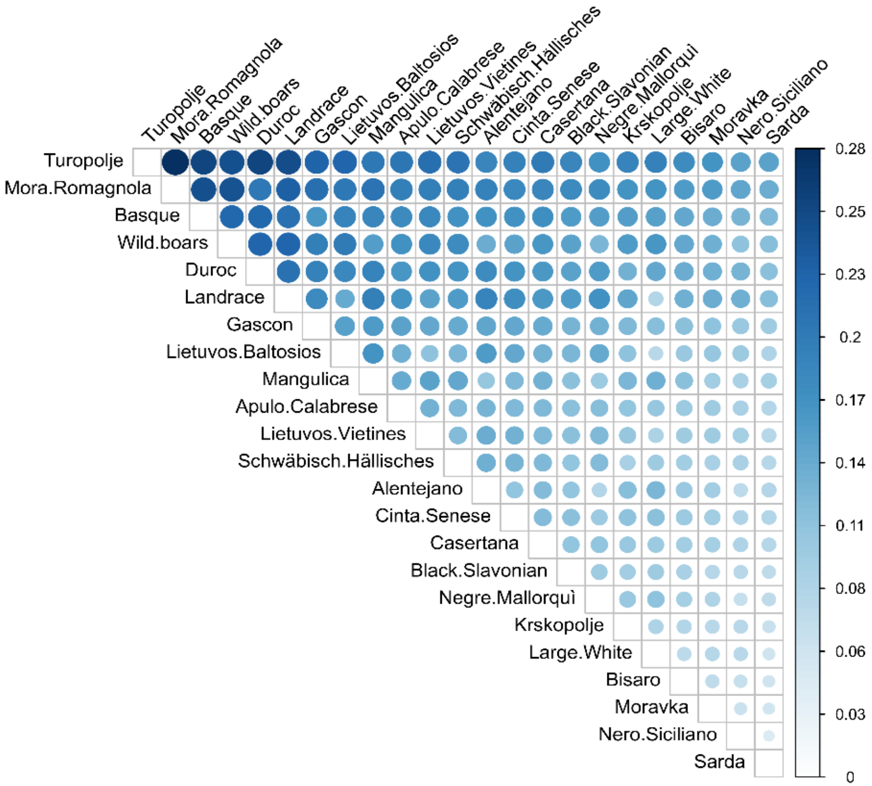
MIN correlation

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

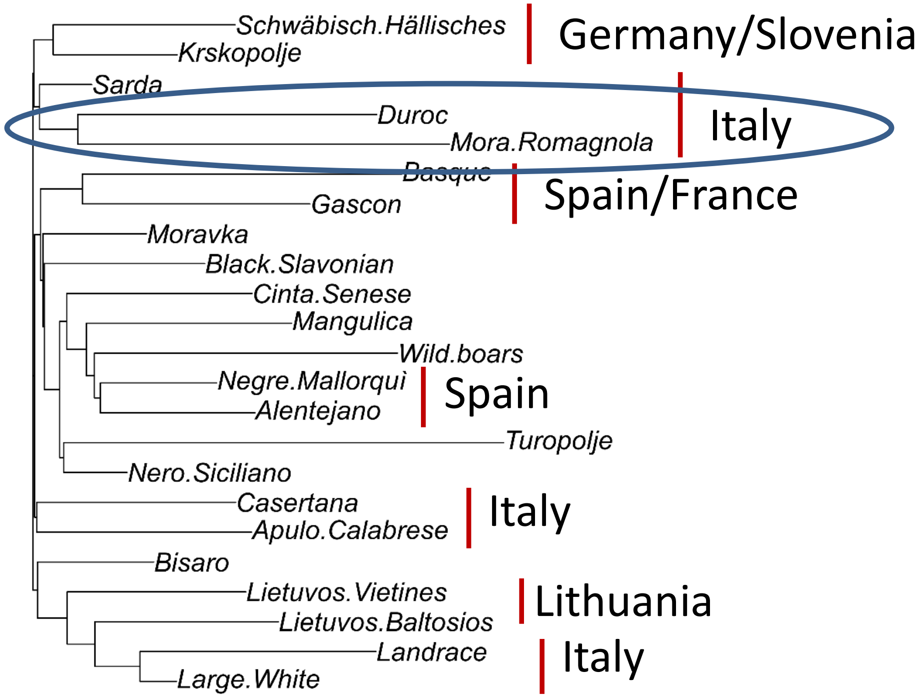
Whole genome sequencing data

Genetic similarity between populations

Heatmap plot of Fst distances



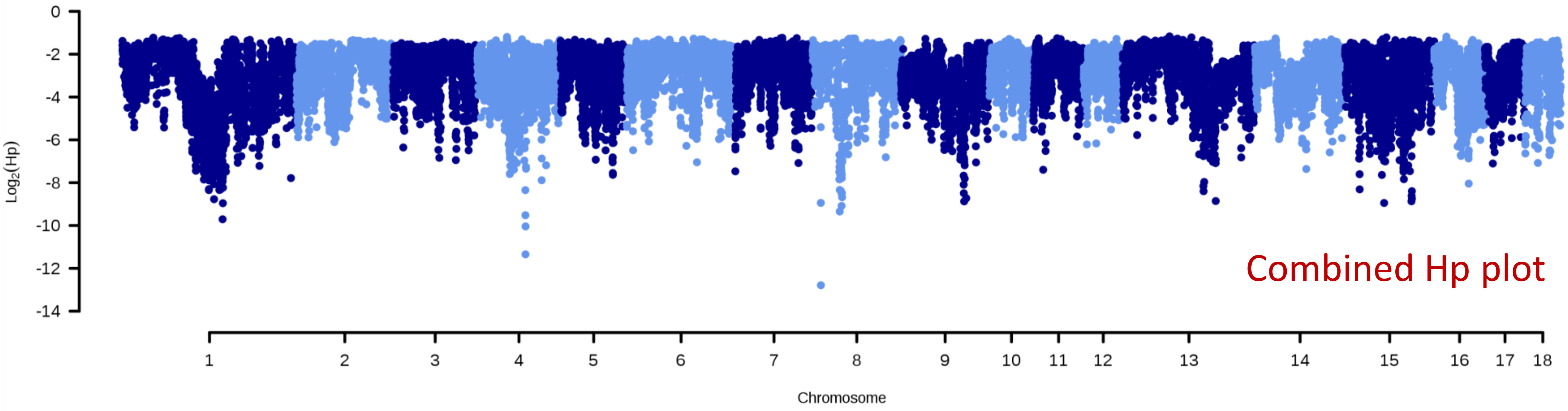
Fst based Neighbour-Joining tree



1 Pooled heterozygosity

Identified genome windows

- A total of 276 outliers windows identified across the 23 populations
- 68 genome windows shared among two or more populations.
- 9 genome windows were shared by four to seven populations.



Combined Hp plot

Genome windows, suggestive for selective sweeps, shared by four or more pig breeds

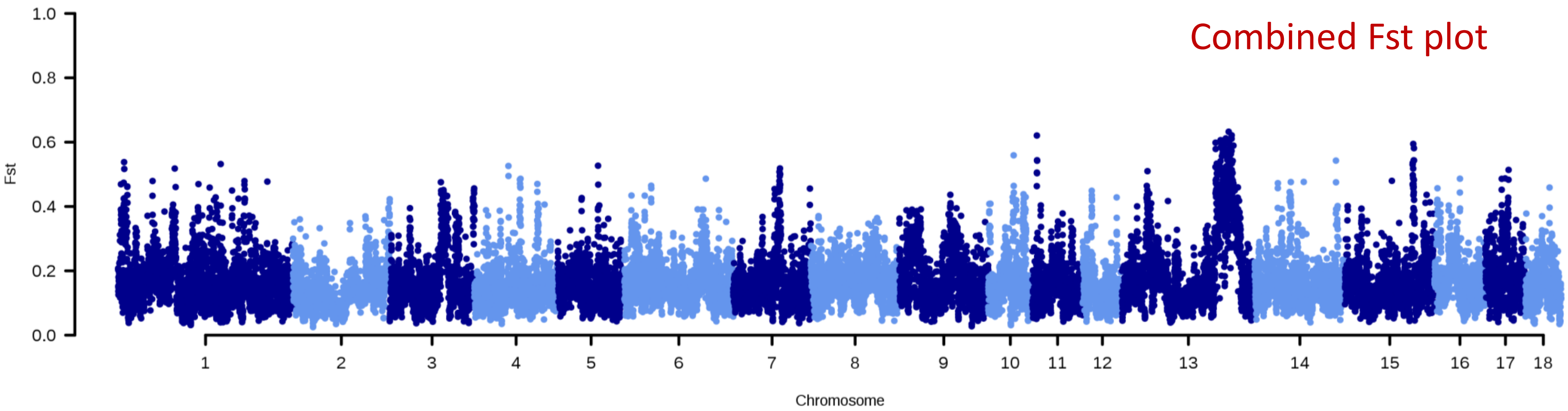
Genome Windows (SSC:start-end)*	Breeds	No. of SNPs	Annotated genes (±200-kb)*
1:170,300,001:170,400,001	Apulo Calabrese, Casertana Krskopolje, Negre Mallorquì	829	na
4:42,900,001:43,000,001	Black Slavonian, Nero Siciliano Krskopolje, Gascon	683	TMEM67; **PDP1 ;** FAM92A; RBM12B
4:75,500,001:75,900,001	Cinta Senese, Apulo Calabrese Krskopolje, Lietuvos Baltosios, Sarda Large White, Landrace	2356	CHCHD7; SDR16C5; MOS; PENK; TMEM68; LOC100626876; TGS1; LYN; LOC106510084; **PLAG1;** XKR4
8:12,900,001:13,000001	Bisaro, Schwäbisch Hällisches, Large White, Landrace, Negre Mallorquì	1067	**NCAPG**; CAF16; FAM184B; **LCORL**
8:42,600,001:42,700,001	Bisaro, Lietuvos Baltosios, Large White, Landrace	683	LOC102162630; LOC100526059; TLL1; LOC100620475
8:45,500,001:45,600,001	Bisaro, Lietuvos Baltosios, Large White, Landrace	1482	**PDGFC** LOC102162630 LOC100526059 **MAP9** TLL1 LOC100620475

2

Fst analysis of single breeds

Fst values

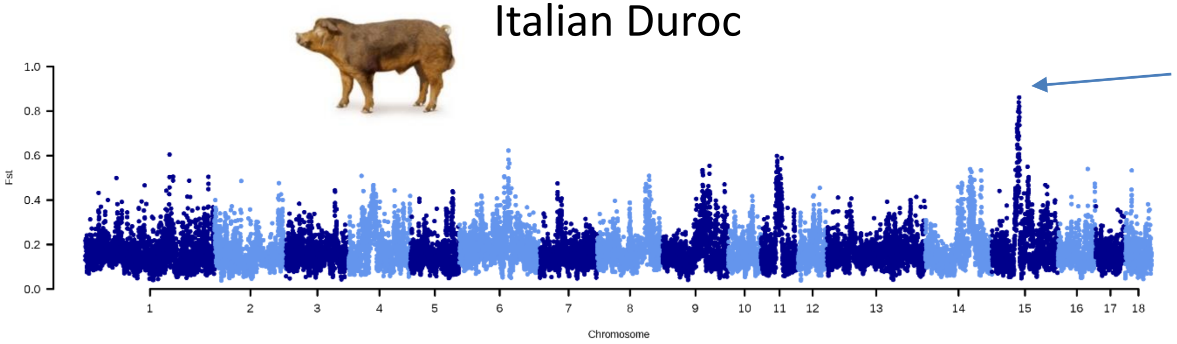
- Fst ranged from 0.0878 (Sarda) to 0.2016 (Turopolje)
- The average Fst value was 0.1347 in agreement with a previous study



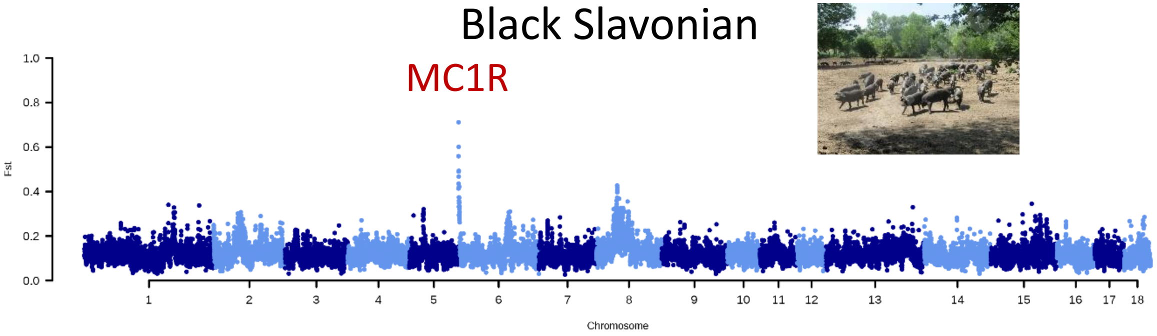
Combined Fst plot

② F_{st} analysis of single breeds

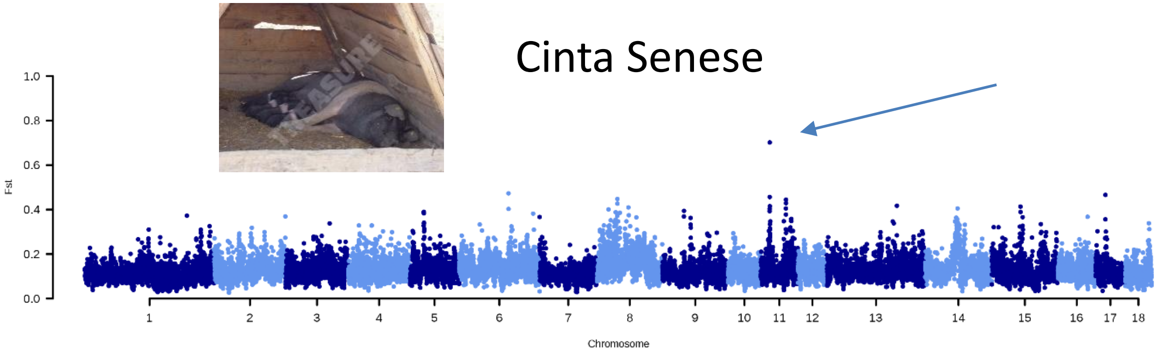
Italian Duroc



Black Slavonian



Cinta Senese



3

F_{ST} analyses of breed groups

Belted and Spotted

Cinta Senese vs All others
Cinta Senese vs solid
Cinta Senese vs Black
Cinta Senese vs spotted
Cinta Senese vs White

Belted vs all Others
Belted vs solid
Belted vs Black
Belted vs Spotted
Belted vs White

Belted non Cinta vs Cinta Senese
Belted non Cinta vs All (no Cinta Senese)
Belted non Cinta vs solid
Belted non Cinta vs Black
Belted non Cinta vs Spotted
Belted non Cinta vs White

Spotted vs Black
Spotted vs White

Colored

White vs Black
White vs Red
Gray vs Black
Gray vs White

Red vs ALL
Duroc vs Mora

Wild boar

Wild boars vs Mora Romagnola
wild Boar vs Mangalitza
wild boar vs All
wild boar vs All (no mangalitza)
Wild boar vs non commercial
Wild boar vs non commercial (no Mangalitza)
Wild boar vs commercial

Size

Medium vs Big
Small vs Medium + Big
Small vs Medium
Small vs Big

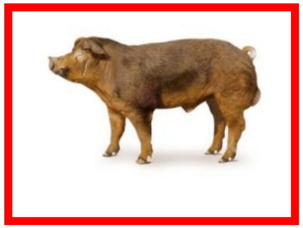
Commercial

Commercial_ITA vs All
Commercial_ITA vs
Non_Commercial_ITA

ANAS
associazione nazionale allevatori suini


Italian Large White


Italian Landrace


Italian Duroc


Mora Romagnola

Cinta Senese

Casertana

Sarda

Apulo Calabrese

Nero Siciliano

3

F_{ST} analyses of breed groups

Italian autochthonous breeds

Cinta Senese

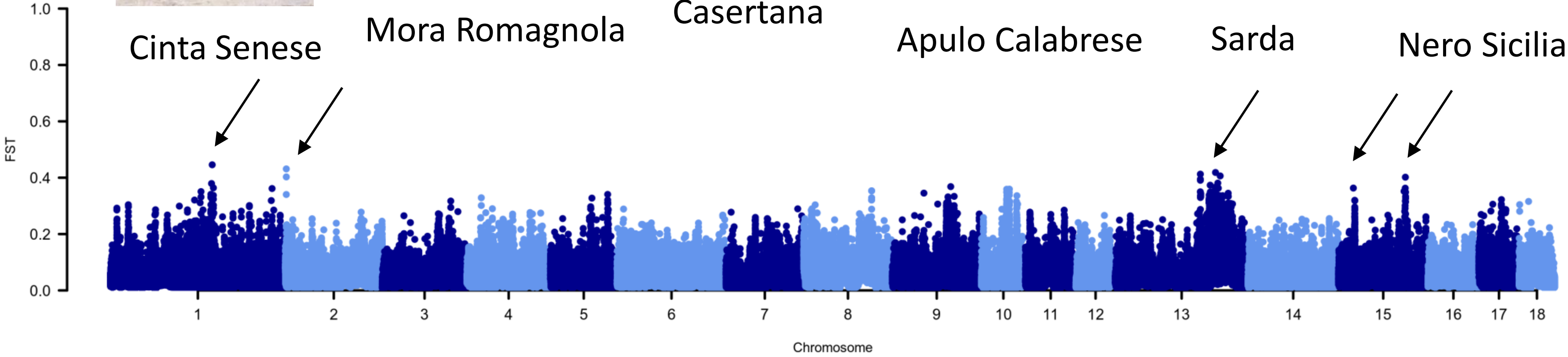
Mora Romagnola

Casertana

Apulo Calabrese

Sarda

Nero Siciliano



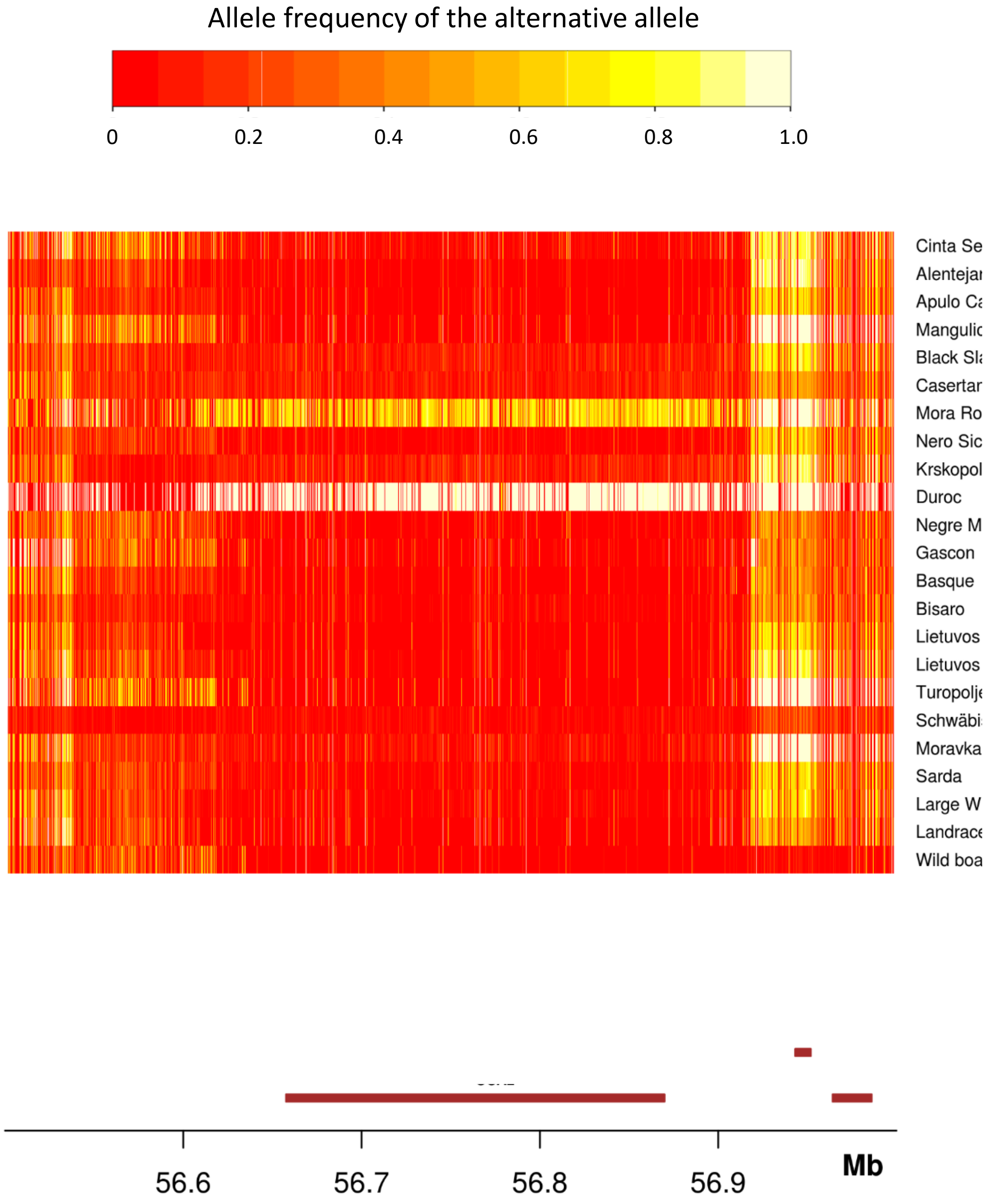
Italian cosmopolitan-derived breeds

Italian Large White

Italian Landrace

Italian Duroc

Red breeds (Italian Duroc + Mora Romagnola) vs all other breeds



Mora Romagnola

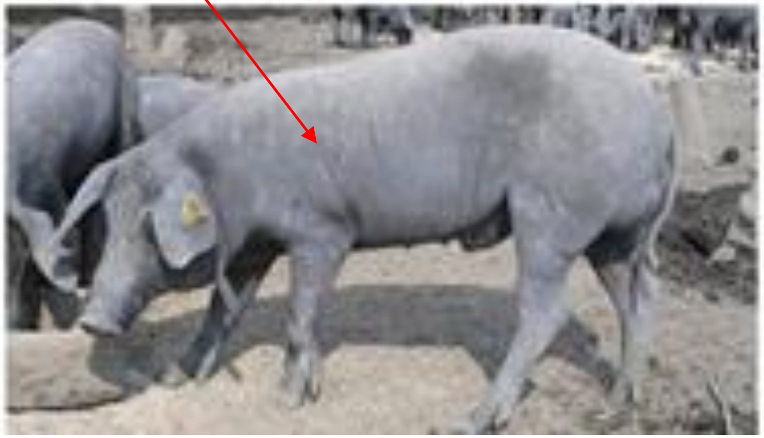
Italian Duroc

Combined SNP genotyping and whole genome sequencing data

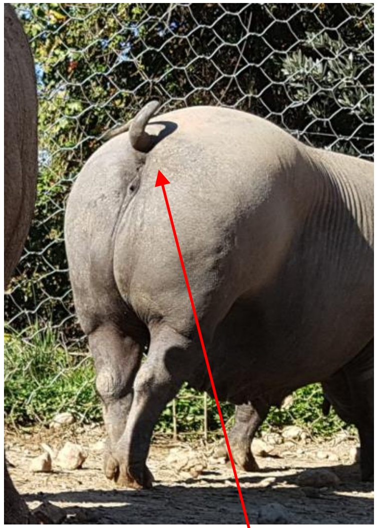

Casertana

Phenotypic heterogeneity in Casertana

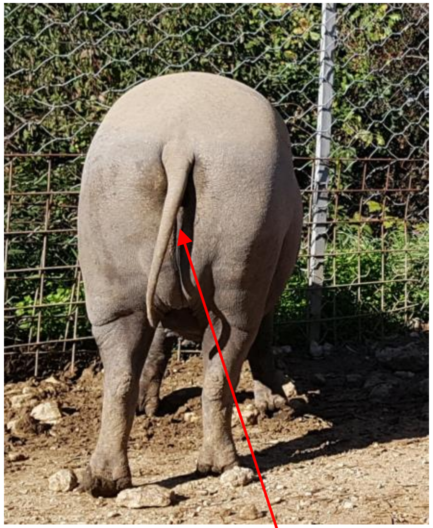
Hairless



Hairy



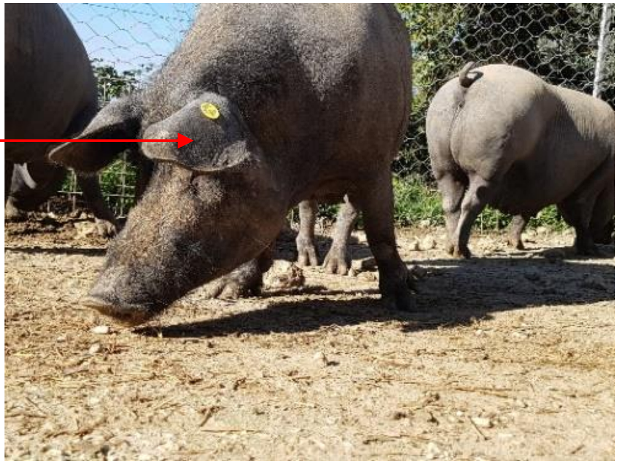
Curly tail

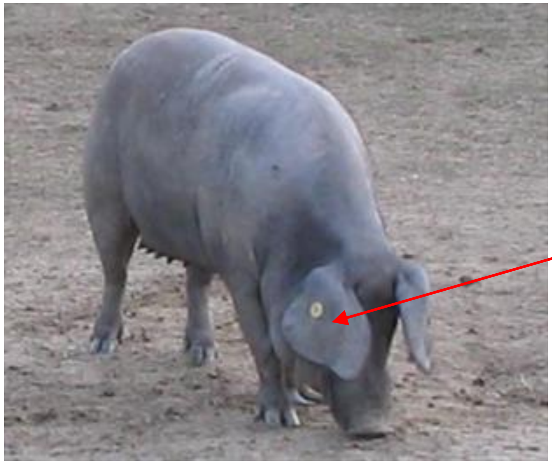


Straight tail

Phenotypic heterogeneity in Casertana

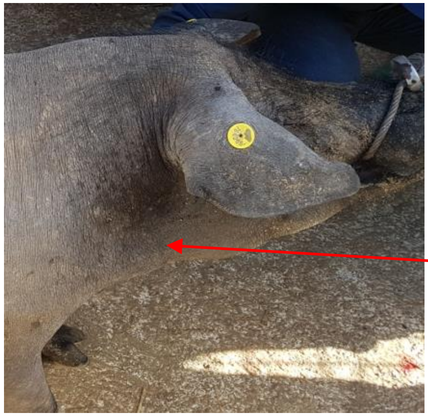
Small-forward ears





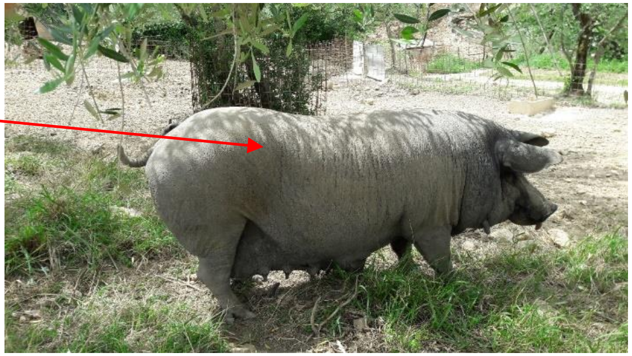
Large-floppy ears

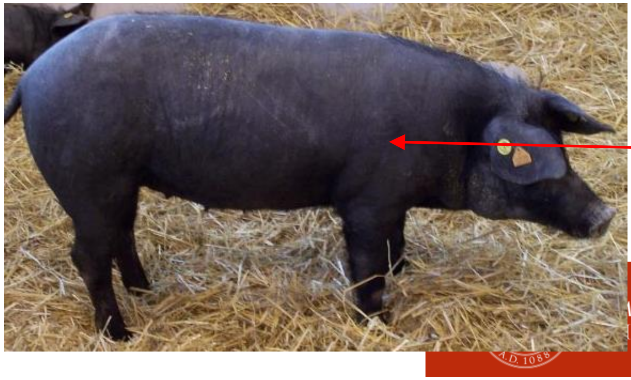
Presence of wattles



Absence of wattles

Slate-grey coat colour

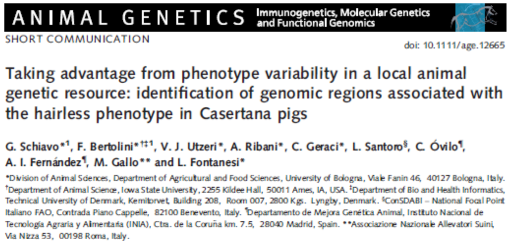




Black coat colour

SNP chip data

Genome Wide Association Study



ANIMAL GENETICS Immunogenetics, Molecular Genetics and Functional Genomics

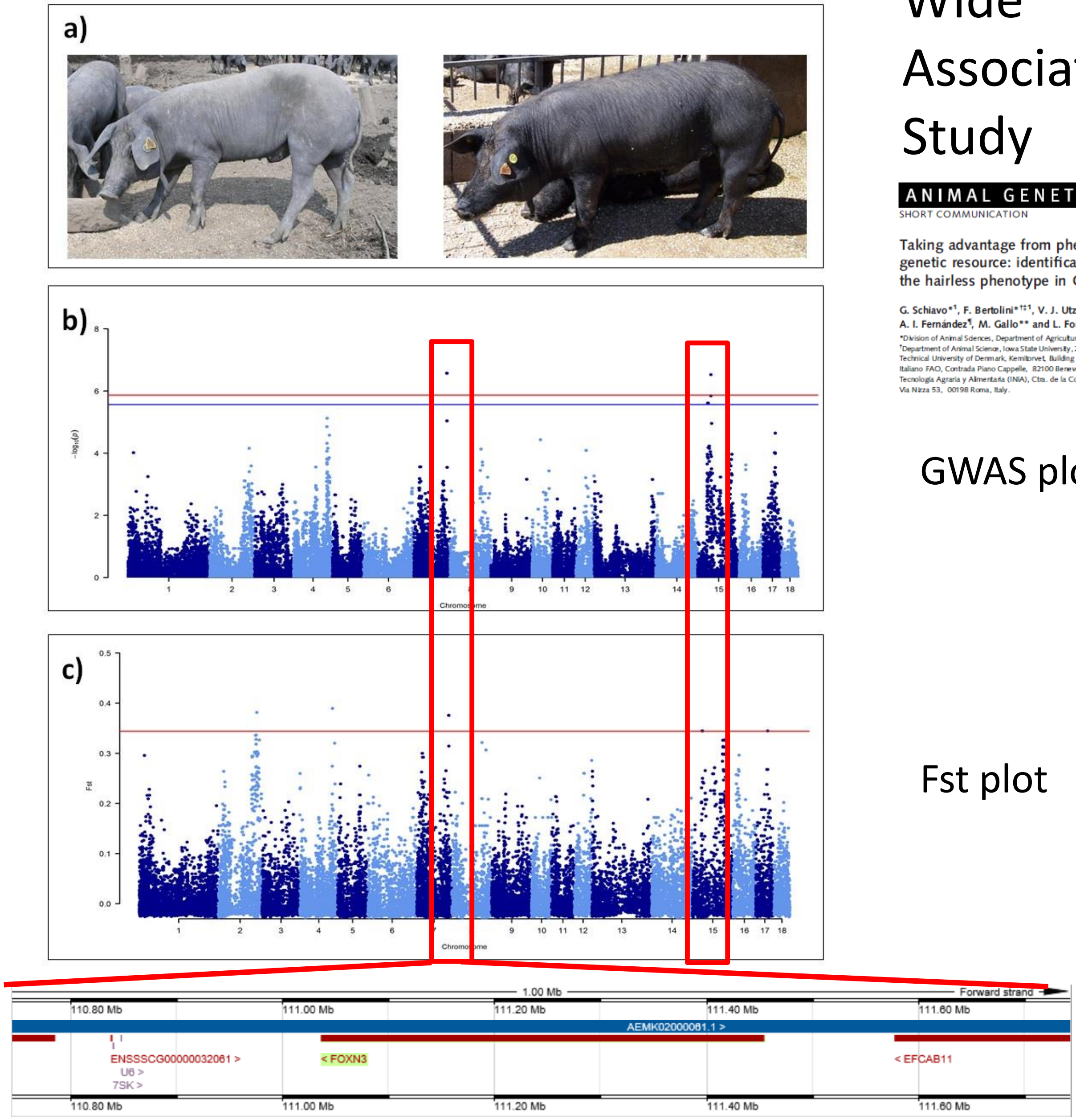
SHORT COMMUNICATION

doi: 10.1111/age.12665

Taking advantage from phenotype variability in a local animal genetic resource: identification of genomic regions associated with the hairless phenotype in Casertana pigs

G. Schiavo*[1], F. Bertolini*[†‡1], V. J. Utzeri*, A. Ribani*, C. Geraci*, L. Santoro[§], C. Óvilo[¶], A. I. Fernández[¶], M. Gallo** and L. Fontanesi*

*Division of Animal Sciences, Department of Agricultural and Food Sciences, University of Bologna, Viale Fanin 46, 40127 Bologna, Italy. [†]Department of Animal Science, Iowa State University, 2255 Kildee Hall, 50011 Ames, IA, USA. [‡]Department of Bio and Health Informatics, Technical University of Denmark, Kemitorvet, Building 208, Room 007, 2800 Kgs. Lyngby, Denmark. [§]ConSDABI – National Focal Point Italiano FAO, Contrada Piano Cappelle, 82100 Benevento, Italy. [¶]Departamento de Mejora Genética Animal, Instituto Nacional de Tecnología Agraria y Alimentaria (INIA), Ctra. de la Coruña km. 7.5, 28040 Madrid, Spain. **Associazione Nazionale Allevatori Suini, Via Nizza 53, 00198 Roma, Italy.



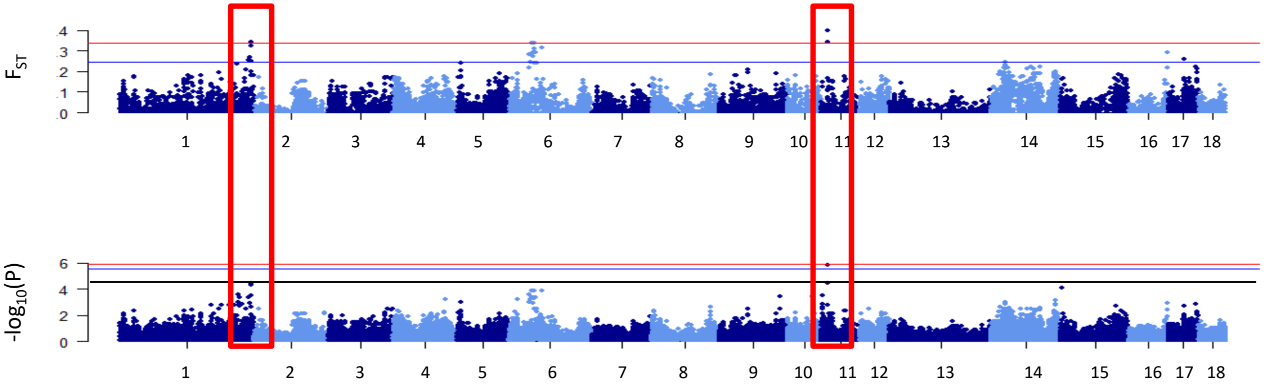
GWAS plot

Fst plot

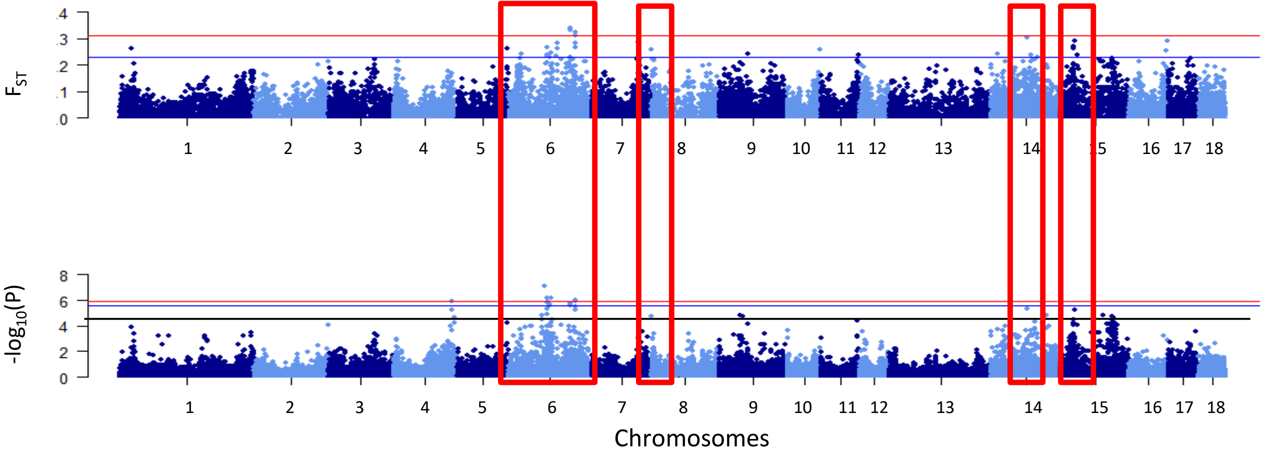
ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

SNP chip data

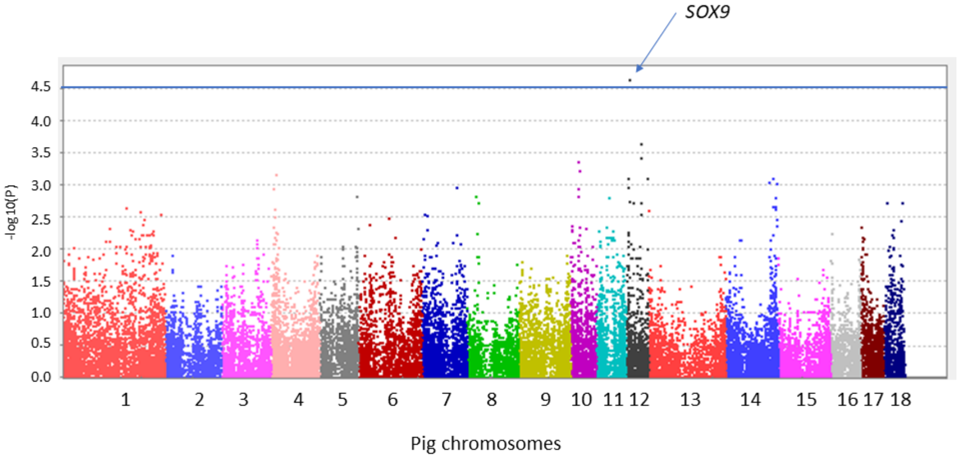
Presence/absence of wattles

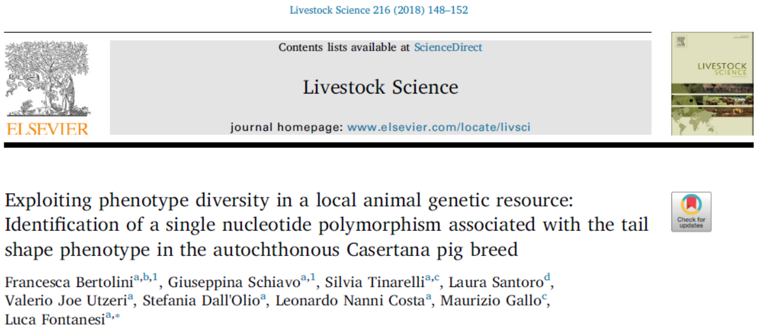


Slate-grey vs black coat colour



SNP chip data




Livestock Science 216 (2018) 148–152

ELSEVIER

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Exploiting phenotype diversity in a local animal genetic resource: Identification of a single nucleotide polymorphism associated with the tail shape phenotype in the autochthonous Casertana pig breed

Francesca Bertolini[a,b,1], Giuseppina Schiavo[a,1], Silvia Tinarelli[a,c], Laura Santoro[d], Valerio Joe Utzeri[a], Stefania Dall'Olio[a], Leonardo Nanni Costa[a], Maurizio Gallo[c], Luca Fontanesi[a,*]

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

Conclusions

- We have characterized genetic variability in European pig breeds
- The large number of comparisons made it possible to detect hundreds of selection signatures
- We are moving from the description to the use of genetic variability in pigs

TREASURE

Diversity of local pig breeds and production systems for
high quality traditional products and sustainable pork chains

Funded by European Union
Horizon 2020
Grant agreement No 634476

SUIS
Suinicoltura
Italiana
Sostenibile

ALMA MATER STUDIORUM A.D. 1088
UNIVERSITÀ DI BOLOGNA

INIA
Instituto Nacional de Investigación
y Tecnología Agraria y Alimentaria

ANAS
associazione nazionale allevatori suini

The TREASURE Consortium

University of Novi Sad

Earlham Institute

ConSDABI - National Focal Point Italiano FAO

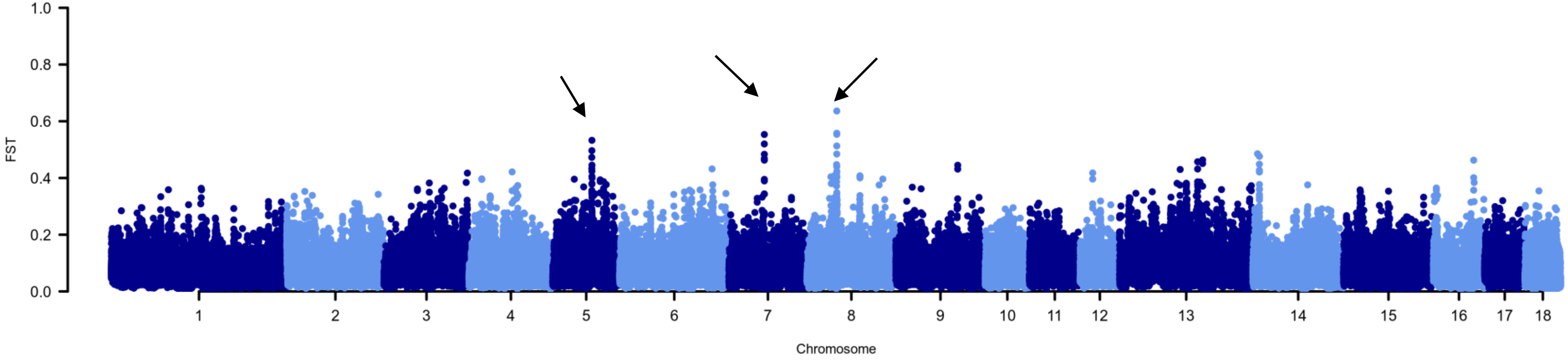
EASTBALKAN PIG
RAISED BY Kuljo Kulen

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

3

F_{ST} analyses of breed groups

All other normal haired breeds



Long-curly haired

Mangulica

Turopolje

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA