63rd Annual Meeting
EAAP 2012
August 27th - 31st, 2012

Bratislava, SLOVAKIA

Discovering the unique values of indigenous livestock populations

The opportunities of genomics

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Why to conserve livestock diversity?

To respond to the production needs, consumer and market demands (short term and long term, predicted and unknown)

Across populations (breeds) perspective

For sustainable continuous breeding improvement – productivity traits (predicted and unknown)

Within populations (breeds) perspective

Strategies

In situ and ex situ in vivo and in vitro

Maximize diversity conserved at minimum cost (monetary and practical)

Livestock conservation genetics

Neutral genetic markers

Microsatellite and AFLP

Conservation of neutral diversity

Characterization

↓

Prioritization

Genetic parameters of relevance to livestock conservation genetics

Populations (ind.) diversity – populations structure – admixture – inbreeding – differentiation and relationships – migration rates – loss of genetic diversity – pedigree/parentages

Livestock conservation genomics

"Full" genome information (HD SNPs chips, full genome sequences)

Conservation of adaptive diversity

Characterization

↓

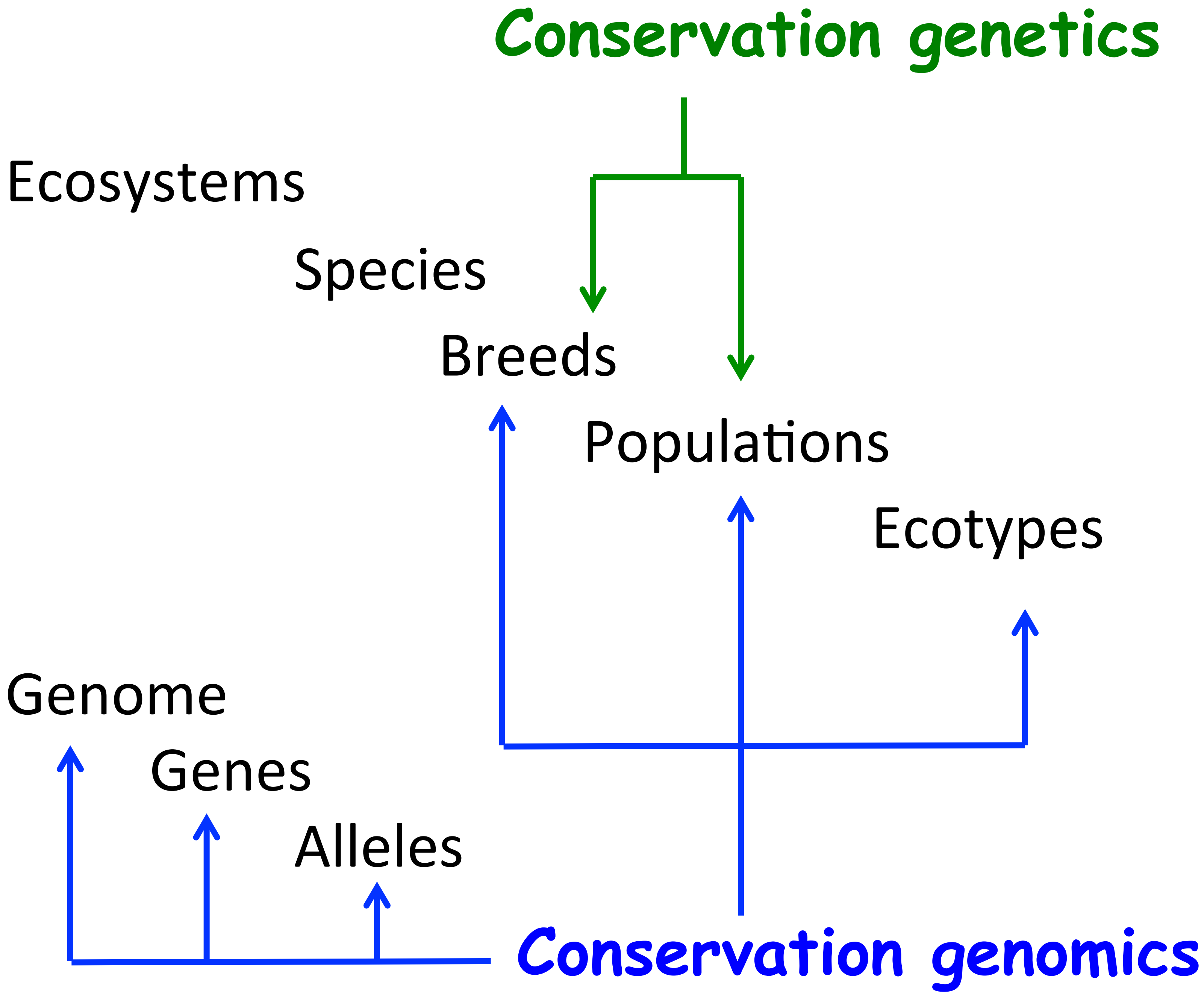
Prioritization

Genetic parameters of relevance to livestock conservation genomics

Conservation genetics

Populations (ind.) diversity – populations structure – admixture – inbreeding – differentiation and relationships – migration rates – loss of genetic diversity – pedigree/parentages

Same as conservation genetics but higher accuracy, greater power + adaptive diversity (e.g. transcriptome variation), introgression, local adaptation – livestock landscape genomics, molecular basis of inbreeding and outbreeding depression, population history, "genomics selection" – ind. adaptive value to a specific environment



Conservation genetics

Ecosystems

Species

Breeds

Populations

Ecotypes

Genome

Genes

Alleles

Conservation genomics

Conservation genomics

Opening the door to all (incl. non-descript livestock populations) to utilisation and conservation (ecotypes, rather than breeds/population)

Developed and 'developing' world

Through the unravelling of the genetic control of adaptation

Discovering the unique values of indigenous livestock – Genomics

European introgression in African indigenous cattle - EASZ

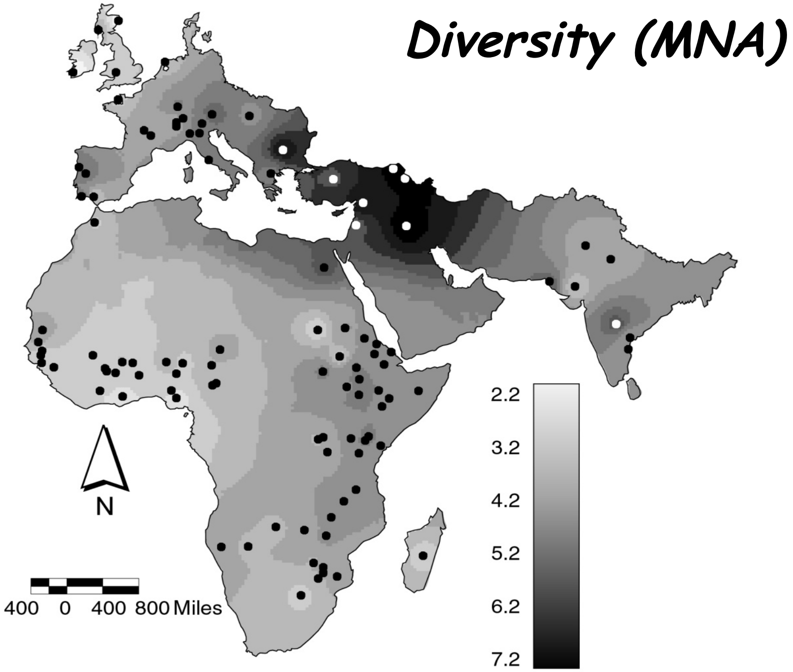
Mary Ndila PhD

East African shorthorn Zebu

Non – descript
Outbreed – Panmictic
'Human selection' –
Natural selection

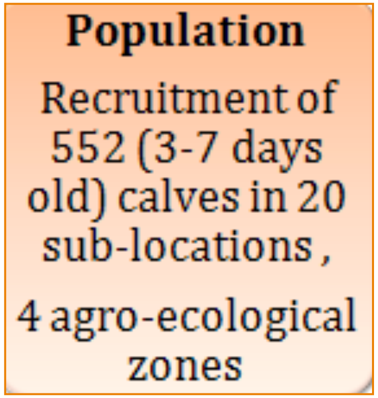
Origin and migration routes of domestic cattle in Africa

Modified from Hanotte et al. Science 2002


Diversity (MNA)

Freeman et al. to Animal Genetics 2005

Genetic objectives



Population

Recruitment of 552 (3-7 days old) calves in 20 sub-locations ,

4 agro-ecological zones

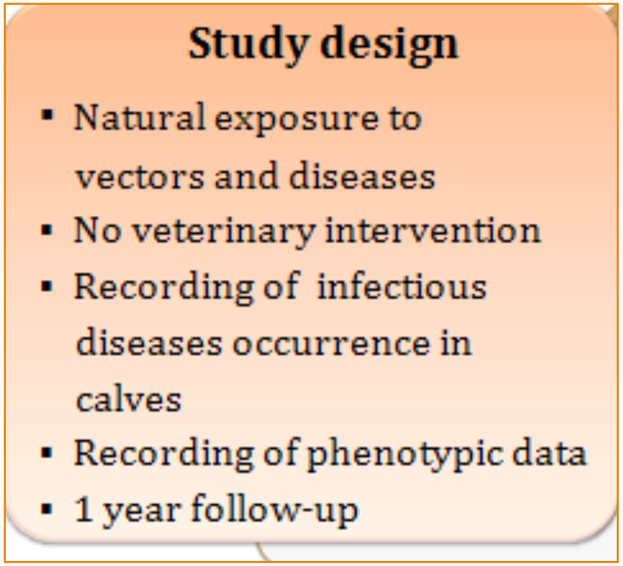
To identify genetic signature of selection of adaptation to environmental challenges (infectious parasitic diseases)

Segregating within the population

Good cow – Bad cow hypothesis

Fixed within the populations

Good population – Bad population hypothesis



Study design

- Natural exposure to vectors and diseases
- No veterinary intervention
- Recording of infectious diseases occurrence in calves
- Recording of phenotypic data
- 1 year follow-up

Recruitment criteria: Pure calves – No exotic blood (farmer interviews)

Genomics tools - data

illumina

BovineSNP50 Genotyping BeadChip

Featuring 54,609 evenly spaced SNP probes that span the bovine genome.

Vr.1

550 Calves + references breeds

BovineHD Genotyping BeadChip

More than 777,000 SNPs that deliver the densest coverage available for the bovine genome

120 pure Calves + references breeds

-

Full genome sequencing : 10 calves (dead/alive)

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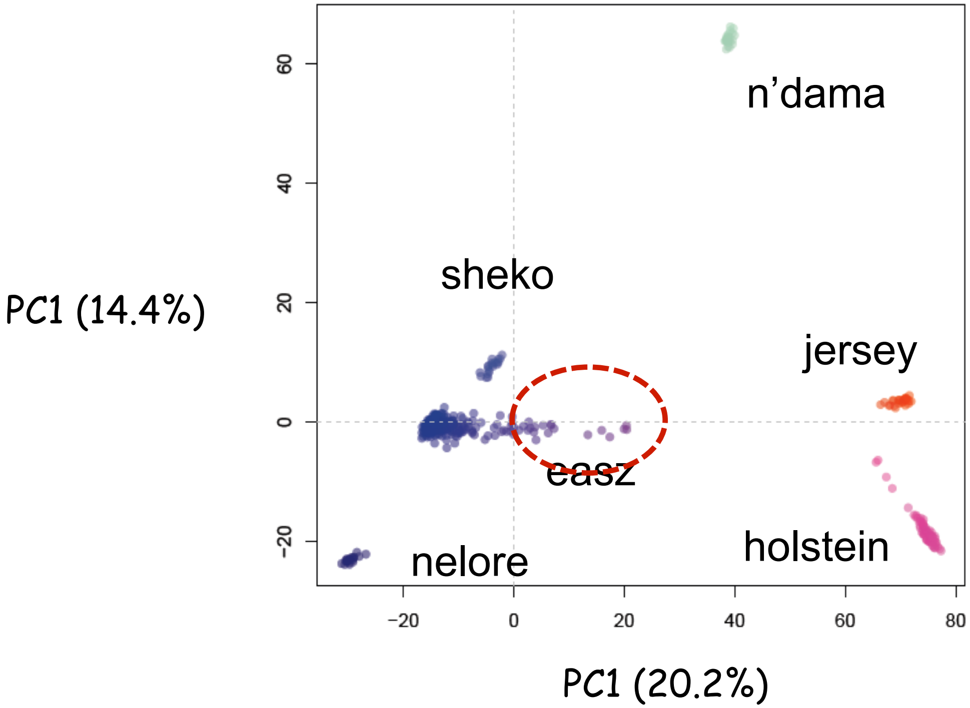
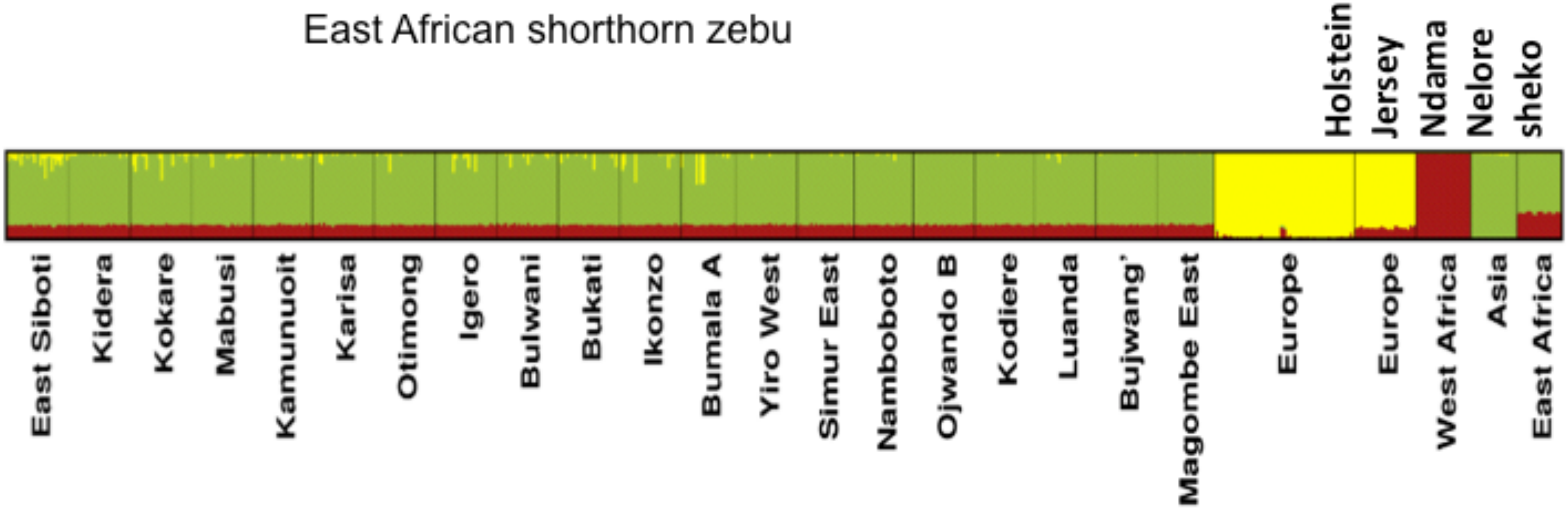
EAST AFRICAN SHORTHORN ZEBU: GENOME ADMIXTURE

Data quality control

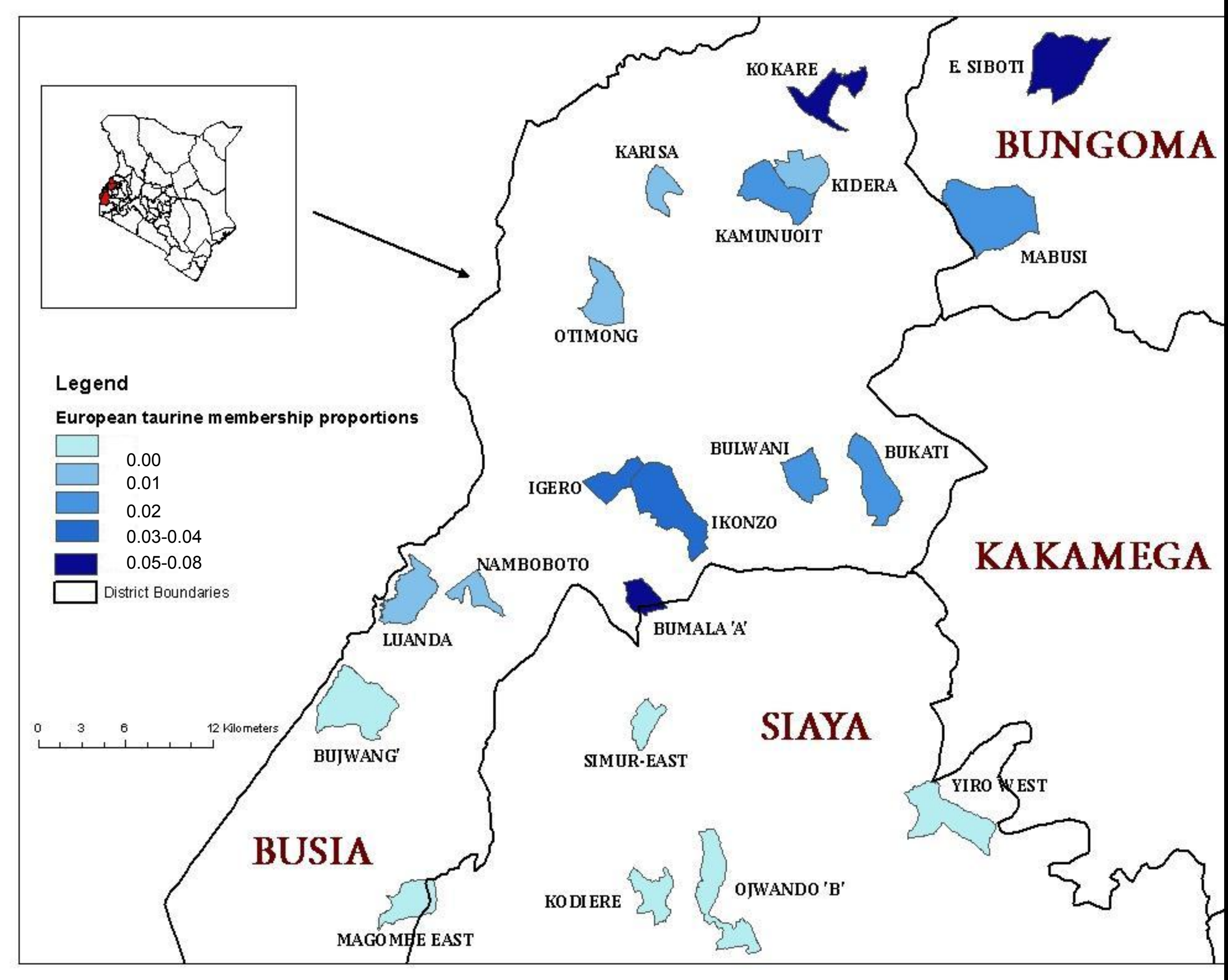
Samples and markers with call rate ≤ 90% excluded

48,903 markers and 550 calves used

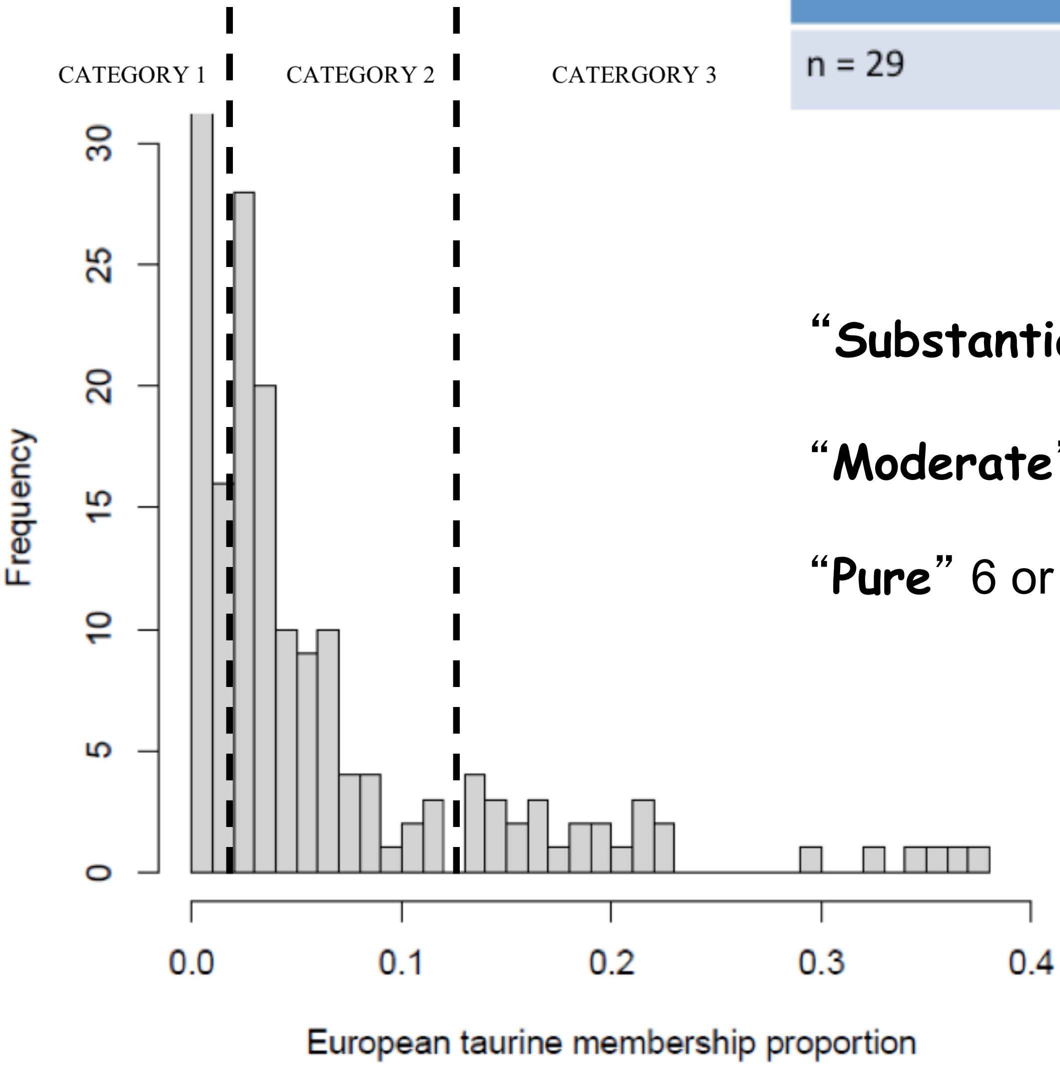




Average proportions of European taurine introgression within sub-locations



Substantial European introgression > 12.5%	Moderate European introgression 1.56% < x ≤12.5%	Pure from European introgression x ≤ 1.56%
n = 29	n = 94	n = 425

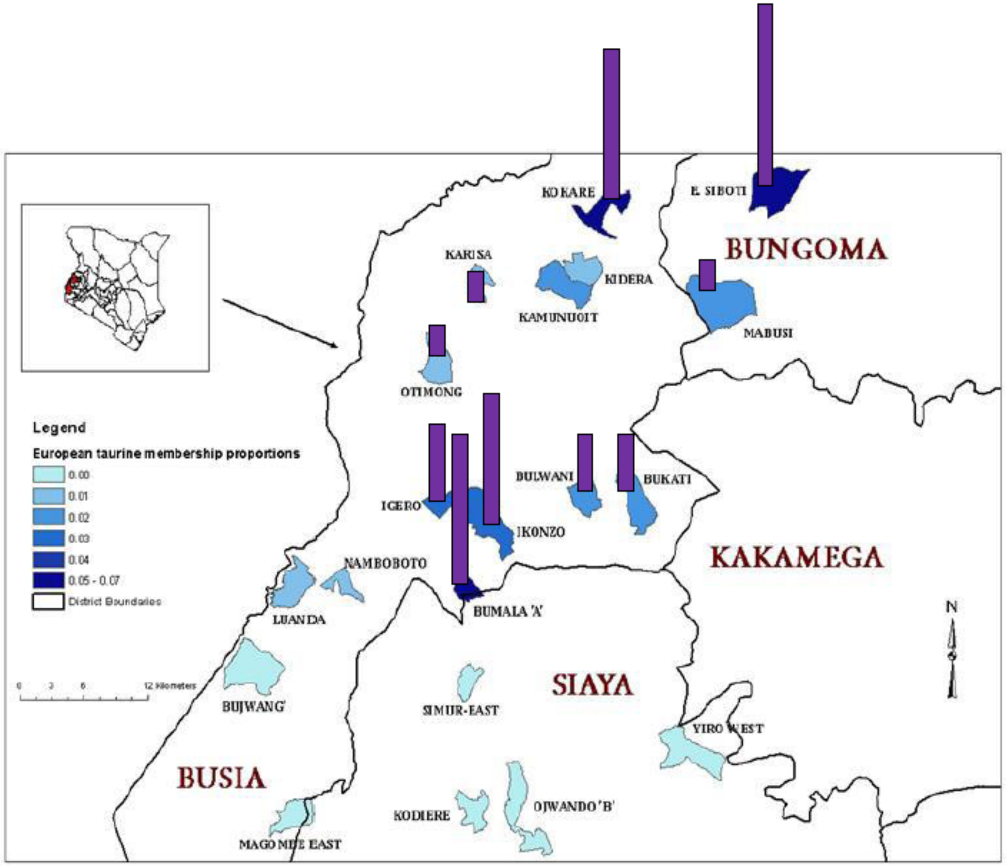


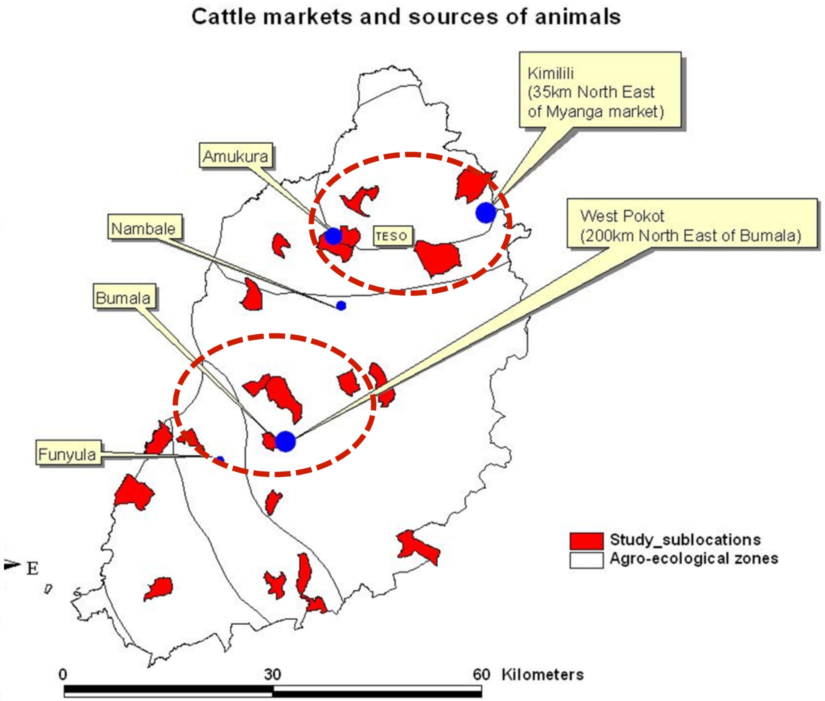
“**Substantial**”; 2-3 ET generations ago

“**Moderate**” 6 or more ET generations ago

“**Pure**” 6 or more ET generations ago

Origin of the European taurine introgression "substantial" > 12.5%




Cattle markets and sources of animals

Livestock markets

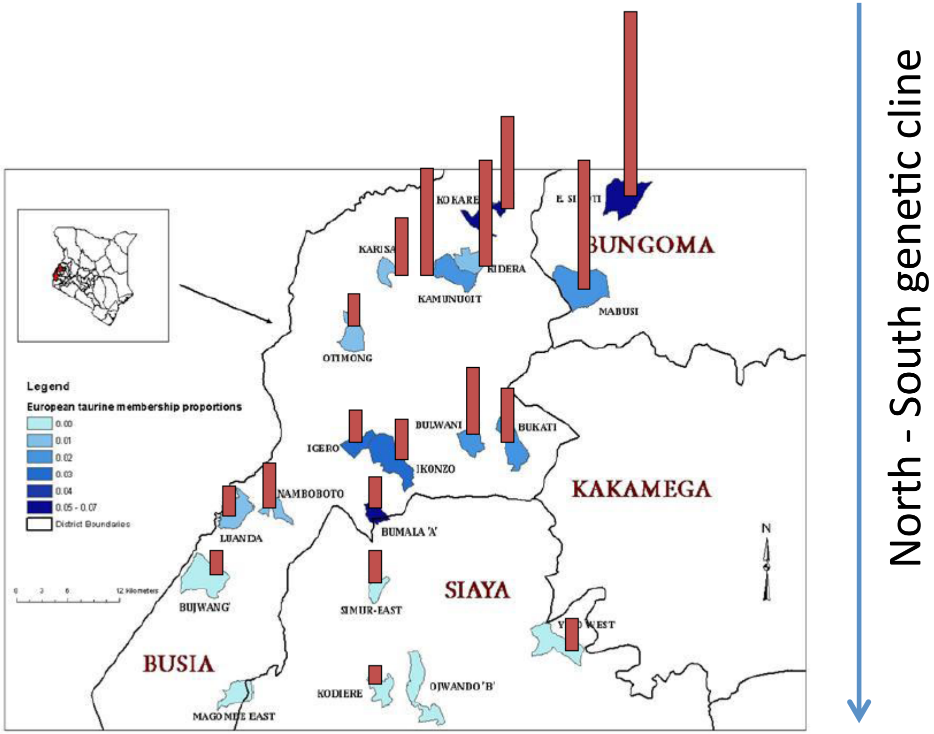
Origin of the European taurine introgression "moderate' , 1.56% < x ,12.5%

Breeding programmes

- Rural Development Project (1979-1989) - Kitinda dairy Bungoma
- Kenyan Dairy project ~1980s
- Kenya – Finland Livestock Development Programme (1991-2003)

Services offered:
AI upon request, in-calf heifers, bull schemes
Breeds used: Holstein, Aryshire, Jersey, Guernsey

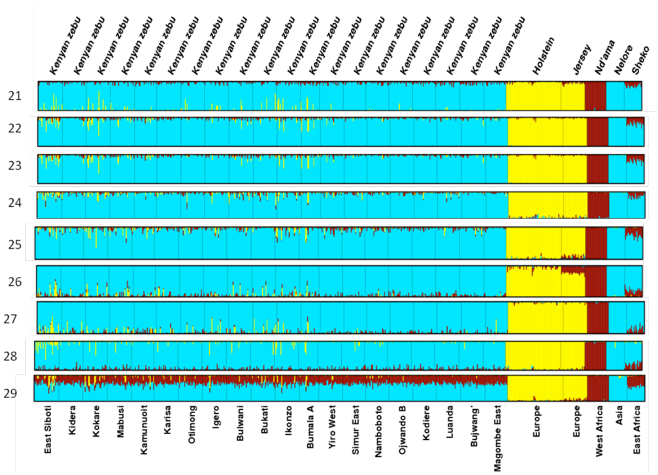
Impacts
Offspring boom 84,749
Bull schemes 138,904



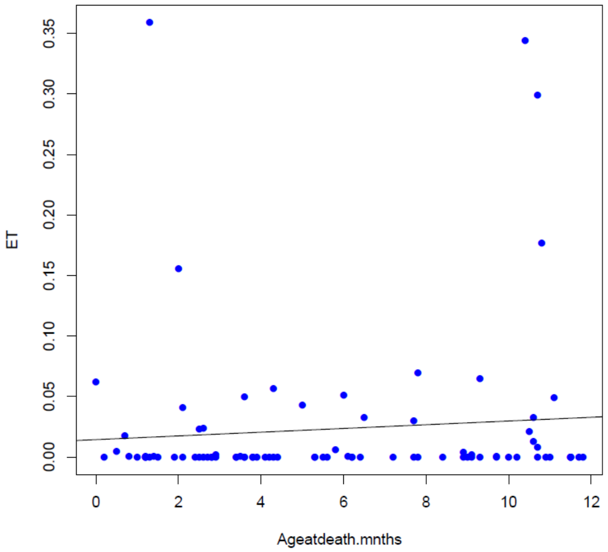
$r = 0.82 \quad P < 0.0005$

Moderate 4-5 generations 1.56% >x ≤ 12.5%

Selection against the European taurine genotypes?



Proportion of ET background similar across chromosomes



No significant relationships between age of dead and proportion of ET background

Origin	r²	P-values
African taurine	0.0089	0.380
European taurine	0.0074	0.426
Asian zebu	0.0104	0.344

Membership proportions	Mean Alive	Mean Dead	*P* values
Alive AZ vrs Dead AZ	0.8278176	0.8221591	0.436
Alive AT vrs Dead AT	0.1534418	0.1544659	0.3677
Alive ET vrs Dead ET	0.01864835	0.02357471	0.5082

No significant difference between the mean proportion of genetic background and the dead or alive phenotype

Discovering the unique value of indigenous livestock

Unravelling the genetic control of adaptation

Egg shell coloration

Dave Wragg PhD

Chicken diversity

Commercial breed

Village chicken

Traditional fancy
chicken breed

Blue egg

Mendelian inheritance
Dominant
Pigment – Biliverdin

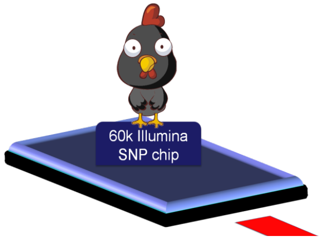
Traditional breeds

Araucana

Village chicken

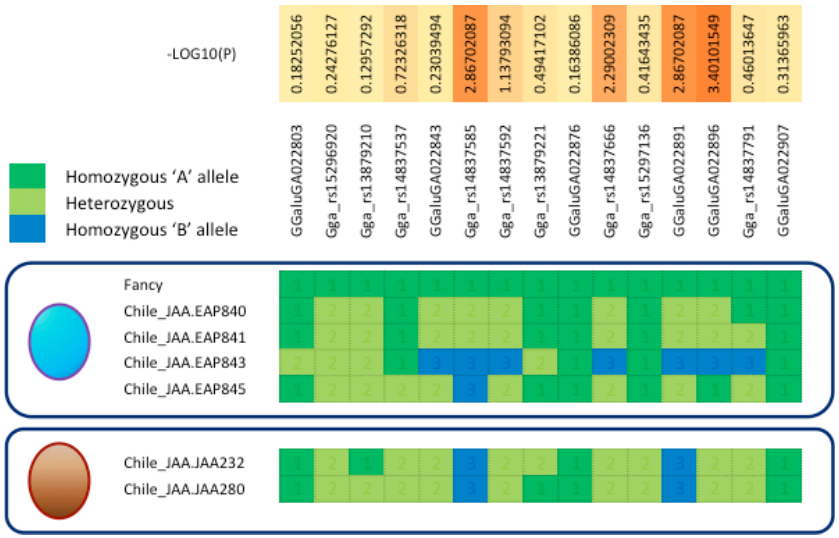
Chile

Strategy: Exploiting the diversity Genomics tools



A. Across fancy breed mapping (case - control)

B. Haplotype analysis village chicken



- 🟩 Homozygous 'A' allele
- 🟩 Heterozygous
- 🟦 Homozygous 'B' allele

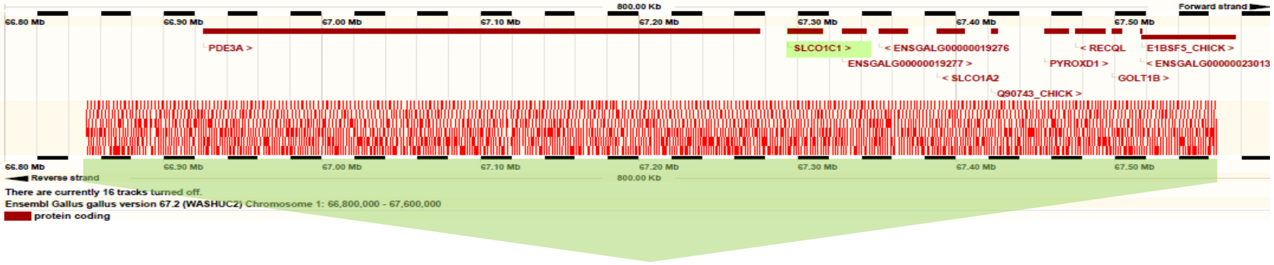
	GGaluGA022803	Gga_rs15296920	Gga_rs13879210	Gga_rs14837537	GGaluGA022843	Gga_rs14837585	Gga_rs14837592	Gga_rs13879221	GGaluGA022876	Gga_rs14837666	Gga_rs15297136	GGaluGA022891	GGaluGA022896	Gga_rs14837791	GGaluGA022907
-LOG10(P)	0.18252056	0.24276127	0.12957292	0.72326318	0.23039494	2.86702087	1.13793094	0.49417102	0.16386086	2.29002309	0.41643435	2.86702087	3.40101549	0.46013647	0.31365963
Fancy	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Chile_JAA.EAP840	1	2	2	1	2	2	2	1	1	2	1	2	2	1	1
Chile_JAA.EAP841	1	2	2	1	2	2	2	1	1	2	1	2	2	2	1
Chile_JAA.EAP843	2	2	2	1	3	3	3	2	1	3	1	3	3	3	1
Chile_JAA.EAP845	1	2	2	2	2	3	2	1	1	2	1	2	1	2	1
Chile_JAA.JAA232	1	2	1	2	2	3	2	2	1	2	2	3	2	2	1
Chile_JAA.JAA280	1	2	2	2	2	3	2	1	1	2	2	3	2	2	1

C. TARGETED SEQUENCING

Target enrichment
(Agilent SureSelect XT 4Mb)

Paired-end sequencing
(2 x 100bp Illumina)

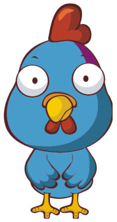
Baits for blue egg
~700Kb



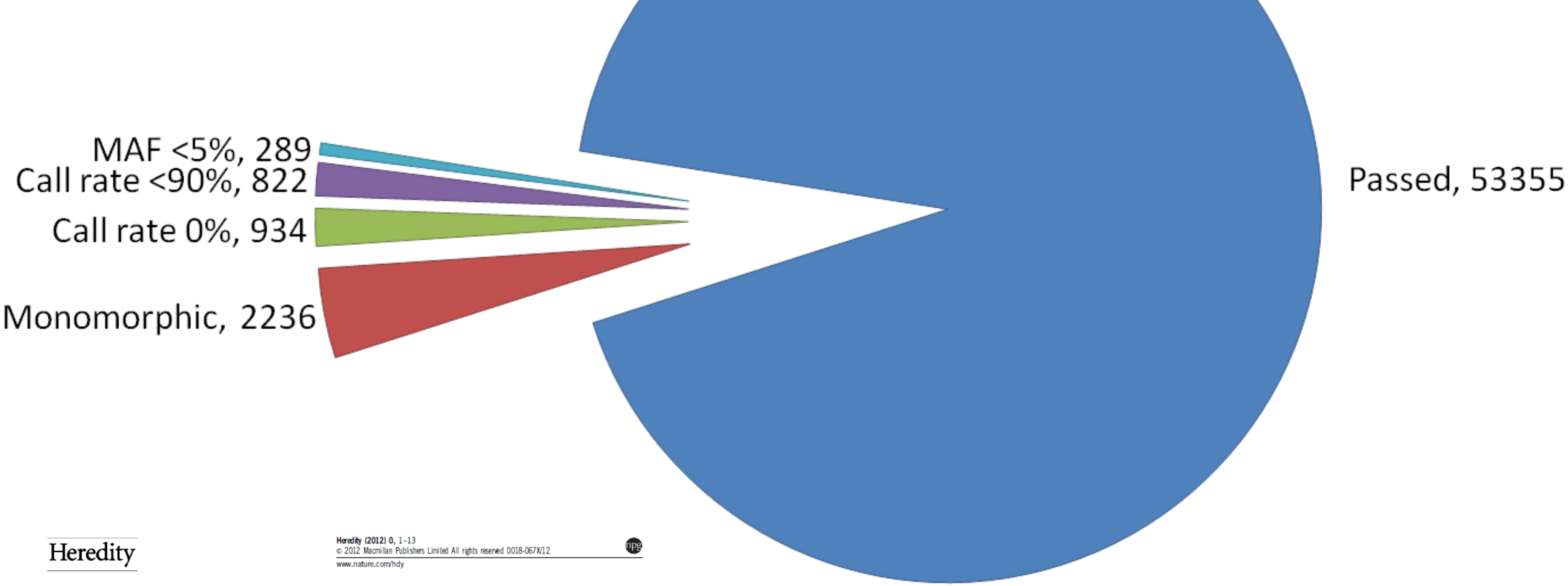
There are currently 16 tracks turned off.
Ensembl Gallus gallus version 67.2 (WASHUC2) Chromosome 1: 66,800,000 - 67,600,000
protein coding

Village chickens
17 Kenya (1 region)
24 Ethiopia (4 regions)
10 Chile (2 regions)

Fancy Breeds
79 birds (34 breeds)



Background: Pruning information



Heredity

Heredity (2012) 0, 1–13
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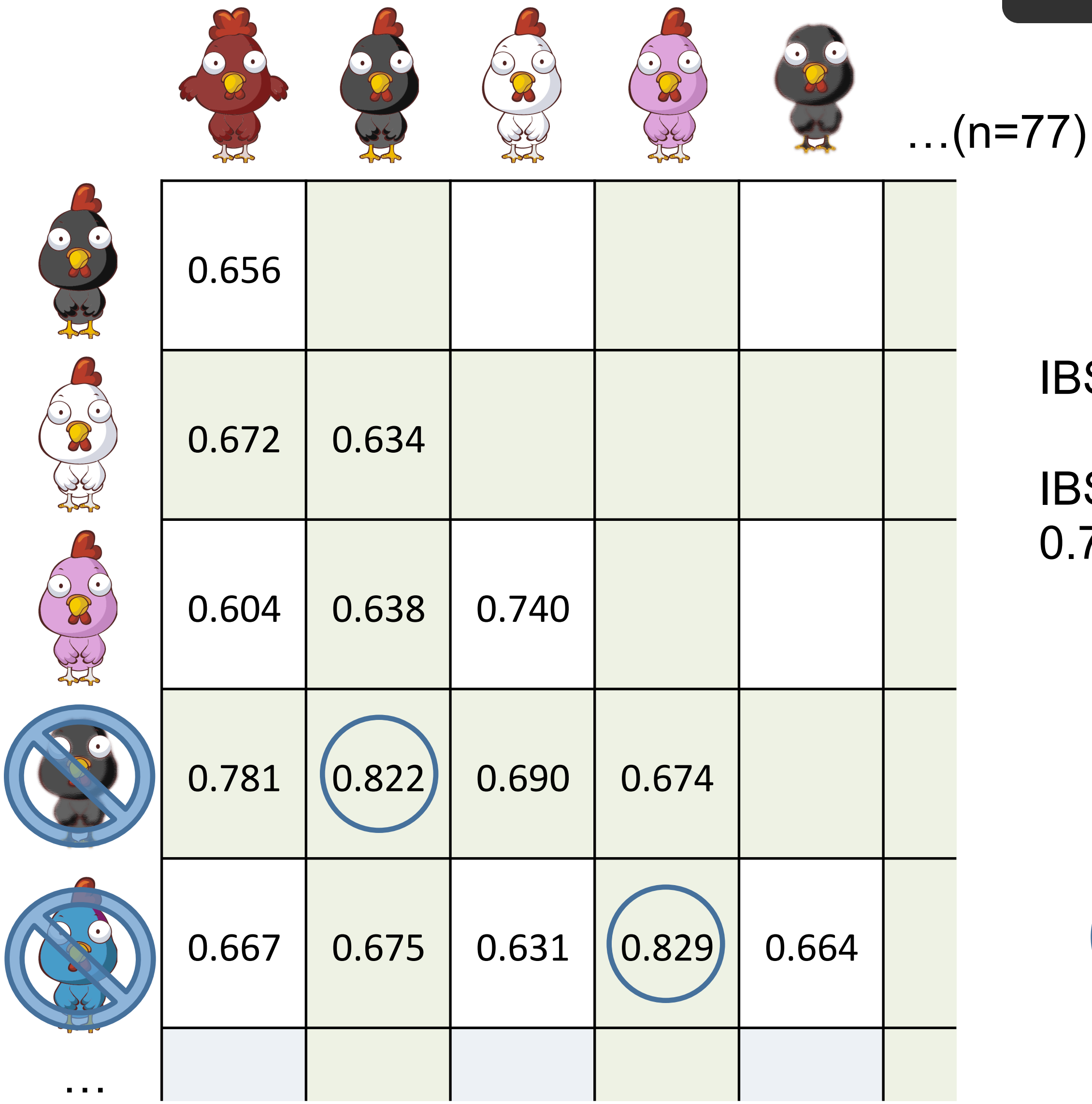
ORIGINAL ARTICLE

Analysis of genome-wide structure, diversity and fine mapping of Mendelian traits in traditional and village chickens

D Wragg[1], JM Mwacharo[1], JA Alcalde[2], PM Hocking[3] and O Hanotte[1]

Identity 'crisis'



…(n=77)

	0.656				
	0.672	0.634			
	0.604	0.638	0.740		
	0.781	0.822	0.690	0.674	
	0.667	0.675	0.631	0.829	0.664
…					

Identity-by-state (IBS) matrix

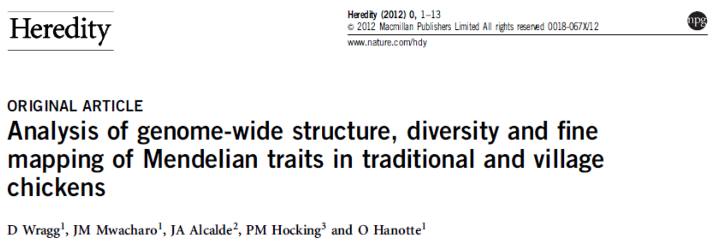
IBS μ = 0.7, σ = 0.03

IBS threhsold = μ + 3σ = 0.79

= 57/79 = 72.2%

= 22/79 = 27.8%

Haplotype blocks



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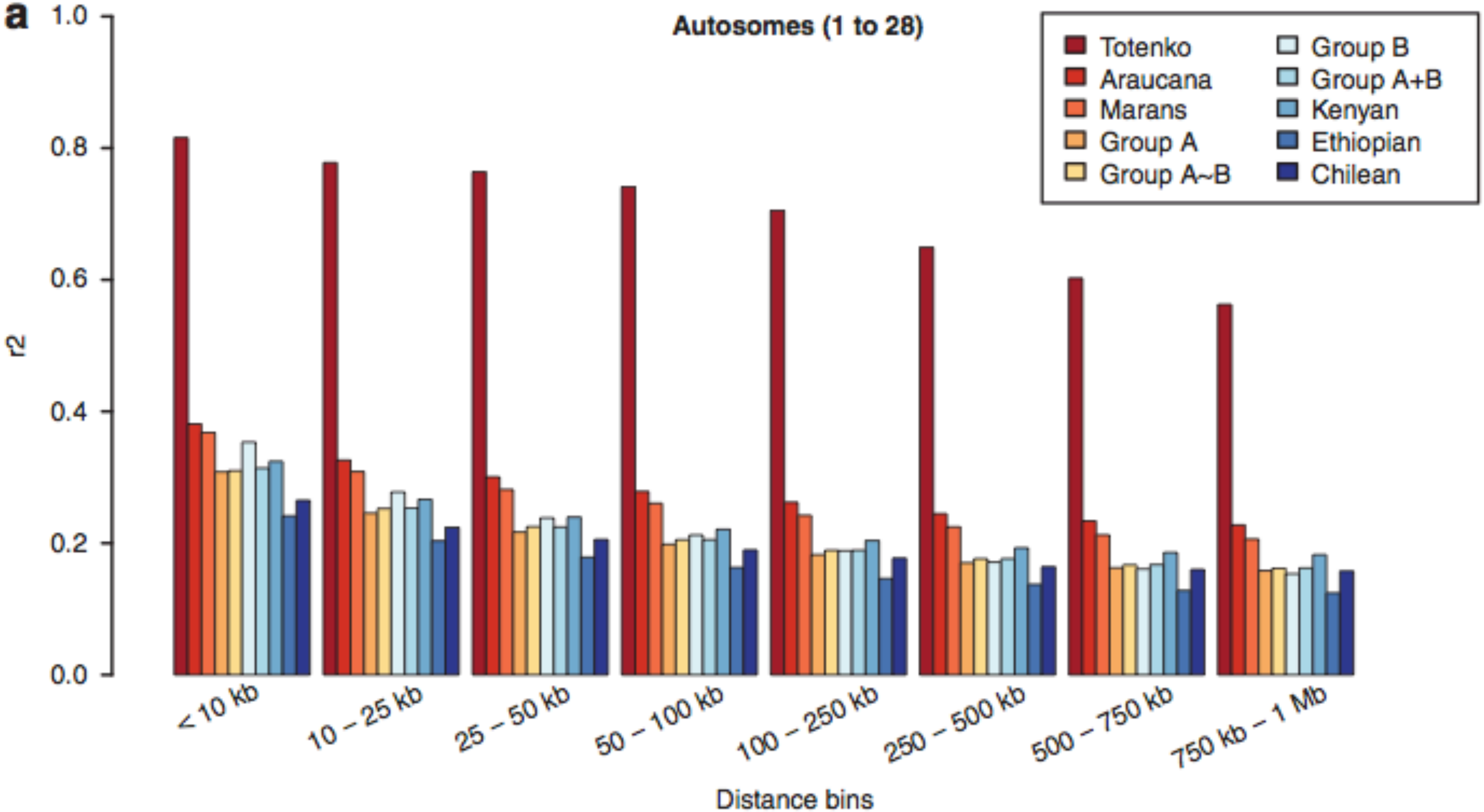
		Fancy breeds (n=40)	**Village Chickens (n=40)**
Mean haplotype block size (kb)	**Autosomes (1 to 28)**	11.87	11.14
	Macro-chromosomes (1 to 5)	12.61	12.80
	Micro-chromosomes (11 to 28)	9.37	9.14

				Shared blocks
Number of haplotype blocks observed per block size bin	**<10 kb**	395	380	184
	10-25 kb	342	278	87
	25-50 kb	65	58	13
	50-100 kb	58	53	7
	100-250 kb	61	63	5
	250-500 kb	11	26	3
	>500 kb	7	6	1

Ancestral *Gallus sp.* ?

Mean binned recombination rates (r2), Fancy and village chicken



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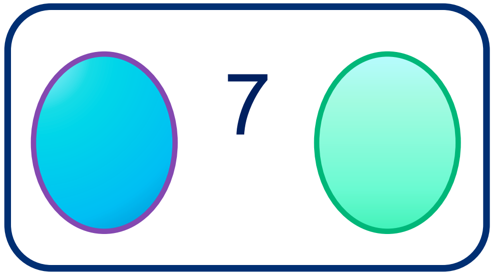

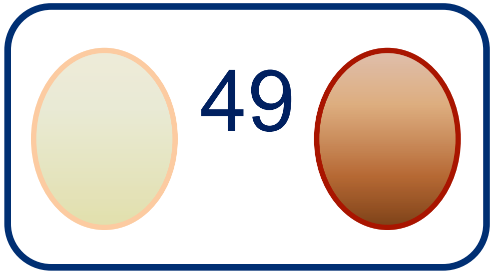

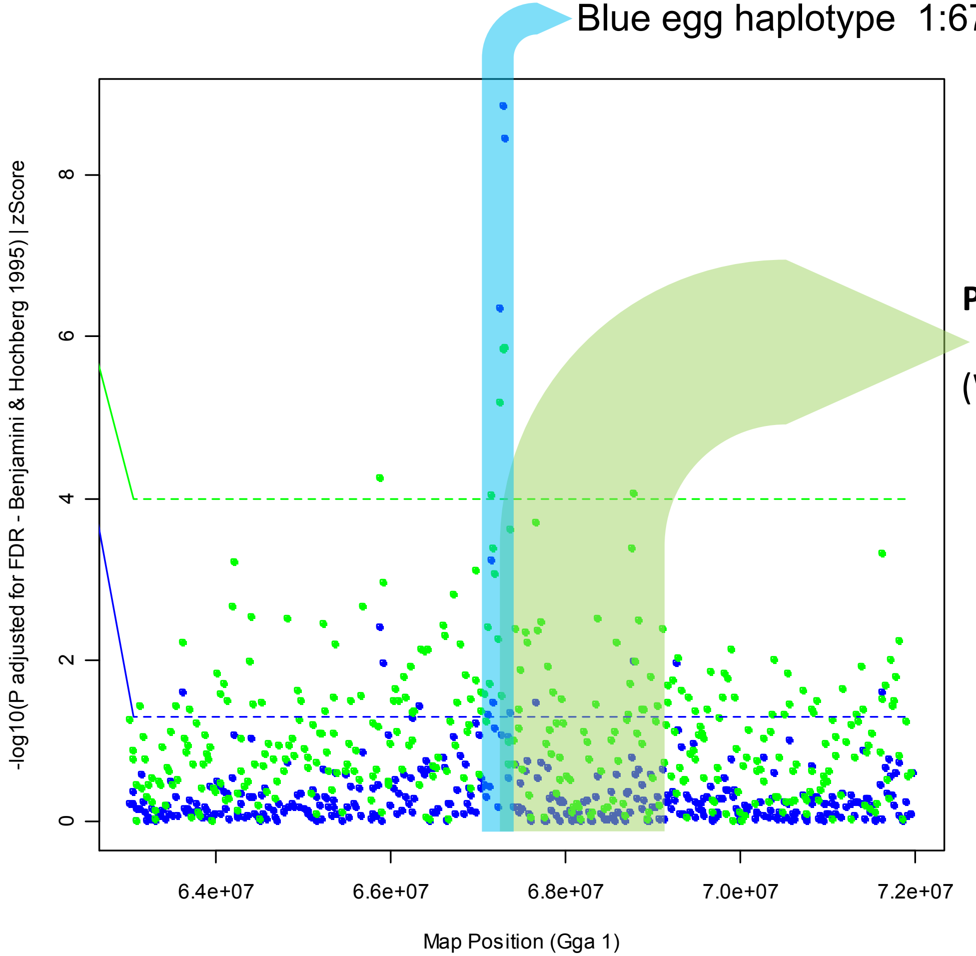

Blue egg haplotype 1:67-67.4 Mb

Previous mapping
1:67.3-69.1 Mb
(Wang *et al*. 2010)

CASE – CONTROL ASSOCIATION STUDIES ≈ 300 Kb

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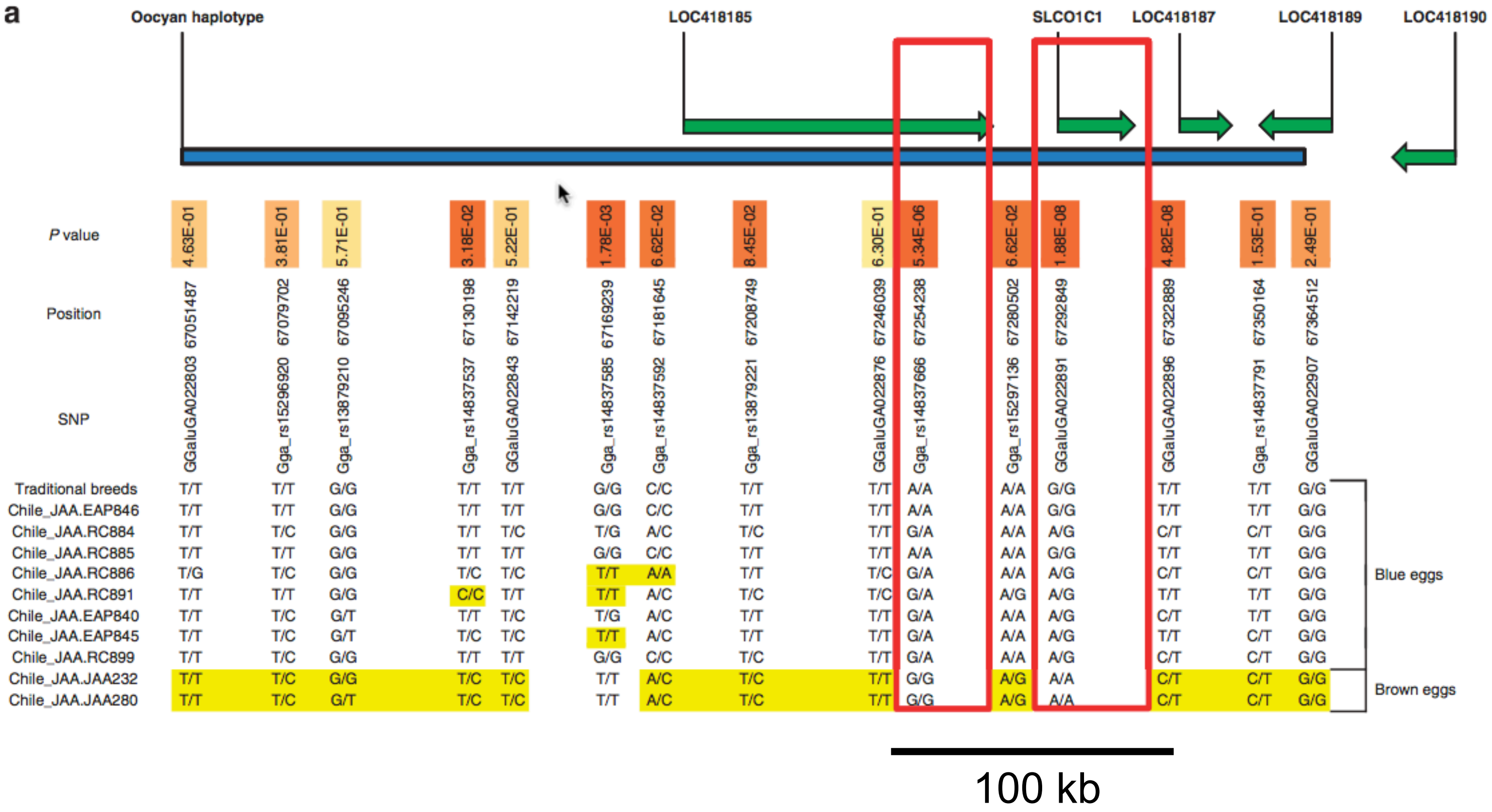
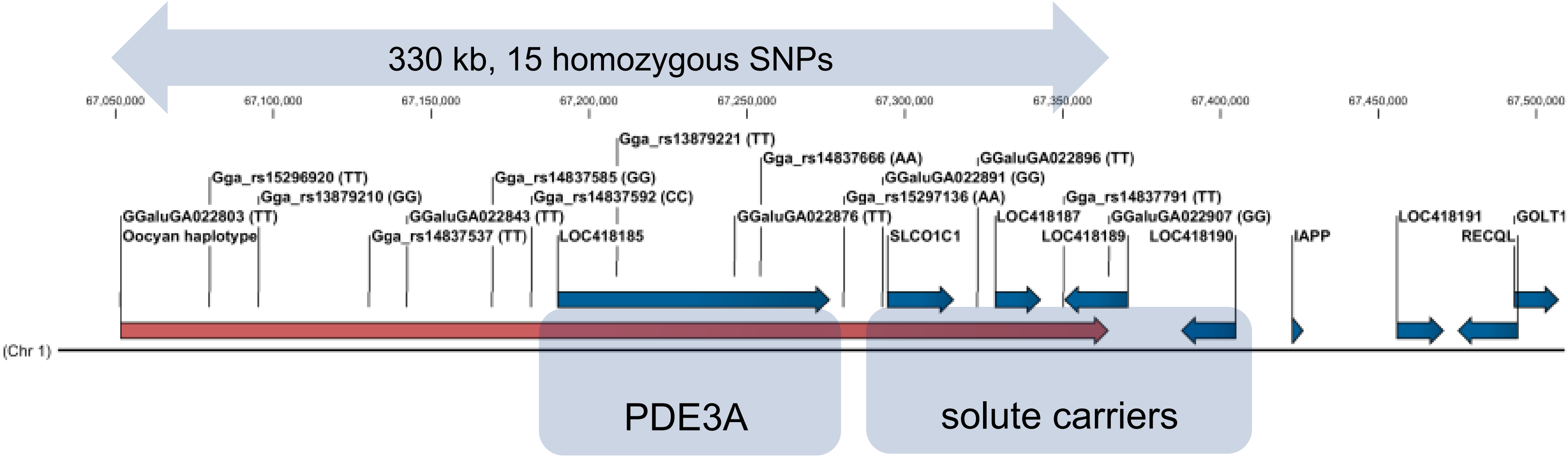


P value	4.63E-01	3.81E-01	5.71E-01	3.18E-02	5.22E-01	1.78E-03	6.62E-02	8.45E-02	6.30E-01	5.34E-06	6.62E-02	1.88E-08	4.82E-08	1.53E-01	2.49E-01	
Position	67051487	67079702	67095246	67130198	67142219	67169239	67181645	67208749	67246039	67254238	67280502	67292849	67322889	67350164	67364512	
SNP	GGaluGA022803	Gga_rs15296920	Gga_rs13879210	Gga_rs14837537	GGaluGA022843	Gga_rs14837585	Gga_rs14837592	Gga_rs13879221	GGaluGA022876	Gga_rs14837666	Gga_rs15297136	GGaluGA022891	GGaluGA022896	Gga_rs14837791	GGaluGA022907	
Traditional breeds	T/T	T/T	G/G	T/T	T/T	G/G	C/C	T/T	T/T	A/A	A/A	G/G	T/T	T/T	G/G	Blue eggs
Chile_JAA.EAP846	T/T	T/T	G/G	T/T	T/T	G/G	C/C	T/T	T/T	A/A	A/A	G/G	T/T	T/T	G/G	
Chile_JAA.RC884	T/T	T/C	G/G	T/T	T/C	T/G	A/C	T/C	T/T	G/A	A/A	A/G	C/T	C/T	G/G	
Chile_JAA.RC885	T/T	T/T	G/G	T/T	T/T	G/G	C/C	T/T	T/T	A/A	A/A	G/G	T/T	T/T	G/G	
Chile_JAA.RC886	T/G	T/C	G/G	T/C	T/C	T/T	A/A	T/T	T/C	G/A	A/A	A/G	C/T	C/T	G/G	
Chile_JAA.RC891	T/T	T/T	G/G	C/C	T/T	T/T	A/C	T/C	T/C	G/A	A/G	A/G	T/T	T/T	G/G	
Chile_JAA.EAP840	T/T	T/C	G/T	T/T	T/C	T/G	A/C	T/T	T/T	G/A	A/A	A/G	C/T	T/T	G/G	
Chile_JAA.EAP845	T/T	T/C	G/T	T/C	T/C	T/T	A/C	T/T	T/T	G/A	A/A	A/G	T/T	C/T	G/G	
Chile_JAA.RC899	T/T	T/C	G/G	T/T	T/T	G/G	C/C	T/C	T/T	G/A	A/A	A/G	C/T	C/T	G/G	
Chile_JAA.JAA232	T/T	T/C	G/G	T/C	T/C	T/T	A/C	T/C	T/T	G/G	A/G	A/A	C/T	C/T	G/G	Brown eggs
Chile_JAA.JAA280	T/T	T/C	G/T	T/C	T/C	T/T	A/C	T/C	T/T	G/G	A/G	A/A	C/T	C/T	G/G	

Haplotype analysis Chilian village chicken: ≈ 100 Kb

Oocyan haplotype



- SLCO1C1 has a role in bile acid transport (Klaassen and Aleksunes, 2010)
- Accumulation of the bile pigment biliverdin gives rise to blue eggs (Wang *et al*, 2009)

Target enrichment

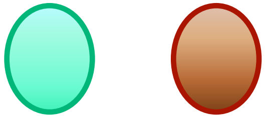
(Agilent SureSelect XT 4Mb)

Paired-end sequencing

(2 x 100bp Illumina)

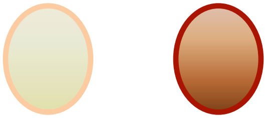
Blue locus

French Araucana
UK Araucana
Pirque (Chile)



Outgroup

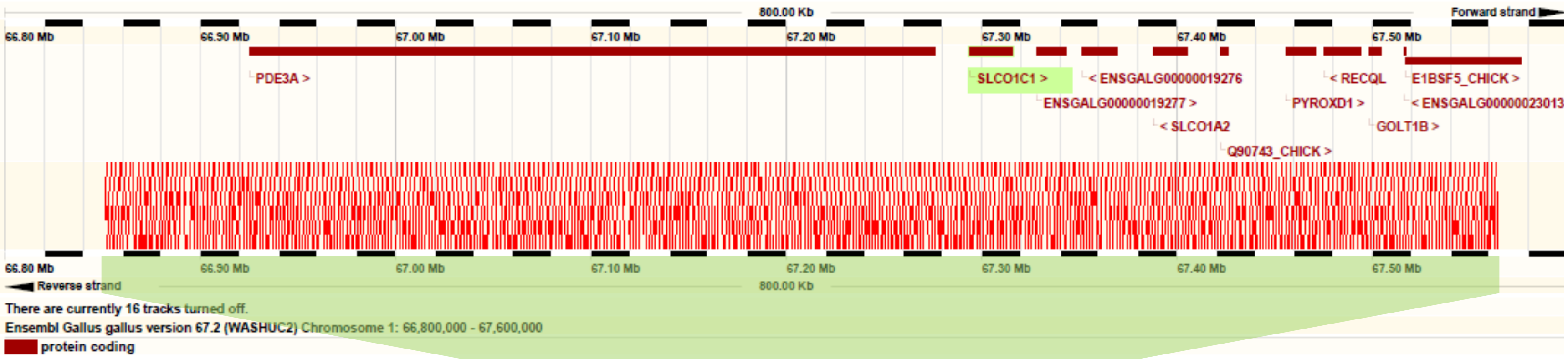
Pheasant (UK)



Wild-type

White Star (UK)
Crevecoeur (UK)
Pirque Kike (Chile)
Pirque (Chile)
Villa Cautin (Chile)
Pirque (Chile)

TARGETED SEQUENCING



There are currently 16 tracks turned off.

Ensembl Gallus gallus version 67.2 (WASHUC2) Chromosome 1: 66,800,000 - 67,600,000

1218 relevant SNPs identified across the entire region (700 Kb) but only 225 present in fine mapped regions

Reduce to 210 SNPs post-filtering against Ensembl and BGI databases.

Consequence	Ensembl Variant Effect Predictor
5PRIME_UTR	1
CODING_UNKNOWN,SPLICE_SITE	3
SPLICE_SITE,INTRONIC	5
CODING_UNKNOWN	15
INTERGENIC	64
UPSTREAM	65
DOWNSTREAM	83
INTRONIC	982
Total	**1218**

NGS SNP SUMMARY

Unravelling the genetic control of adaptation

Detection of signature of selection in African indigenous cattle

Harry Clifford,
MSc
Hussain
Bahbahani, PhD

Identifying fixed signature selection could provide insight into genome requirements for survival in the African environments………..

The University of Nottingham
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Signature of selection in East African indigenous Cattle Genome wide scan analysis

QC: MAF= 0.005, SNP call rate = 0.95, ibs.threshold = 0.95.

45227 Autosomal SNPs

Samples

EASZ: 421 pure calves (18 sublocations)

Friesian: 62 animals

Jersey: 28 animals

Nelore: 21 animals

Sheko: 20 animals

Analysis

Between pair of populations

Between "group of populations

Within population

Allele frequencies / Haplotype segregation

Extended Haplotype Homozygosity (EHH)

Identify region with unusually longe range of haplotype and high pop. frequency

|iHs| *"integrated Haplotype Score" (Voight et al. 2006)*

Comparison of EHH between derived and ancestral alleles

Rbs *Across populations (Tang et al. 2007)*

fastPHASE 1.4 (Scheet and Stephens, 2006): ***Haplotypes***

Ancestral states*: Bovinae subfamily data (Decker et al. 2009) applied the BovineSNP50 13 of the 70 species (Bovinae subfamily), Most common allele inferred as ancestral.*

iHS Manhattan plots

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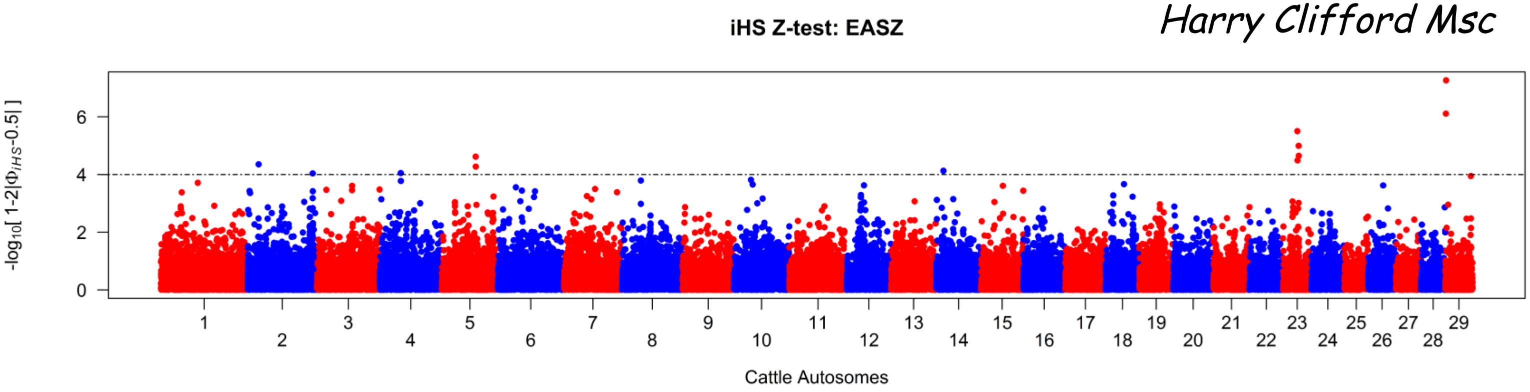

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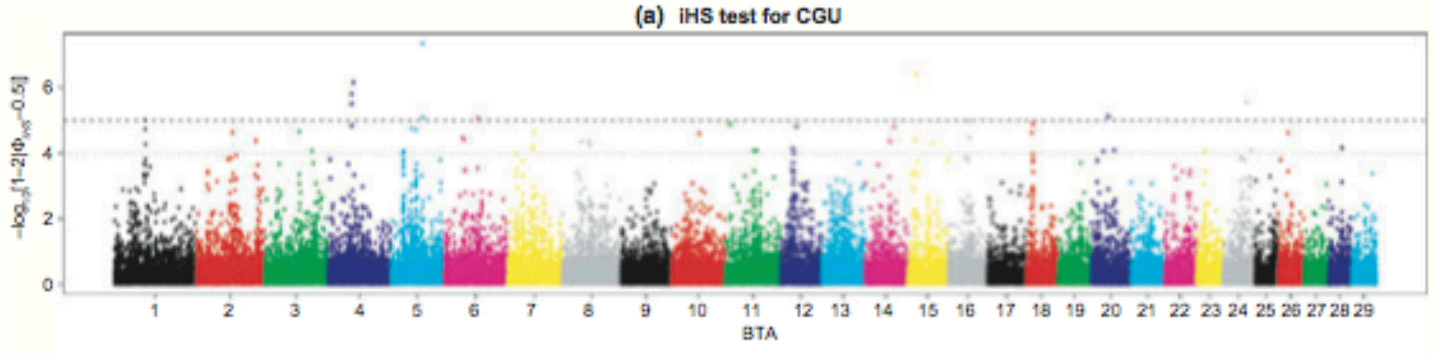
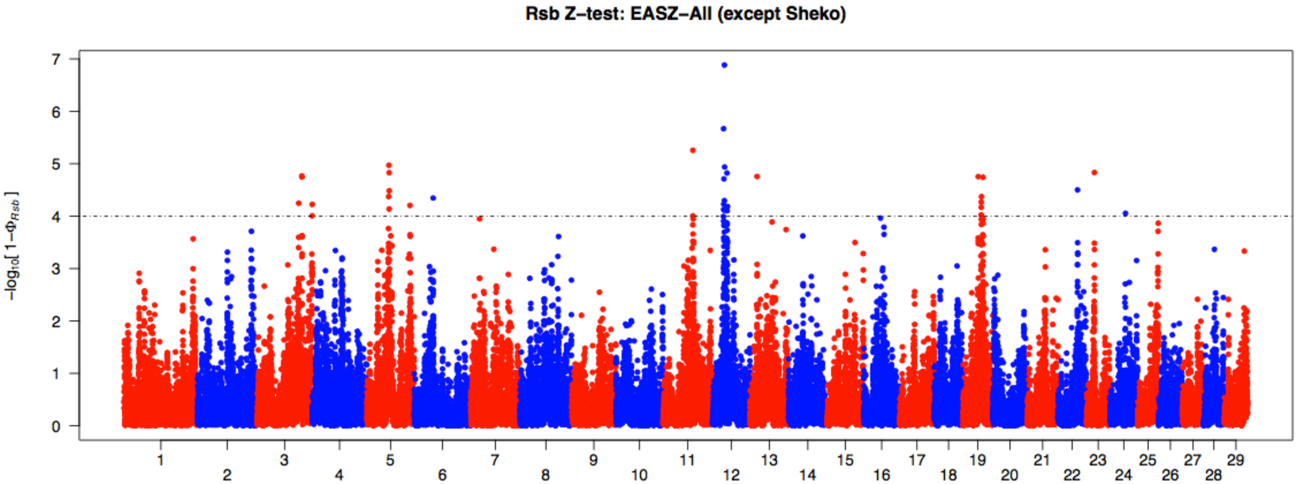


No overlap……


Rsb Z-test: EASZ-All (except Sheko)

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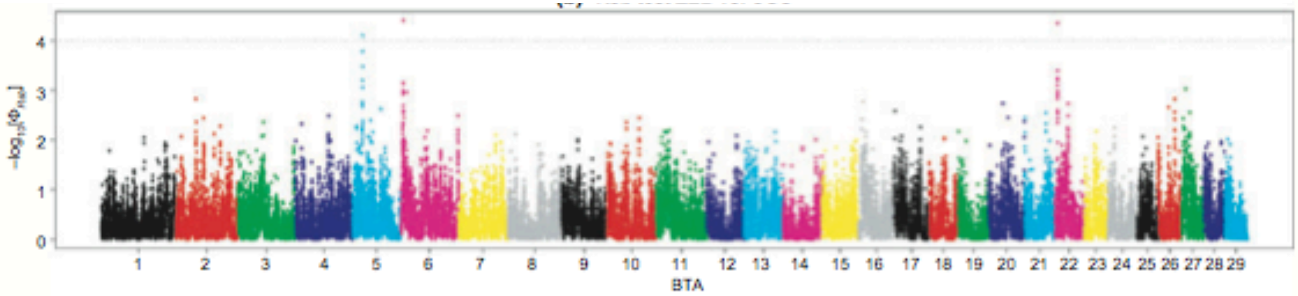
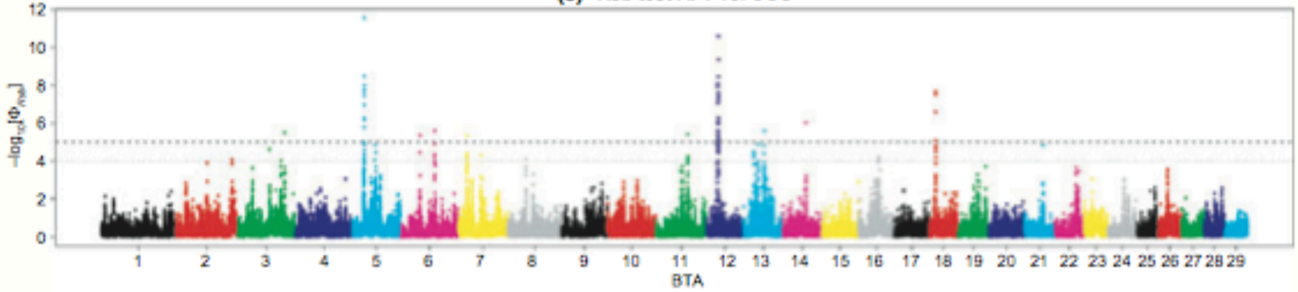
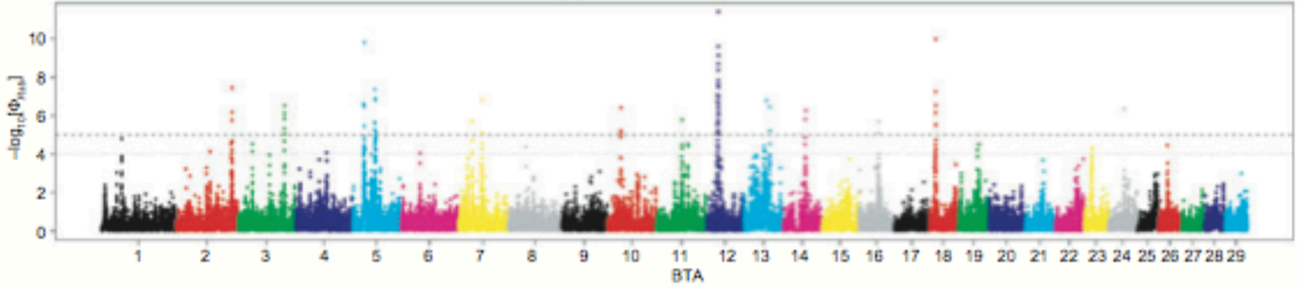


(c) Rsb test AFT vs. CGU



(d) Rsb test EUT vs. CGU



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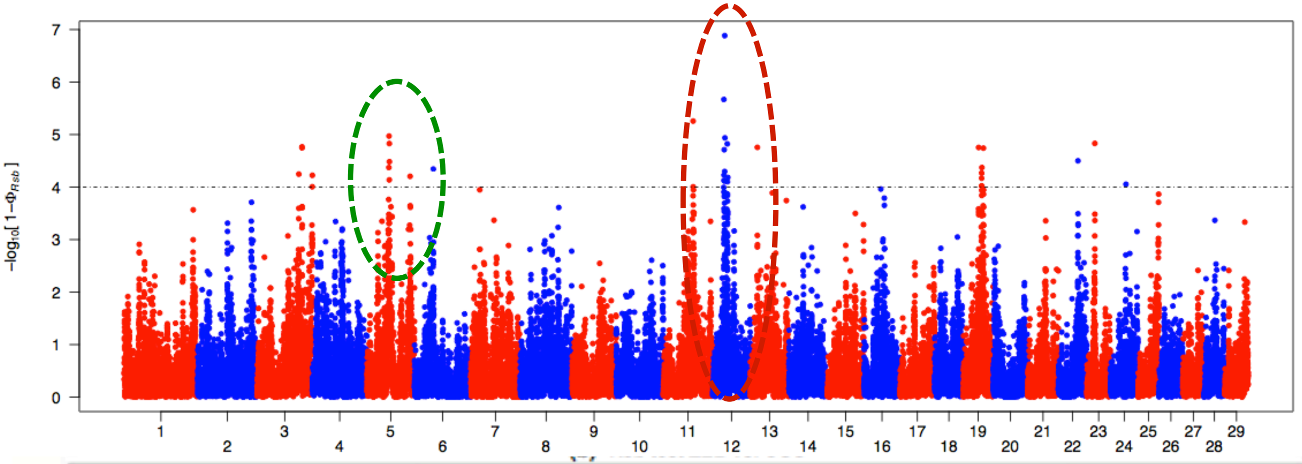

Rsb Z–test: EASZ–All (except Sheko)

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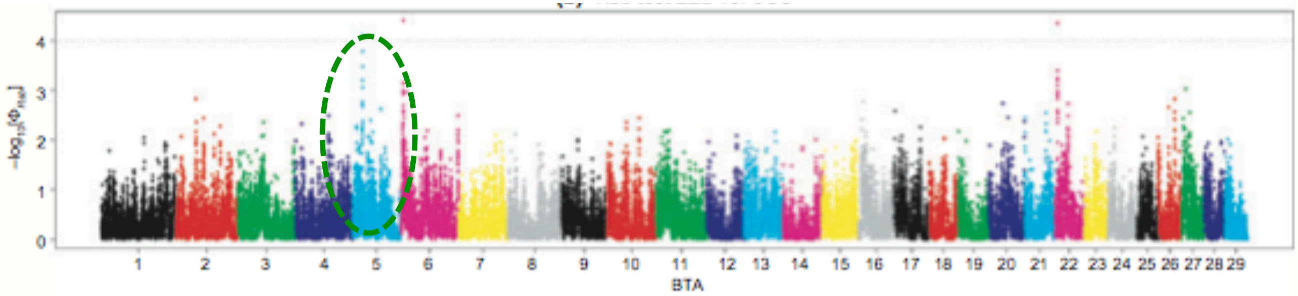
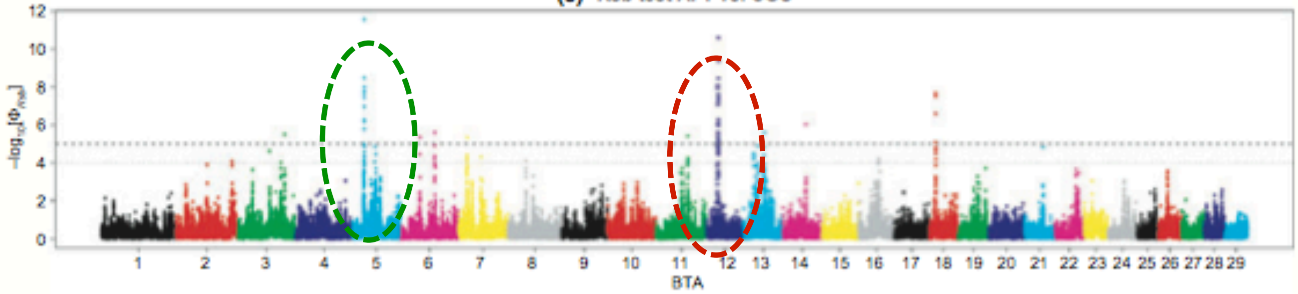


(c) Rsb test AFT vs. CGU



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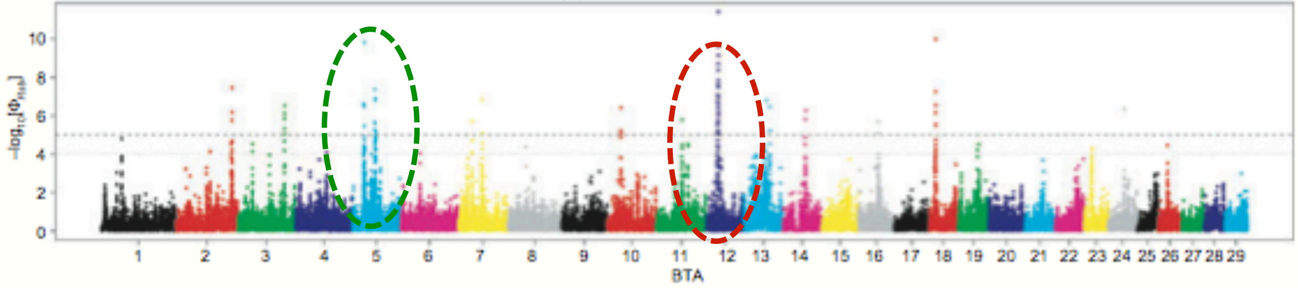
(d) Rsb test EUT vs. CGU



Candidates genes

BTA05: SNRPF - Small nuclear ribonucleoprotein polypep. F

BTA12: Relaxin/insulin-like family peptide receptor 2

(LGR8, GREAT and INSL3)

RXFP2: male fertility, control of the transabdominal stage of early development testicular descent and cryptorchidism. Physiological adaptation (endothermic mammals) to avoid potential impairment of spermatogenesis body temperature reached a threshold of 34– 35 °C (Park et al. 2008).

Signal of selection results from favorable variant for adaptation to warm conditions in male reproductive function

Acknowledgements funders

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