



The genetic basis of breed diversification: signatures of selection in pig breeds

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Lu ZH, Megens H-J, Archibald AL, Haley CS, Jackson IJ, Groenen MAM, Crooijmans RP, Ogden R, Wiener P

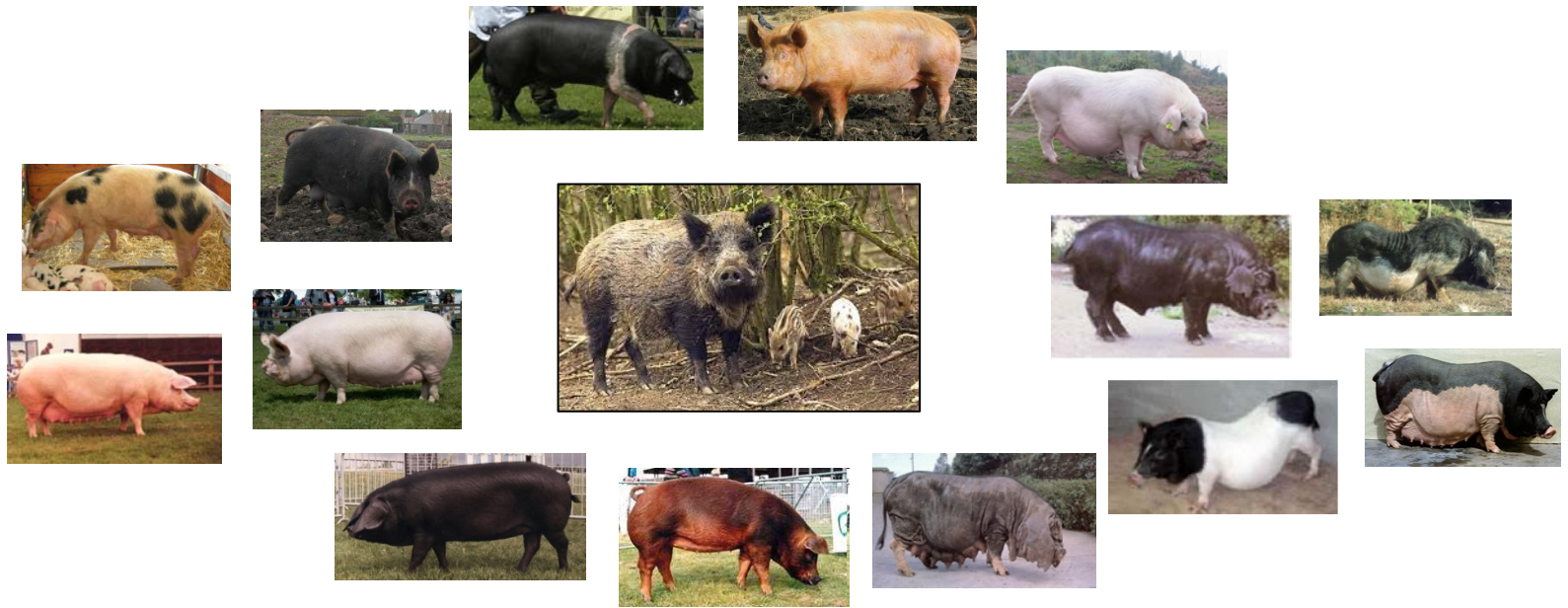


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Breed diversification

- Domestication and selective breeding has produced a diversity of pig breeds



- What is the genetic architecture underlying this variation?

History of UK pig breeds

Unique and interesting history of selective breeding



1. Selective breeding from 18th century
 - Breeds-crossed, selection for fat
2. Introgression from Asian breeds
 - Growth, prolificacy, early maturing



contemporary breeds formed

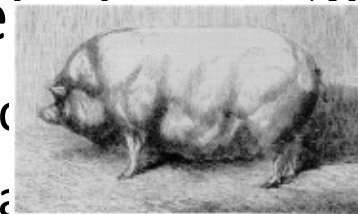
3. Pig breed societies set up

“consequence of so much crossing, some well-known breeds have undergone rapid changes; thus, according to Nathusius, the Berkshire breed of 1780 is quite different from that of 1810” (Darwin 1868).

4



A Sanders Spencer Large White boar 'Holysell Jimmy'



The famous Middle White sow 'Miss Emily', the first 'Pig of the Year'



A Tamworth pig from Mr G Allender

Strong and recent genetic change

- Resultant UK pig breed diversity
 - Numerous breeds
 - Broad and distinct phenotypic diversity
 - High breed genetic differentiation
 - Many commercial breeds originated from UK

Review. Genetics of animal domestication P. Wiener & S. Wilkinson 3163

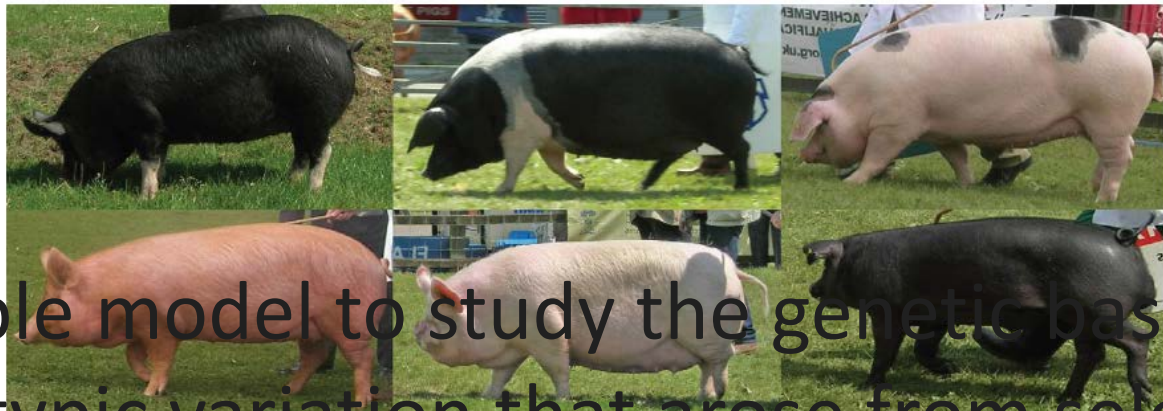


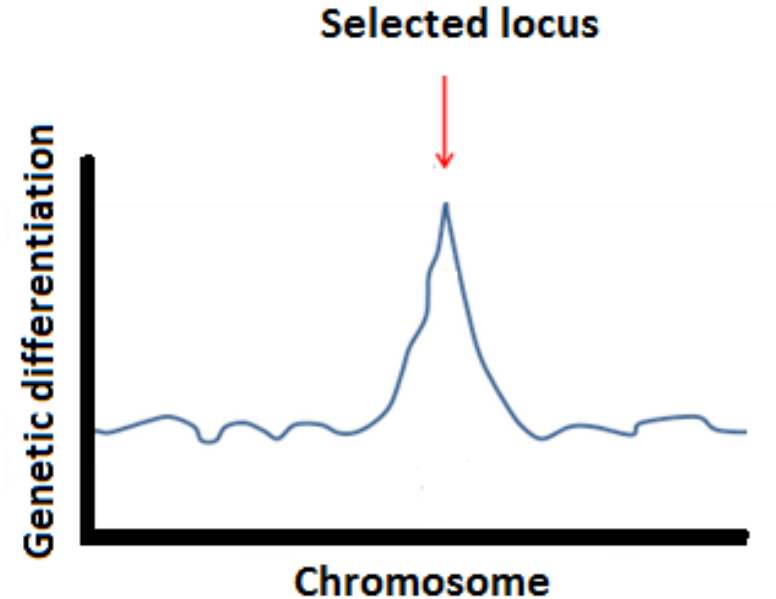
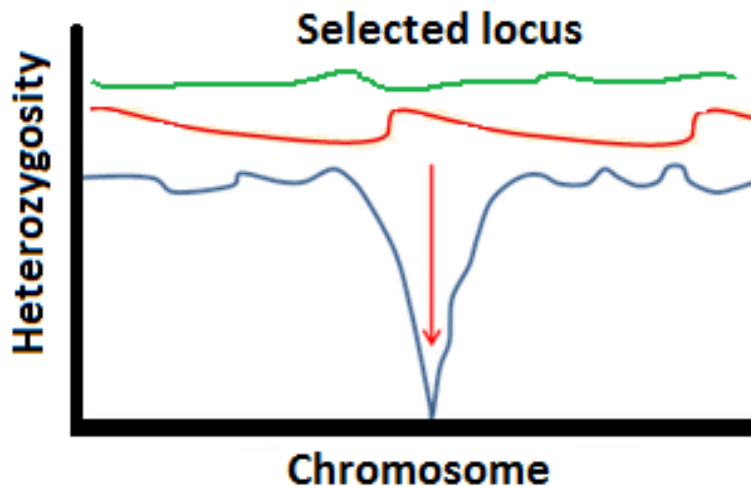
Figure 2. Coat colour variation in pig breeds. Clockwise from top-left: Berkshire, British Saddleback, Gloucestershire Old Spots, Large Black, Middle White and Tamworth (photos: S. Wilkinson).

Large Black
British Saddleback Berkshire Gloucestershire Old Spots

➤ Valuable model to study the genetic basis of phenotypic variation that arose from selection

Selection

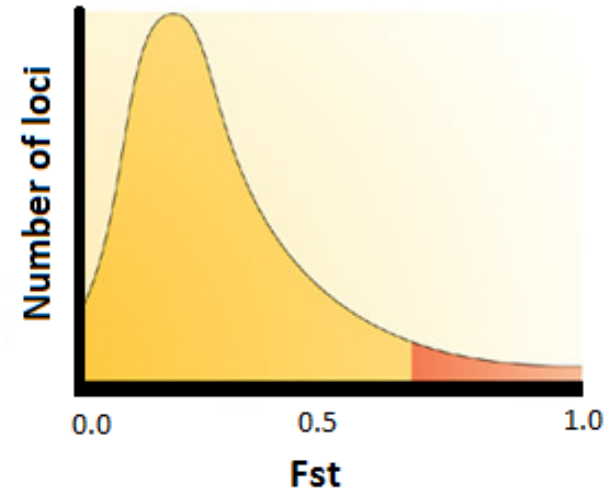
- Theory: regions under selection display patterns that depart from neutral expectations



Selection mapping

- Measuring population genetic differentiation (F_{ST})

$$F_{ST} = \frac{\text{var}(\mathbf{p})}{p(1-p)}$$



- With '000s SNPs - can scan genome of populations for signatures of diversifying selection



Detect signatures of selection associated with pig breed diversification by mapping breed genetic differentiation

QUESTIONS:

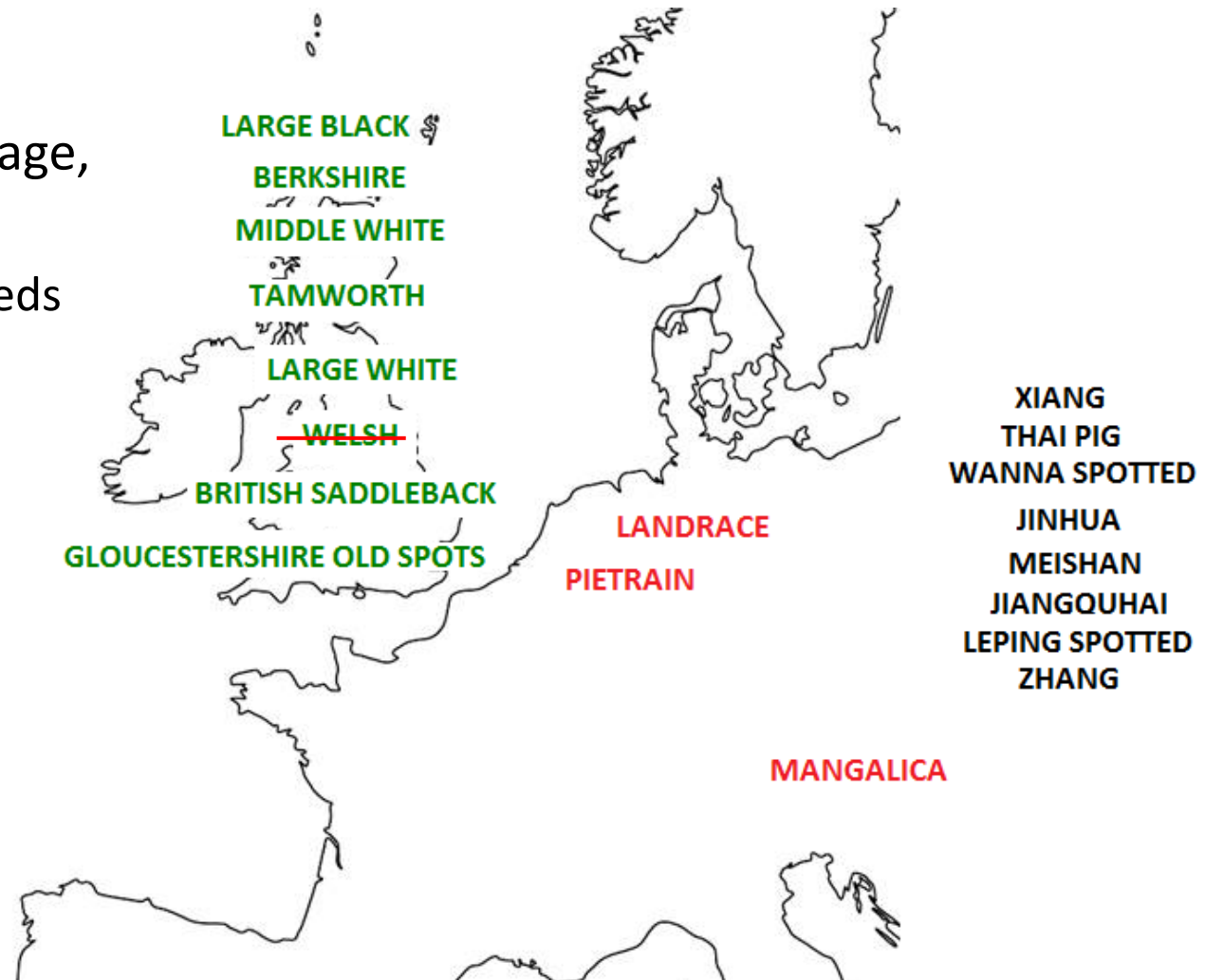
1. Do signatures of selection overlap regions already associated with phenotypic traits (QTLs and genes)?
2. What types of traits are associated with the signatures of selection?

What does this tell us about the history of selective breeding and genetic characteristics underlying phenotypic diversity in pig breeds?

Porcine Data

- SNPs (PorcineSNP60 chip: 60,000)
 - 13 UK/Europe breeds - 372 individuals
 - 1 Asian breed

- Sequence (10x coverage, Illumina)
 - 12 UK/Europe breeds - 52 individuals
 - 8 Asian breeds - 24 individuals



Analysis pipeline

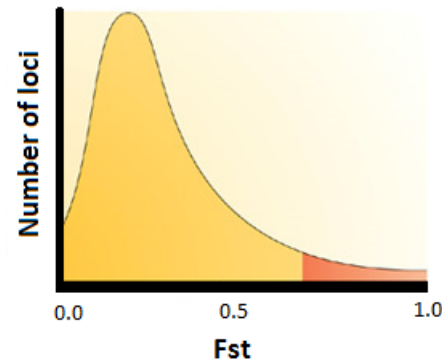
After quality control 49,260 SNPs remained

For each breed, locus-by-locus F_{ST} estimated

For each breed, moving average estimated using 13-SNP sliding window



For each breed, a 99th percentile was imposed on the F_{ST} distribution

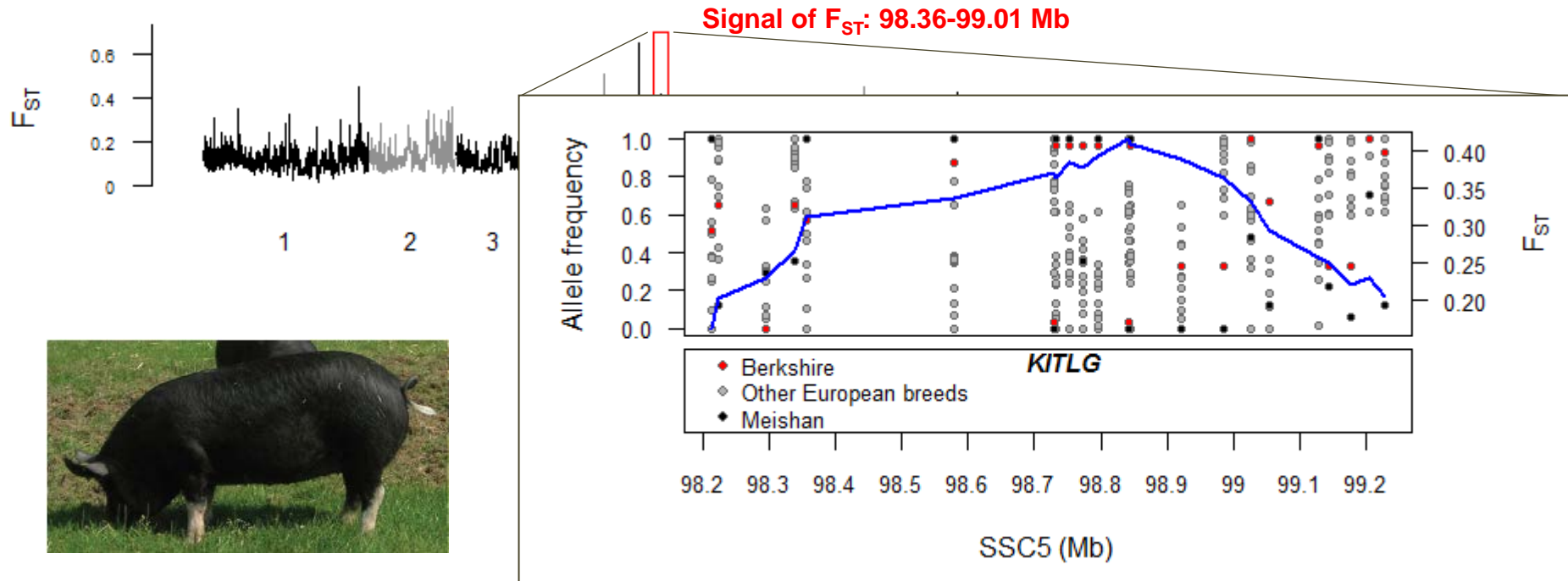


Breed specific signatures of diversifying selection

RESULTS

Coat colour: *KITLG*

in BERKSHIRE: black animal with 6 white points



- *KITLG* is involved in melanocyte production
 - Investigated for role in pig colouration (Hadjiconstantouras et al. 2008; Okumura et al, 2010)
 - Pigmentation in mice, humans

Variation at *KITLG*

Differentiation region on SSC5 (98.0-99.0 Mb) searched for sequence variants unique to Berkshire

- Key variants found:
 - 1 SNP on the 3'-UTR of *KITLG* in Berkshire
 - not in other European breeds; in 1 Asian breed, the Jiangquahai
 - 2 non-synonymous variants in *KITLG* in Berkshire
 - 3/50 European and 16/24 Asian individuals, respectively
 - Similar results by Okamura et al (2008)
- An Asian origin of *KITLG* in Berkshire
- *MC1R* implicated in Berkshire coat phenotype
 - E^P allele: 2bp insertion
- Interactions between the different coat loci?

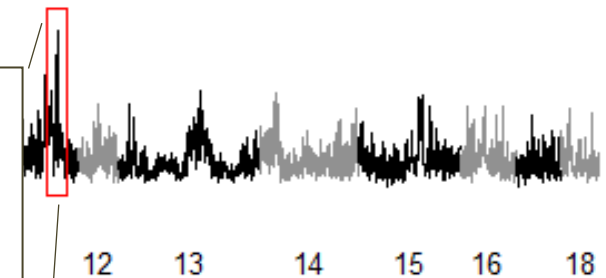
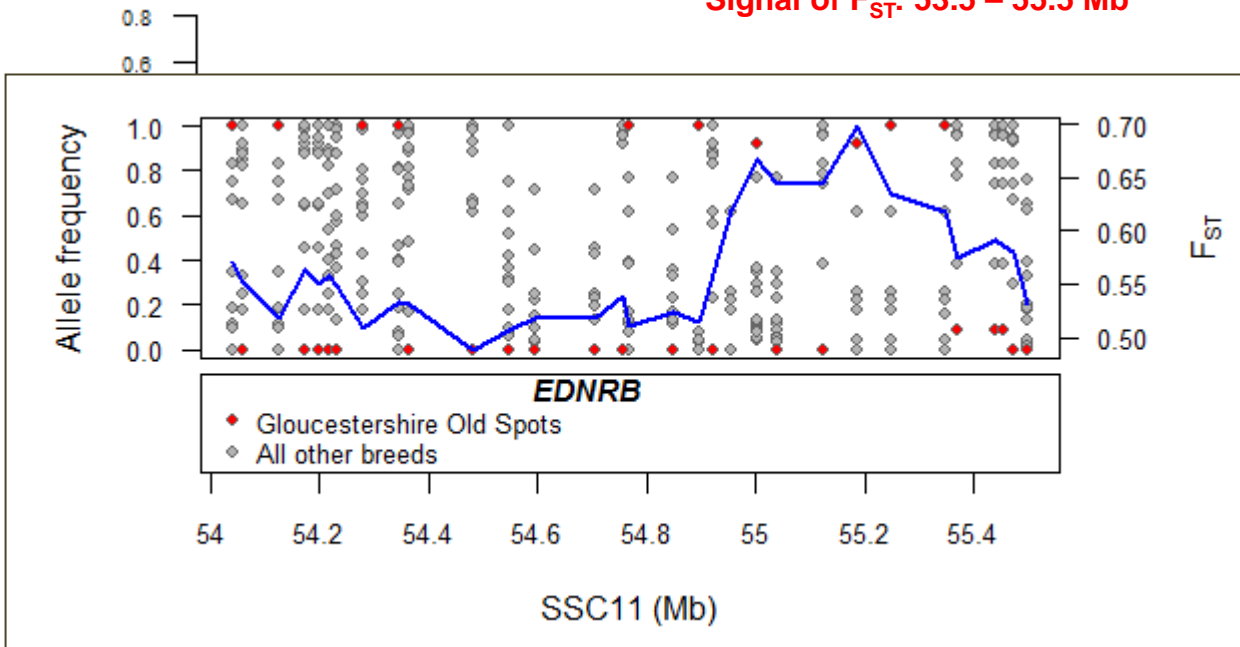
Jiangquahai:



Coat colour: *EDNRB*

in GLOUCESTERSHIRE OLD SPOTS: white animal with black spots

Signal of F_{ST} : 53.5 – 55.5 Mb



- *EDNRB* involved in melanocyte development
 - White coat-spotting (mice, horses)

Variation at *EDNRB*



Residue 17	European breeds	Asian breeds
Leucine	Gloucestershire Old Spots	Xiang, Jiangquahai
Phenylalanine	All other European breeds	All other Asian breeds

Residue 68	European breeds	Asian breeds
Phenylalanine	Gloucestershire Old Spots	Xiang
Serine	All other European breeds	All other Asian breeds

Reference			DTFK : 102
GOS	..	E	: 102
Berkshire	..		: 102
British_Saddleback	..		: 102
Duroc	..		: 102
Large_Black	..		: 102
Hampshire	..		: 102
Landrace	..		: 102
Large_White	..		: 102
Mangalica	..		: 102
Middle_White	..		: 102
Pietrain	..		: 102
Tamworth	..		: 102
Jiangquahai	E		: 102
Jinhua	..	S	: 102
LepingSpotted	..		: 102
Meishan	..		: 102
Thai_Pig	..		: 102
Wannan_Spotted	..		: 102
Xiang	E		: 102
Zang	X		: 102
Microbat	..P...H...L.S...G...AT...L.GAR...T...SRTA...W.W...A...T...A...P...A.PHA--RP...I... : 102		
Tarsier	..		: 102
Hedgehog	..		: 102
Panda	..Q.WSA...L...LG...S...L.L.GP...TT...EVS...G...A.W...SS...A...A...A.P.PPKF...K...E... : 104		
Mouse	..SPA.R...L...FL.WG.K...Q...LSL.G.K...T...S.T...S.M...A.A...T...G...V.P...F...RN...SK... : 101		
Tasmanian_devil	..TVH...CIL..L..FL..YTLE.WG..NELLSSRD.L.T.EA...TT.TV.AA...AA...Q.-LE..I..G-----S...TL.S.RT..E... : 92		
Cow	..P...L...A...E...Q.LPG...ET...E.S.GR...D...AT...I.P...D...E... : 100		
Human	..PP...L...SR.WG...DR.-L.Q.A...T...L...A...IA.A...D.T.S.P...E... : 101		

Signal peptide

N-terminal extracellular domain

Mutations at *EDNRB*



- Mutations in *EDNRB* lead to reduced expression or partial/complete loss-of-function
 - Hirschsprung's disease

	*	20	*	40	*	60	*	80	*	100																	
Ref_Pig	:										: 102																
GOS	:		L					F			: 102																
Berkshire	:										: 102																
British_Saddleback	:										: 102																
Duroc	:										: 102																
Large_Black	:										: 102																
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Tamworth	:										: 102																
Jiangquahai	:		L								: 102																
Jinhua	:							S			: 102																
LepingSpotted	:										: 102																
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Thai_Pig	:										: 102																
Wannan_Spotted	:										: 102																
Xiang	:		L					F			: 102																
Zang	:		X								: 102																
Microbat	:	P	H	L	S	G	AT	L	GAR	T	SRT	A	W	W	A	T	A	P	A	PHA	RP	I	: 102				
Tarsier	:																						: 102				
Hedgehog	:																						: 102				
Panda	:	Q	WSA	L	LG	S	L	GP	TT	EVS	G	A	W	SS	A	A	A	P	PKF	K	E	: 104					
Mouse	:	SPA	R	L	FL	WG	K	Q	LSL	G	K	T	S	M	A	A	T	G	V	P	F	RN	SK	: 101			
Tasmanian_devil	:	TVH	CIL	L	FL	Y	TLE	WG	NELL	SSRD	L	T	EA	TT	TV	AA	PA	Q	LP	L	G	S	TL	S	RT	E	: 92
Cow	:	P	L	A	E	Q	LPG	ET	E	S	GF	D	AT	I	P	D	E	: 100									
Human	:	PP	L	SR	WG	DR	L	QA	T	L	A	LA	A	D	T	S	P	E	: 101								

EDNRB and pig spotting



Gloucestershire Old Spots



Xiang



Jiangquahai

- *MC1R* – E^P allele: 2bp insertion
 - Other European coat phenotypes also associated with E^P allele
 - At low frequency or absent in Asian breeds
- Melanocortin – Endothelin signalling
 - Complex melanocortin-endothelin signalling in cats (Kaelin et al 2012)
 - *MC1R* variant interacts with partial loss-of-function *EDNRB* to give Gloucestershire Old Spots its spots?

Ear phenotype variation

Prick-eared breeds

Berkshire
Hampshire
Large White
Middle White
Pietrain
Tamworth



Intermediate-eared breeds

Duroc
Landrace
Welsh



Flat-eared breeds

British Saddleback
Gloucestershire
Old Spots
Large Black
Mangalica



- Assess genetic divergence between the different ear phenotypes:

F_{ST} :

Prick-eared breeds vs flat-eared breeds

Prick-eared breeds vs intermediate-eared breeds

Intermediate-eared breeds vs flat-eared breeds

Signals for ear phenotype

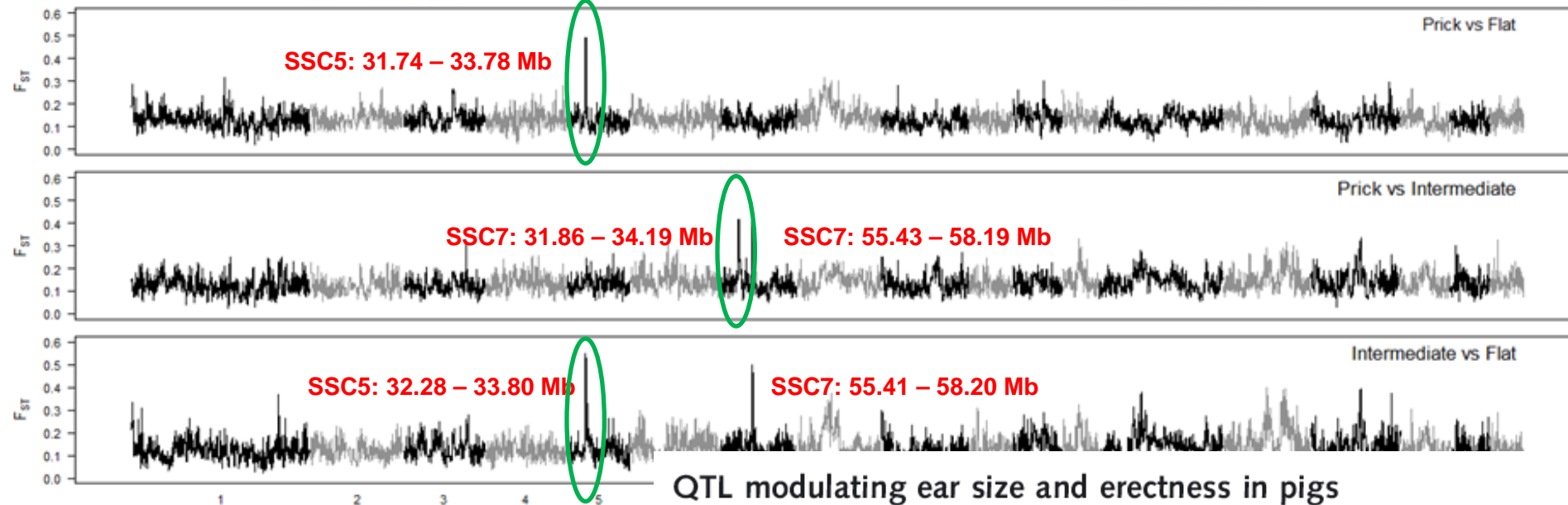
Prick-eared pig phenotype:



Intermediate-eared pig phenotype:



Flat-eared pig phenotype:



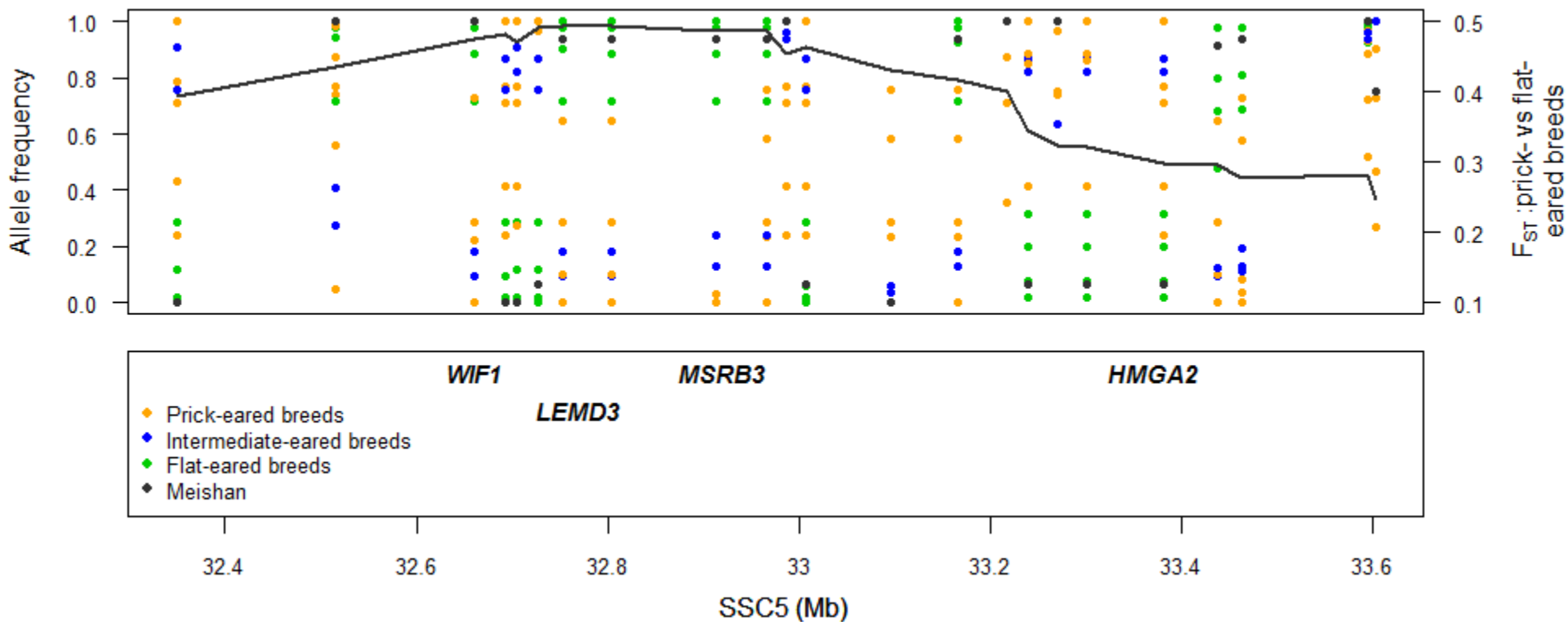
W. H. Wei, D. J. de Koning, J. C. Penman, H. A. Finlayson, A. L. Archibald and C. S. Haley
Roslin Institute (Edinburgh), Roslin, Midlothian EH25 9PS, Scotland, UK

- Significant QTL on SSC7 overlaps the signal
- Significant QTL on SSC5 is ~10Mb upstream of the signal

Signal on SSC5

Associated with contrast between prick or intermediate ears and large flat ears

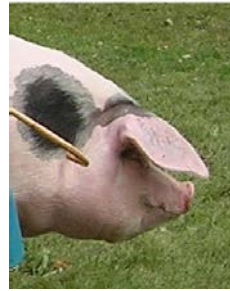
- Syntenic to a region in the dog genome associated with ear morphology (Boyko et al. 2010, Vaysse et al 2011)



Variation at SCC5 signal



Differentiation region on SSC5 (31.0-34.0 Mb) searched for variants shared by flat-eared breeds vs prick-eared breeds:

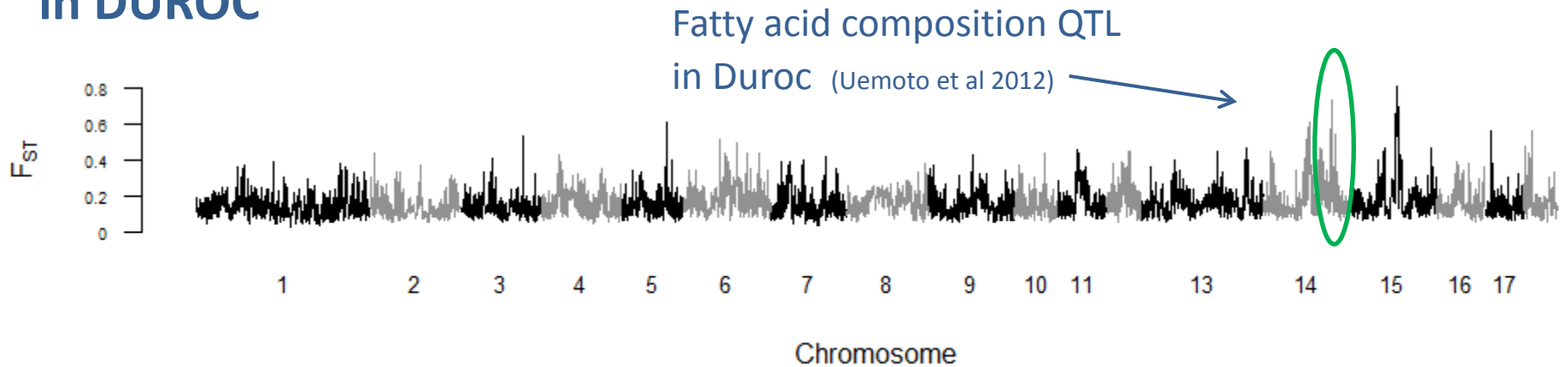


- No non-synonymous differences
- Variants in non-protein coding sequence
 - SNPs in regulatory elements or miRNA genes may be responsible?

Selection mapping of QTLs



in DUROC



GWAS study found a significant SNP in this region (Yang et al 2013)

Genes with a role of fatty acid synthesis found

in the region: *SCD* (120.90-121.30 Mb) ; *EVOLVL3* (123.08-123.083 Mb)



- Duroc has a unique meat quality composition
 - high intramuscular fat content
 - high concentrations of saturated and mono-unsaturated fatty acids

Conclusions

- Pig breeds display signatures of selection associated with highly visible phenotypic differences

- Gloucestershire Old Spots and Berkshire selected for distinct coat phenotypes



- *EDNRB* amino acid differences
- *KITLG* variation

- 3 genomic regions are associated with ear phenotype variation in pigs



- Influence of Asian alleles on the UK phenotypic diversity

- Asian breeds influenced highly visible phenotypic differences as well as production traits



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Thank you ... Questions?



- **Journal article on this study:**

Wilkinson S, Lu ZH, Megens H-J, Archibald AL, Haley CS, Jackson IJ, Groenen MAM, Crooijmans RPMA, Ogden R, Wiener P (2013) Signatures of diversifying selection in European pig breeds. *PLoS Genetics* 9(4): e1003453.

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