



Genetics of pre-disposition to boar taint

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Aim

- to use a genomics approach to study the genetic control of boar taint in entire male pigs



Boar taint

Boar taint

- Strong urine / faecal smell
- ~10% intact males

Solutions

- Castrate
- Slaughter younger

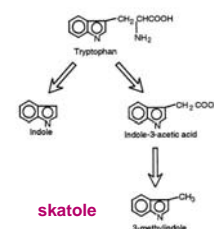
Impacts

- Animal welfare
- Product quality
 - taste, fat/lean
- Sustainability
 - feed efficiency
 - environmental
 - economic



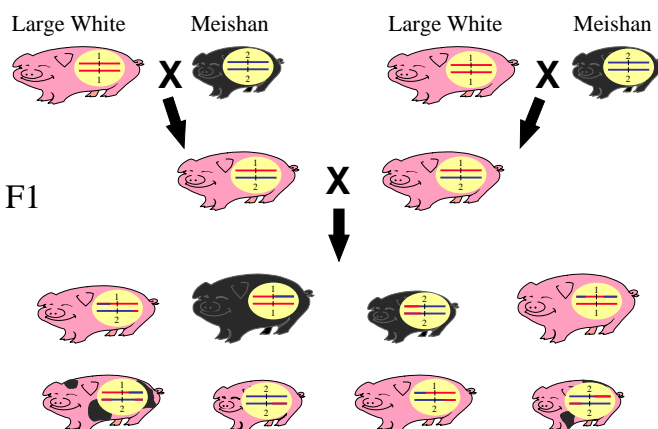
The offending molecules

- Skatole, Indole
 - produced by bacteria in hind gut
 - absorbed into fat
- Androstenone
 - steroid / pheromone



Genome scan version 1

- Pedigreed animals recorded for traits
- Genotype with polymorphic genetic markers
- Statistical tools for analysis
- Search for association between performance and inheritance of marked chromosomal regions



Extreme cross - pigs



- Meishan superior reproductive performance
- Large White superior growth and carcass traits
- Differences in taint traits?



Genome scan results

- No evidence of a major gene
- Evidence for QTL for
 - abnormal flavour in fat
 - abnormal flavour in lean
 - abnormal odour
 - boar flavour in fat
 - boar flavour in lean



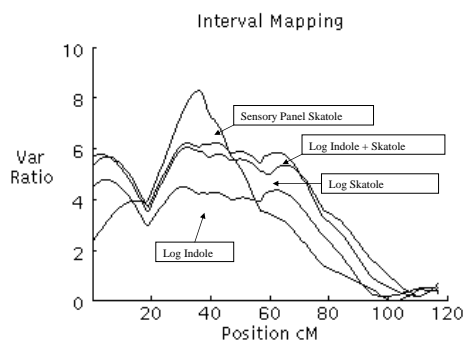
Genome scan results

- Evidence for QTL for
 - androstenone levels (lab)
 - indole level (lab)
 - skatole level (lab)
 - androstenone (taste panel)
 - skatole (taste panel)
- Most significant QTL
 - chromosome 14
 - chromosome 6

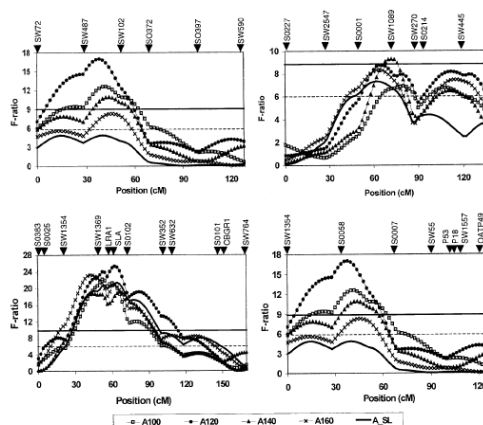
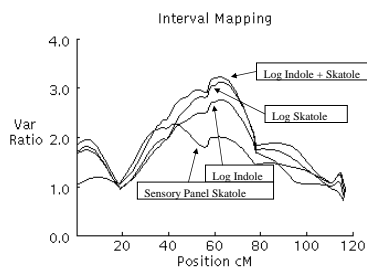
Lee et al., 2004.
Animal Genetics 36, 14-22



Between breed QTL analyses



Within breed QTL analyses

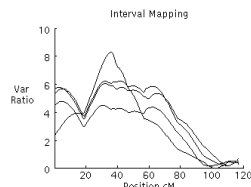


Quintanilla et al., 2003. *J Anim Sci* 81, 385-394

Figure 1. Profile of F-ratios (L.C model) throughout chromosomes 3 (upper left), 4 (upper right), 7 (lower left), and 14 (lower right) for fat androstenone levels at 100 (A100), 120 (A120), 140 (A140), and 160 (A160) d of age and at slaughter (A_SL) when a single QTL is fitted. The horizontal lines denote the 5% genome-wide (solid line) and chromosome-wide (dashed line) thresholds.

Genetics, genome scan

- Large White / Meishan
- No evidence for major gene
- QTL for taint
 - skatole, indole
 - androstenone
- Are there plausible candidate genes?

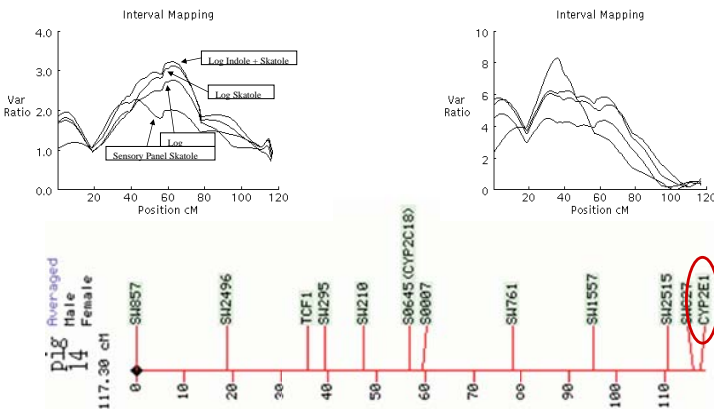


Lee *et al.*, 2005 *Anim Genet* 36: 14



Cytochrome P450 2E1 - CYP2E1

- Physiological candidate gene
- Comparative positional candidate gene?
- Mapped to SSC14
- Isolate genomic clone (BAC)
- Develop genetic markers
- Add to linkage / QTL map
- Scan promoter for polymorphism



CYP2E

- Mechanistic link
- Physiological candidate

BUT

- Not positional candidate in *Large White / Meishan* population

Skinner *et al.*, 2005. *Animal Genetics* 36, 417-422



Physiological candidates

- Squires *et al.*, Sygen-PIC
- Multiple genes
 - Lin *et al.*, 2004a. *Anim Genet* 35, 314-316; Lin *et al.*, 2004b. *Mamm Genome* 15, 218-226; Lin *et al.*, 2005. *Mamm Genome* 16, 367-373
- Patented, but surrendered by Genus to Squires
- No independent confirmation
 - Small sample, confounded by breed?

Skinner *et al.*, 2006. *Animal Genetics* 37, 521-522

WP9: Product Quality (Boar taint)

- Perform genome wide scan to confirm known QTL and identify new QTL
- Identify new candidate genes for skatole and androstenone by comparative genome-wide expression analysis and comparative proteomic studies
- Integrate mapping, expression and proteomic data, target causative genes and identify predictive SNPs
- Confirm SNPs in experimental and commercial populations



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SABRE CUTTING EDGE GENOMICS FOR SUSTAINABLE ANIMAL BREEDING

Genome scan version 2

- ❑ Genome-wide association studies
- ❑ Require
 - 1000's genetic markers
 - Single Nucleotide Polymorphisms (SNPs)
 - Trait recorded animals + DNA source

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Genome scan - population

- ❑ Danish Landrace
- ❑ Danske Slagterier in-line skatole testing
- ❑ ~6,000 samples
 - select full sib pairs
 - 500 high skatole
 - 500 low skatole
- ❑ Full phenotypes – growth, fat, pedigree
- ❑ Androstenone levels assayed - Norway

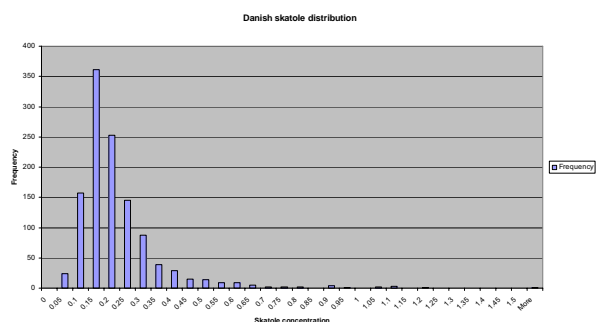
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Taint data

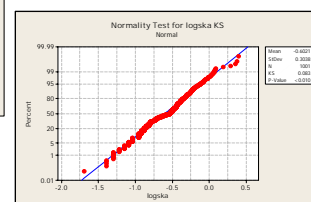
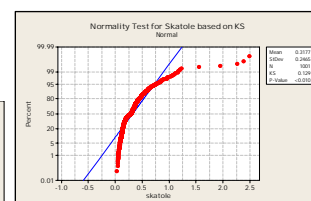
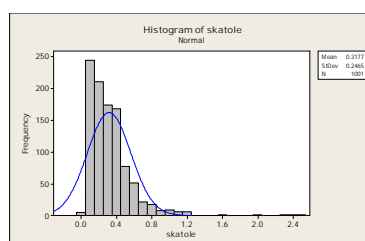
	Number of animals	Mean (SD)	Range (min-max)
Skatole (µg/g) (unselected)	6178	0.20 (0.14)	0.02 – 2.86
Skatole (µg/g) (selected)	1001	0.32 (0.25)	0.02 – 2.49
Androstenone (selected)	1002	1.04 (0.97)	0.0 – 10.62

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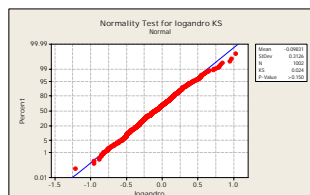
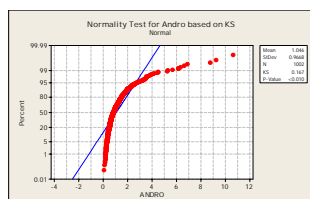
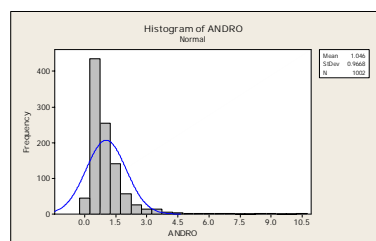
Danish Landrace samples



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SNP discovery

- Re-sequence ~6,500 STS (BES)
- Panel – 22 unrelated pigs, incl.
 - Duroc, Hampshire, Large White (old, contemporary), Landrace, Berkshire, Pietrain, Meishan, Meishan/Large White synthetic, LR,LW,Du,Pi synthetic
- Comparative map spacing (2 per Mbp)
- Random selection of BES



SNP discovery to SNP chips

- Roslin/Sanger SNP discovery, phase 1
 - 1,728 BES/STS sequenced
 - 2,258 SNPs discovered
- Pooled SNP data with Bendixen, DIAS, DK
- First generation pig SNP chip designed

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7K SNP chip

- Candidate SNPs
 - 1,635 (23.2%) targeted re-sequencing of BES STS (Roslin/Sanger)
 - 2,695 (38.3%) targeted re-sequencing of cDNA (Aarhus / UMB)
 - 2,712 (38.5%) *in silico* mining EST data (Aarhus)
 - 7,042 submitted for design
 - 6,523 Illumina iSelect / Infinium II assays



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Validation (1)

- 1584 samples
 - Duplicates
 - Nuclear families
 - Different breeds



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Validation (2)

	Resequence BES STS (RI/Sanger)	Resequence cDNA (AU/UMB)	<i>In silico</i> mining EST data	Total
Perfect SNPs	1,385	2,042	1,166	4,593
Homozygous SNPs	41	194	491	726
Partially genotyped	52	113	99	264
Possible SNPs	21	84	158	263
Bad SNPs	26	46	605	677

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Data analyses

- Identification of fixed effects
 - Step-wise regression (Minitab 2006)
 - Slaughter week, herd, age, year_born,....
- Estimation of heritabilities and genetic correlations
 - Univariate and multivariate animal models ASREML (Gilmour *et al.*, 2007)
- Association analyses
 - GenABEL (Aulchenko *et al.*, 2007)



	Androstenone	Skatole	Unselected Skatole
Androstenone	0.56(0.10)		
Skatole		0.04-0.56 (0.03-0.10)	
Unselected Skatole			0.35(0.06)

Heritability (on diagonal), phenotypic (upper diagonal) and genetic correlation estimates for androstenone, skatole and unselected skatole.



	Androstenone	Skatole	Unselected Skatole
Androstenone		0.35-0.37 (0.03-0.03)	0.29 (0.03)
Skatole			
Unselected Skatole			

Heritability (on diagonal), **phenotypic (above diagonal)** and genetic **correlation estimates** for androstenone, skatole and unselected skatole.



	Androstenone	Skatole	Unselected Skatole
Androstenone			
Skatole	0.29-0.92 (NA)		NA
Unselected Skatole	0.42(0.11)	NA	

Heritability (on diagonal), phenotypic (upper diagonal) and **genetic correlation estimates** for androstenone, skatole and unselected skatole.



	Androstenone	Skatole	Unselected Skatole
Androstenone	0.56(0.10)	0.35-0.37 (0.03-0.03)	0.29(0.03)
Skatole	0.29-0.92 (NA)	0.04-0.56 (0.03-0.10)	NA
Unselected Skatole	0.42(0.11)	NA	0.35(0.06)

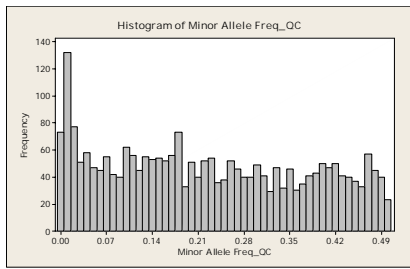
Heritability (on diagonal), phenotypic (upper diagonal) and genetic correlation estimates for androstenone, skatole and unselected skatole.



Data quality control

- Remove
 - Markers with very low MAF (< 0.02)
 - Markers with call rates < 0.95
 - Individuals with call rates < 0.95
 - Individuals with high autosomal heterozygosities
 - Individuals with high levels of homozygosity
- Number of markers 2753 (was 6523)
- Number of animals 884 (was 993)

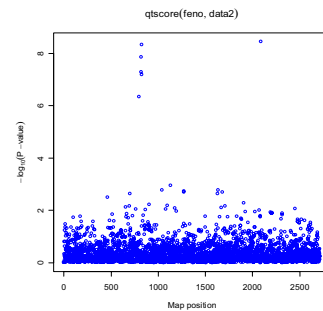




	$X \leq 0.01$	$0.01 < X \leq 0.05$	$0.05 < X \leq 0.1$	$0.1 < X \leq 0.2$	$X > 0.2$
Nu	152	261	231	542	1278
Prob	0.062	0.106	0.094	0.22	0.519

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TOP 3 MARKERS based on SIGNIFICANCE LEVEL RANKINGS (QC)



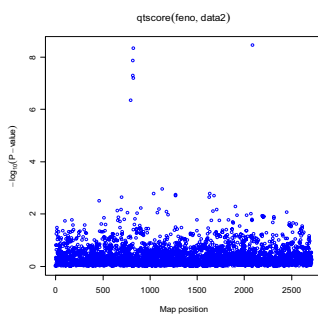
MARKER	P-VAL
sNG1354670_307	1E-10
Chr14_CYP2E1_3	1E-10
Chr14_CYP2E1_2	3E-10

TOP 3 MARKERS based on EXPERIMENT WISE RANKINGS (QC)

MARKER	P-VAL
Chr14_CYP2E1_1	9.999e-05
Chr14_CYP2E1_2	9.999e-05
Chr14_CYP2E1_3	9.999e-05

CYP2E1

TOP 3 MARKERS based on SIGNIFICANCE LEVEL RANKINGS (QC)



MARKER	P-VAL
sNG1354670_307	1E-10
Chr14_CYP2E1_3	1E-10
Chr14_CYP2E1_2	3E-10

TOP 3 MARKERS based on EXPERIMENT WISE RANKINGS (QC)

MARKER	P-VAL
Chr14_CYP2E1_1	9.999e-05
Chr14_CYP2E1_2	9.999e-05
Chr14_CYP2E1_3	9.999e-05

CYP2E1

Linking androstenone and skatole

- Correlation between androstenone and skatole
 - Inconsistent
- Bristol work suggests mechanisms
 - Androstenone inhibits CYP2E1 expression

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Future work

- Complete genome scan analyses
 - Androstenone
 - Different models
- Characterisation of candidate genes
 - CYP2E1,....
- Validation in other populations / samples
- Fine mapping / genome scan with 60K SNPs

SABRE CUTTING EDGE GENOMICS FOR SUSTAINABLE ANIMAL BREEDING

7K SNP chip development

- Roslin Institute
 - Alan Archibald
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- PIC
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 - Maren Moe
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SABRE CUTTING EDGE GENOMICS FOR SUSTAINABLE ANIMAL BREEDING

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