SABRE

CUTTING EDGE GENOMICS FOR SUSTAINABLE ANIMAL BREEDING

An integrated genomics approach to unravel the genetic basis of boar taint

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Food Quality and Safety

The offending molecules

- Androstenone

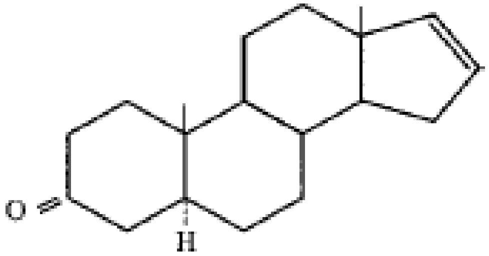
  steroid / pheromone produced in testes

- Skatole, Indole

  tryptophane degradation by bacteria in hind gut

absorbed into fat

- Other factors



5α-androst-16-en-3-one

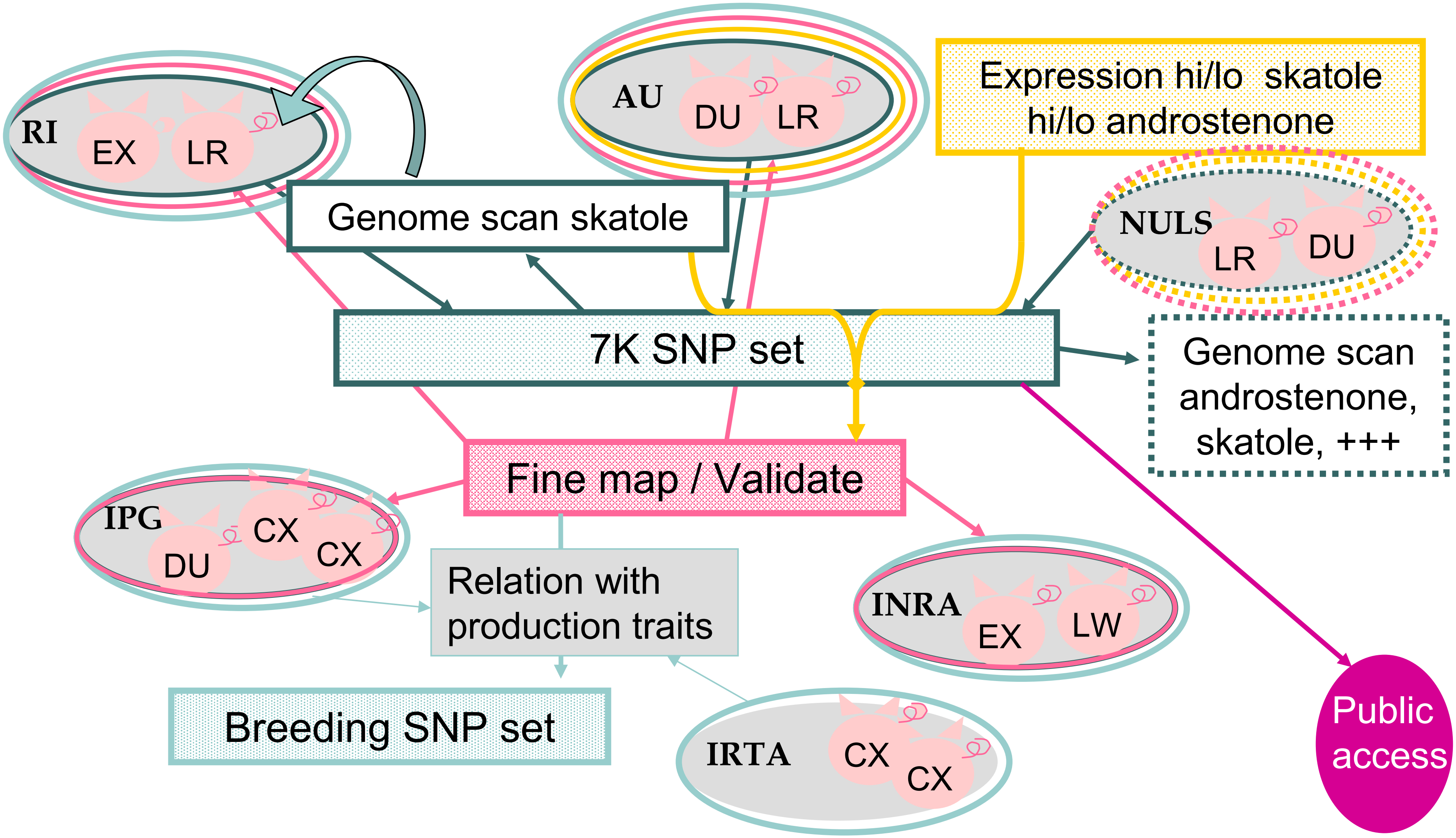
Goals and Tools of WP9

- Avoid boar taint by breeding
- Identify genetic mechanisms of accumulation of skatole and androstenone in fat
- Combine high-throughput expression analyses with QTL information

Objectives

- Confirm known QTL and identify new QTL
  - Establish porcine SNP panel √
  - Perform genome wide scan √
- Identify new candidate genes
  - Comparative gene expression: RNA & Protein √
  - Integrate mapping and comparative expression results
- Fine-map QTL regions (started)
  - Target causative genes
  - Identify predictive SNPs
- Confirmation studies (started)
  - Validate SNPs in commercial populations
  - relationship with other important production traits

The Partnership



RI EX LR

AU DU LR

Expression hi/lo skatole
hi/lo androstenone

Genome scan skatole

NULS LR DU

7K SNP set

Genome scan
androstenone,
skatole, +++

Fine map / Validate

IPG DU CX CX

Relation with
production traits

INRA EX LW

Breeding SNP set

IRTA CX CX

Public
access

7K SNP set designed for genome scan

- Candidate SNPs
  - 1,635 (23.2%) re-sequencing of BAC ends (RI/Sanger)
  - 2,695 (38.3%) re-sequencing of cDNA (AU,NULS)
  - 2,712 (38.5%) *in silico* mining EST data (AU)
  - 7,042 submitted for design
  - 6,523 Illumina iSelect / Infinium assays

Characterize 7K SNP set on 8 breeds

5.482 loci genotyped:
728 loci monomorphic

→ 4.754 SNPs

1 SNP / cM ≈ 1 MS / 5cM

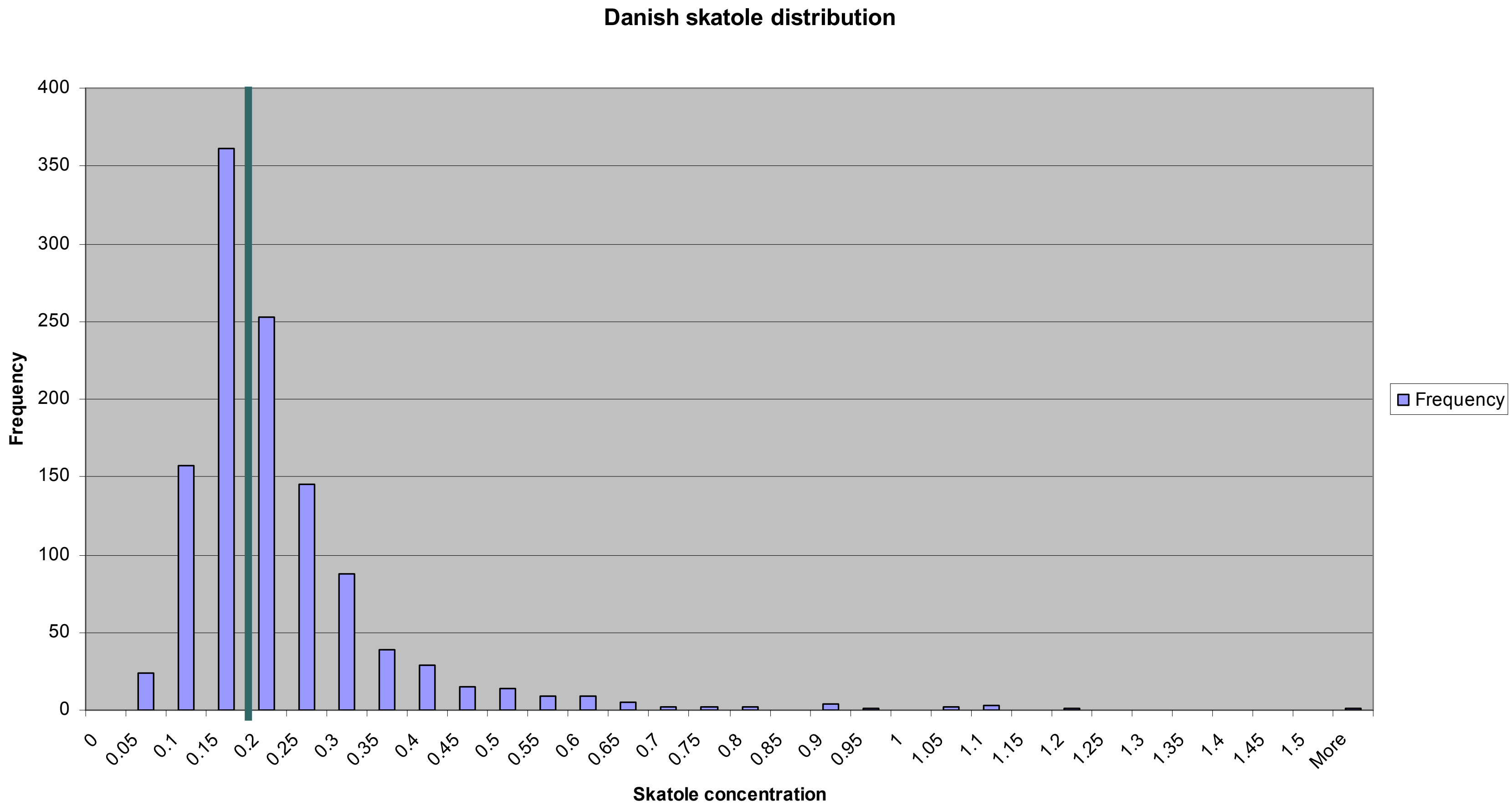
→ dense genome scan

	n	MAF >15%
Landrace	30	2082
Large White	24	2069
Duroc	23	2633
Pietrain	51	2263
Hampshire	50	1899
D synthetic	24	2699
E synthetic	24	2045
Meishan	36	1609
Mean		2163

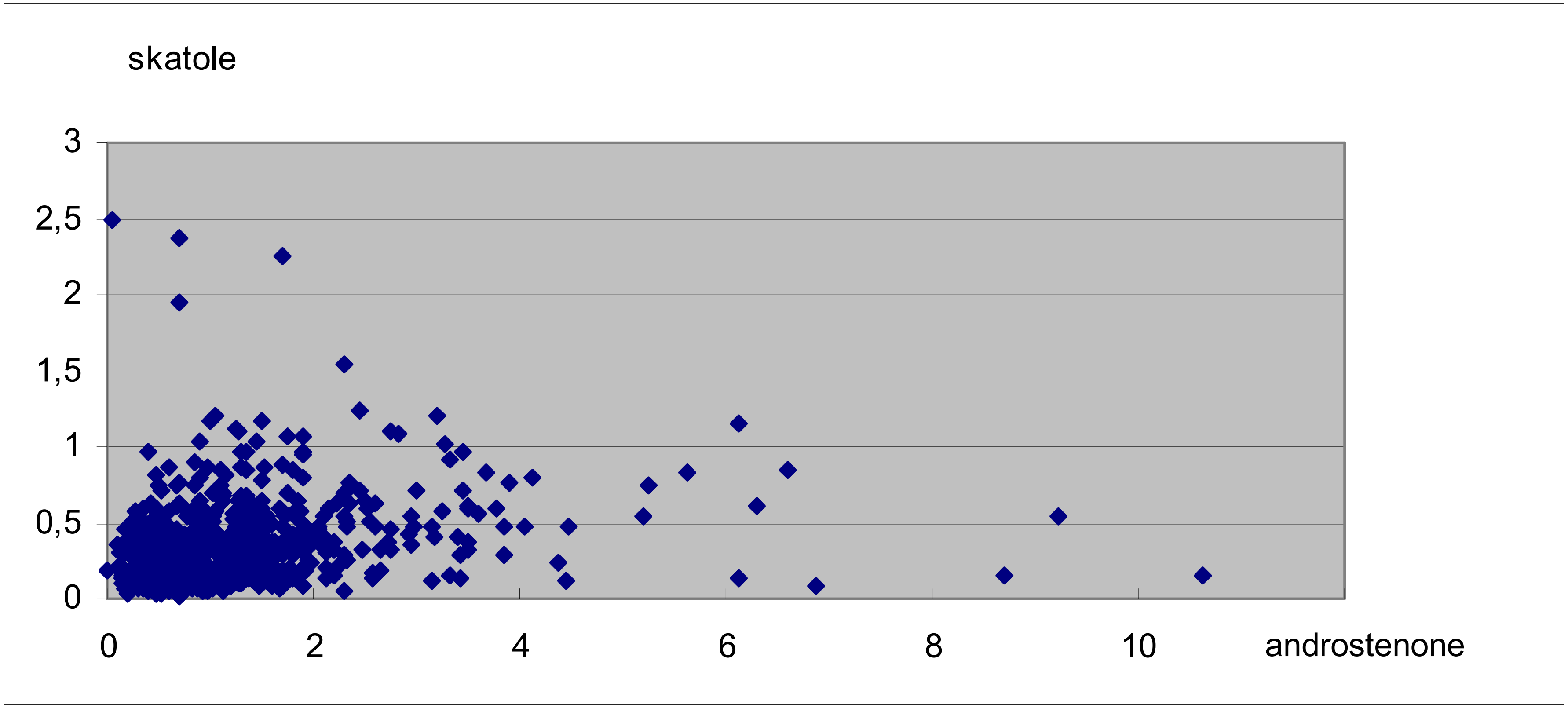
Genome scan for skatole in Landrace (RI)

- Danish Landrace (Danske Slagterier in-line skatole testing)
- ~6,000 samples
    - full sib pairs: 500 high skatole / 500 low skatole
- Phenotypes: growth, fat, pedigree, androstenone
- Genotyping completed Apr 2008
- Statistical analysis in progress

Danish Landrace samples for genome scan (RI)

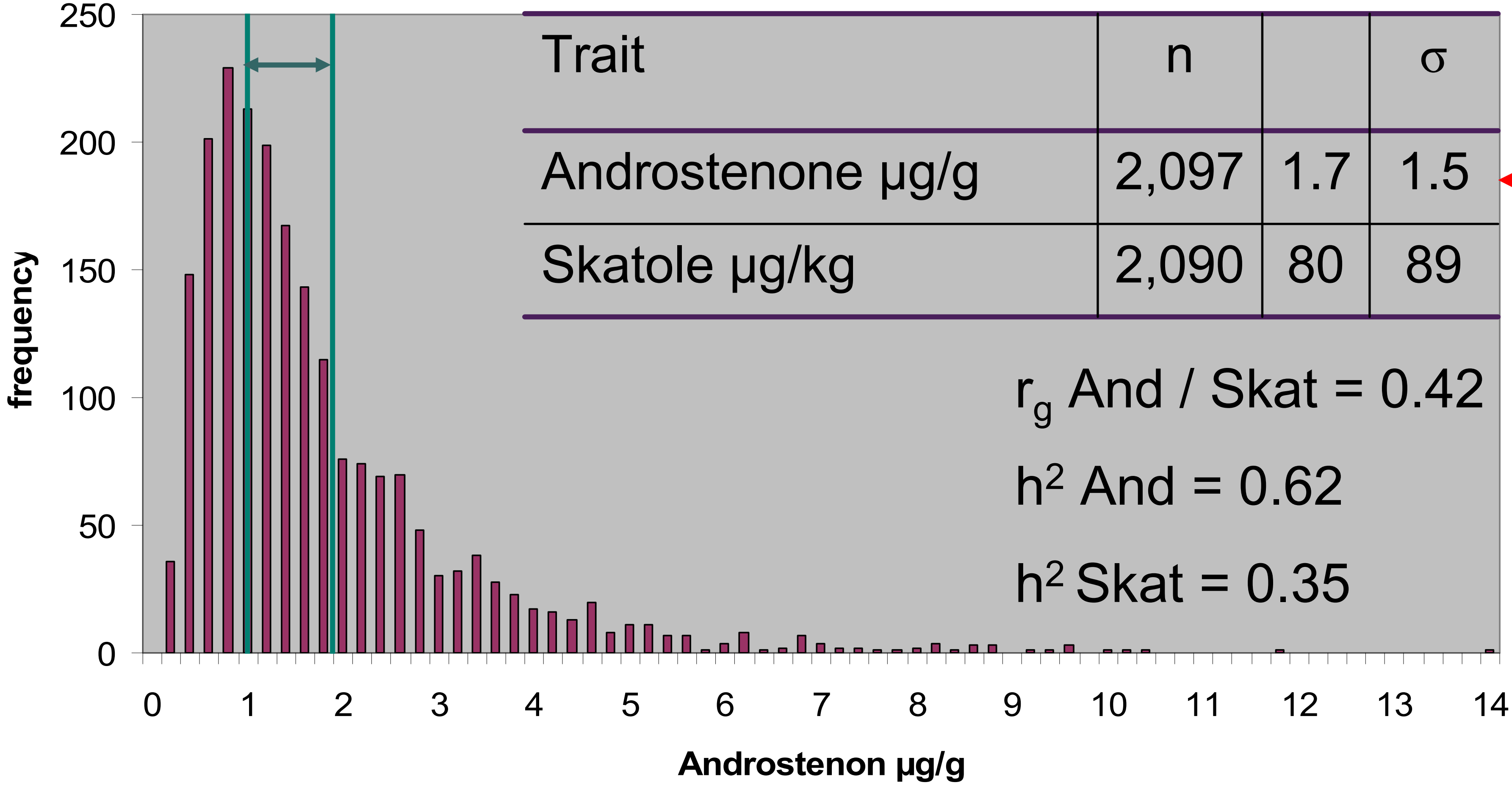


Skatole vs. Androstenone: r = 0.27



h^2 And = 0.56 h^2 Skat = 0.35

Duroc synthetic line sampled (IPG)



Trait	n		σ
Androstenone µg/g	2,097	1.7	1.5
Skatole µg/kg	2,090	80	89

r_g And / Skat = 0.42

h^2 And = 0.62

h^2 Skat = 0.35

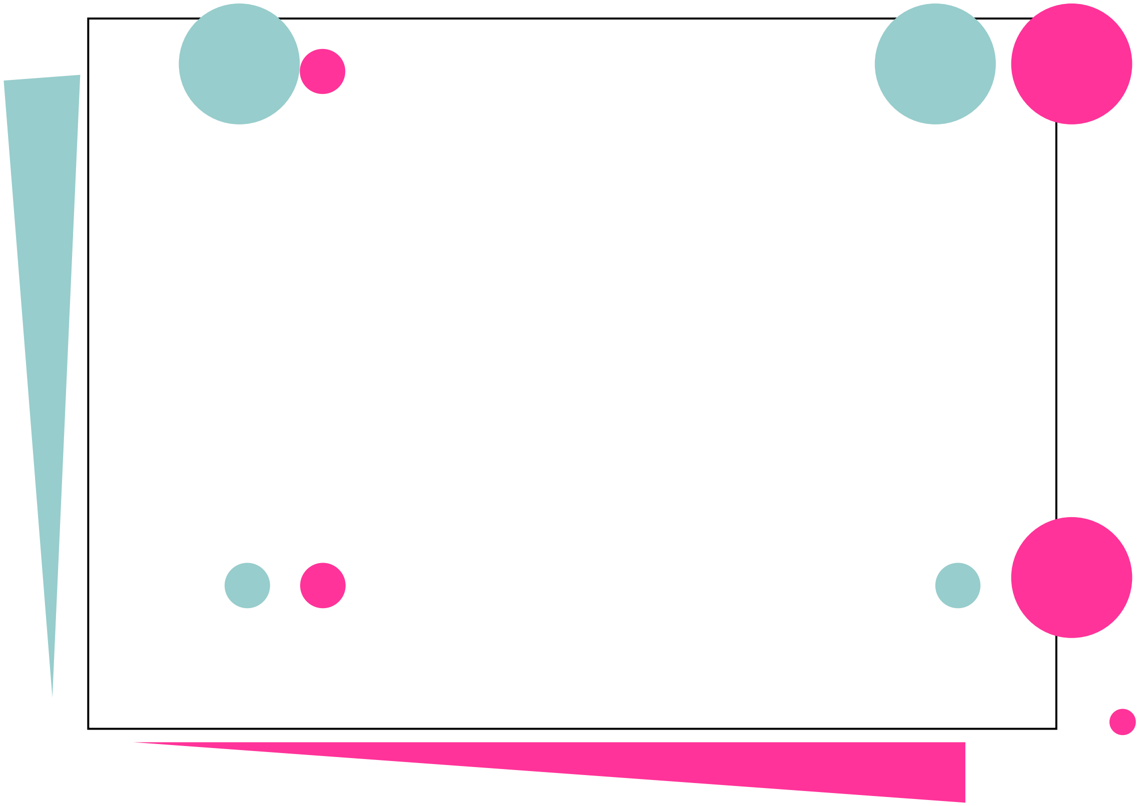
Breed differences for genetic back ground of boar taint compounds ?

Possible reasons for increase in boar taint compounds

Synthesis (testis), storage (fat) ↑

Metabolism, clearance (liver) ↓

● androstenone



● skatole

Analyse gene expression (AU)

- 27K cDNA porcine microarray on high/low androstenone

  60 Duroc and 60 Landrace (AU, NULS)

  testis / liver *(Moe et al. 2007, Moe et al. 2008)*

- 20K 70-mer oligo-array on high/low skatole

  60 Landrace + 60 Duroc/Yorkshire (AU, NULS)

  liver

Compare with protein expression (AU)

- Genes identified:
  - Involved in androstenone biosynthesis (CYP17, CYB5, FTL ...)
  - Involved in skatole metabolism, known genes (e.g. CYP2E, CYP2A) but many new genes strongly upregulated in high-skatole animals
  - Breed differences in expression profiles

- iTraq based proteomics: liver samples (40 LR high/low skatole)
  - good overlap to oligo-array study
  - large number of new differentially expressed proteins

Start fine mapping (INRA)

- Fine mapping of SSC7 QTL in Large White x Meishan backcross families

Previous QTL analysis→ QTL androstenone on SSC7

Construction of BC_4 animals

Fine mapping of this Androstenone QTL (A: LW a:MS)

Characterization of a region of 137 genes

Characterization (in silico) of all transcripts of each gene

137 genes in this region - 25 pseudogenes

- 26 genes without ESTs

86 genes with their transcripts

Development of PCR
specific for each transcript
for 13 testis-genes
testes/fat/liver

Confirmation of variation by qRT-PCR

Success with gene 73

- Meishan allele reduces androstenone
- 3 different transcripts indentified
- Increased transcription from Meishan allele
- Potential causal mutation in the promoter region under investigation

Sample collection for confirmation studies

- Collect samples from other breeds and measure boar taint :

Partner	Breed / cross	Boars
INRA	Large White	455
IPG	D line (Duroc based)	2000
	commercial crosses	400
IRTA	Commercial crosses	256
AU	Landrace, Duroc	1200
Total		4322

- and production traits: growth, feed efficiency, fertility, behaviour, carcass composition

Comparison of androstenone methods

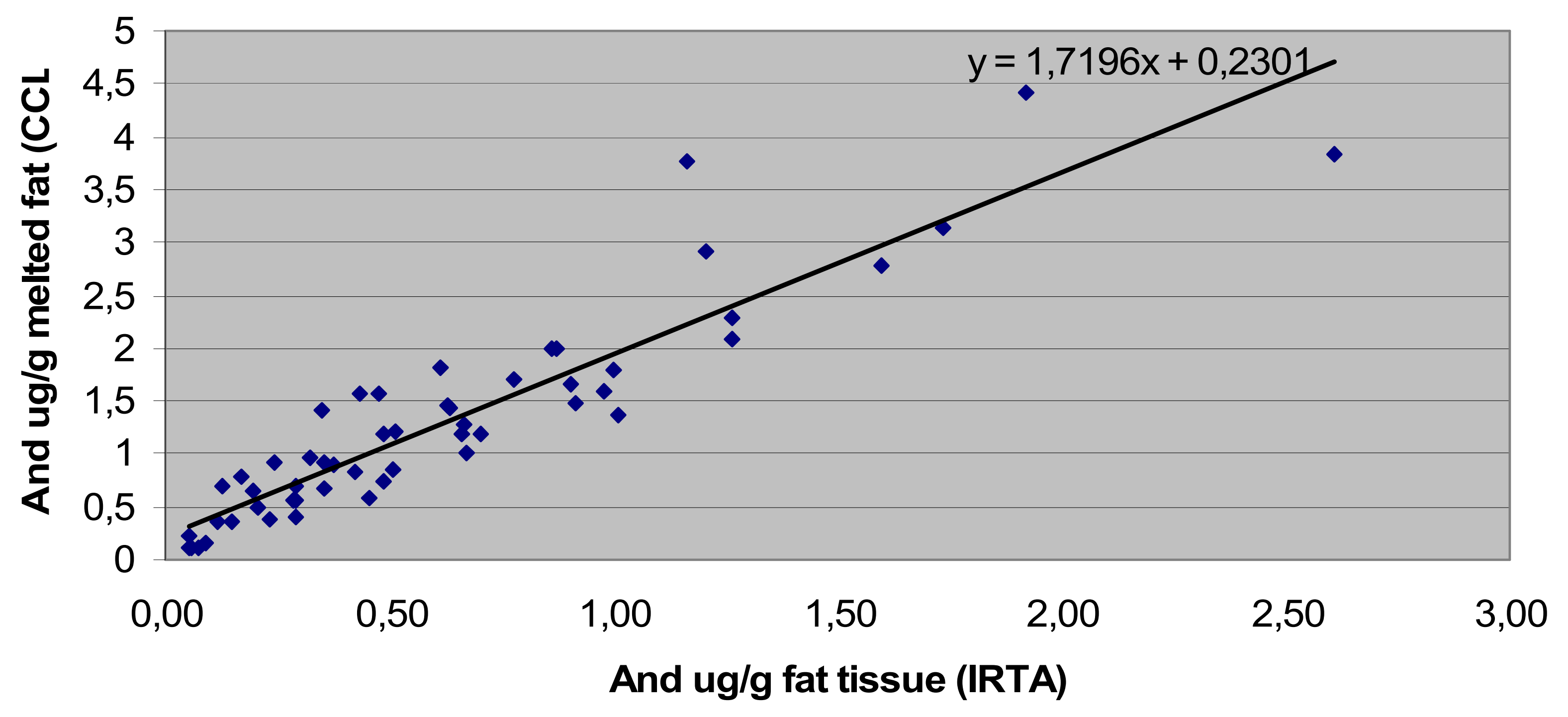
- 53 fat samples from 1 slaughter day:

Ship on dry ice to	method	sensitivity
NSVS	Fluoro-IA	0,05 µg/g
IRTA	GC-MS	0,1 µg/g
CCL	GC-MS	0,2 µg/g

correlations are high: 0.91– 0.82

… but levels differ

Pure melted fat vs total fat tissue



Relevant for comparison between studies and determination of consumer acceptance thresholds!

Do I really smell that bad??