Global gene expression analysis of liver for androstenone and skatole production in young boars

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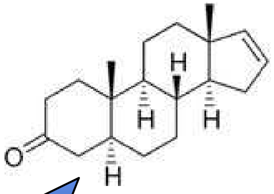
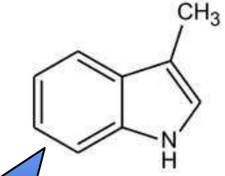
What is boar taint?

- ….sensory trait, which plays a major role in the acceptance of food products

- Boar taint (urine-, musk-, faecal-like):

Androsten-one, 3α,β –ol + skatole + other substances





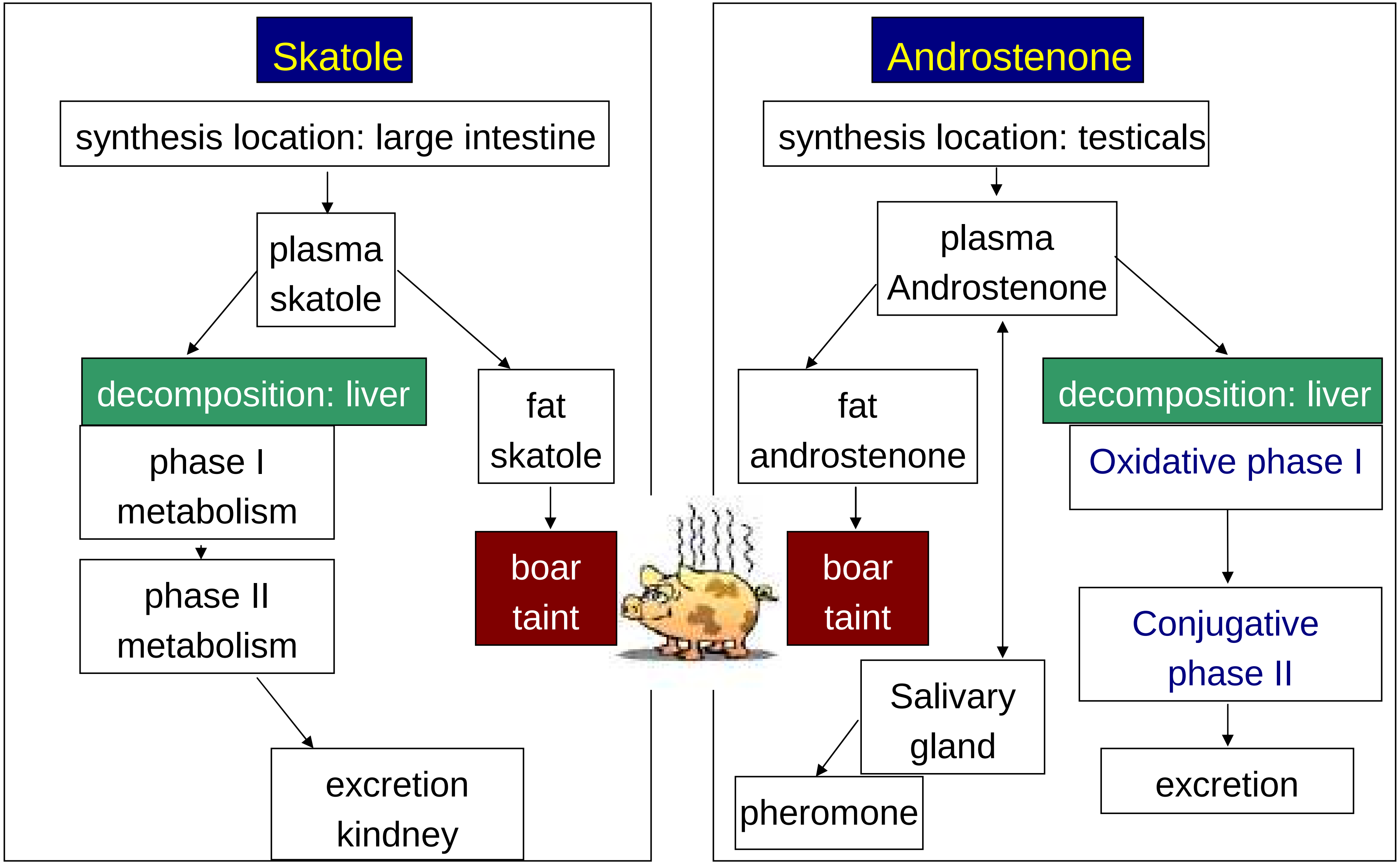
sexual pheromone
fat-soluble

tryptopan metabolite
fat-soluble
colon (sows and boars)

Indole, phenole
aldehyde
short-cain fatty acid

- Odour perception dependents on: gender, age, culture….

Metabolism



Skatole

synthesis location: large intestine

plasma skatole

decomposition: liver

phase I metabolism

phase II metabolism

excretion kindney

fat skatole

boar taint

Androstenone

synthesis location: testicals

plasma Androstenone

fat androstenone

boar taint

Salivary gland

pheromone

decomposition: liver

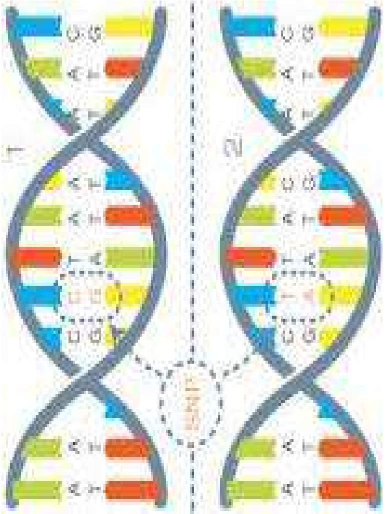
Oxidative phase I

Conjugative phase II

excretion

"Genome and Transcriptome"

- **QTL in fat:**
  - For androstenone on SSC 2, 4, 6, 7, 9 and 14 *(Lee et al. 2005; Quintanilla et al. 2003)*
  - For skatole on SSC 6, 7, 12 and X *(Varona et al. 2005; Lee et al. 2005)*



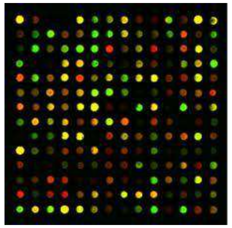
- **SNP – Arrays for Boar taint:**
  - Duroc: 227 SNPs in 123 candidate genes *(Grindflek, 2008)*
  - Landrace: 137 SNPs in 60 candidate genes *(Grindflek, 2008)*
  - Danishes Landrace: 7000 SNPs *(Archibald, 2008)*

- **Candidate genes:**
  - Androstenone biosynthesis (CYP2E, CYP2A,…)

    (Skinner et al. 2005; Lin et al. 2006; Grindflek 2008)
  - Skatole-, androstenone decomposition (SULT1A1, SULT2E1,….)

    (Lin et al. 2004; Grinflek 2008)
  - Androstenone retention in fat (CYB5,…)

    (Lin et al. 2004; Peacock et al. 2007; Zamaratskaia et al. 2008)



Relationship between androstenone and skatole yielded conflicting results

Phenotypic correlation close to	0	*(Zamaratskaia et al. 2005)*
Positive correlation	0.36 – 0.62	*(Tajet et al. 2006)*
Genetic correlation	0.32	*(Bergsma et al. 2007)*

- Physiological relationship between skatole and androstenone levels are not fully understood
    - „Androstenone inhibits the action of the CYP2E1 protein which is involved in skatole degradation in the liver *(Doran et al. 2002)*“.

Problem and aim of this study

Problem

- Castration prohibiting
- Boar meat products yes/no
- Breed-specific characteristics
- Number of relevant genes

Aim

To identify genetic factors affecting levels of boar taint compounds (androstenone and skatole) and their interaction

Materials

101 Animale (Piètrain x F1)

20 F_2 boars

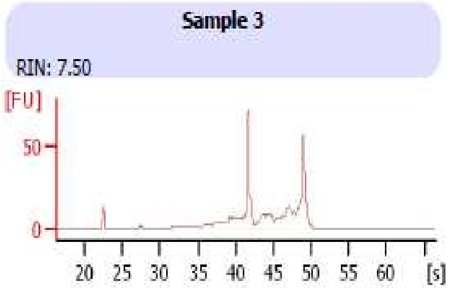
5 boars high androstenone/ low skatole	5 boars high androstenone/ high skatole	5 boars low androstenone/ high skatole	5 boars low androstenone/ low skatole

mg and.	**mg ska.**
> 500.5	**< 142.9**

mg and.	**mg ska.**
> 437.7	**> 339.9**

mg and.	**mg ska.**
< 97	**> 282.8**

mg and.	**mg ska.**
< 169.7	**< 145.1**



RNA isolation and quality control

Method

Microarray
porcine Affymetrix Chip

Creation of
transcriptome profiles

Data analysis

Normalization and
background correction

gcRMA

Linear contrasts among the factor levels,

(F-test)

P-Wert ≤ 0,05, FC ≥ 1, FDR ≤ 0,3

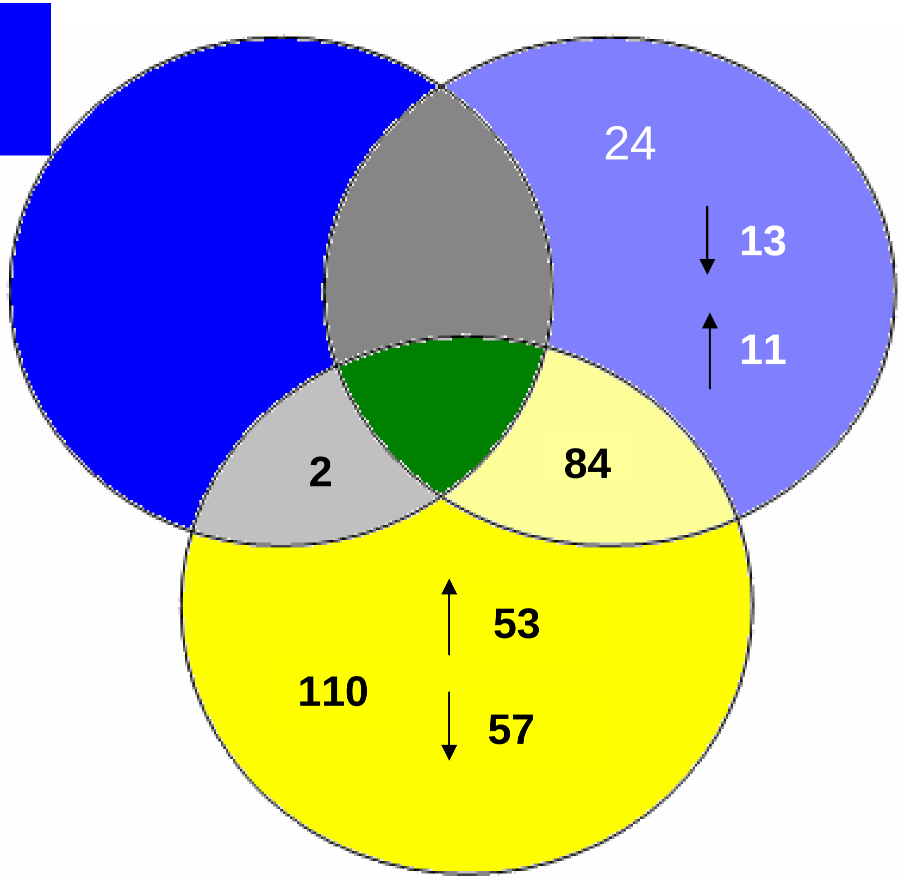
LIMMA

Bioinformatic
analyses

Results

Androstenone high/ Androstenone low

Skatole high/ Skatole low



Interaction

Comparison of high and low levels of androstenone

- potentially differentially expressed genes

Gene name		log FC	P-Value	FDR
CYP3A7	Cytochrome P450 3A7	1.34	0.0017	0.84
INHBA	Inhibin beta A chain precursor	-1.07	0.0019	0.84

- CYP3A is one of the main subfamily involved in the biotransformation *(Guengerich, 2005)*

- INHBA
  - Negative impact on hepatocyte cell growth *(Mei et al. 2006)*
  - Expression in fetal interstitium in mouse testes *(Denise et al. 2010)*
  - The first factor produced by fetal Leydig cell *(Denise et al. 2010)*

Comparison of high and low levels of skatole

- 108 genes differentially expressed
- 59 up-regulated and 49 down regulated in liver tissue

Gene name		log FC	P-Value	FDR
CYP4A11	**Cytochrome P450 4A11**	**-3.11**	**0.00011**	**0.05**
DHRS3	**Short-chain dehydrogenase/reductase 3**	**-1.01**	**0.00136**	**0.15**
UGT2B17	**UDP-glucuronosyltransferase 2B17**	**-1.24**	**0.00294**	**0.22**
GSTT1	**Glutathione S-transferase theta 1**	**-1.14**	**0.00295**	**0.22**
DHRS4	**Dehydrogenase/reductase SDR family member 4**	**2.08**	**0.00356**	**0.24**
CYP39A1	**Cytochrome P450 39A1**	**-1.01**	**0.00378**	**0.24**

Comparison of high and low levels of skatole

- Significantly differentially expressed genes in stage I and II of liver metabolism
    - Cytochrom P450 Familie
    - UGT Familie *(Moe et al. 2008)*

- GSTT1
    - Catalytic effect in the conjugation reactions
    - Intracellular transport of steroids *(Listowsky et al. 1988)*
    - GST gene ⟶ breed-specific differences in expression

      (Moe et al. 2008)

- DHRS3 & DHRS4
    - Catalyze the oxidation / reduction of retinoids and steroids

      (Haeseleer and Palczewski, 2000)

Interaction between skatole and androstenone

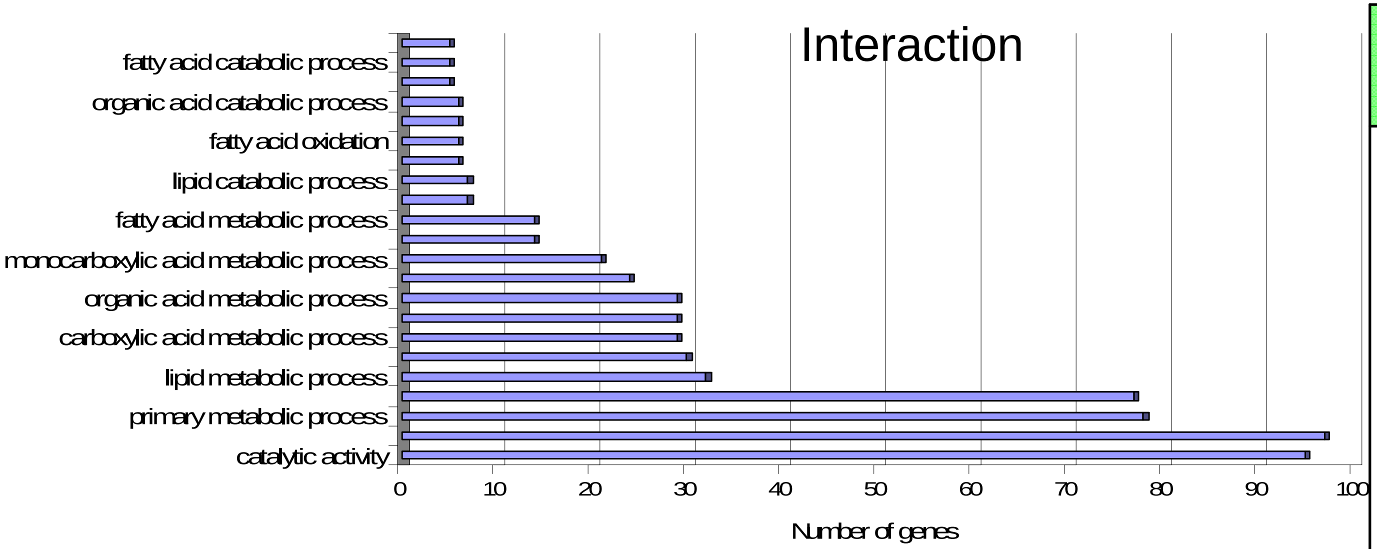
- 196 genes are differentially expressed
- 94 down represented and 103 over represented

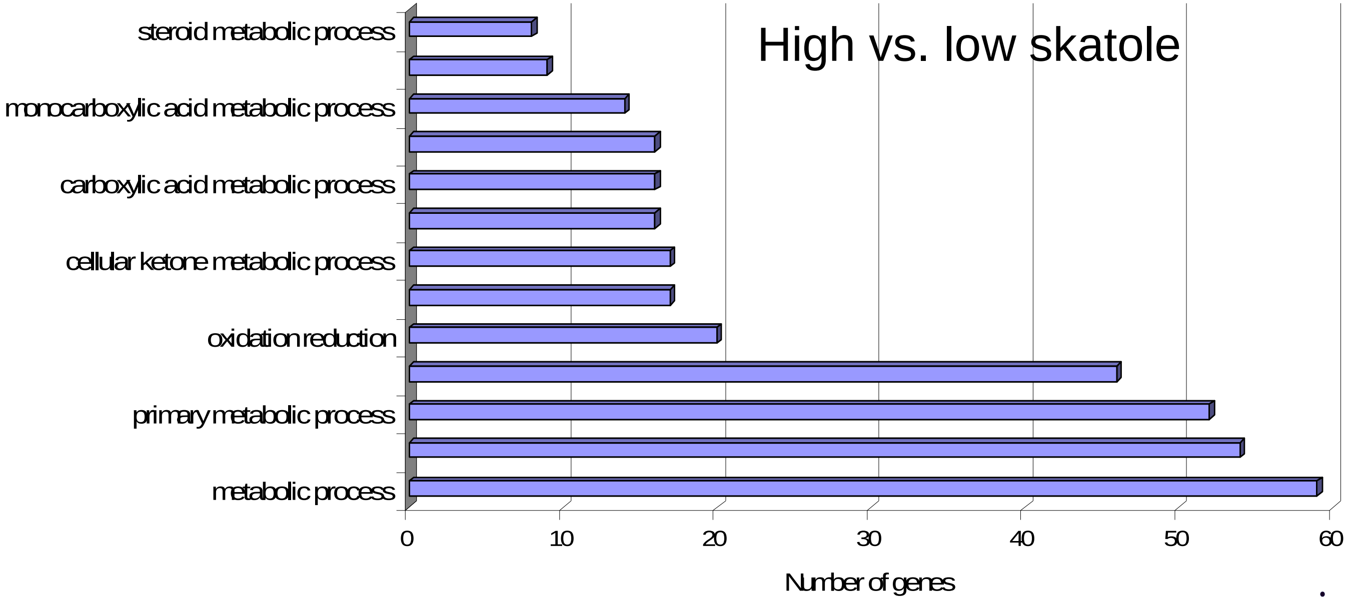
Gene name		**logFC**	**P-Value**	**FDR**
CYP3A7	**Cytochrome P450 3A7**	**-2.04**	**0.00010**	**0.04**
CYP4A11	**Cytochrome P450 4A11**	**-2.90**	**0.00018**	**0.06**
CYP2D6	**Cytochrome P450 2D6**	**-1.06**	**0.0096**	**0.29**
Q5TCH4	**Cytochrome P450, family 4, subfamily A, polypeptide 22**	**-3.42**	**0.00055**	**0.09**
DHRS7	**Dehydrogenase/reductase SDR family member 7 precursor**	**-1.55**	**0.0014**	**0.12**
DHRS3	**Short-chain dehydrogenase/reductase 3**	**-1.07**	**0.00095**	**0.11**
GSTT1	**Glutathione S-transferase theta 1**	**-1.23**	**0.0019**	**0.14**
Q9BT58	**Hydroxysteroid dehydrogenase like 2**	**1.05**	**0.0029**	**0.18**
HSD17B12	**Steroid dehydrogenase homolog; 3-ketoacyl-CoA reductase**	**-1.51**	**0.0077**	**0.26**

Interaction between skatole and androstenone

- Transcriptes from phase I and II of liver metabolism
- Genes from the cytochrome P450, GST and SDR family
- HSDs belong to the SDR and AKR subfamilies *(Moe et. al 2008)*
  - involved in steroid metabolism *(Moe et al. 2008, Grindflek et al. 2011)*
  - central role in the biosynthesis and inactivation of all steroid hormones *(Penning 1997)*

Gene ontology


Interaction


High vs. low skatole

Pathway	**Molecules**
Fatty acid-Metabolism	ACAA1 ACSL1 ACSL5 ACADVL ADH4 CPT1A CYP4A11 HADHA
Retinol – metabolism	ADH4 ALDH1A1 CYP3A7 CYP4A11 DHRS3 DHRS4 RDH11

Summary

- Genes from two phases of liver metabolism
  - Phase I : gene of Cytochrome P450 family
  - Phase II : gene of UGT, GST and SDR family

- The results of the interaction analysis suggested that there is a interaction between androstenone and skatole

- Further work

  - Validation of the results in different breeds

  - Identification of polymorphisms

Thank you for your attention

- Federal Ministry of Food, Agriculture and Consumer Protection Germany (BMELV)

Bundesministerium für
Ernährung, Landwirtschaft
und Verbraucherschutz

- State of North Rhine Westphalia with the programme USL "Umweltverträgliche und Standortgerechte Landwirtschaft"

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