ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

PigPhenomics

Re-Livestock
RESILIENT FARMING SYSTEMS

How heritable are metabolomic features? An omic based approach in pigs

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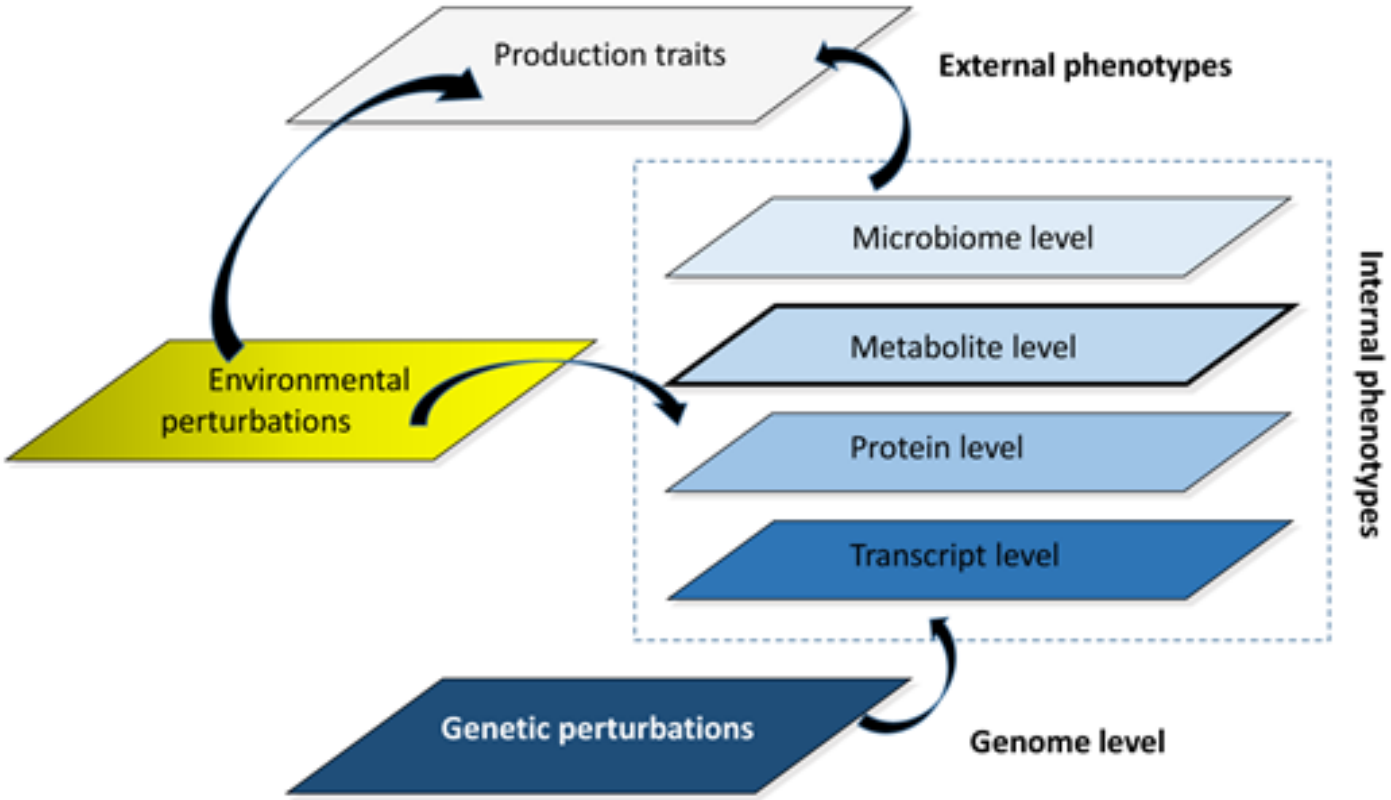
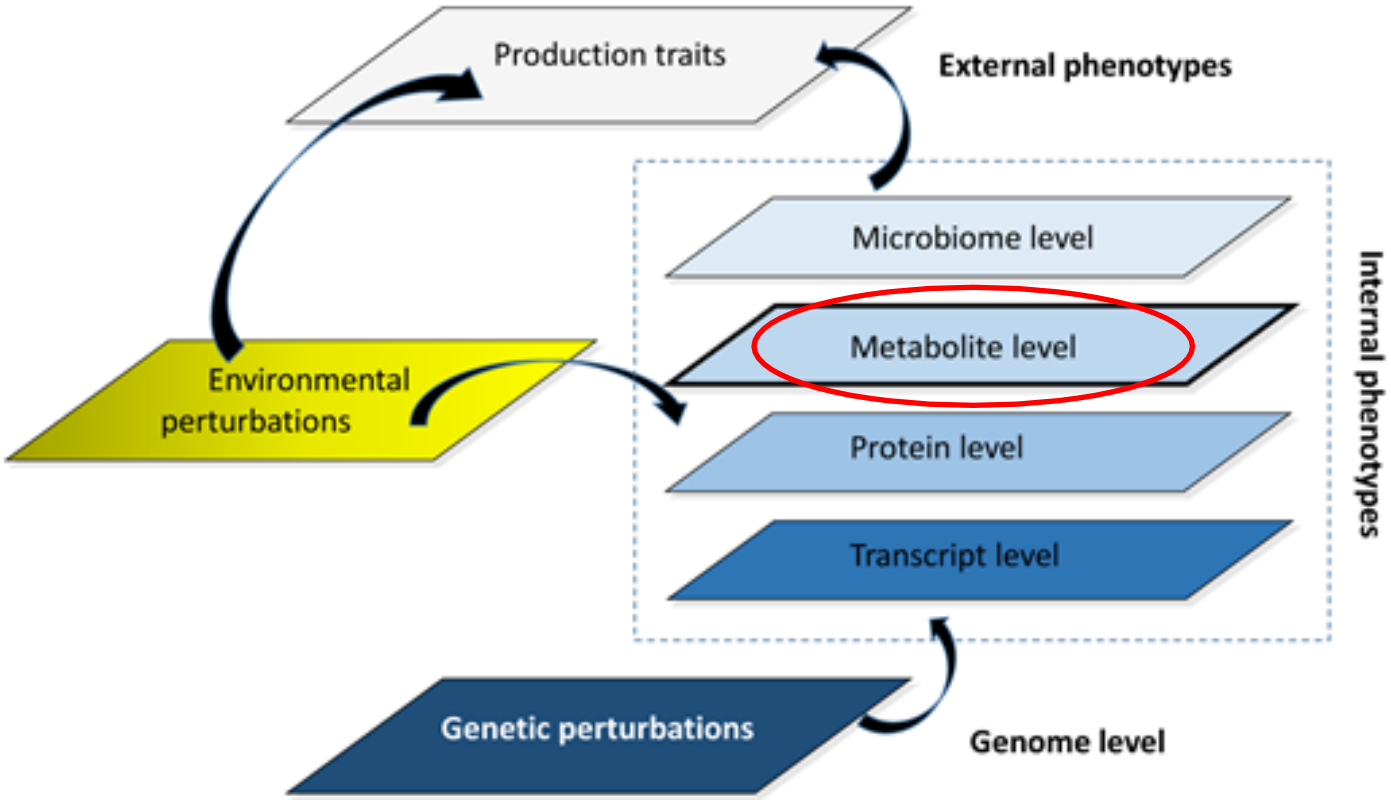
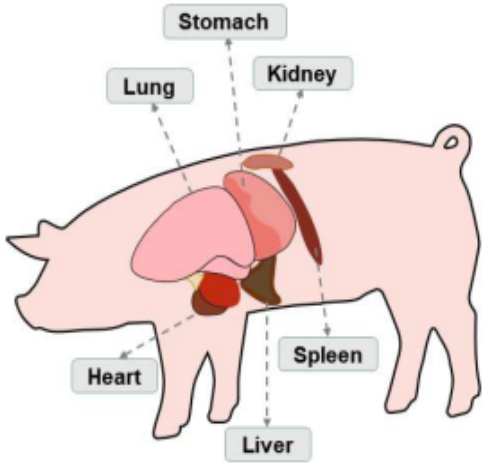
Biological complexity: an overview



Biological complexity: an overview



Blood senses the whole organism physiology



Metabolomics in pigs: What do we know?

- Studies mainly exploited untargeted metabolomics
- Plasma as main analysed biofluid
- Small fraction of the metabolome is usually analysed
- Few studies applied more extensive metabolomics
- Few information about the genetic architecture of metabolomic traits

What we did:

Targeted metabolomics
Biocrates AbsoluteIDQ p180 Kit

Metabolite classes	No.	Biological relevance (selected)
Acylcarnitines	40	Energy metabolism fatty acid transport
Amino acids	21	Amino acid metabolism, **neurotransmitter metabolism**
Biogenic amines	19	**Neurological disorders** DNA stability, oxidative stress
Hexoses	1	Carbohydrate metabolism
Glycerophospholipids	90	
- lysoPhosphatidylcholine acyl – lysoPC a Cx:x	14	Degradation of phospholipids fatty acid profile
- Phosphatidylcholine diacyl – PC aa Cx:x	38	Dyslipidemia, Membrane composition and damage
- Phosphatidylcholine acyl-alkyl – PC ae Cx:x	38	
Sphingolipids	15	Signalling cascades membrane damage

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ANAS
associazione nazionale allevatori suini

Italian Large White

900 pigs

Italian Duroc

400 pigs

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Illumina 60K SNP chip

Data quality control

R, Python

Metabolomics

- Removal of metabolites with CV over replicates > 25%
- Samples 30% of metabolites failing QC (1.5 times the inter-quartile)
- Box-Cox transformations
- Removal sex, weight, environmental and technical factors/effects via LM


ILW: 169/186 metabolites (residuals)


IDU: 164/186 metabolites (residuals)

R, Python

Pedigree (4 generations)

Check of the records:

- Sex
- Date of birth
- Parents

ILW: 2,236 records 

- 85 sires, 347 dams
- …

IDU: 865 records 

- 48 sires, 140 dams
- …

Plink

Genomics

Inclusion of samples and SNPs

- Missing rate < 0.10
- Call rate >0.95
- MAF > 0.05


ILW: 45,423 SNPs


IDU: 38,361 SNPs

Heritability estimation

R tool
library(kinship2)
library(regress)
library(gap)
GEMMA tool

Additive model:

$$y_i = \beta_0 + u_i + \varepsilon_i$$

y_i is the phenotype, β_0 is an intercept, u_i is an additive genetic effect, ε_i is a the of non genetic effect.
i = 1 to i = n (n = number of animals).

$$\mathbf{y} = \mathbf{1}\beta_0 + \mathbf{u} + \varepsilon$$

y is a vector of phenotype values (residuals), **u** is a vector additive genetic effects, ε is a vector residuals

$\mathbf{u} = \mathbf{a} \sim N(\mathbf{0}, \mathbf{A}^*\sigma^2_a)$ → A, pedigree-based relationship matrix;
$\mathbf{u} = \mathbf{g} \sim N(\mathbf{0}, \mathbf{G} * \sigma^2_g)$ → G, SNP-based (genomic) relationship matrix (GRM);

Narrow sense heritability

Ratio of the genetic variance to the total variance

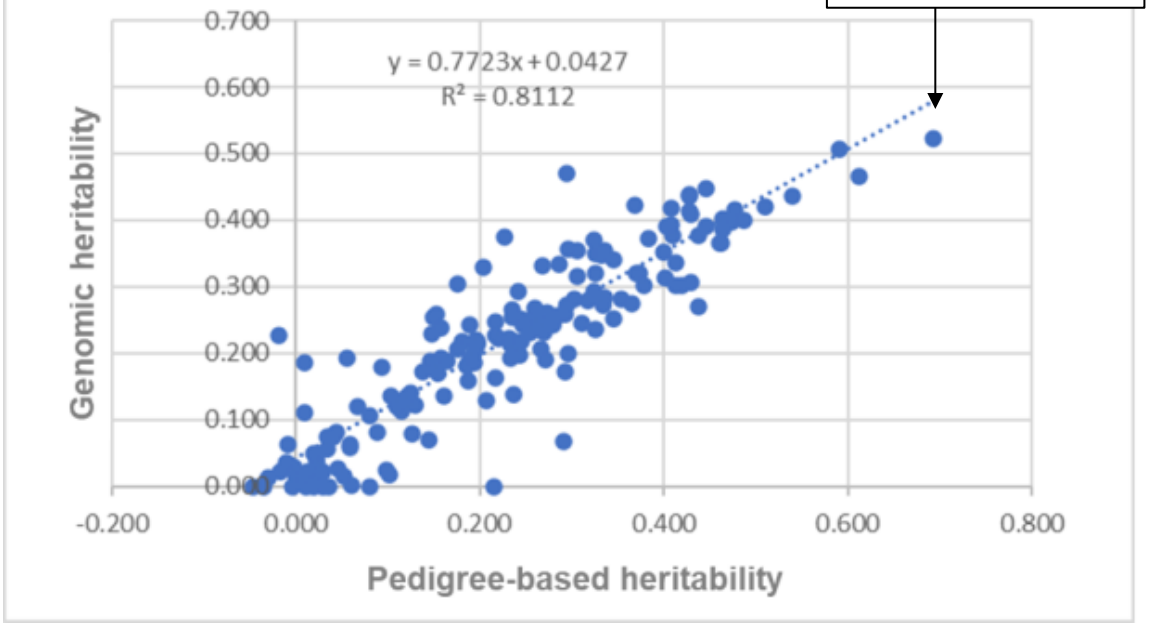
$$h^2_a = \frac{\sigma^2_a}{\sigma^2_a + \sigma^2_\varepsilon} \qquad h^2_g = \frac{\sigma^2_g}{\sigma^2_g + \sigma^2_\varepsilon}$$

Genomic hereditability vs pedigree-based heritability

h^2_a and h^2_G estimates are similar

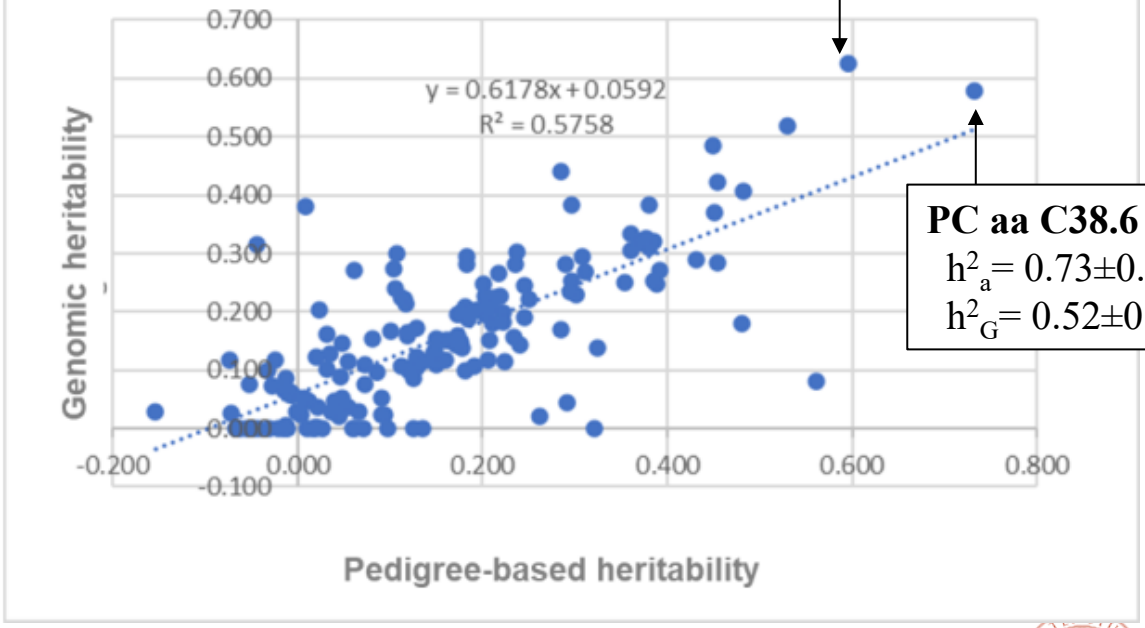
Italian Large White, corr = 0.90 - Italian Duroc, corr = 0.76

PC aa C40:5
h^2_a= 0.69±0.10
h^2_G= 0.52±0.06



PC aa C40.6
h^2_a= 0.60±0.16
h^2_G= 0.63±0.11

PC aa C38.6
h^2_a= 0.73±0.16
h^2_G= 0.52±0.06



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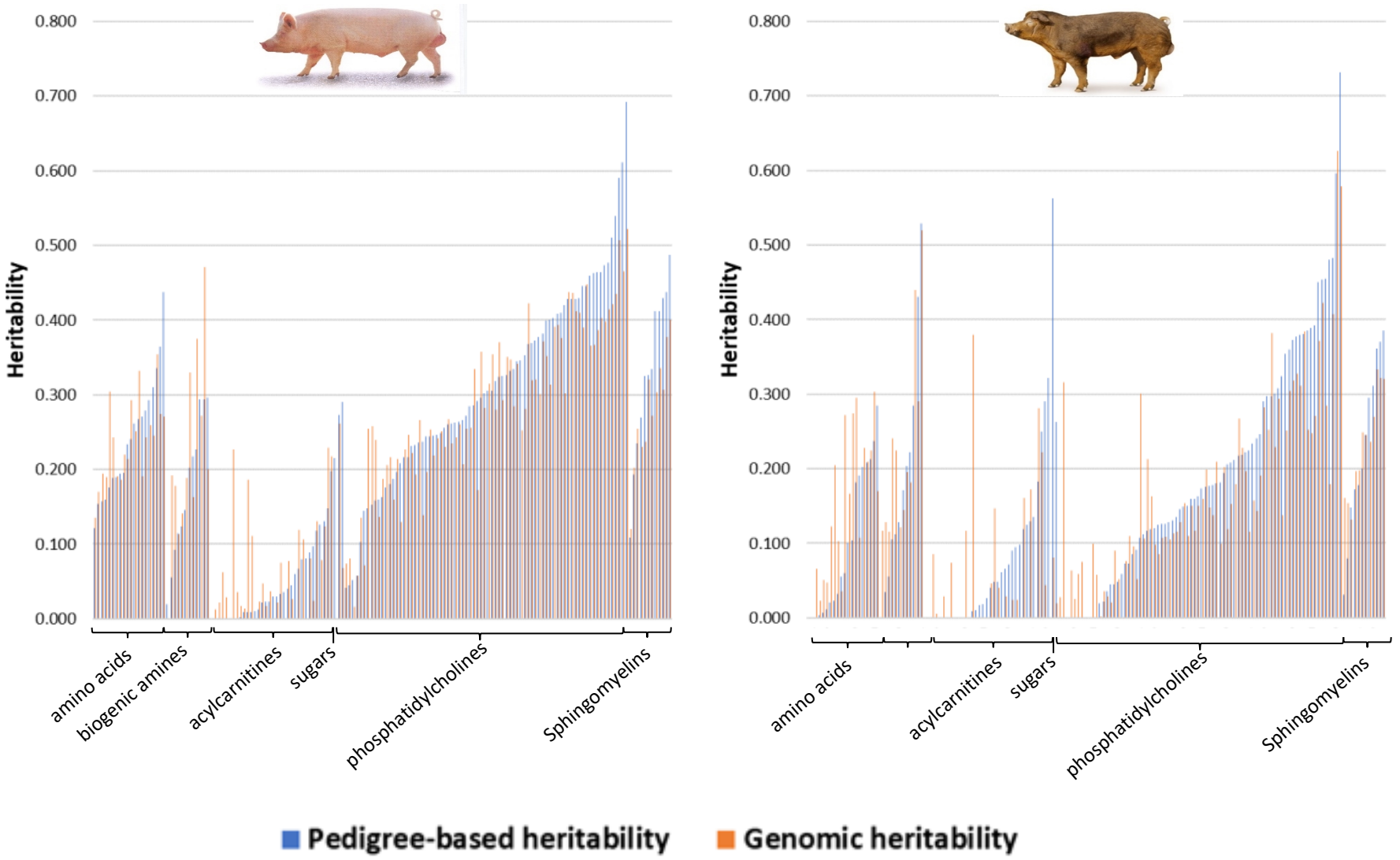
Overview of the heritability of the different metabolites



Overview of the heritability of the different metabolite classes

	Italian Large White		Italian Duroc	
Classes	h^2_a	h^2_G	h^2_a	h^2_G
Amino acids	0.24±0.08	0.24±0.06	0.09±0.09	0.15±0.10
Essential (n . 9)	0.22±0.08	0.21±0.06	0.06±0.08	0.11±0.10
Nonessential (n. 11)	0.26±0.08	0.26±0.06	0.12±0.10	0.18±0.10
Acylcarnitines	0.05±0.05	0.06±0.04	0.06±0.08	0.05±0.09
Biogenic amines	0.19±0.07	0.23±0.06	0.21±0.12	0.24±0.11
Phosphatidylcholines	0.30±0.08	0.28±0.06	0.19±0.11	0.17±0.13
Sphingomyelins	0.33±0.09	0.28±0.06	0.20±0.12	0.22±0.12
Sugars	0.29±0.11	0.07±0.04	0.26±0.18	0.02±0.02

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Heritability estimates: focus on amino acids

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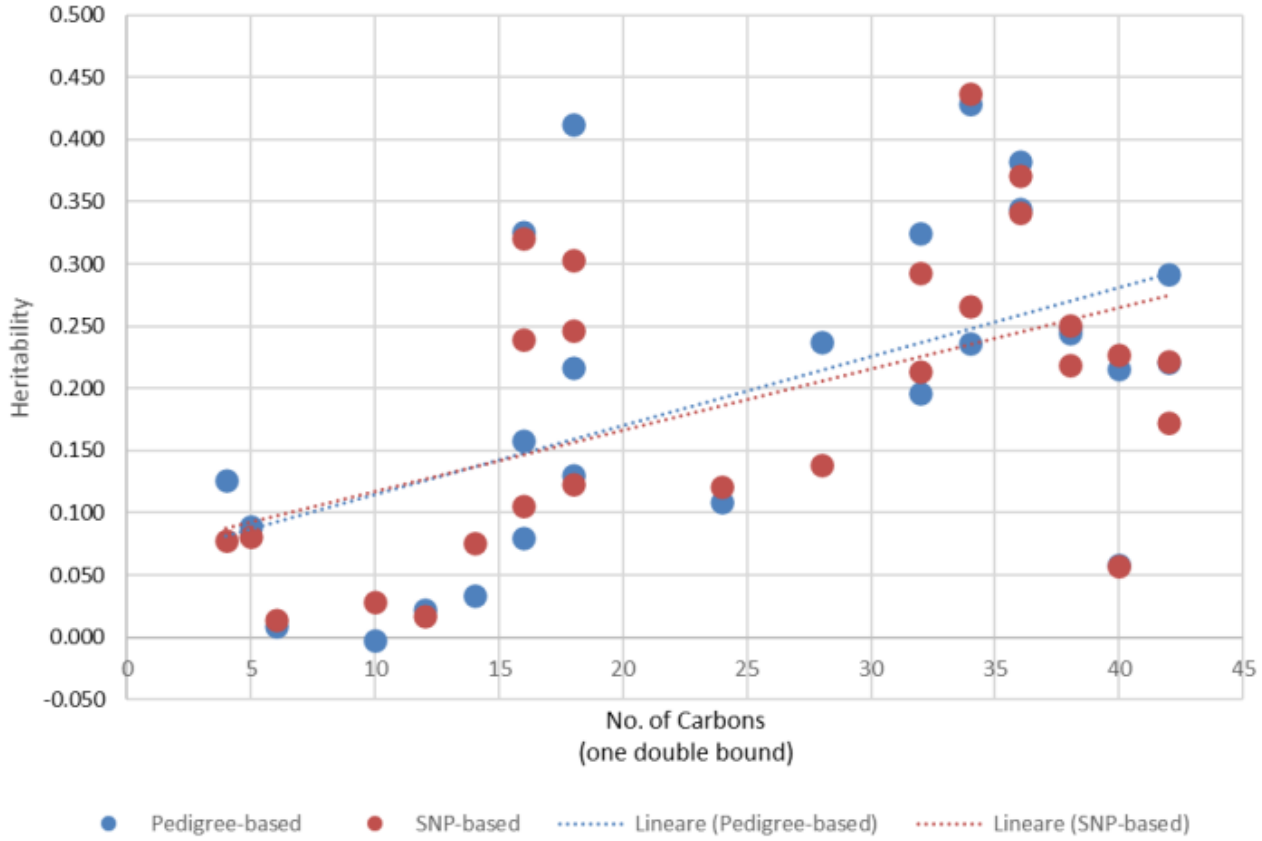
* Including a non-protein amino acid

Heritability of metabolites based on the number of carbon atoms

Acylcarnitine, Glycerophospholipids, Sphingolipids



Positive correlation with an increasing number of carbon when we considered all metabolites with only one double bound

- $r = 0.55$ with h^2_P
- $r = 0.53$ with h^2_{SNP}

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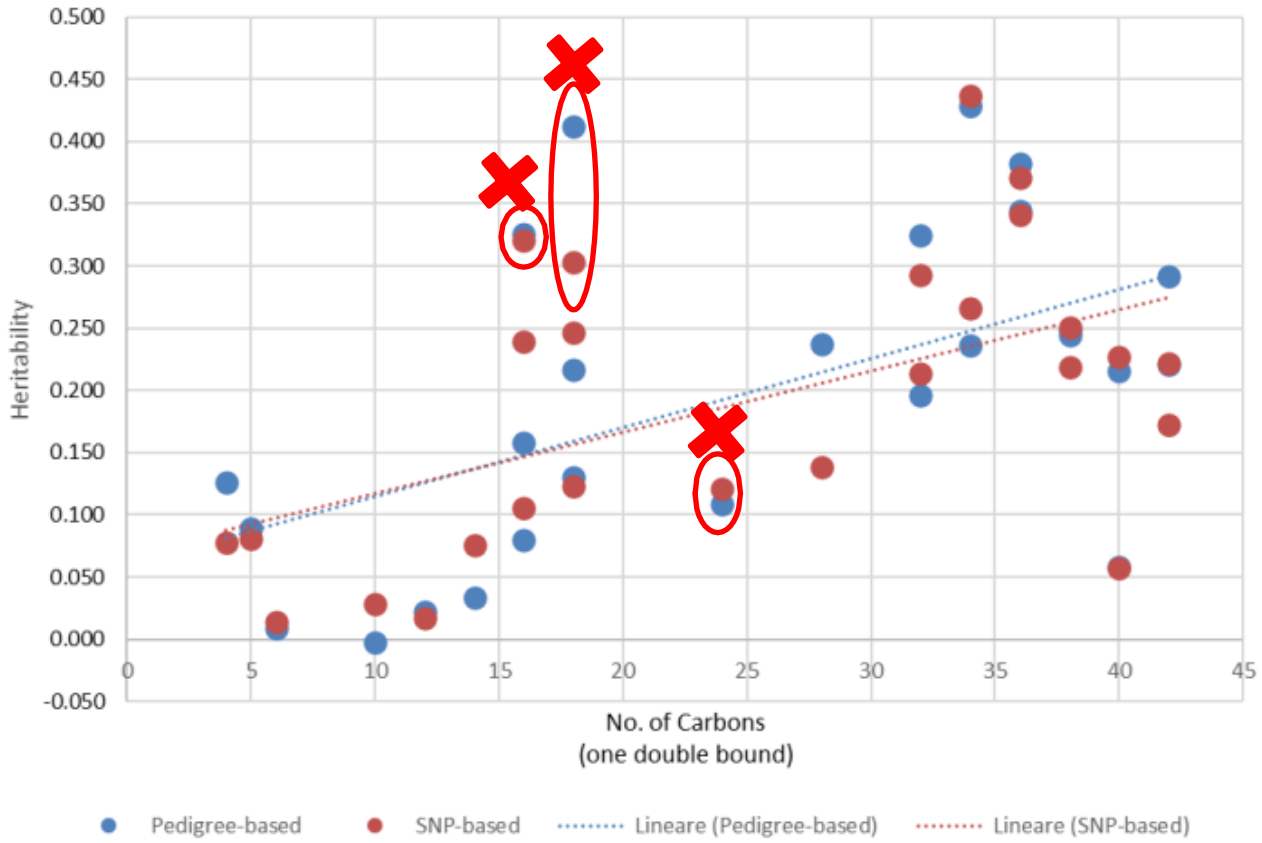

Positive correlation with an increasing number of carbon when we considered all metabolites with only one double bound

- $r = 0.55$ with h^2_P
- $r = 0.53$ with h^2_{SNP}

✖ When sphingomyelins where not considered

- $r = 0.70$ with h^2_P
- $r = 0.63$ with h^2_{SNP}

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Other trends of heritability estimates

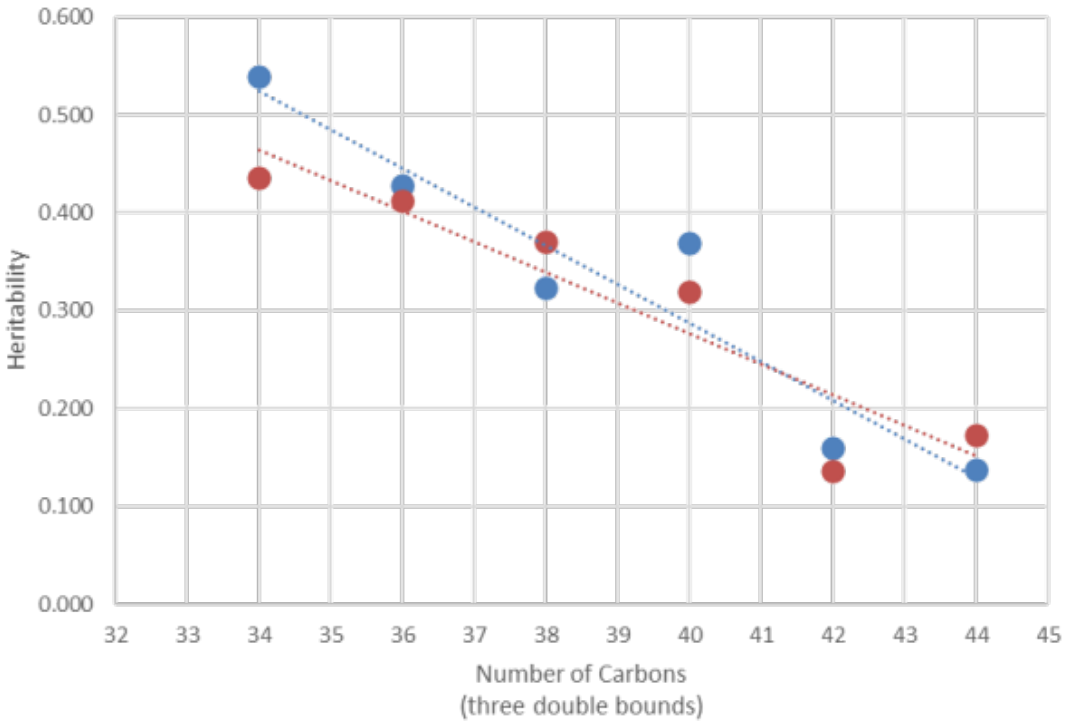
Within an analyte class (PC ae)

- Different no. of Carbons
- Three double bonds each metabolite

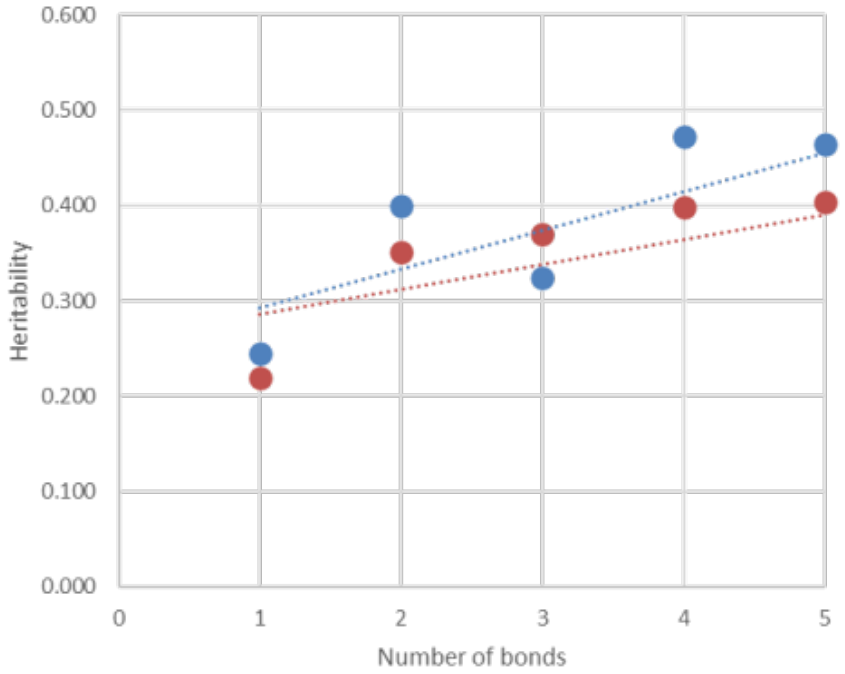
Within a specific metabolite: PC ae C38

- Same no. of Carbons
- Variable number of bounds

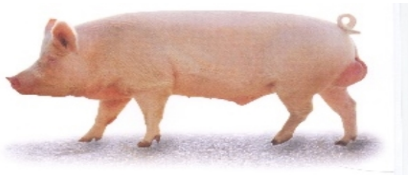
Phosphatidylcholine acyl-akyls (PC ae)



PC ae C38



Metabolomics in Pigs: let's scale up to phenomics



Targeted metabolomics
- 186 compounds
- 5 metabolite classes

Untargeted metabolomics
- >700 known compounds

SUPER_PATHWAY
Lipid
Amino Acid
UNNAMED
Xenobiotics
Nucleotide
Peptide
Cofactors and Vitamins
Carbohydrate
Energy
Partially Characterized Molecules

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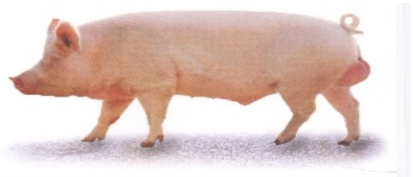
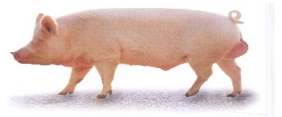


Correlation between platforms (some metabolites)

Metabolite	Class	Correlation
C2	acylcarnitines	0.975998345
C3	acylcarnitines	0.88026814
C16	acylcarnitines	0.853506255
H1	sugars	0.850376421
Kynurenine	biogenic amines	0.848239497
Asp	aminoacids	0.82522752
Thr	aminoacids	0.806543427
Taurine	biogenic amines	0.79144597
Cit	aminoacids	0.784755164
Pro	aminoacids	0.764434112
Serotonin	biogenic amines	0.755764553
Ala	aminoacids	0.738146684
Glu	aminoacids	0.735538107
Gly	aminoacids	0.710684447
Tyr	aminoacids	0.698004144
Arg	aminoacids	0.691249584
Met	aminoacids	0.684752848
Orn	aminoacids	0.663771832
alpha-AAA	biogenic amines	0.661253721
Phe	aminoacids	0.635076333
Trp	aminoacids	0.626816334
Ser	aminoacids	0.623964926
Gln	aminoacids	0.599885051
lysoPC a C18:1	glycerophospholipids	0.594866502
Asn	aminoacids	0.537954759
Met-SO	biogenic amines	0.518967723
Val	aminoacids	0.517217768
Lys	aminoacids	0.512279164
C4	acylcarnitines	0.436621761
His	aminoacids	0.41798508
C8	acylcarnitines	-0.050185106

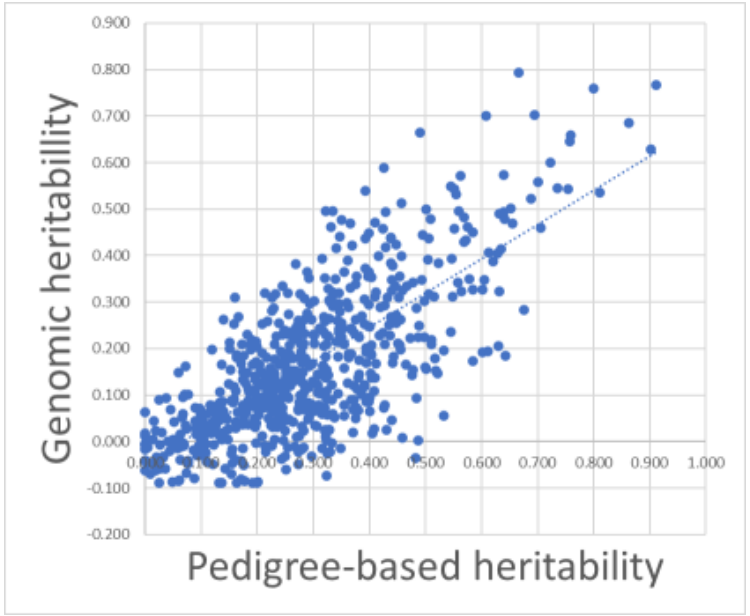
Heritability estimates



h^2_a and h^2_g estimates are similar

ILW, corr = 0.74

Classes	h^2_a	s.e.(h^2_a)	h^2_G	s.e.(h^2_G)
Amino Acid	0.198	0.106	0.323	0.095
Carbohydrate	0.076	0.081	0.266	0.098
Cofactors and Vitamins	0.156	0.098	0.249	0.093
Energy	0.039	0.072	0.188	0.092
Lipid	0.096	0.086	0.257	0.095
Nucleotide	0.104	0.088	0.255	0.094
Partially Characterized Molecules	0.138	0.094	0.238	0.087
Peptide	0.241	0.112	0.369	0.082
Unnamed	0.123	0.092	0.266	0.089



Xenobiotics not included

Average moderate values (h^2_a~ 0.16, h^2_G~ 0.35)

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Conclusions

- A broad range of heritability
- Metabolite class and pathway depended estimates
- Effect of different platforms
- Metabolites can be useful internal phenotypes
- We are already using some metabolites in breeding programmes

https://www.cost.eu/actions/CA22112/

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