# On host-microbiota interactions for the determinism of livestock phenotypes: an overview of recent results in pigs

**Jordi Estellé**

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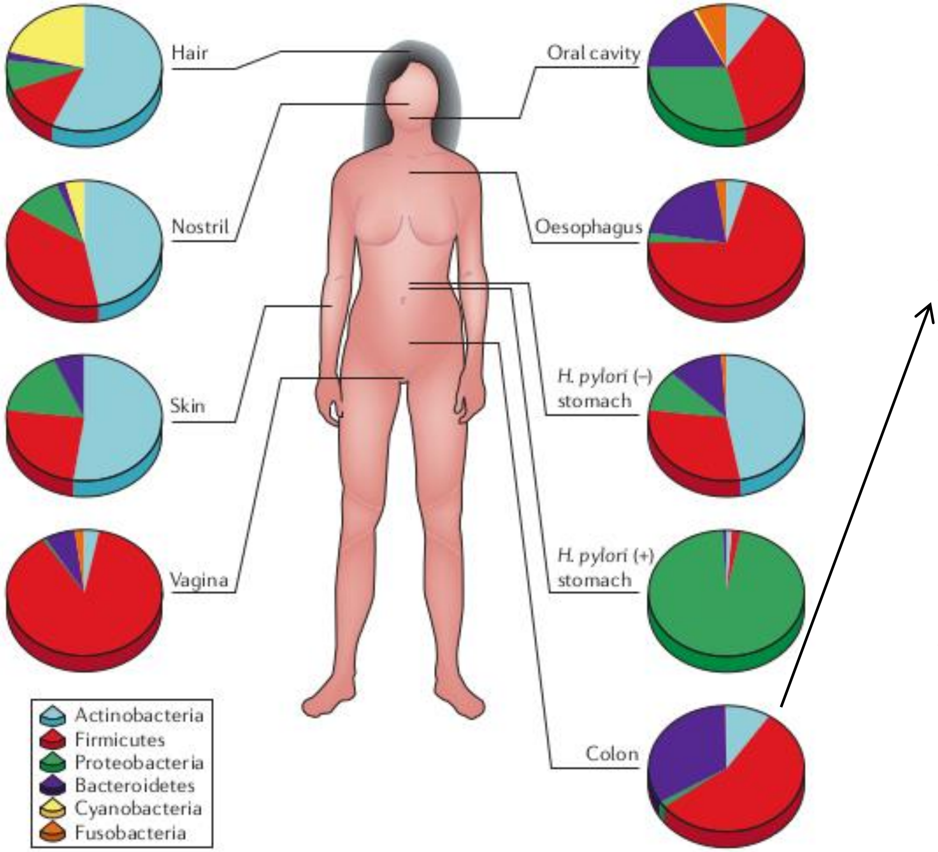
# Symbiotic microbiota



## Microbiota

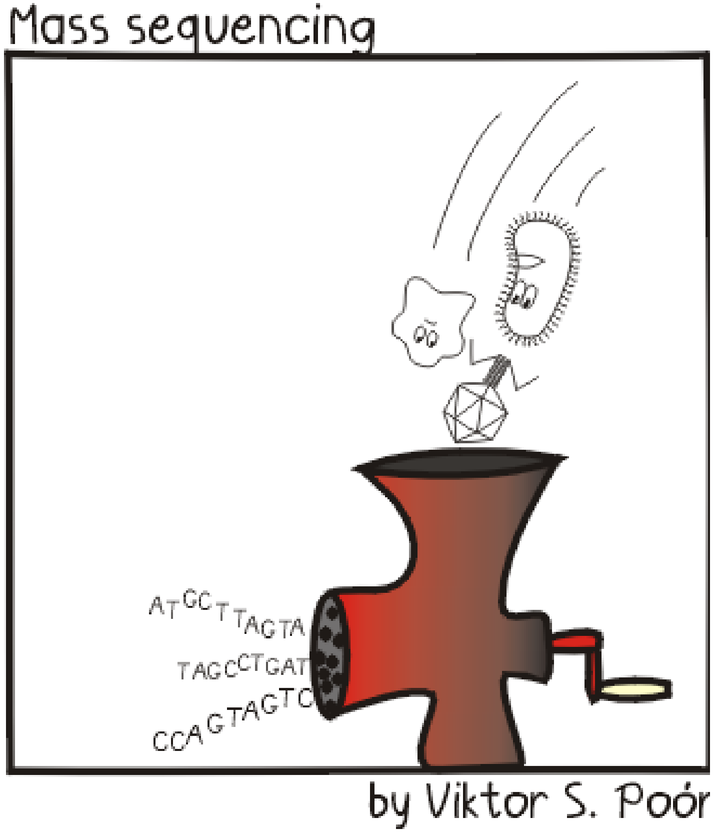
- Population of microorganisms living in symbiosis with its host
- Intestine:
  - $10^{14}$ microorganisms (~ 1,5 Kg)
  - Implantation at early age
  - Relative stability along host's life

## Dysbiosis

- Disequilirium of microbiota composition that can led to pathology

# How to look at microbiota?

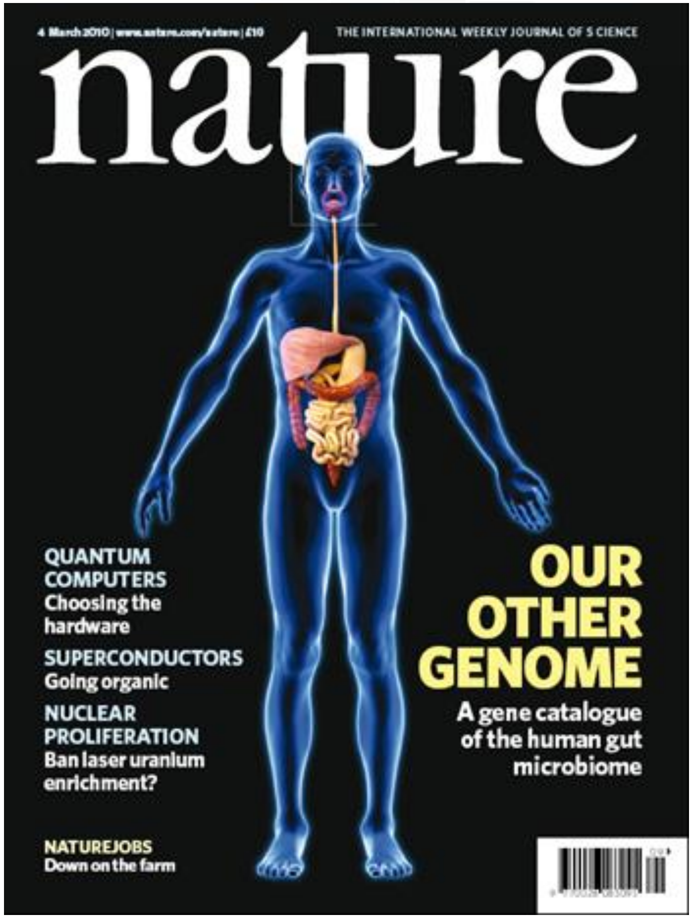
VS.



“Shotgun”

16S

# The first metagenome catalogue



4 March 2010 | www.nature.com/nature | £10

THE INTERNATIONAL WEEKLY JOURNAL OF SCIENCE

nature

QUANTUM COMPUTERS
Choosing the hardware

SUPERCONDUCTORS
Going organic

NUCLEAR PROLIFERATION
Ban laser uranium enrichment?

NATUREJOBS
Down on the farm

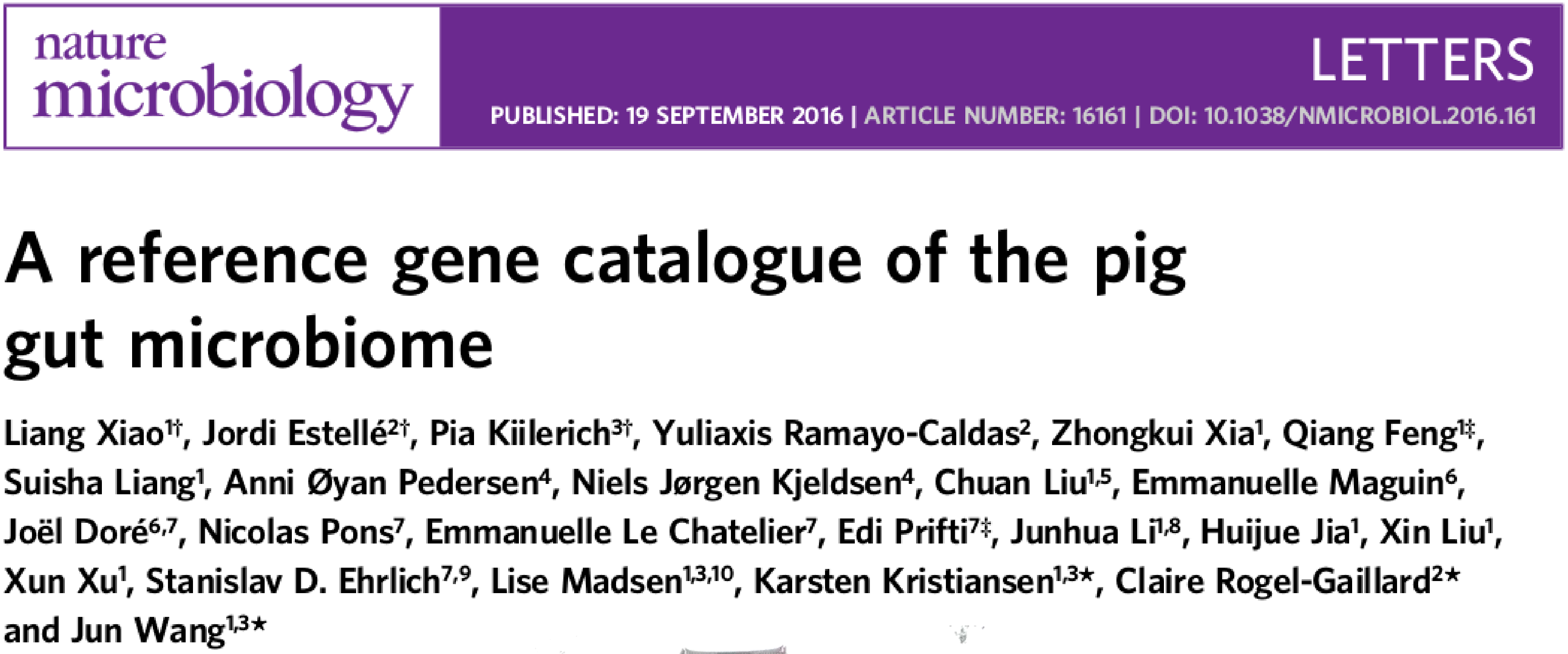
OUR OTHER GENOME
A gene catalogue of the human gut microbiome

Qin *et al.*, Nature, 2010

Intestinal human microbiota:

- A catalogue including over 3.3 million bacterial genes (x150 times the human genome)
- A common «shared» core of 540000 genes
- At least three major enterotypes (Arumugam et al., Nature, 2011)
- Up to 9.9M genes in an integrated catalogue (Li et al. Nat. Biotech, 2014)
- Opens the possibility of shotgun metagenomics by re-sequencing human microbiomes

# The pig other genome



nature microbiology LETTERS
PUBLISHED: 19 SEPTEMBER 2016 | ARTICLE NUMBER: 16161 | DOI: 10.1038/NMICROBIOL.2016.161

## A reference gene catalogue of the pig gut microbiome

Liang Xiao[1†], Jordi Estellé[2†], Pia Kiilerich[3†], Yuliaxis Ramayo-Caldas[2], Zhongkui Xia[1], Qiang Feng[1‡], Suisha Liang[1], Anni Øyan Pedersen[4], Niels Jørgen Kjeldsen[4], Chuan Liu[1,5], Emmanuelle Maguin[6], Joël Doré[6,7], Nicolas Pons[7], Emmanuelle Le Chatelier[7], Edi Prifti[7‡], Junhua Li[1,8], Huijue Jia[1], Xin Liu[1], Xun Xu[1], Stanislav D. Ehrlich[7,9], Lise Madsen[1,3,10], Karsten Kristiansen[1,3*], Claire Rogel-Gaillard[2*] and Jun Wang[1,3*]

# The pig other genome

- **INRA – Univ. Copenhagen – BGI consortium**
  - – Whole porcine metagenome sequencing
  - – *De novo* assembly and annotation by using:
    - 100 French pigs from different breeds and locations
    - 100 Danish pigs with different nutrition conditions
    - 87 Chinese pigs from intensive and extensive farms

**to allow quantitative metagenomics in pigs**

# Samples representative of diversity:

**287 animals:** 17 breeds or selected lines, 11 farms, various ages and diets, 3 countries

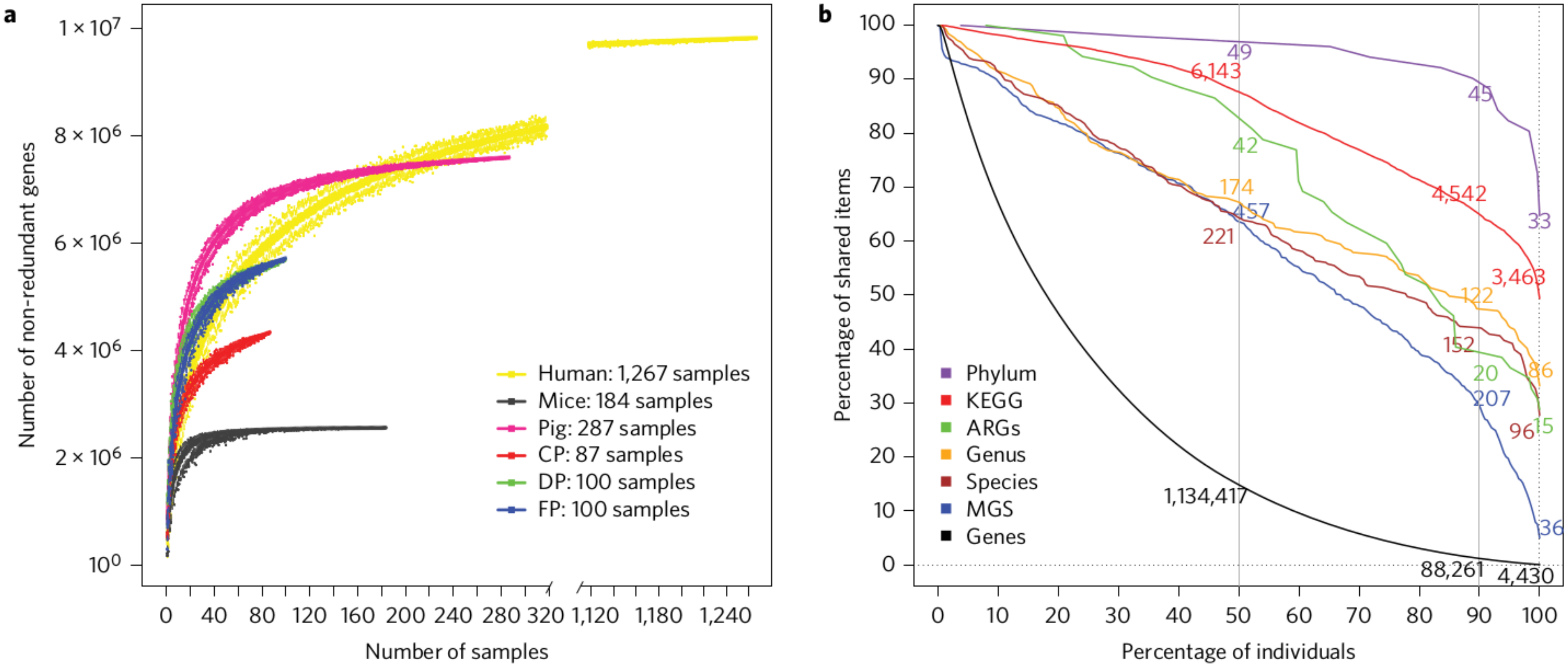
- Native pigs:
  - France, West Indies: Creole pigs
  - China: Bama, Ba Ring, Tibetan

- Selected breeds
  - France: Large White (LW), Large White X Landrace, (Large White X Landrace) X Pietrain, Pietrain, Meishan
  - Denmark: (Landrace x Yorkshire) X Duroc
  - China: Large White, binary mixed, tertiary mixed

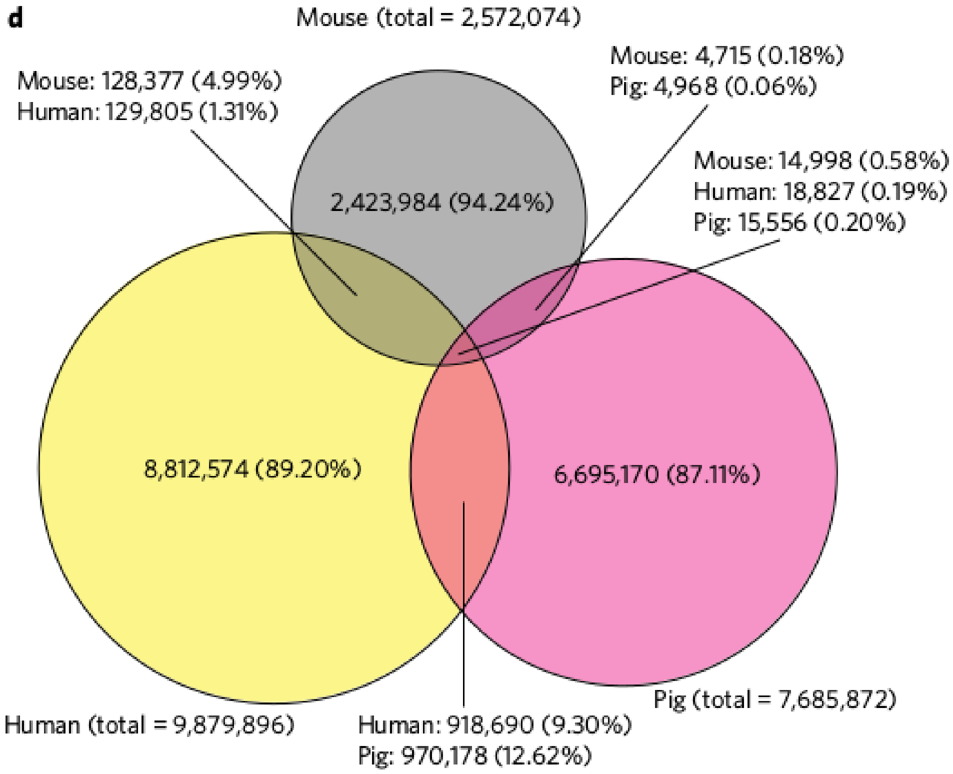
- Miniature model pigs
  - Yucatan
  - Pitman-Moore
  - MeLiM
  - Vietnamese

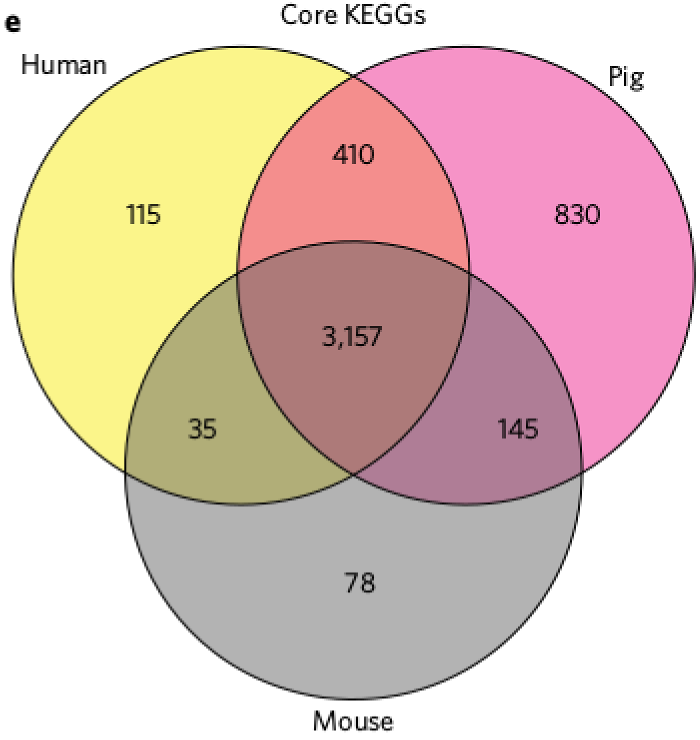
# The pig other genome

- A catalogue of 7.6M genes with 287 pigs



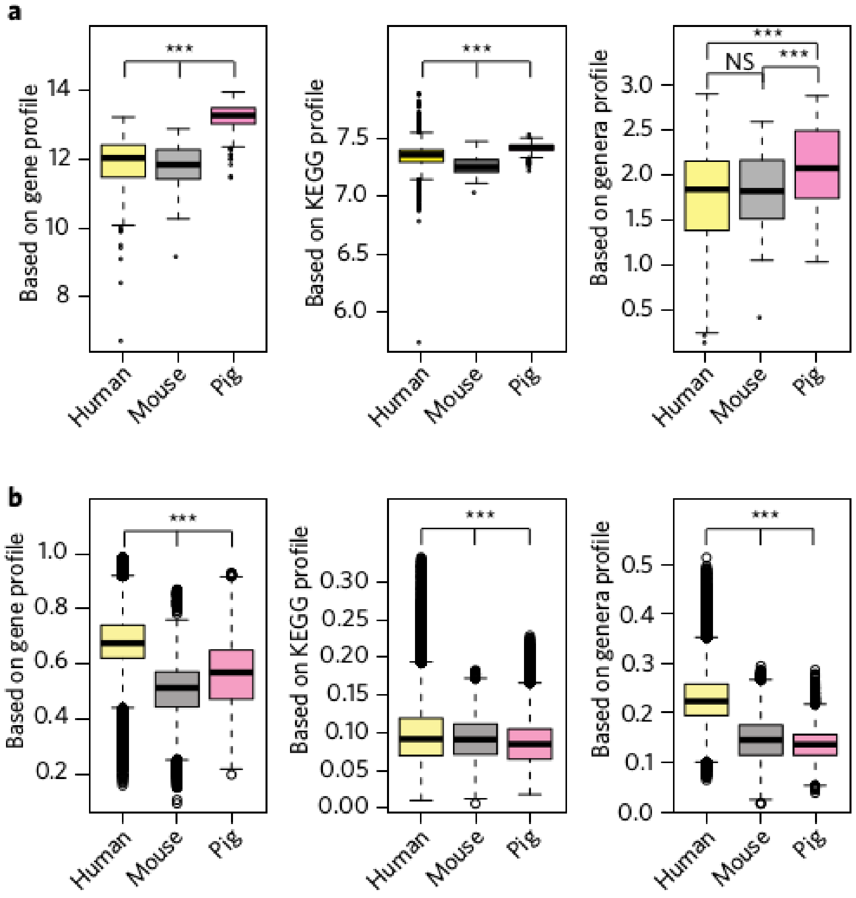
# Comparison with human and mice

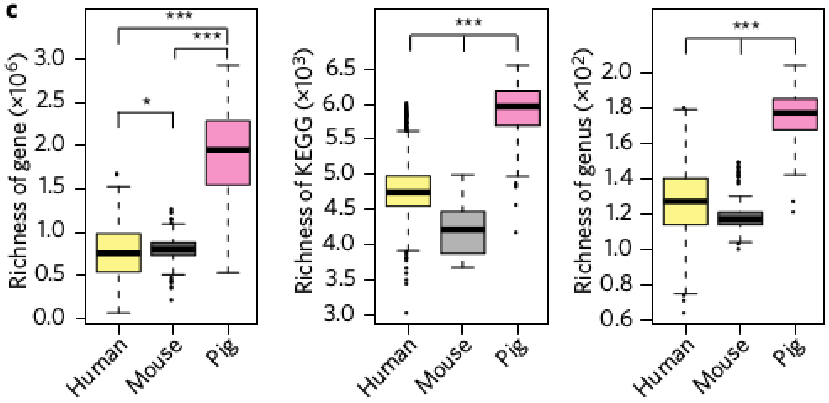




Greater overlap between pig and human than between mice and humans.
At KEGG there much greater overlap: different microbiota but same functions

# Comparison with human and mice

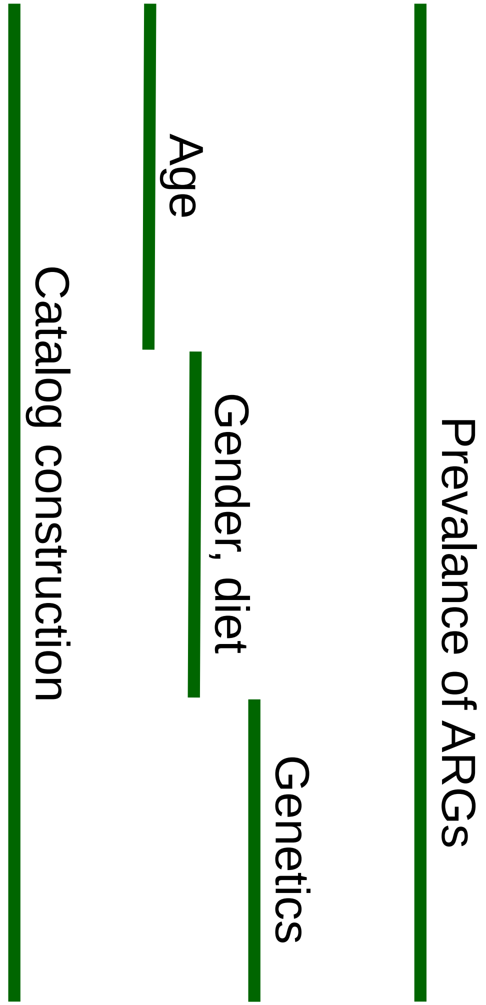




Pigs are richer, and
richer is always better?

# Questions for pig production:

- French set of pigs : 9 or 10 pigs / group
  - 10 breeds or lines from 7 farms
  - Females, males, castrated males
  - Age: 55 to 240 days
- Danish set of pigs: 25 or 50 pigs / group
  - 1 commercial line
  - Three farms
  - Various diets
  - Females, males, castrated males
- Chinese set of pigs: 12 to 19 pigs / group
  - 6 breeds or lines
  - 1 farm
  - Females



Catalog construction

Age

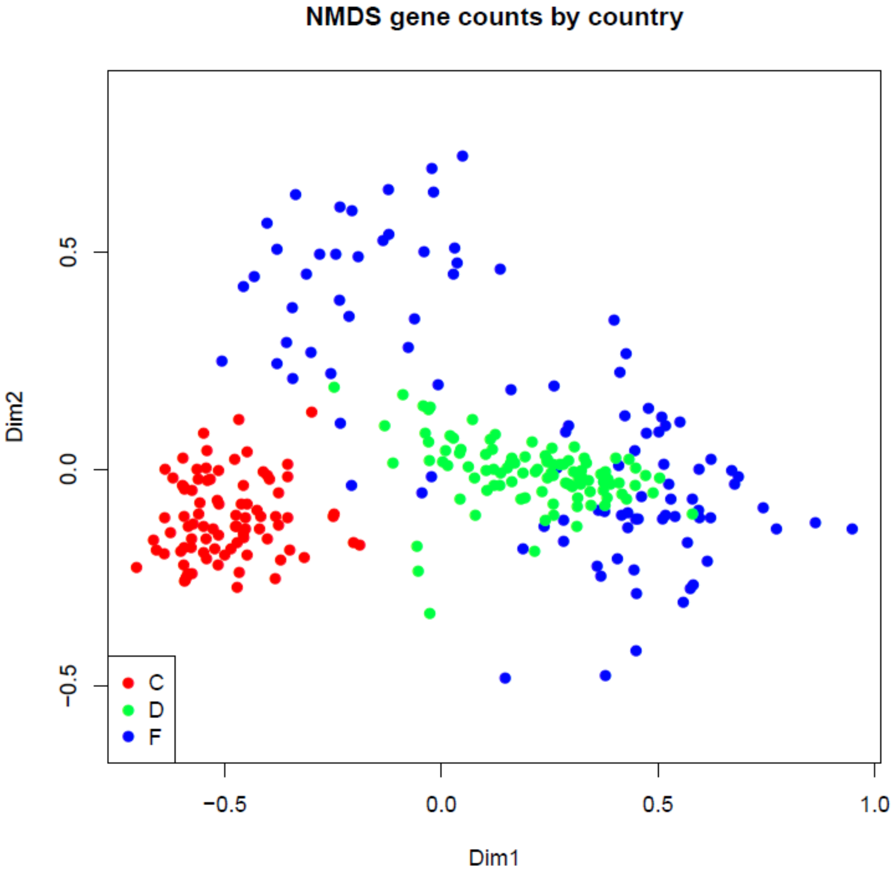
Gender, diet

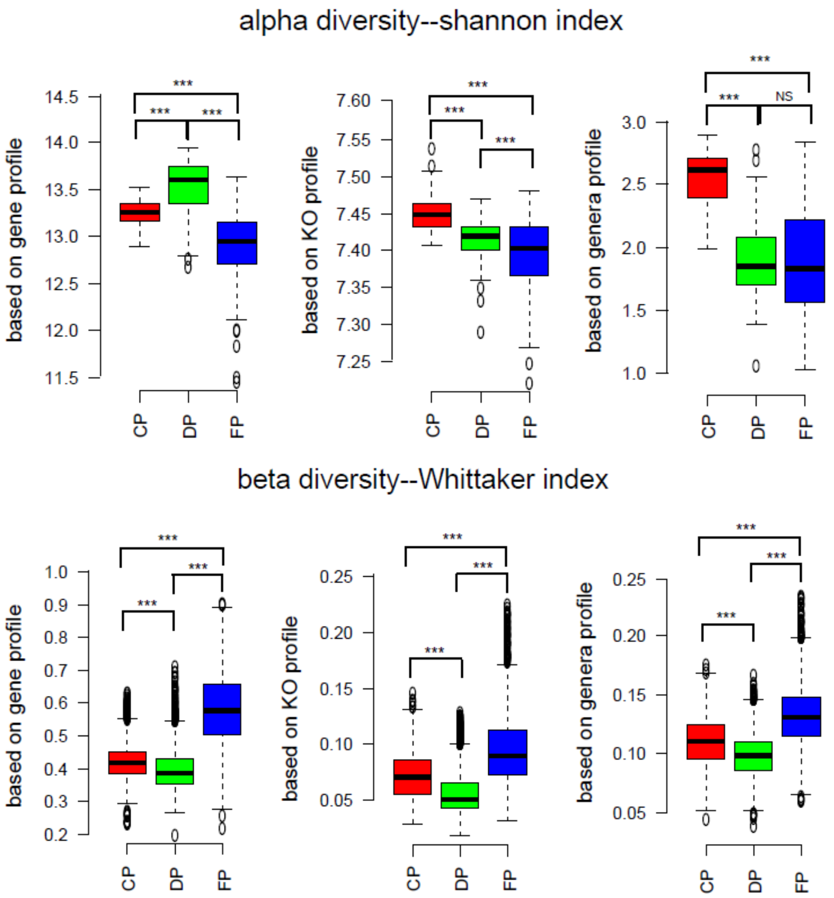
Genetics

Prevalance of ARGs

# The pig other genome

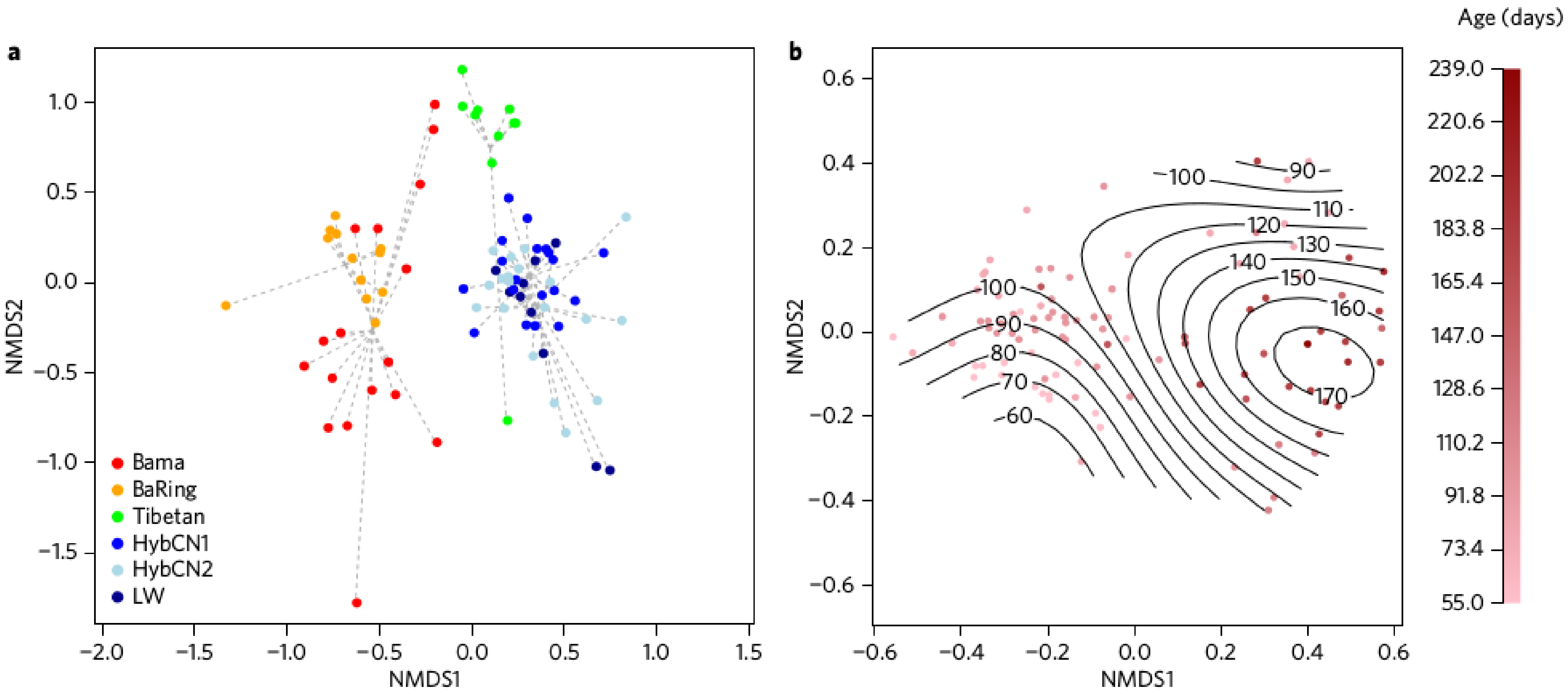
- Stratification of samples according to country of origin



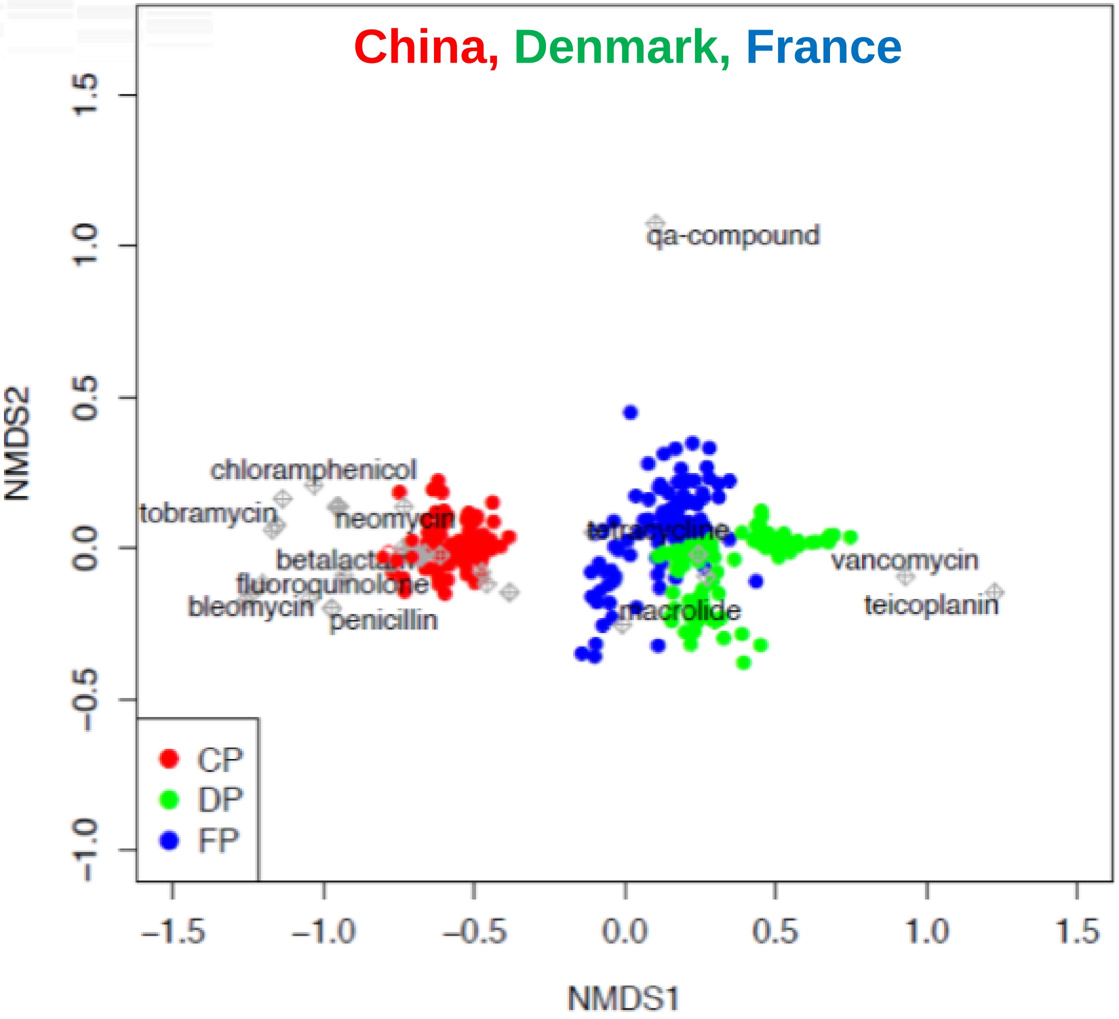


# The swine other genome

- On host genetics and age effects

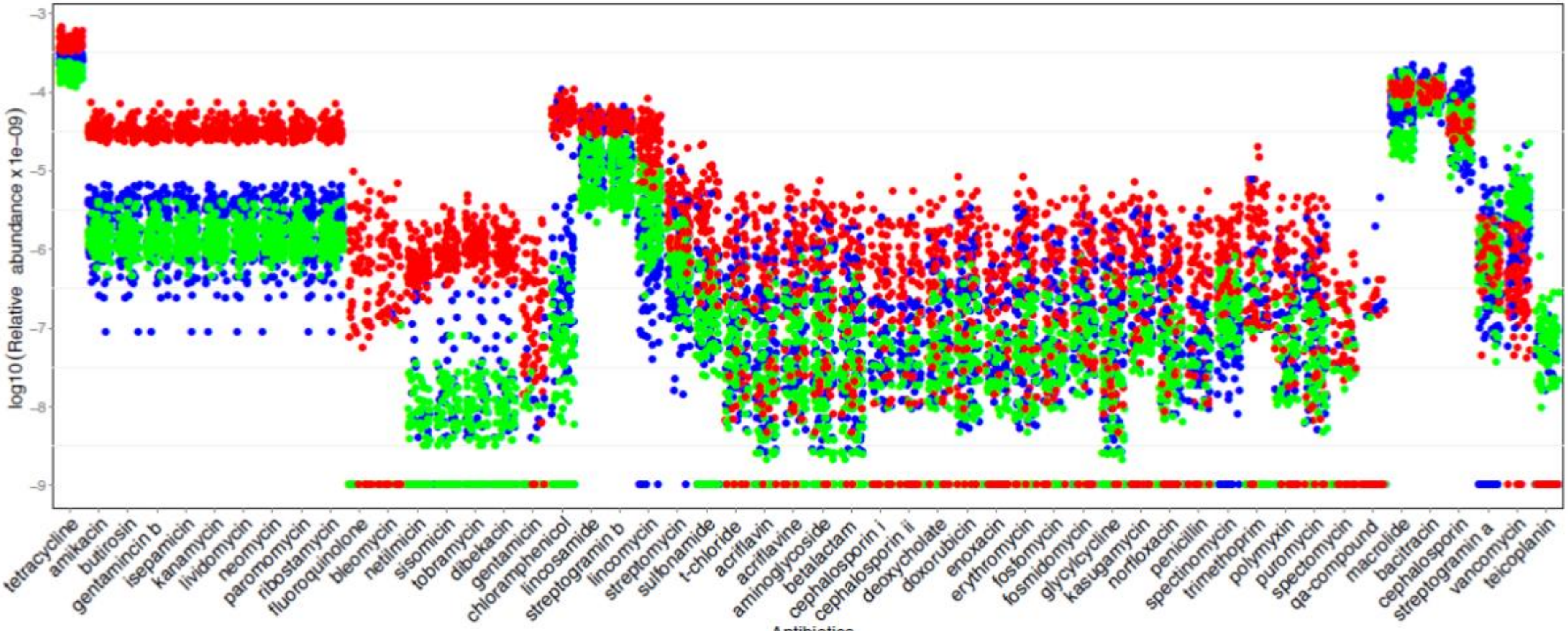


# Prevalence of Ab resistance genes

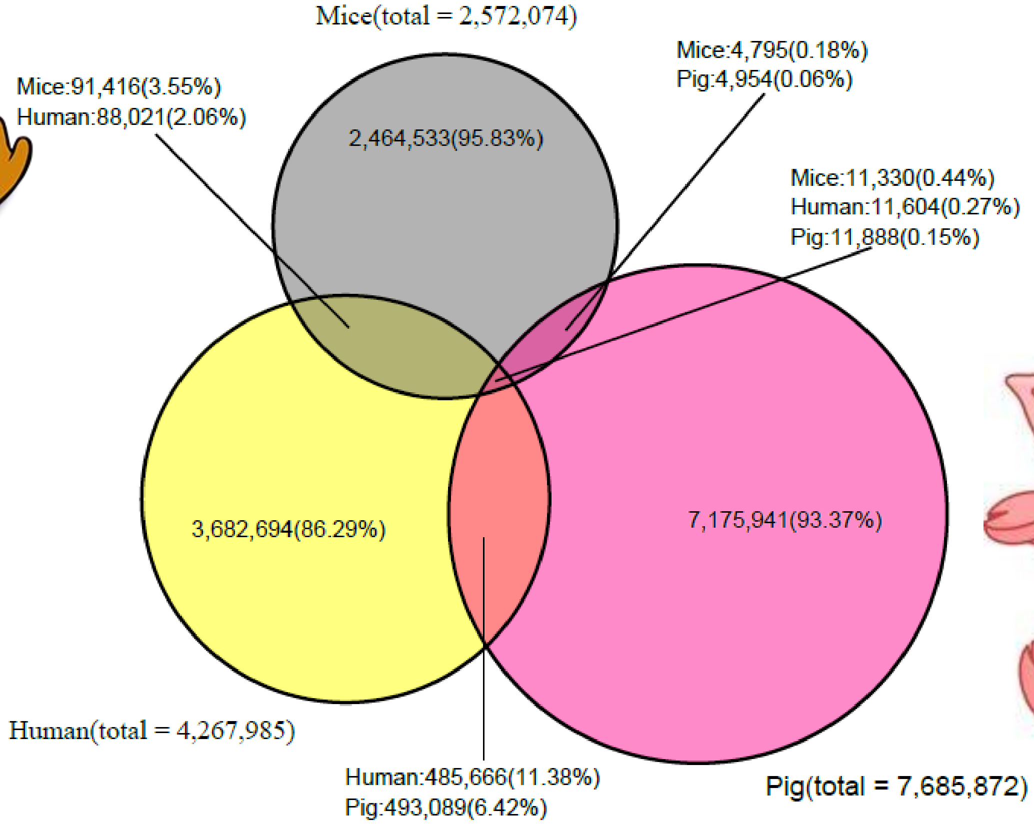


# Prevalence of Ab resistance genes

**China, Denmark, France**



# Pigs or mice as better models for human microbiota?



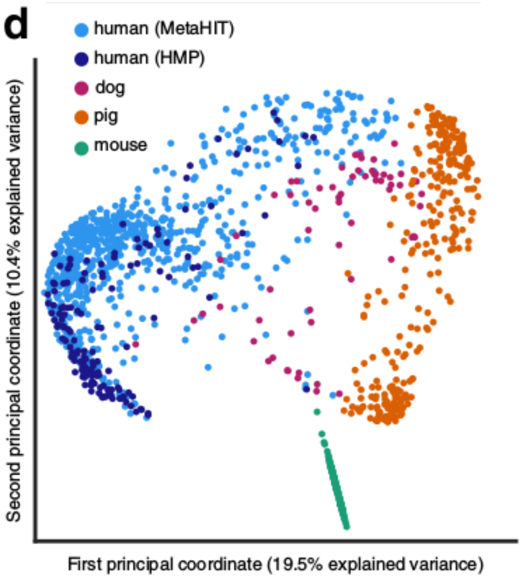
Coelho *et al. Microbiome* (2018) 6:72
https://doi.org/10.1186/s40168-018-0450-3

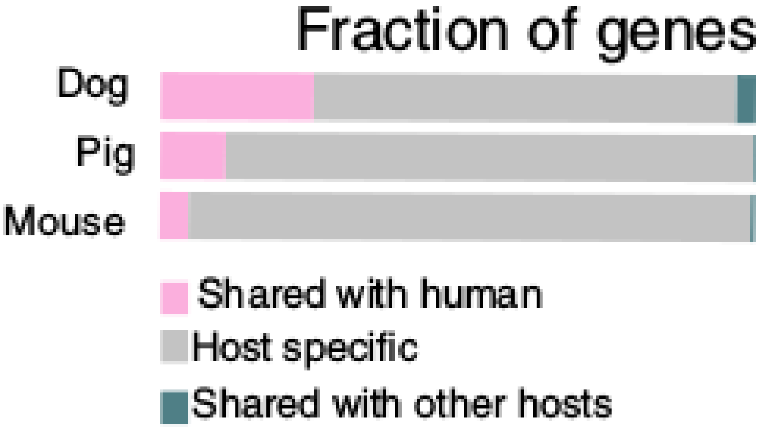
Microbiome

**RESEARCH** **Open Access**

# Similarity of the dog and human gut microbiomes in gene content and response to diet

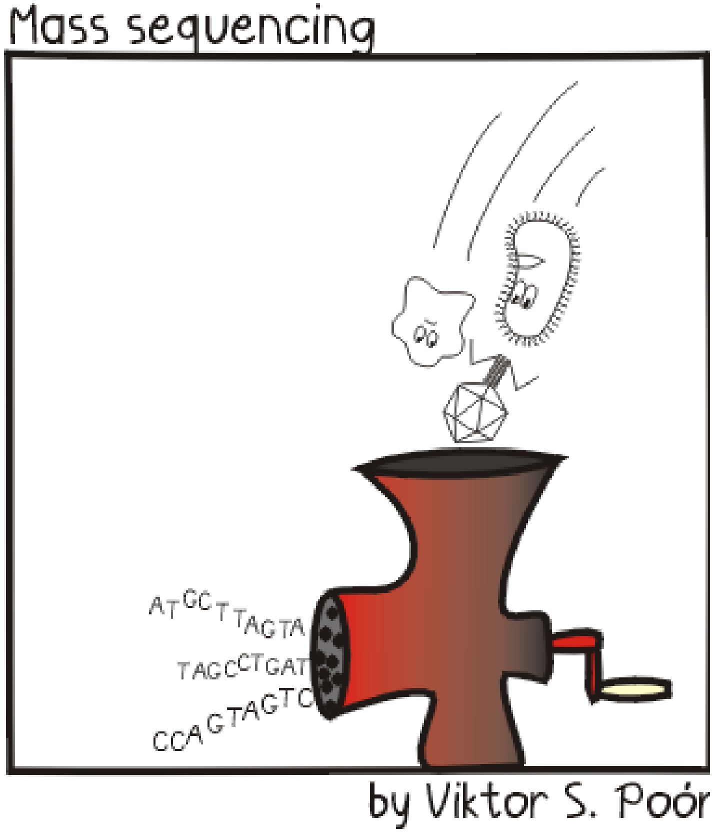
Luis Pedro Coelho[1], Jens Roat Kultima[1], Paul Igor Costea[1], Coralie Fournier[3], Yuanlong Pan[2],
Gail Czarnecki-Maulden[2], Matthew Robert Hayward[1], Sofia K. Forslund[1], Thomas Sebastian Benedikt Schmidt[1],
Patrick Descombes[3], Janet R. Jackson[2], Qinghong Li[2*†] and Peer Bork[1,4,5*†]





# How to look at microbiota?

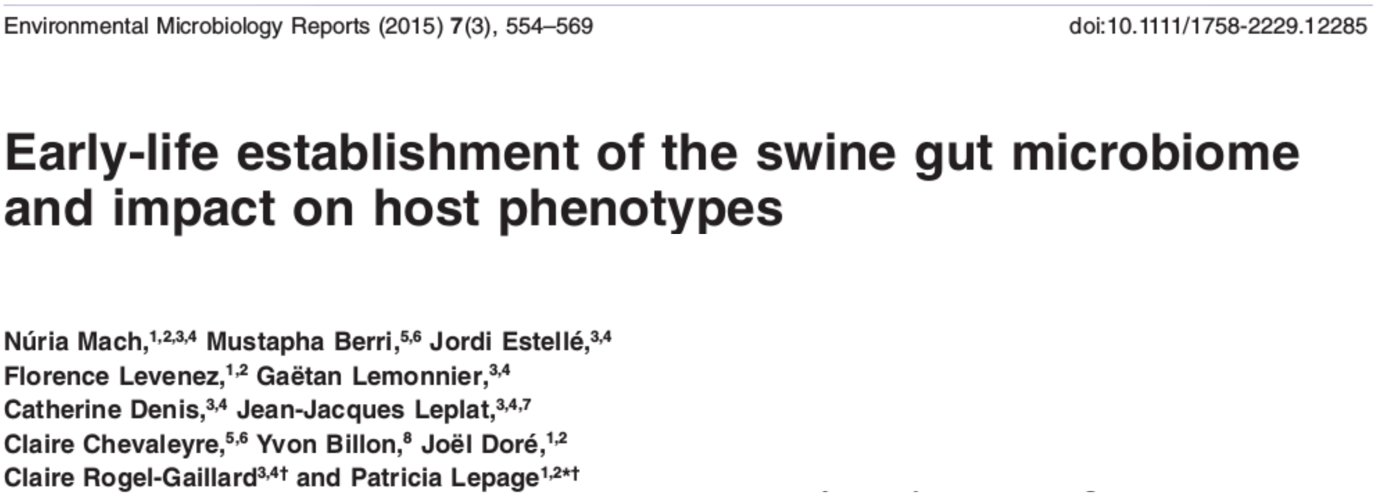
VS.



“shotgun”

**16S**

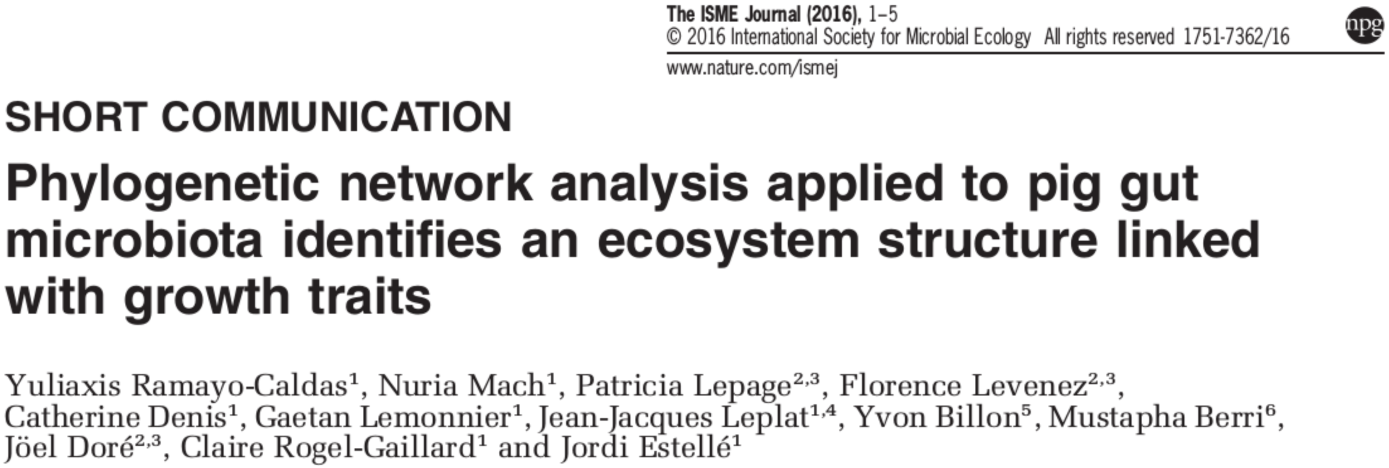
# SUS_FLORA ANR project:
# 16S fecal microbiota composition



Environmental Microbiology Reports (2015) 7(3), 554–569 doi:10.1111/1758-2229.12285

**Early-life establishment of the swine gut microbiome and impact on host phenotypes**

Núria Mach,[1,2,3,4] Mustapha Berri,[5,6] Jordi Estellé,[3,4]
Florence Levenez,[1,2] Gaëtan Lemonnier,[3,4]
Catherine Denis,[3,4] Jean-Jacques Leplat,[3,4,7]
Claire Chevaleyre,[5,6] Yvon Billon,[8] Joël Doré,[1,2]
Claire Rogel-Gaillard[3,4†] and Patricia Lepage[1,2*†]



The ISME Journal (2016), 1–5
© 2016 International Society for Microbial Ecology All rights reserved 1751-7362/16
www.nature.com/ismej

**SHORT COMMUNICATION**

**Phylogenetic network analysis applied to pig gut microbiota identifies an ecosystem structure linked with growth traits**

Yuliaxis Ramayo-Caldas[1], Nuria Mach[1], Patricia Lepage[2,3], Florence Levenez[2,3], Catherine Denis[1], Gaetan Lemonnier[1], Jean-Jacques Leplat[1,4], Yvon Billon[5], Mustapha Berri[6], Jöel Doré[2,3], Claire Rogel-Gaillard[1] and Jordi Estellé[1]

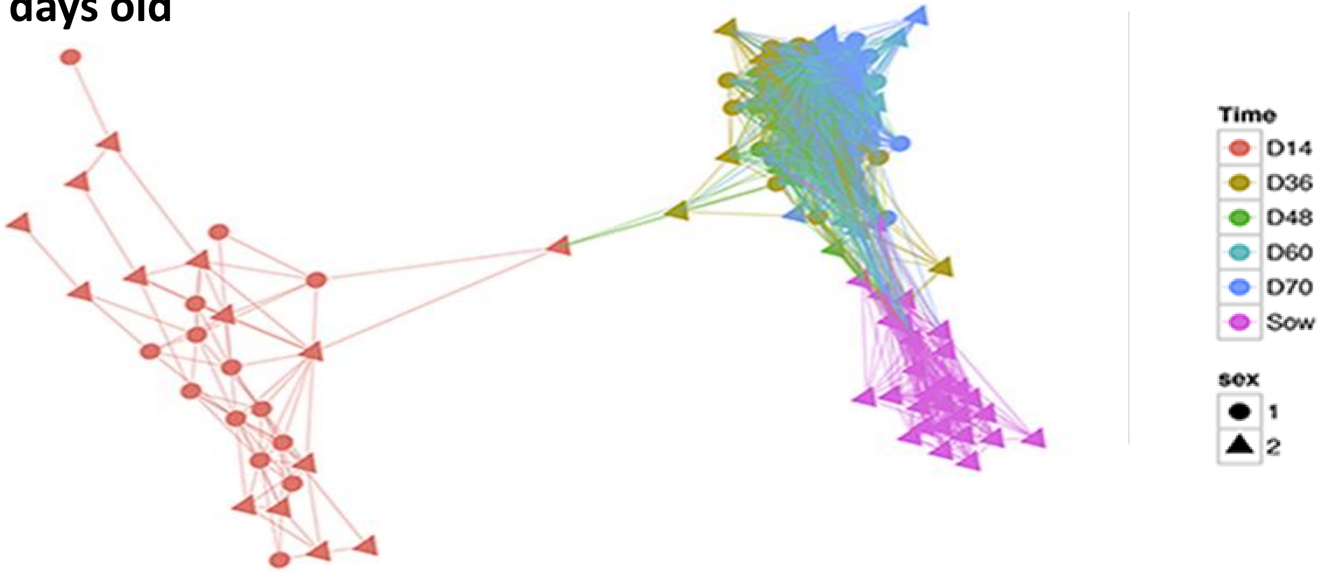
# SUS_FLORA project

- INRA GABI, Jouy
  - **J. Estellé**, **Y Ramayo-Caldas**, **N Mach**, G Lemonnier, C Denis, S Bouet, F Andreoletti, JP Bidanel, **C Larzul**, **C Rogel-Gaillard**
- CEA LREG, Jouy
  - G Piton, JJ Leplat, **S Vincent-Naulleau**
- INRA MICALIS, Jouy
  - **P Lepage, N Mach**, F Levenez, **J Doré**
- INRA TOXALIM, Toulouse
  - L Guzylack, **IP Oswald**
- INRA ISP, Tours
  - **M Berri,** F Meurens
- INRA VIM, Jouy
  - **N Bertho**
- INRA GENESI, Le Magneraud
  - J Bailly, **Y Billon**
- BIOPORC, Le Rheu
  - **MJ Mercat**

# SUS_FLORA: dynamics of implantation

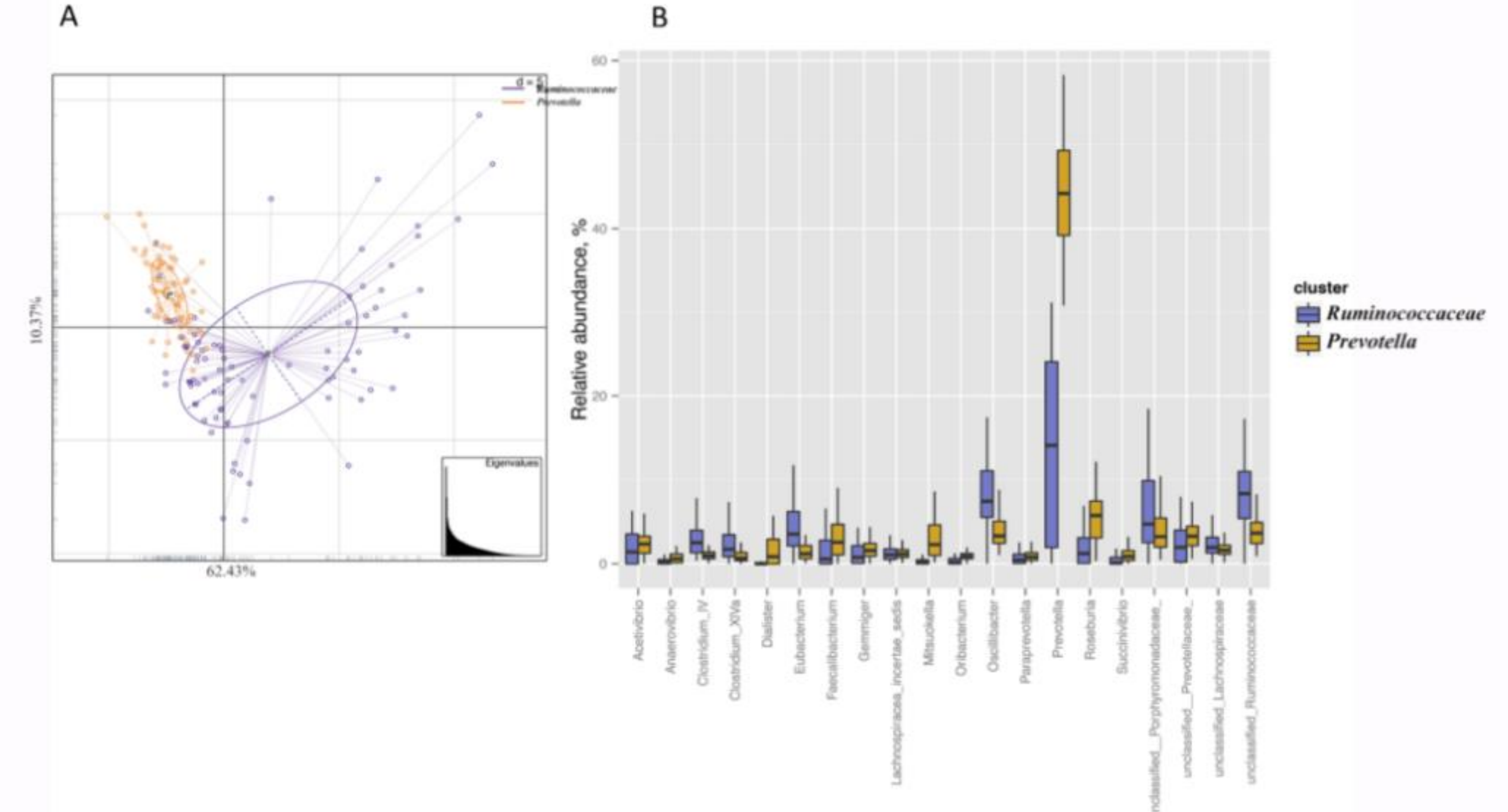
**Older piglets and their mothers**

**Piglets at 14 days old**



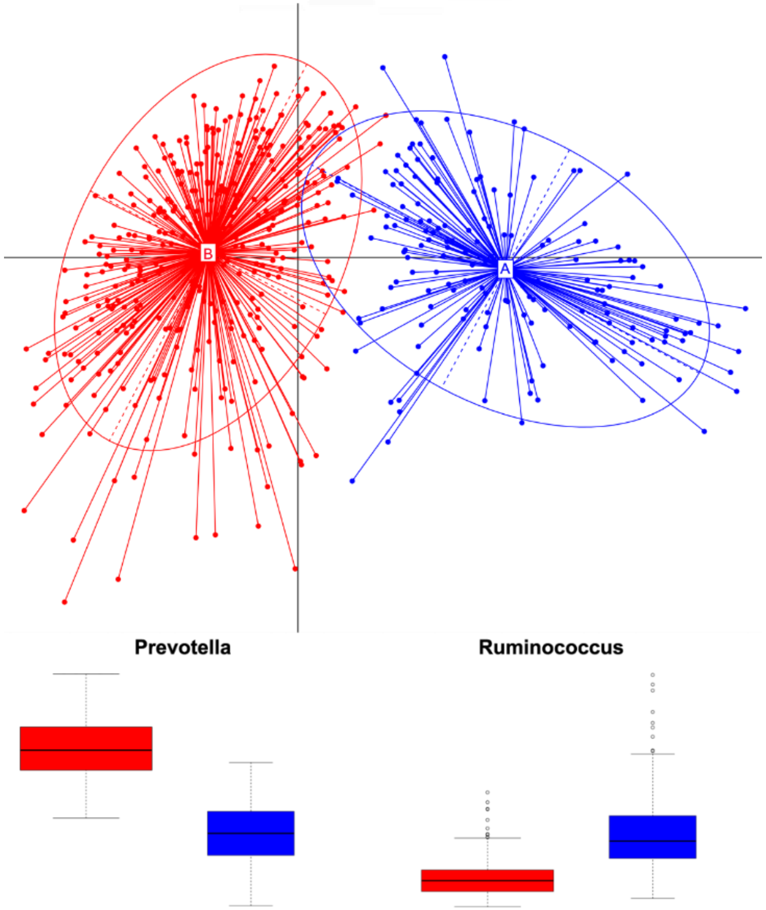
Mach et al., 2015, Environ Microbiol Rep

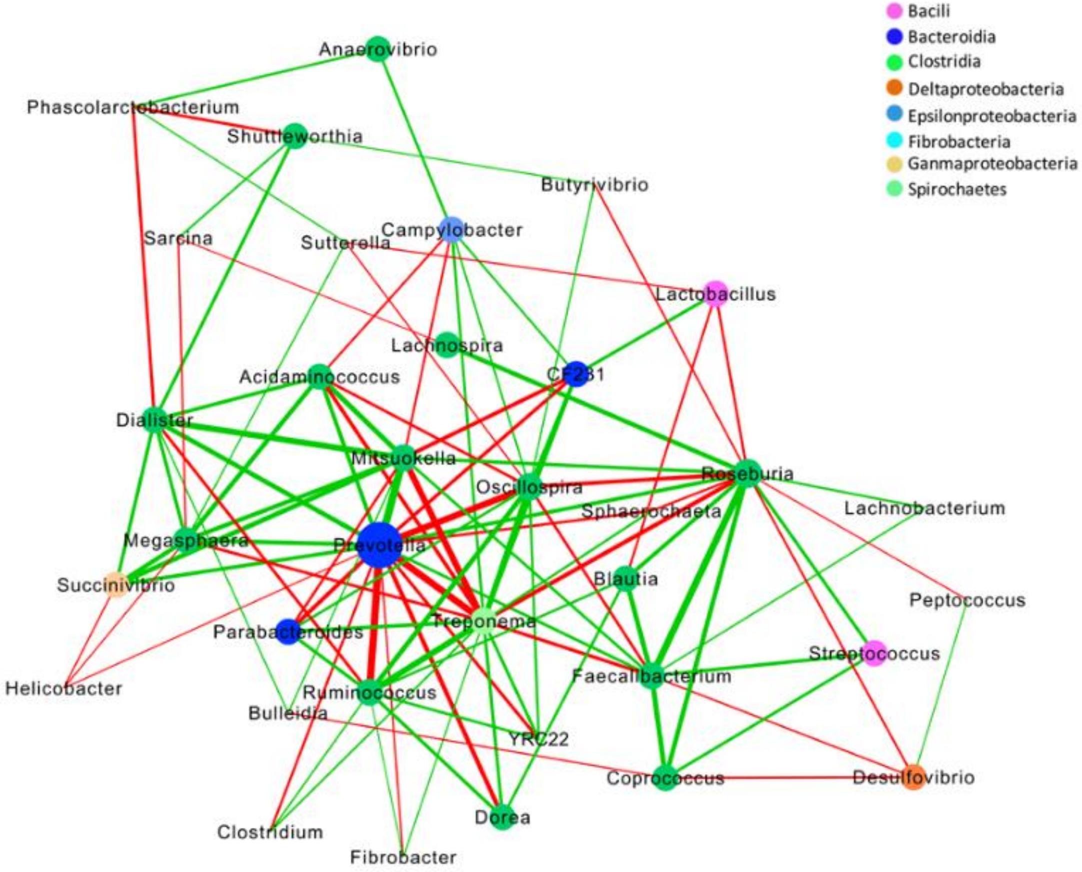
# Two "enterotype-like" clusters detected in young piglets



Mach et al., 2015, Environ Microbiol Rep

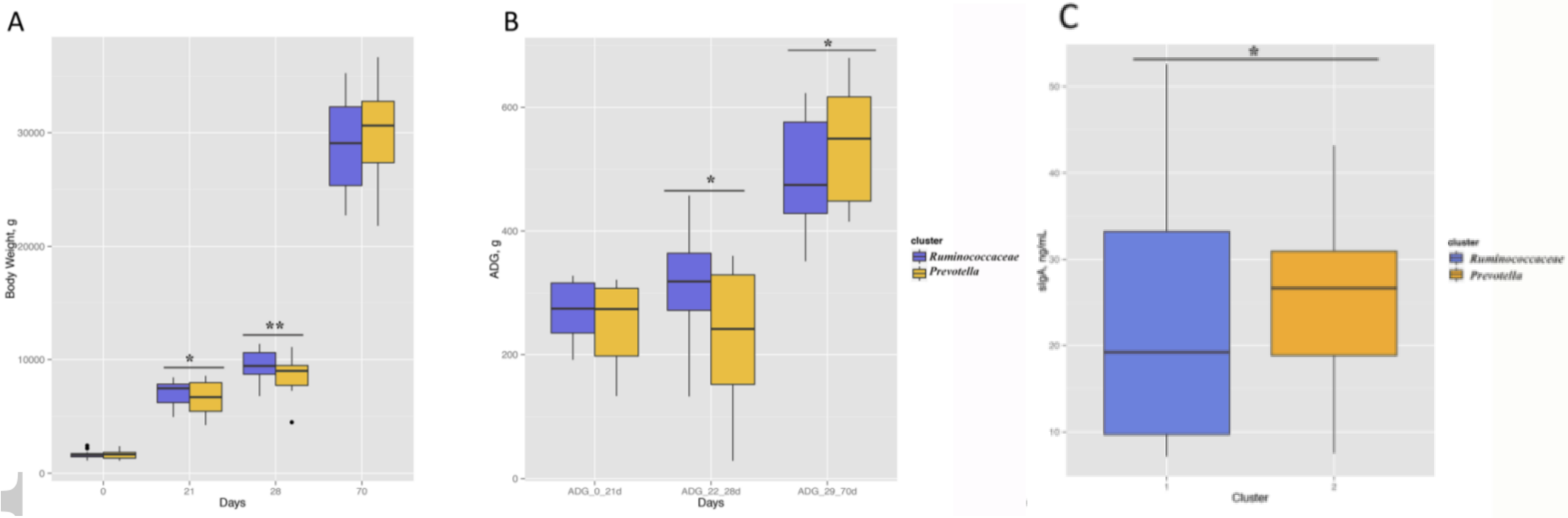
# Enterotype clusters and network analysis in the whole population (60 days old pigs)





Ramayo-Caldas et al., 2016, ISME Journal

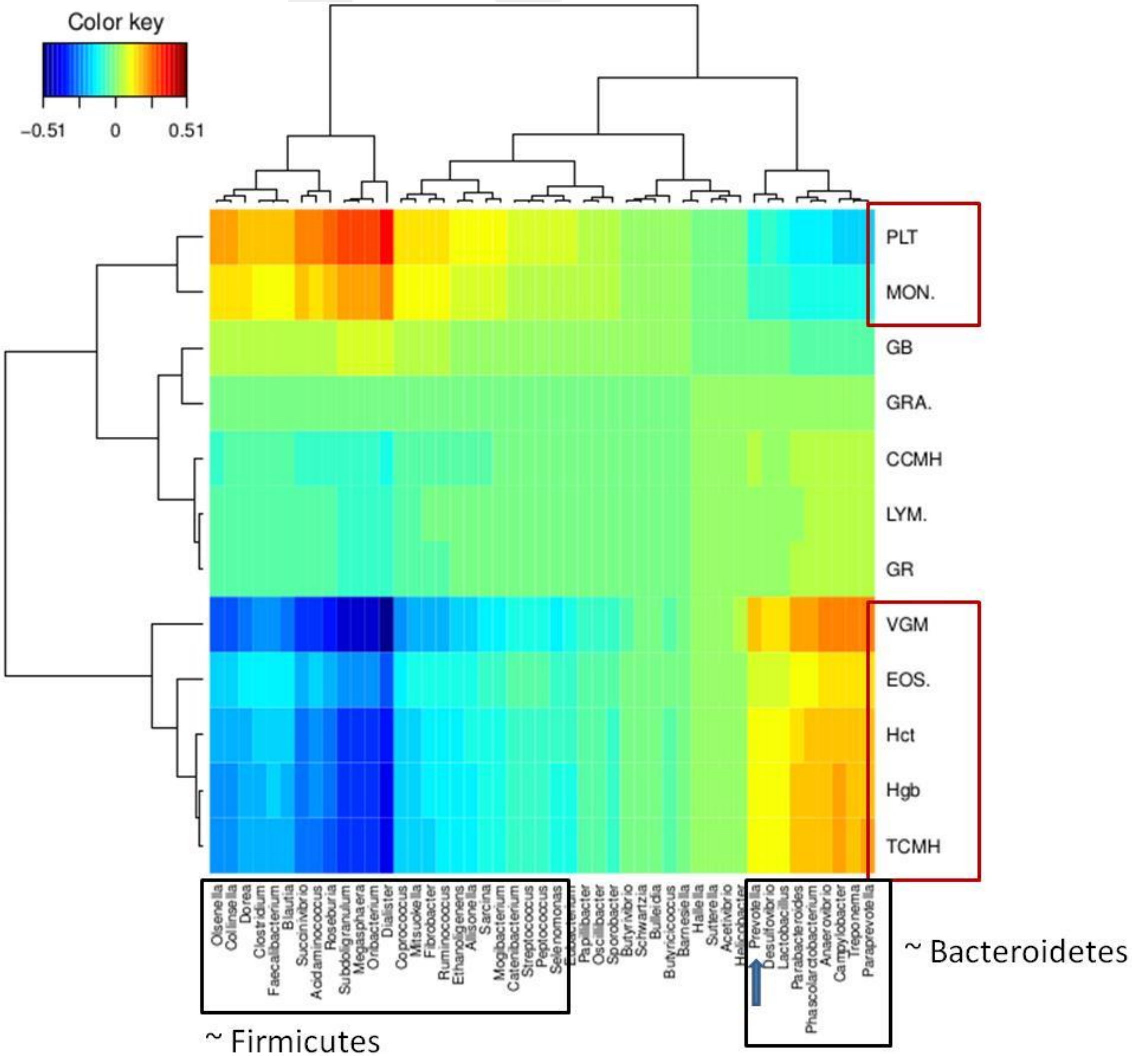
# “Enterotypes” linked to growth and intestine luminal IgA abundance



**Validated at 60 days old cohort with 520 pigs**

Mach et al., 2015, Environ Microbiol Rep
Ramayo-Caldas et al., 2016, ISME Journal

# SUS_FLORA: links with health traits



## rCCA ANALYSIS

PLT: platelets
MON: monocytes
GB: white blood cells
GRA: granulocytes;
CCMH: mean cell hemoglobin conc
LYM: lymphocytes
GR: red blood cells
VGM: red cell distribution width
EOS: eosinophils
Hct: hematocrit
Hgb: hemoglobin
TCMH: mean cell  hemoglobin

# Pig gut microbiota and feed efficiency

Applied and Environmental Microbiology

**Exploring a possible link between the intestinal microbiota and feed efficiency in pigs**

McCormack UM, Curião T, Buzoianu SG, Prieto ML, Ryan T, Varley P, Crispie F, Magowan E, Metzler-Zebeli BU, Berry D, O'Sullivan O, Cotter PD, Gardiner GE, Lawlor PG

**Higher abundance of “beneficial” bacteria:**

- *Prevotella* and *Butyrivibrio* (faeces)
- *Brevibacterium* and *Anaeroplasma* (ileum)
- *Lactobacillus* (caecal content)

↓

Higher ileal isobutyric acid concentrations

↓

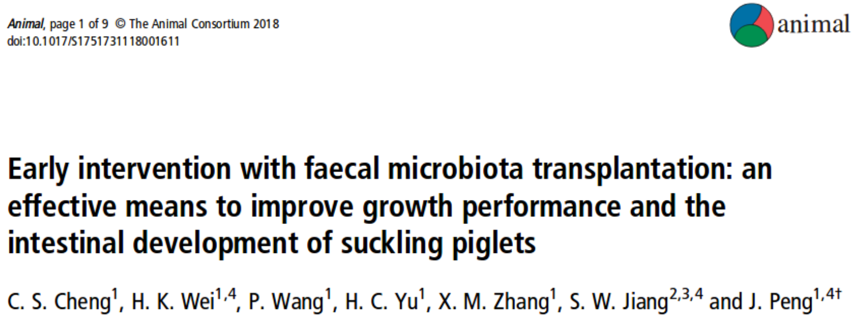
**Improvement of metabolic capabilities**

## Fecal transplant modifies growth of piglets:

RESEARCH ARTICLE
Host-Microbe Biology

**Fecal Microbiota Transplantation in Gestating Sows and Neonatal Offspring Alters Lifetime Intestinal Microbiota and Growth in Offspring**

Ursula M. McCormack,[a,b] Tânia Curião,[a] Toby Wilkinson,[c] Barbara U. Metzler-Zebeli,[d] Henry Reyer,[e] Tomas Ryan,[a] Julia A. Calderon-Diaz,[a,f] Fiona Crispie,[g,h] Paul D. Cotter,[g,h] Christopher J. Creevey,[c] Gillian E. Gardiner,[b] Peadar G. Lawlor[a]



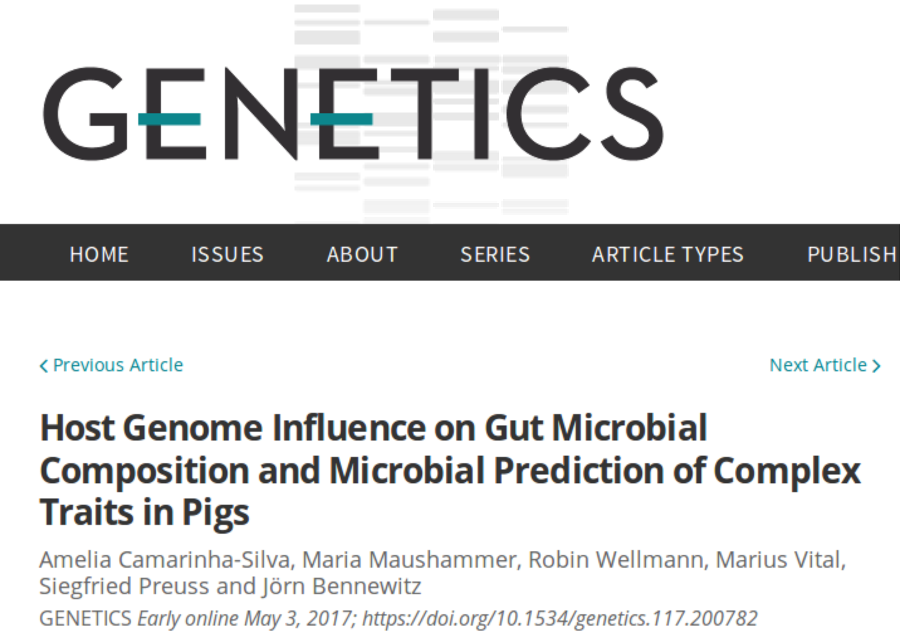
*Animal*, page 1 of 9 © The Animal Consortium 2018
doi:10.1017/S1751731118001611

animal

**Early intervention with faecal microbiota transplantation: an effective means to improve growth performance and the intestinal development of suckling piglets**

C. S. Cheng[1], H. K. Wei[1,4], P. Wang[1], H. C. Yu[1], X. M. Zhang[1], S. W. Jiang[2,3,4] and J. Peng[1,4†]

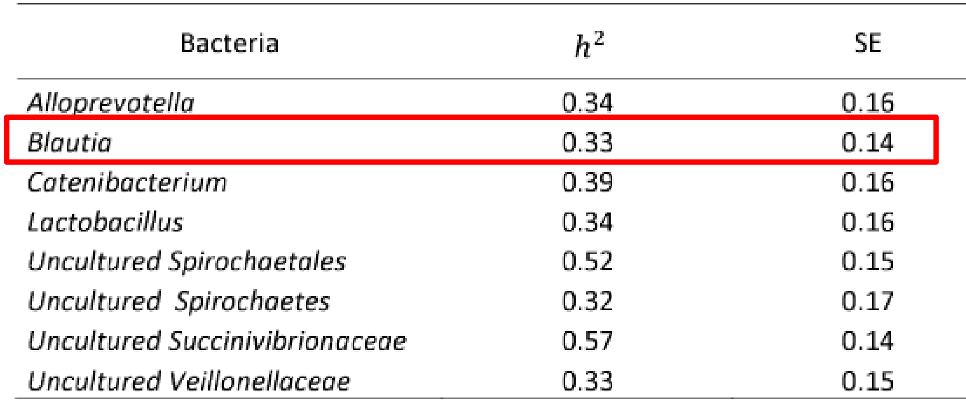
# Host genetics and microbiota:



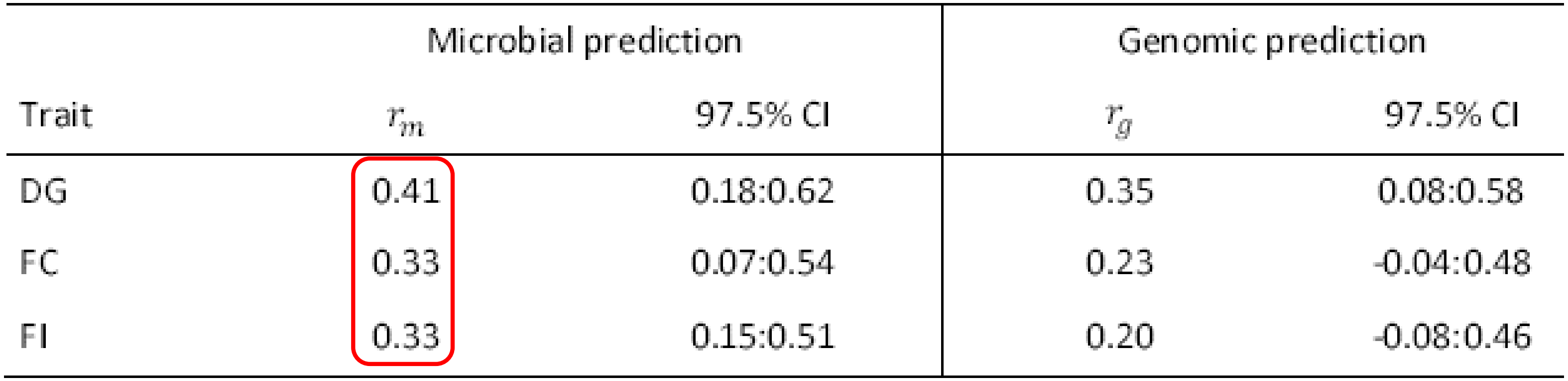
**Host Genome Influence on Gut Microbial Composition and Microbial Prediction of Complex Traits in Pigs**

Amelia Camarinha-Silva, Maria Maushammer, Robin Wellmann, Marius Vital, Siegfried Preuss and Jörn Bennewitz

GENETICS *Early online May 3, 2017; https://doi.org/10.1534/genetics.117.200782*



| Bacteria | $h^2$ | SE |
|---|---|---|
| *Alloprevotella* | 0.34 | 0.16 |
| *Blautia* | 0.33 | 0.14 |
| *Catenibacterium* | 0.39 | 0.16 |
| *Lactobacillus* | 0.34 | 0.16 |
| *Uncultured Spirochaetales* | 0.52 | 0.15 |
| *Uncultured Spirochaetes* | 0.32 | 0.17 |
| *Uncultured Succinivibrionaceae* | 0.57 | 0.14 |
| *Uncultured Veillonellaceae* | 0.33 | 0.15 |



|  | Microbial prediction |  | Genomic prediction |  |
|---|---|---|---|---|
| Trait | $r_m$ | 97.5% CI | $r_g$ | 97.5% CI |
| DG | 0.41 | 0.18:0.62 | 0.35 | 0.08:0.58 |
| FC | 0.33 | 0.07:0.54 | 0.23 | -0.04:0.48 |
| FI | 0.33 | 0.15:0.51 | 0.20 | -0.08:0.46 |

- Microbiota information improve the accuracy of FE prediction

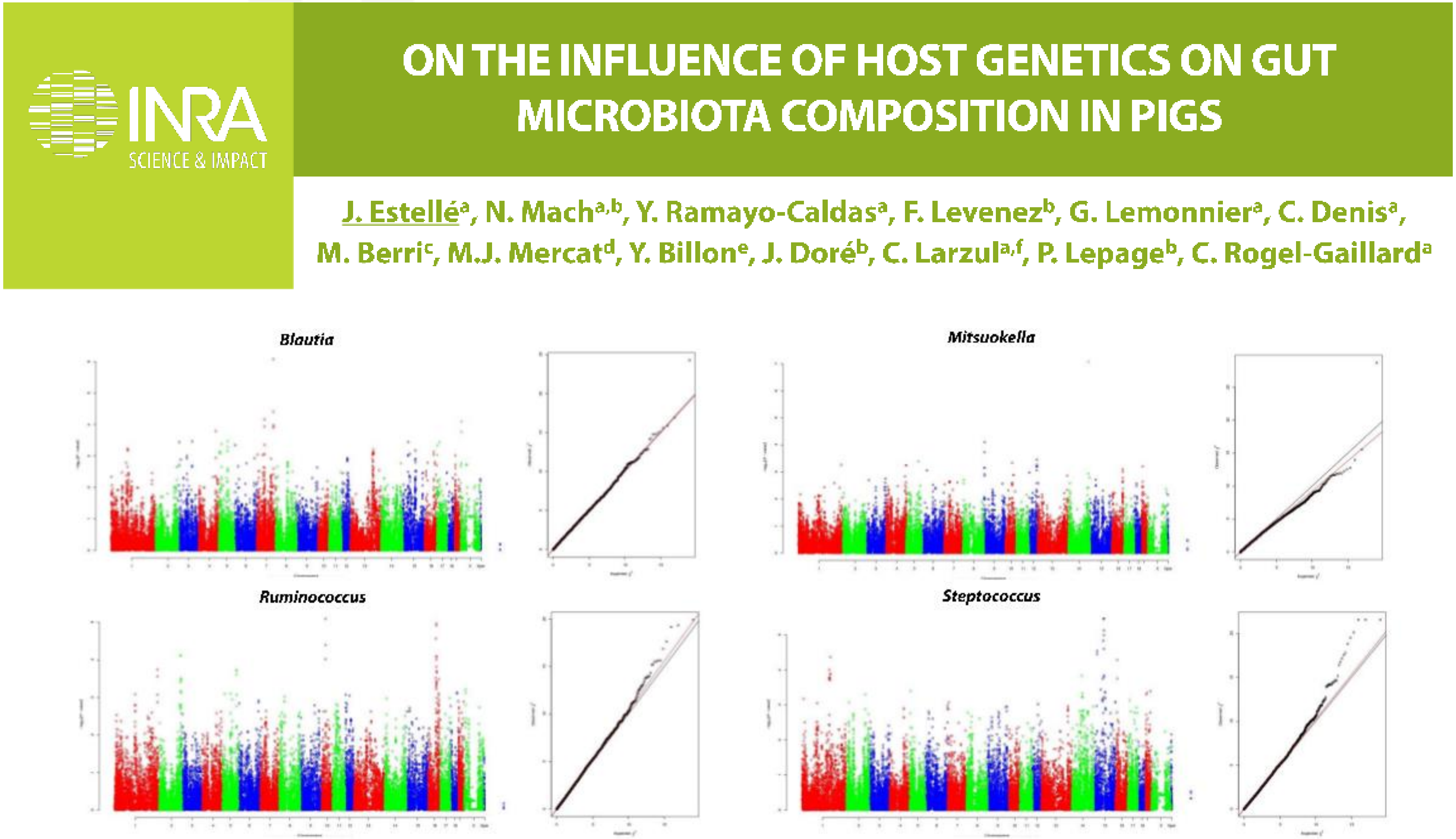
- Microbial explained 28%, 21% and 16% of DG, FC and FI variances (**= “microbiability”**)

# Poster SUSFLORA: genetic control

| Genus | $h^2$ | SD |
|---|---|---|
| *Anaerostipes* | 0,13 | 0,08 |
| *Anaerovibrio* | 0,26 | 0,17 |
| ***Blautia*** | **0,24** | **0,09** |
| *Bulleidia* | 0,02 | 0,06 |
| *Butyrivibrio* | 0,04 | 0,06 |
| *Campylobacter* | 0,34 | 0,10 |
| *Catenibacterium* | 0,16 | 0,08 |
| *CF231* | 0,29 | 0,31 |
| *Clostridium* | 0,11 | 0,32 |
| *Desulfovibrio* | 0,09 | 0,43 |
| *Dorea* | 0,31 | 0,23 |
| *Faecalibacterium* | 0,25 | 0,28 |
| *Fibrobacter* | 0,14 | 0,43 |
| *Flexispira* | 0,08 | 0,11 |
| *Helicobacter* | 0,13 | 0,08 |
| *Lachnobacterium* | 0,04 | 0,14 |

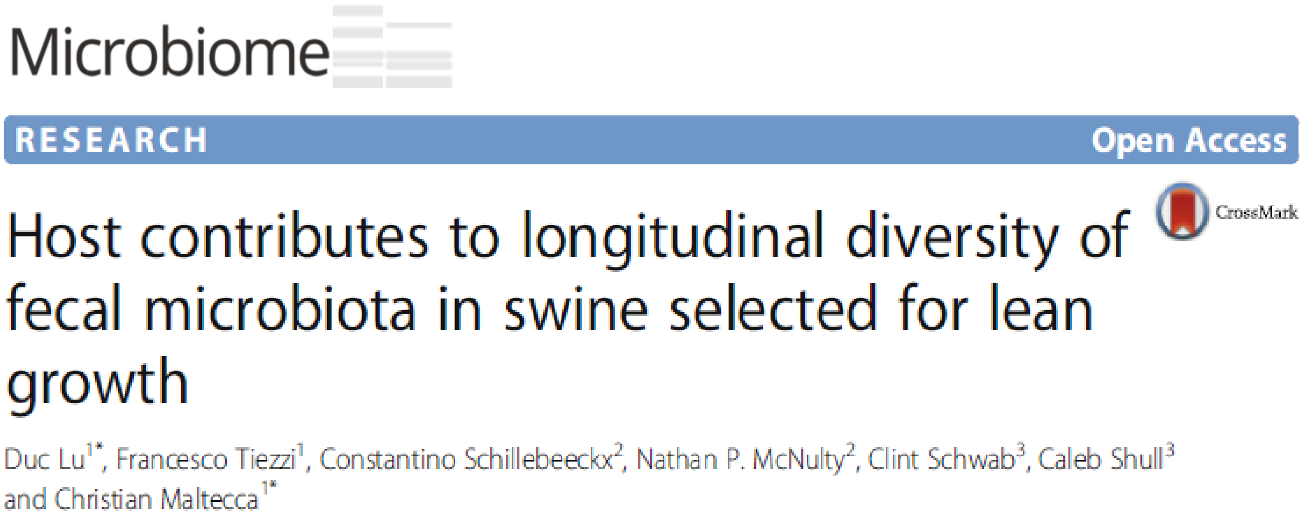
| Genus | $h^2$ | SD |
|---|---|---|
| *Lactobacillus* | 0,18 | 0,23 |
| ***Mitsuokella*** | **0** | **0** |
| *Oscillospira* | 0,11 | 0,21 |
| *Parabacteroides* | 0,22 | 0,30 |
| *Peptococcus* | 0,14 | 0,08 |
| *Phascolarctobact* | 0,02 | 0,31 |
| *Prevotella* | 0,37 | 0,27 |
| *rc4-4* | 0,17 | 0,07 |
| ***Ruminococcus*** | **0,55** | **0,16** |
| *Shuttleworthia* | 0,01 | 0,06 |
| *Sphaerochaeta* | 0,07 | 0,13 |
| ***Streptococcus*** | **0,13** | **0,10** |
| *Succiniclasticum* | 0,05 | 0,18 |
| *Sutterella* | 0,01 | 0,05 |
| *Treponema* | 0,29 | 0,03 |
| *YRC22* | 0,36 | 0,10 |

# Poster SUSFLORA: genetic control



## ON THE INFLUENCE OF HOST GENETICS ON GUT MICROBIOTA COMPOSITION IN PIGS

J. Estellé[a], N. Mach[a,b], Y. Ramayo-Caldas[a], F. Levenez[b], G. Lemonnier[a], C. Denis[a], M. Berri[c], M.J. Mercat[d], Y. Billon[e], J. Doré[b], C. Larzul[a,f], P. Lepage[b], C. Rogel-Gaillard[a]

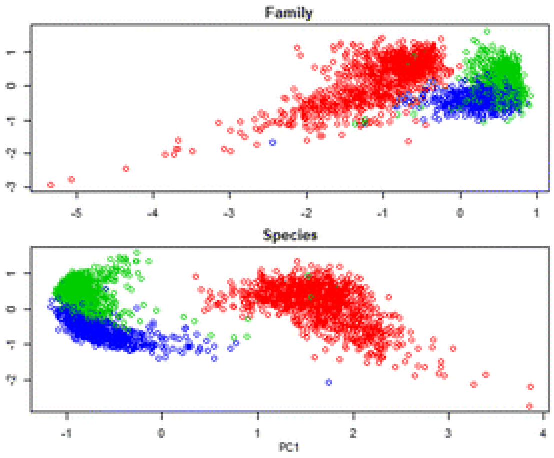


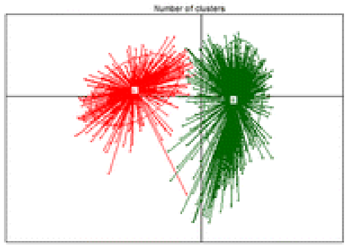
Microbiome

RESEARCH Open Access

Host contributes to longitudinal diversity of fecal microbiota in swine selected for lean growth

Duc Lu[1*], Francesco Tiezzi[1], Constantino Schillebeeckx[2], Nathan P. McNulty[2], Clint Schwab[3], Caleb Shull[3] and Christian Maltecca[1*]





weaning, week 15, and ~week 28

- Alpha diversity and OTU richness were moderately heritable (0.15 – 0.33)

- Negative genetic correlations between alpha diversity BF (-0.53) and ADG (-0.45)

-Microbiota diversity can be used as an indicator to improve host traits

**-Results are not homogeneous along time points = relevant age effect!**

# So: which age, sex, diet, location, …?

Frese *et al. Microbiome* (2015) 3:28
DOI 10.1186/s40168-015-0091-8

*Microbiome*

**RESEARCH** Open Access

CrossMark

Diet shapes the gut microbiome of pigs during nursing and weaning

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**Fecal microbial composition associated with variation in feed efficiency in pigs depends on diet and sex**[1]

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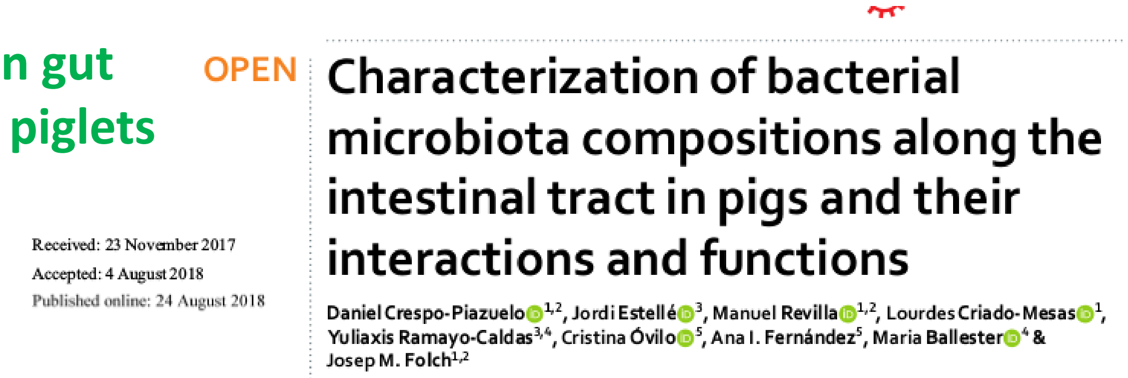
**Talk session 58 (Thursday morning): Massacci et al.**

**Impact of weaning age on gut microbiota composition in piglets**



OPEN

**Characterization of bacterial microbiota compositions along the intestinal tract in pigs and their interactions and functions**

Daniel Crespo-Piazuelo[1,2], Jordi Estellé[3], Manuel Revilla[1,2], Lourdes Criado-Mesas[1], Yuliaxis Ramayo-Caldas[3,4], Cristina Óvilo[5], Ana I. Fernández[5], Maria Ballester[4] & Josep M. Folch[1,2]

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- Different target phenotypes might have different optimal solutions for microbiota sampling and analysis!

# Future in animal breeding: the best holobiont for a given environment!

- Integrative biology and advanced models necessary to define:
    - The best host (**genetics**) and the best intestinal microbiota (**metagenomics**) in a given environment (*e.g.* **nutrition**!) in order to optimize:
        - Production, immunocompetence, food efficiency, enviromental impact, robustness, …
- Microbiota is not “only” a new phenotype, neither an environmental factor: P = G + M + E

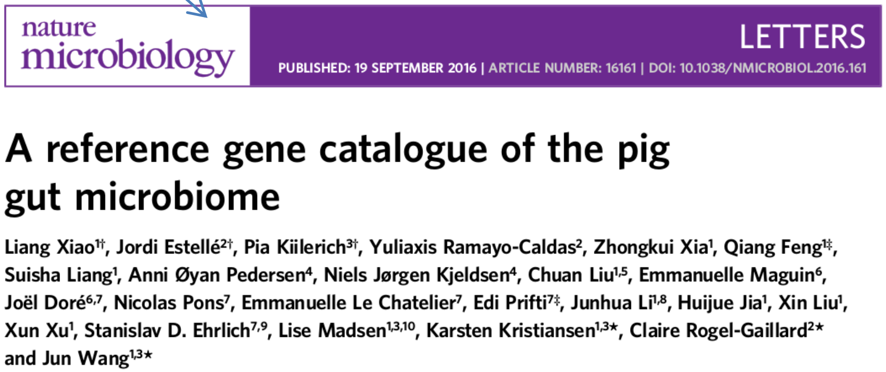
# P = G + M + E

ARTICLE

doi:10.1038/nature11622

**Analyses of pig genomes provide insight into porcine demography and evolution**

A list of authors and their affiliations appears at the end of the paper



nature microbiology

LETTERS

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**A reference gene catalogue of the pig gut microbiome**

Liang Xiao[1†], Jordi Estellé[2†], Pia Kiilerich[3†], Yuliaxis Ramayo-Caldas[2], Zhongkui Xia[1], Qiang Feng[1‡], Suisha Liang[1], Anni Øyan Pedersen[4], Niels Jørgen Kjeldsen[4], Chuan Liu[1,5], Emmanuelle Maguin[6], Joël Doré[6,7], Nicolas Pons[7], Emmanuelle Le Chatelier[7], Edi Prifti[7‡], Junhua Li[1,8], Huijue Jia[1], Xin Liu[1], Xun Xu[1], Stanislav D. Ehrlich[7,9], Lise Madsen[1,3,10], Karsten Kristiansen[1,3*], Claire Rogel-Gaillard[2*] and Jun Wang[1,3*]

+ G*M + G*E + M*E

+G*M*E + e

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INRA SCIENCE & IMPACT

# Thanks for your attention!