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Molecular genetics of the adrenocortical axis and breeding for robustness

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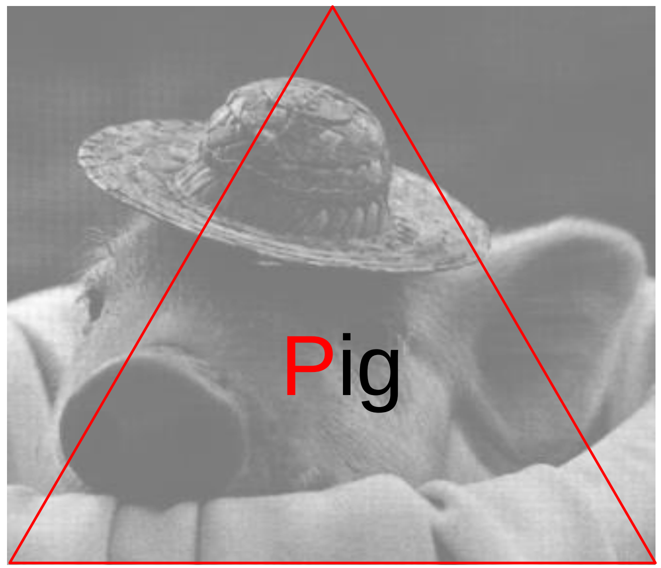
Diet, agriculture, environment

New challenges for farm animal breeding

Sustainability

Reduced production costs
-feed efficiency
-less labour (independent animals)
-adaptability to environmental constraints

Profit



-environmental footprint (GHG, N, P)
-antibiotics, heavy metals & hormones

-food safety
-product quality
-animal welfare
-rural lifestyle

Planet

People

Main breeding objectives: feed efficiency, robustness, product quality

ROBUSTNESS

- The concept of robustness refers to the ability of the animals to express their production potential in a wide range of environmental conditions without compromising their physical health and welfare (Knap 2005)

- Includes several components
  - Sensitivity of the production potential
    - ✓ to diverse commercial environments
    - ✓ and to external stressors (temperature)
  - Functional traits
    - ✓ quality of supportive tissues (e.g. leg soundness)
    - ✓ newborn survival
    - ✓ resistance to diseases (efficiency of the immune system)
    - ✓ productive longevity

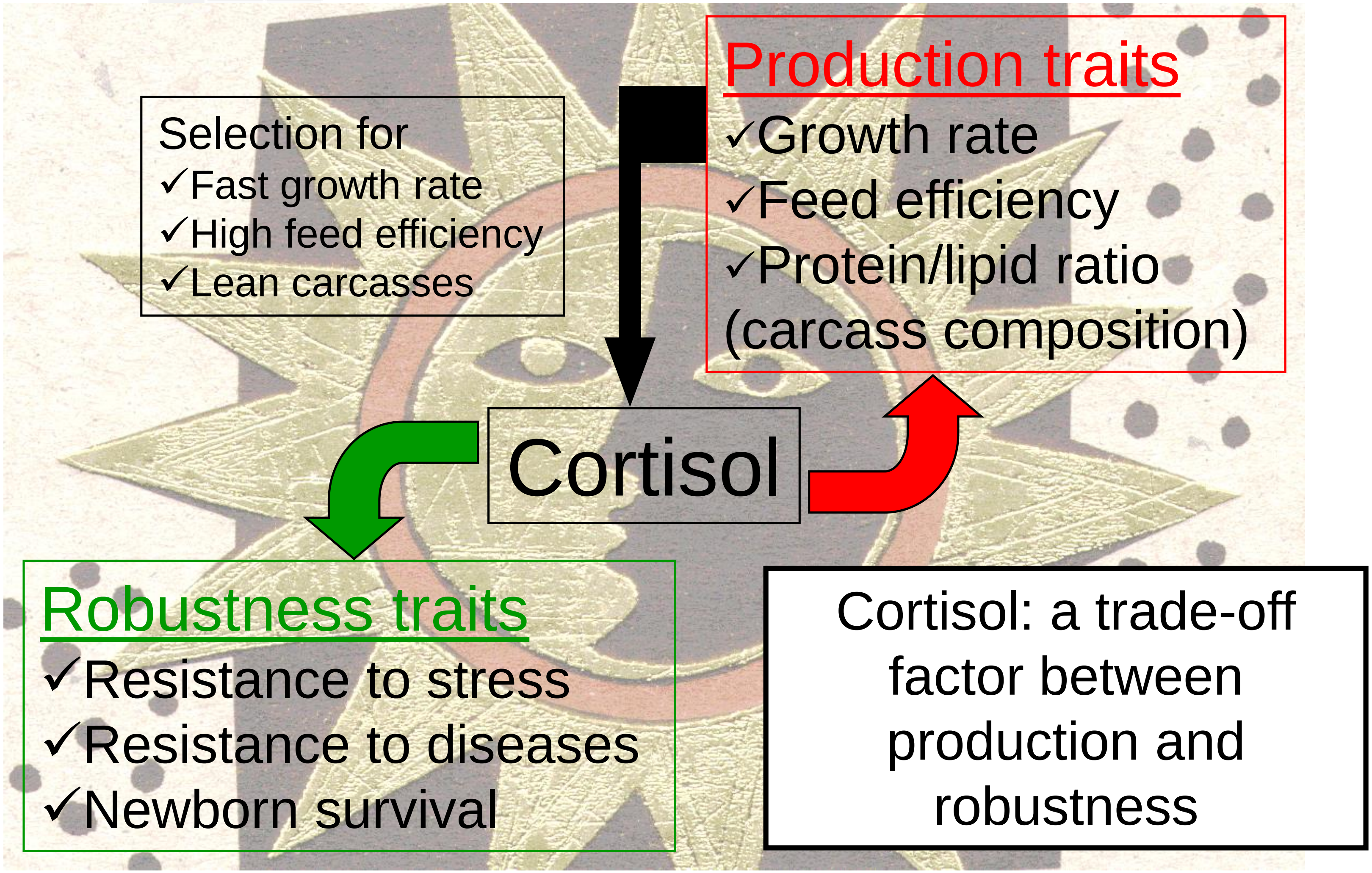
Robustness as a breeding objective

‘Sustainable breeding goals combine robustness traits with production traits to such an extent that selection balances genetic change in production potential with genetic change in environmental sensitivity’ (Knap 2009)

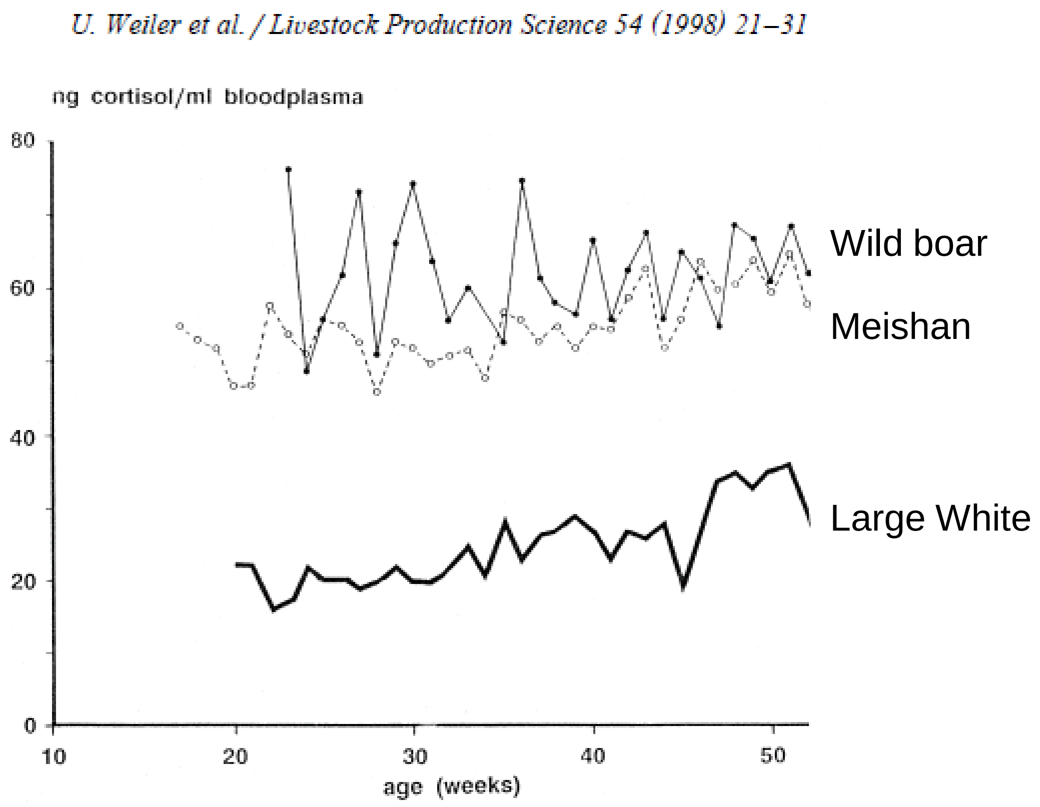
Selection strategies

1. Inclusion of directly measurable **functional traits** in the breeding objective and in the selection index (BLUP) – leg soundness, newborn survival rate, fertility, cellularity of milk …
2. **Reaction norm** analysis – global sensitivity to the environment (Lynch and Walsh 1998) – low heritability
3. **Canalizing selection** for a reduced variance
4. Genetics of **stress responses**
   behavioural and **neuroendocrine**

Effects of cortisol on production and robustness traits



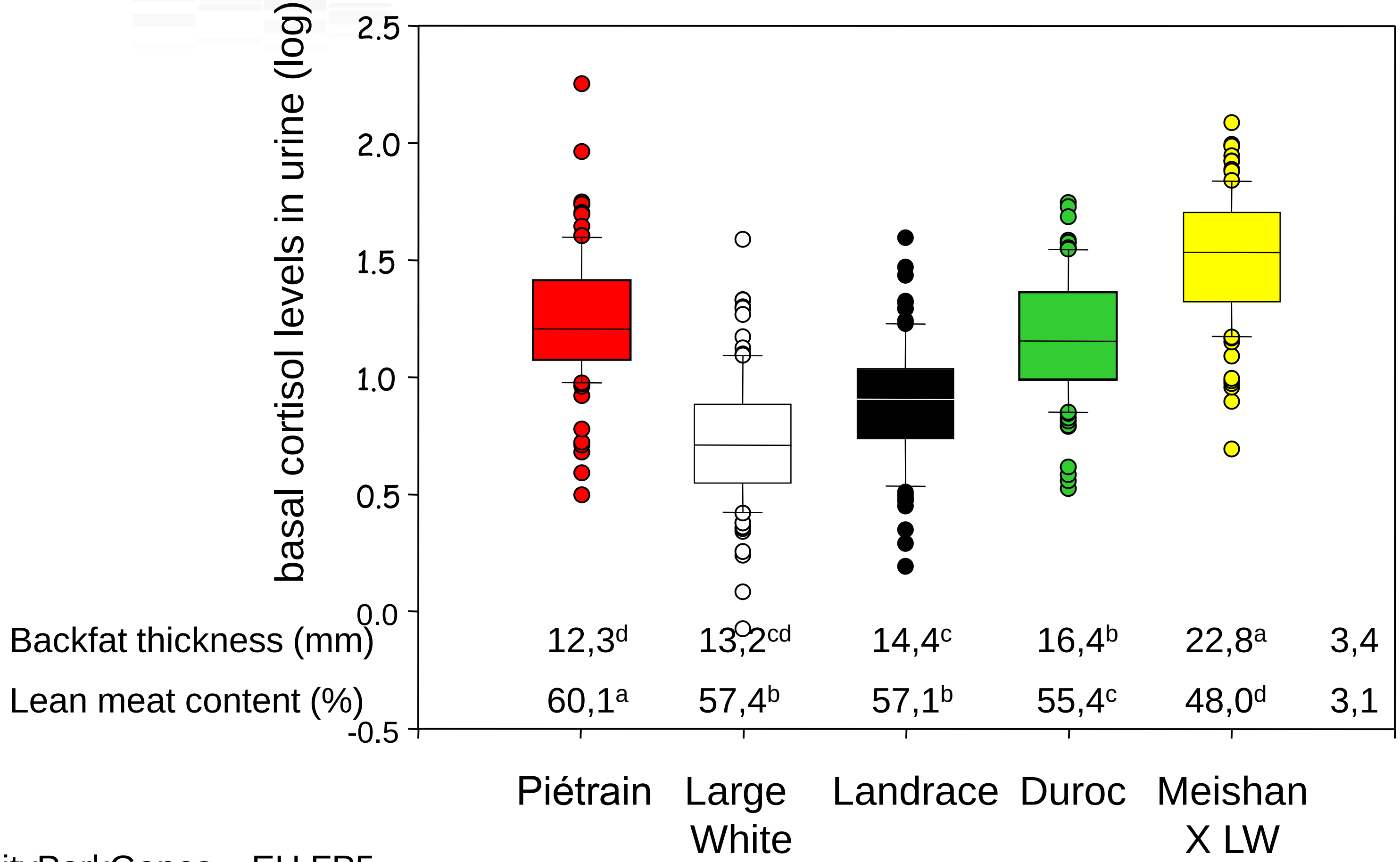
Domestication and the HPA axis



Breeding objectives to increase robustness by selection of a stronger HPA axis

- Selection for fast growth rate, high feed efficiency and lean carcasses may have reduced HPA axis activity with negative consequences on robustness
- Working hypothesis : selection for a more active / reactive HPA axis could improve robustness with (hopefully) limited effects on production traits.
- Strong genetic influences on HPA axis activity
  - Differences between genetic stocks (e.g. Large White / Meishan)
  - Divergent genetic selection in chicken (ACTH, social stress), turkey (cold), duck (suspension), trout (confinement), mouse (restraint).
- Several sources of genetic variation
  - Cortisol production (sensitivity to ACTH)
  - Bioavailability
  - Receptor function

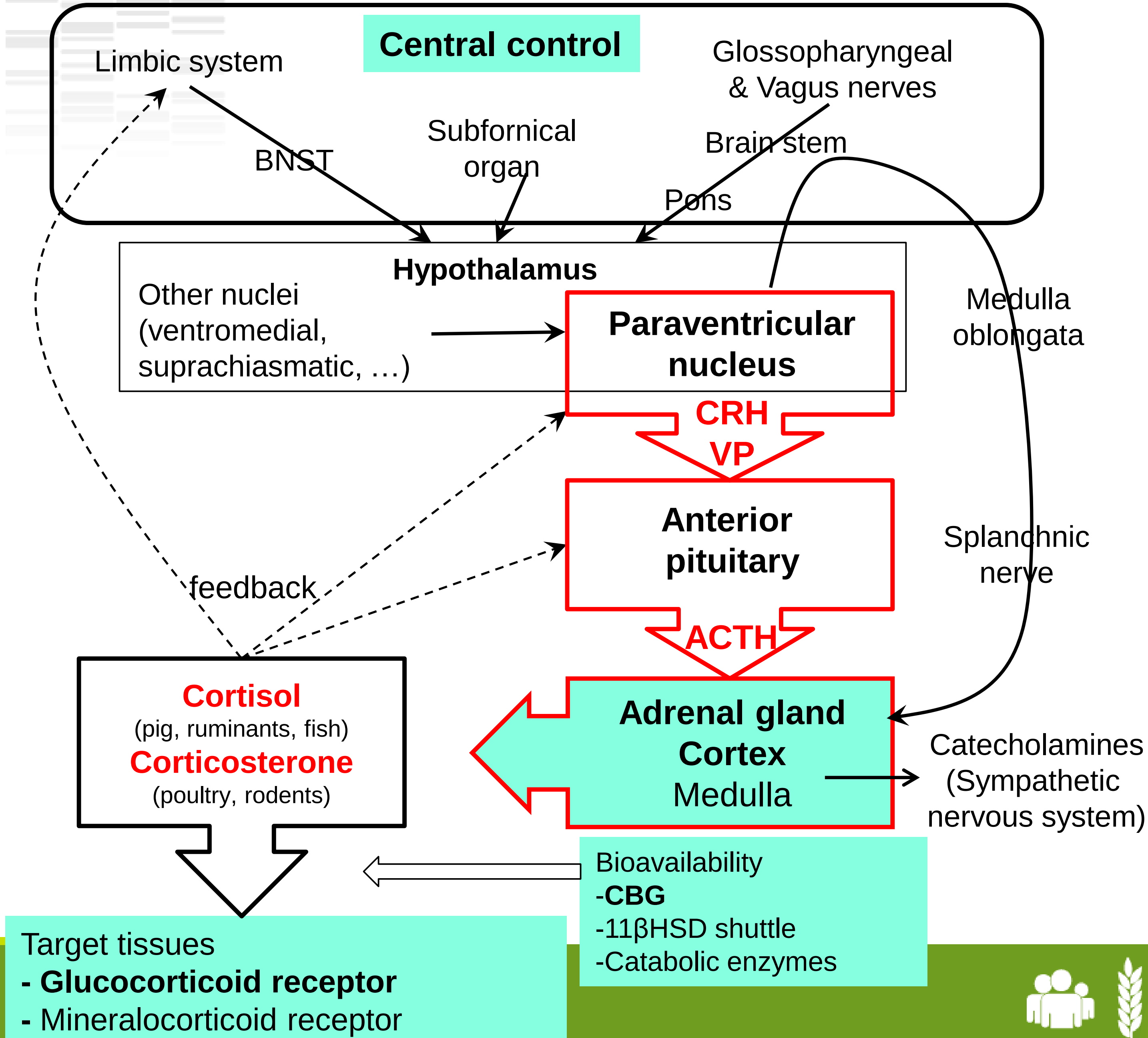
Between- and within-breed variation of cortisol production



QualityPorkGenes – EU FP5
Foury et al. Animal 1:973, 2007

N = 100 female pigs/ breed

HPA axis, sources of genetic variability



Sources of genetic variability in the HPA axis

CORTICOSTEROID HORMONE PRODUCTION
- Response of the adrenal cortex to ACTH

- ❖ Is the main source of genetic variation in cortisol production
- ❖ Is an individual trait (stable across time)
  - – Pigs Hennessy et al. 1988
  - – Humans - 'Adrenal phenotype' Bertagna et al. 1994; Coste et al. 1994
- ❖ Is heritable
  - – $h^2 = 0.68$ - analysis in 30 litters of Large White pigs
    - Larzul et al. Animal 9:1929, 2015
  - – Divergent selection
    - Direct selection in chicken, response to ACTH (Edens et Siegel, 1975)
    - Main trait selected in response to stress (trout, duck)
- ❖ Molecular genomics
  - Hazard et al. BMC Genomics 9:101, 2008
  - Jouffe et al. BMC Proceedings 3:S14, 2009

Adrenals: differentially expressed genes

- No difference
  - In the **ACTH signaling pathway** (i.e. ACTH receptor, G protein, Adenylate Cyclase, Protein Kinase A and MAPK, ERK1, ERK2) except *Crem* (cAMP response element modulator) that activates genes involved in steroidogenesis
  - In **steroidogenic enzymes** (*i.e. Cyp11a1, Cyp17, 3βhsd, Cyp21, Cyp11b*)
- Differentially expressed
  - **Cholesterol transport** (Star, Ldlr) and several kinases involved in phosphorylation of StAR (Snf1lk)
  - **Tricarboxylic acid cycle** (Mdh2, Sdha and Suclg2) involved in heme biosyntheis (Alas1)
  - Still to be characterized transcripts / genes

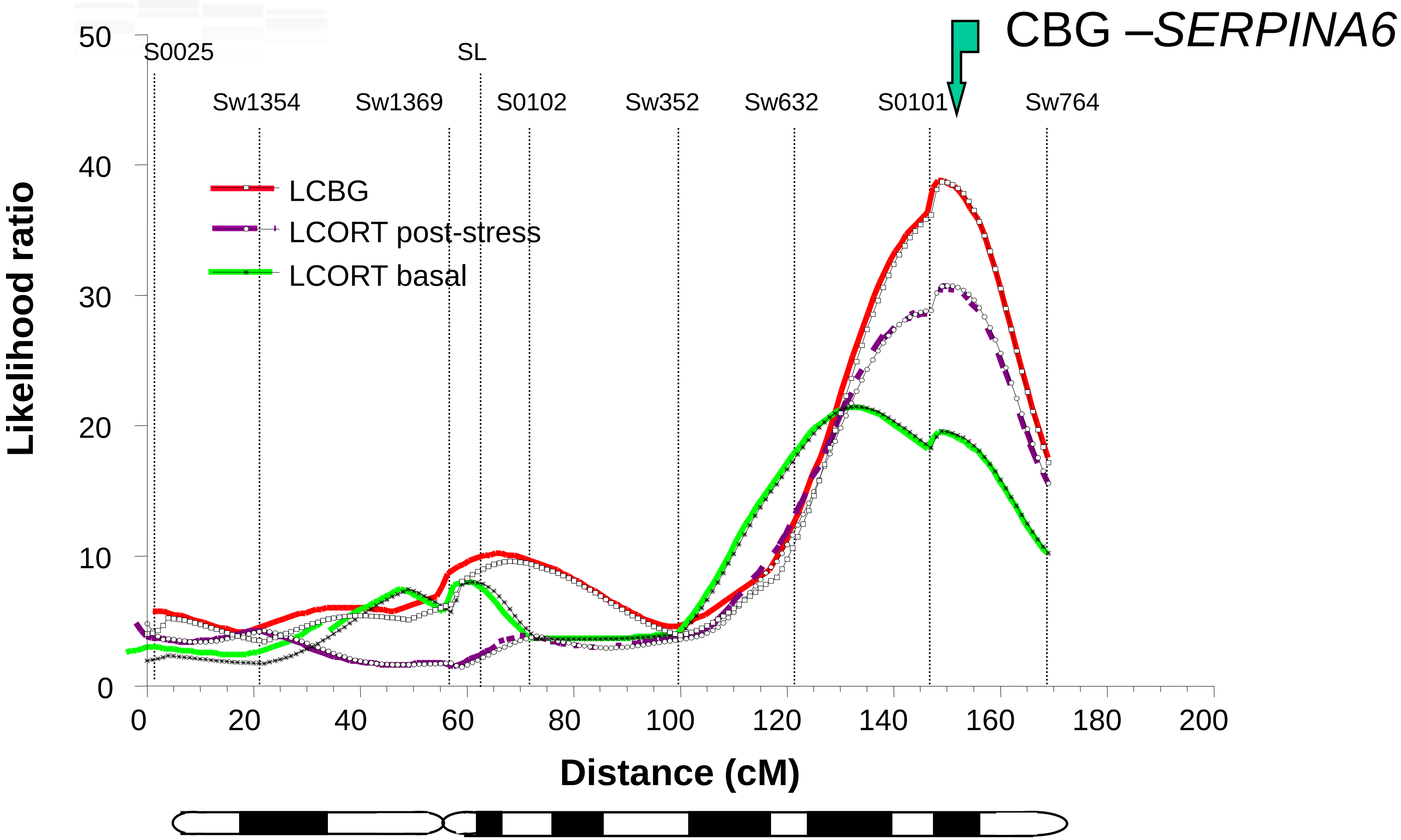
Hazard *et al. BMC Genomics* **9**:101, 2008

Sources of genetic variability in the HPA axis

BIOAVAILABILITY
- Corticosteroid-binding globulin (CBG)
- 11β-hydroxysteroid dehydrogenases

❖ Corticosteroid-binding globulin (CBG or transcortin)
- Glycoprotein synthesized in the liver and carrying cortisol in plasma
  Plays a dual role of sequestration of cortisol in plasma and delivery to the target tissues
  - Moisan Mol Cell Endocr 316:35, 2013
- Genetic determinant of cortisol levels by linkage study
  in a Large White x Meishan F2 population
  - Désautés et al. J Anim Sci 80:2276, 2002
  - Confirmed in rats (Solberg et al. 2006)
    and humans (Bolton et al. PLoS Genetics 2014)
- Molecular polymorphisms influencing protein properties
  with consequences on adiposity and meat quality
  - Ousova et al. Mol Endocrinol 18:1687, 2004
  - Geverink et al. J Anim Sci 84:204, 2006

QTL cortisol / CBG on Ssr 7 (F2 LWxMS)



Désautés *et al. J Anim Sci* **80**:2276 (2002)
Ousova *et al. Mol Endo* **18**:1687 (2004)

CBG and production traits

Pearson Correlation Coefficients between Carcass Composition Traits, Plasma CBG Capacity, and Total Plasma Control

	CBG	Cortisol (Basal)	Cortisol (Post Stress)
Backfat weight	r = 0.39	r = −0.18	r = 0.11
	P = 0.014	*P* = 0.28	*P* = 0.50
% (Ham+ loin)	r = −0.40	r = 0.18	r = −0.11
	P = 0.012	*P* = 0.27	*P* = 0.52
% (Back+ leaf fat)	r = 0.42	r = −0.17	r = 0.12
	P = 0.008	*P* = 0.29	*P* = 0.45
Estimated muscle content	r = −0.43	r = 0.16	r = −0.13
	P = 0.0064	*P* = 0.33	*P* = 0.41

Each cell of the table contains the Pearson correlation (r) coefficient and the probability (*P*). The number of observations is n = 39 in all cases.

Ousova *et al. Mol Endo* **18**:1687 (2004)

Sources of genetic variability in the HPA axis

BIOAVAILABILITY
- Corticosteroid-binding globulin (CBG)
- 11β-hydroxysteroid dehydrogenases

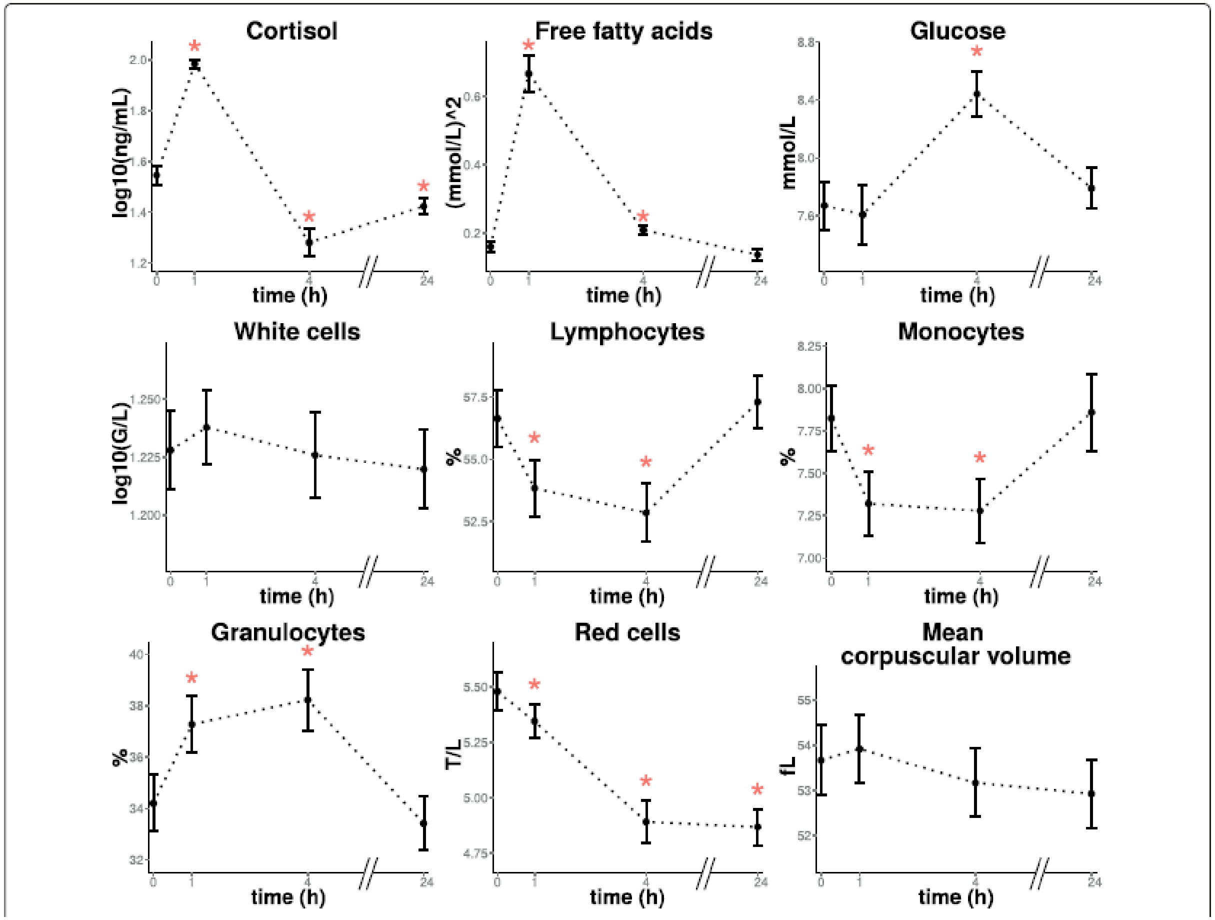
- Corticosteroid-binding globulin (CBG or transcortin)
- Hormone metabolism
  - Cortisol – cortisone shuttle (hydroxysteroid dehydrogenases 1 & 2)
  - Local tissue bioavailability of cortisol may influence adiposity
  - Linkage/association between HSD11B1 polymorphism and carcass length and adiposity in pigs
    - Otieno et al. Anim Genet 36:36, 2004

Sources of genetic variability in the HPA axis

ACTION ON TARGET TISSUES
- Receptors or transduction pathways

- Cortisol receptors are transduction factors
  - Glucocorticoid receptors, GR, gene NR3C1
  - Mineralocorticoid receptors, MR, gene NR3C2
- Genetic influences and gene polymorphisms well documented in humans and experimental animals
- Little information in farm animals
  - Functional differences LW/MS pigs
    Perreau et al. Life Sci 17:1501, 1999
  - Association studies (pigs)
    Murani et al. BMC Genetics 11:74, 2010; Terenina et al. DAE 44:81, 2013
  - Naturally occurring gain-of-function mutation Ala610Val
    Murani et al. 2012, 2016
- Functional genomic fingerprint of cortisol effects (e.g. circulating white blood cells)

Response to ACTH –
biological measures in peripheral blood



Sautron *et al. BMC Genomics* 16:961, 2015

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Response to ACTH - differentially expressed genes in circulating leucocytes

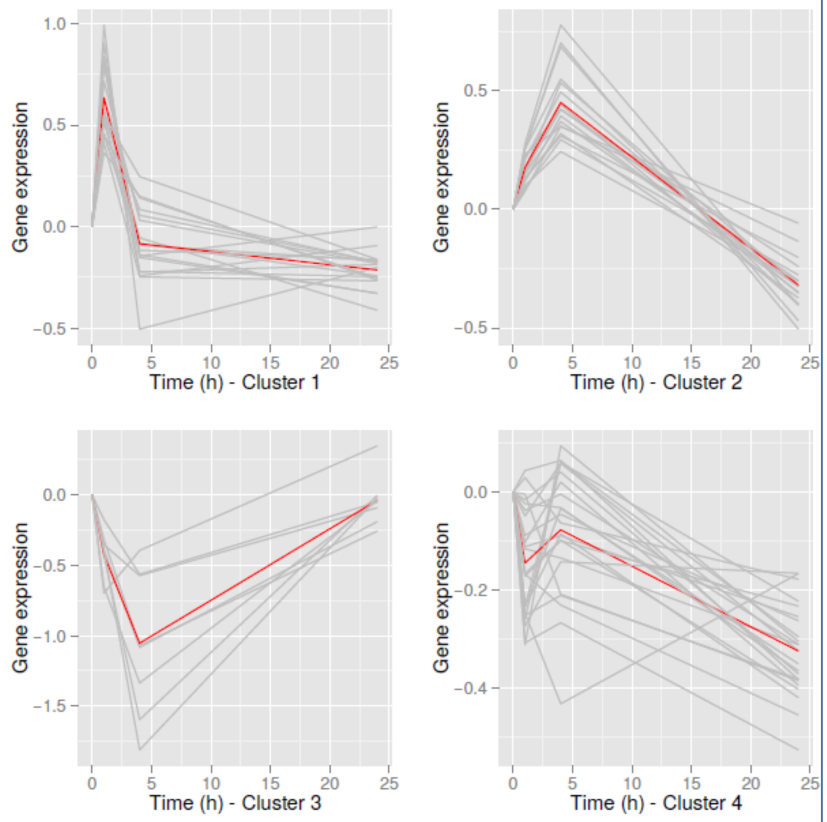
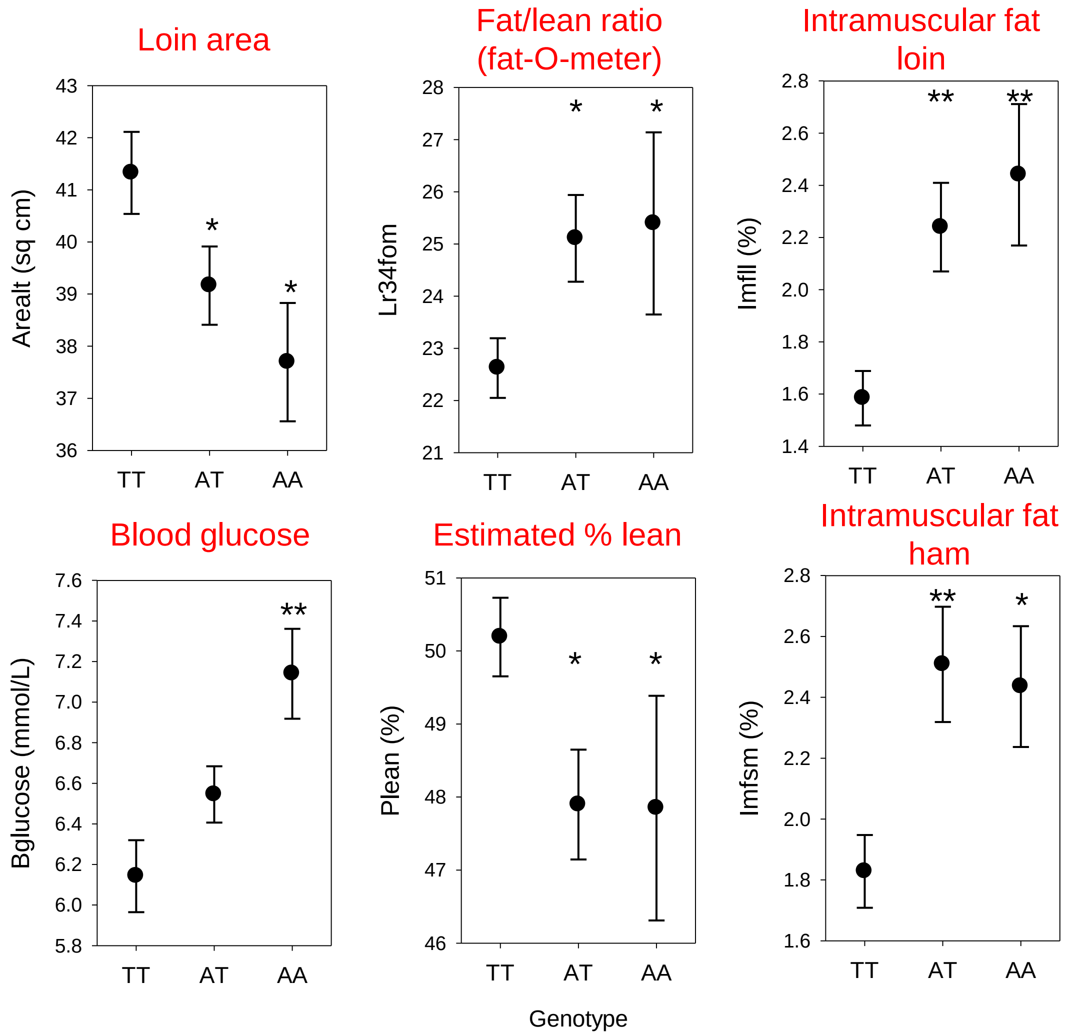


Figure 5 Average evolution of the genes in each of the cluster identified by the HAC on the 66 unique DE genes Evolution of each gene is translated to that it is equal to 0 at $t = 0$; Gray: Average evolution of each of the genes in the cluster. Red: Average evolution over all genes in the cluster.

Sautron *et al. BMC Genomics* (2015) 16:961

Phenotypic effect of the MAOA(-430A>T) SNP in the promoter region of monoamine oxidase A gene
Association study

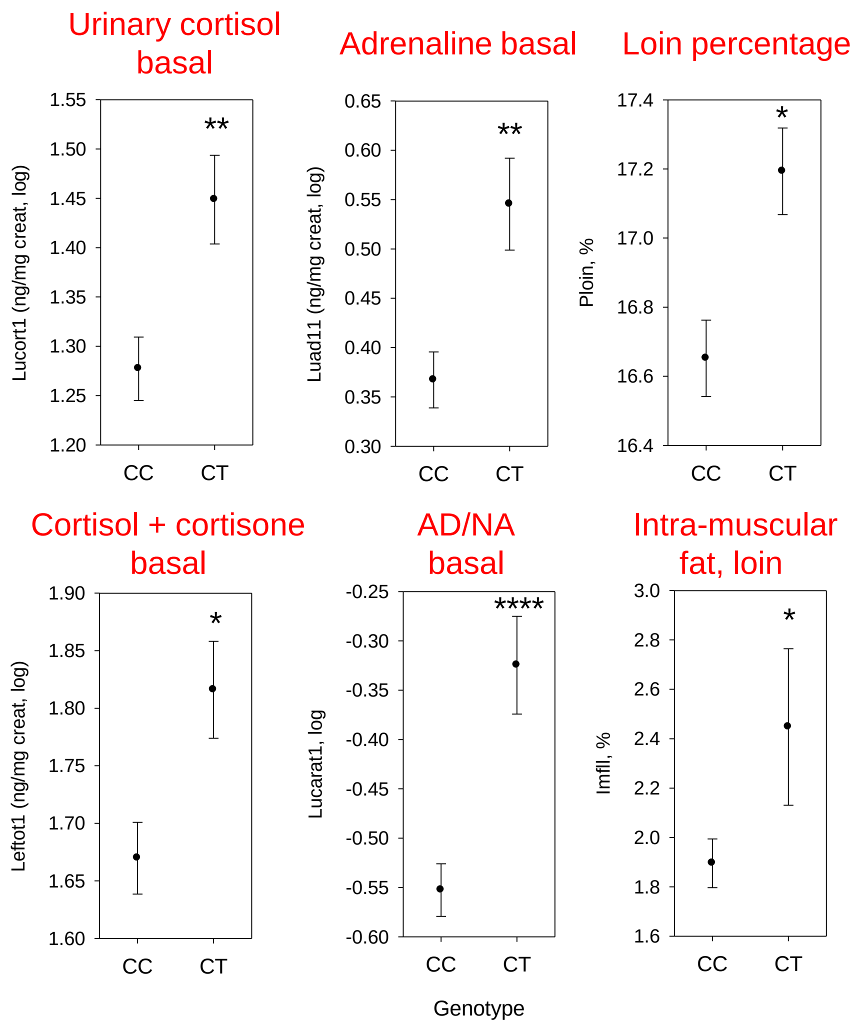


Female pigs (n = 100) from an advanced intercross line (F9-F11) between Large White and Meishan

TT n = 40
AT n = 41
AT n = 11-13

Terenina et al. DAE 44:81, 2013

Phenotypic effect of the DRD3(-680C>T) SNP in the promoter region of dopamine receptor D3 gene Association study



Female pigs (n = 100) from an advanced intercross line (F9-F11) between Large White and Meishan

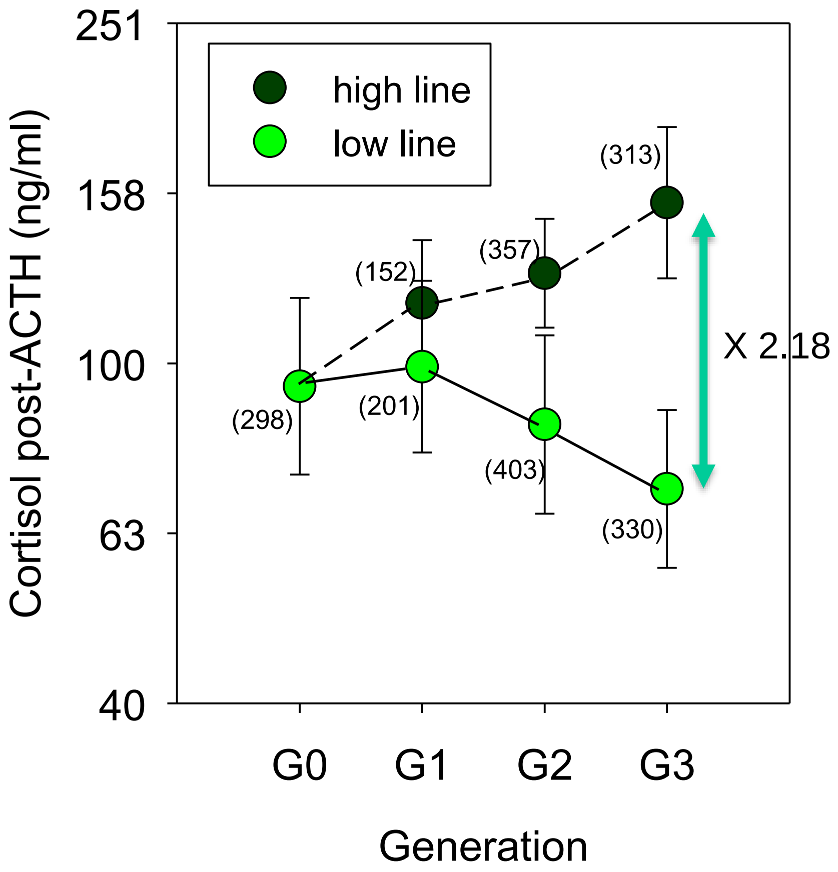
CC n = 66-68
CT n = 13-20
TT n = 3 (not shown)

Terenina et al. DAE 44:81, 2013

HPA axis and robustness - proof of concept
divergent selection for the response to ACTH in Large White pigs

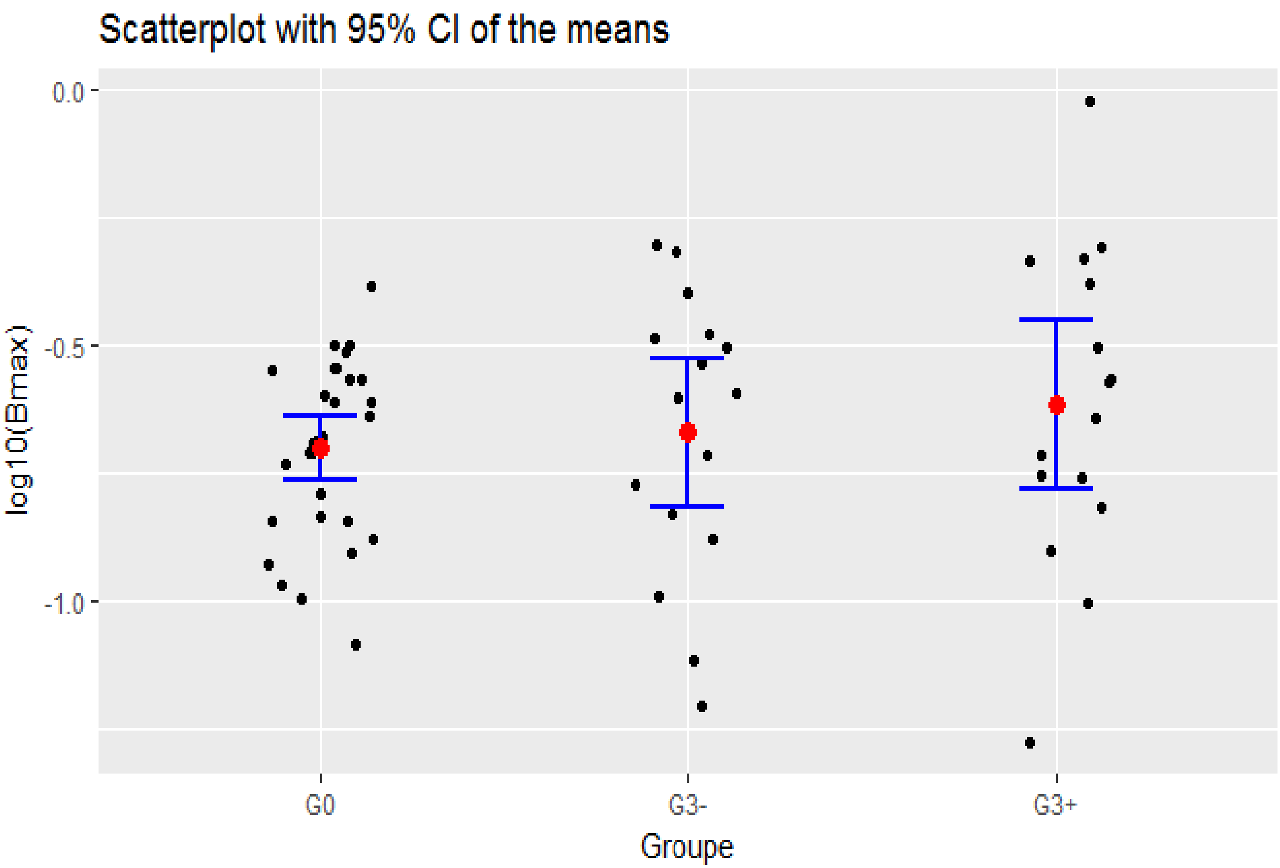
ACTH

6 weeks

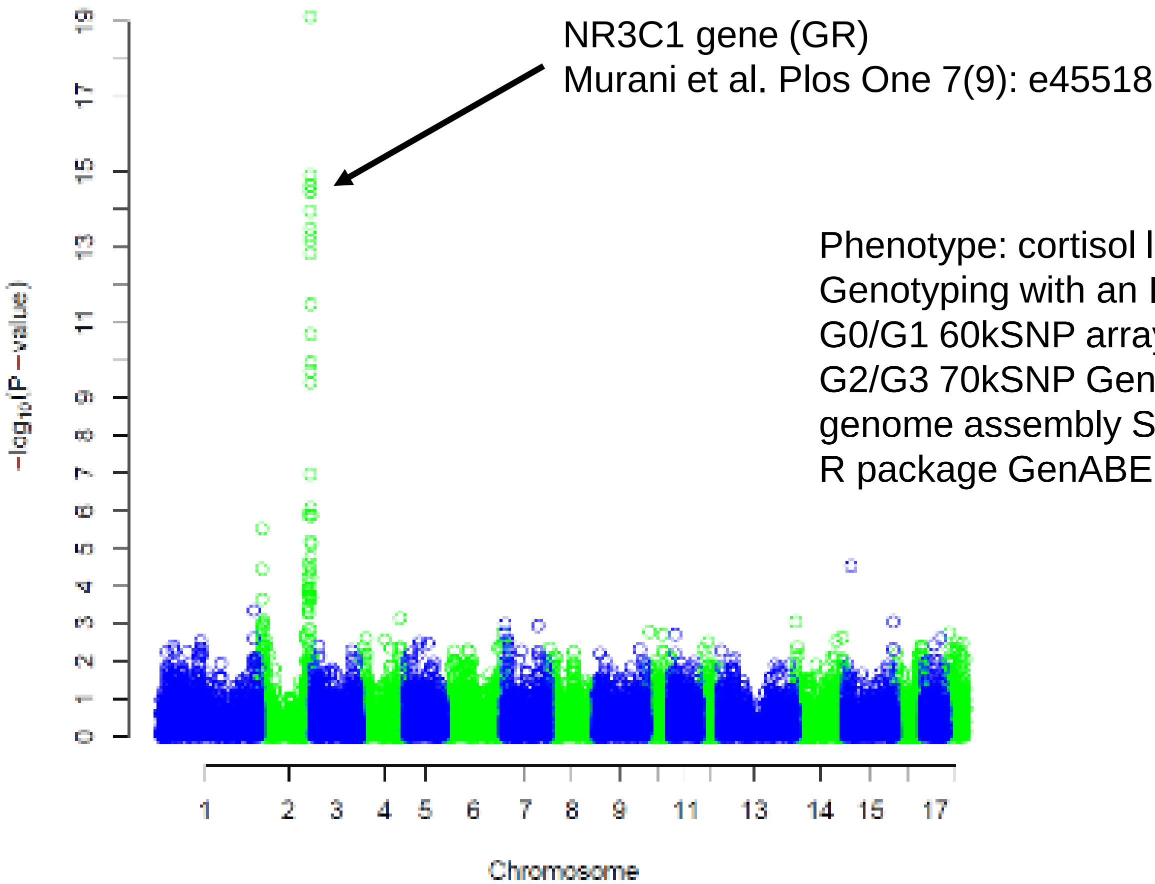


Realized heritability $h^2 = 0.68 \pm 0.12$

CBG (cortisol-binding globulin) in selected lines

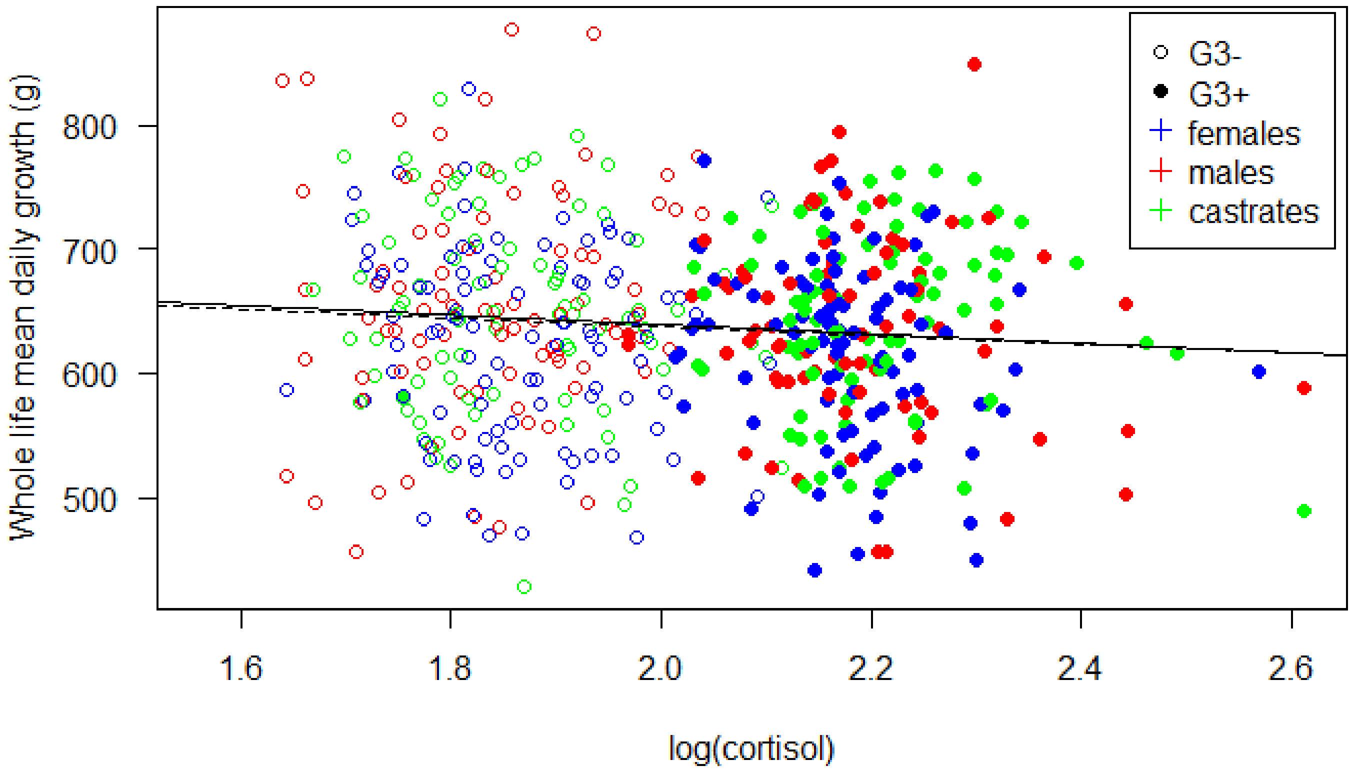


Genome-wide association study



Phenotype: cortisol level 1 h post-ACTH
Genotyping with an Illumina Beadchip array
G0/G1 60kSNP array
G2/G3 70kSNP GenSeek array
genome assembly Sscrofa11.1
R package GenABEL

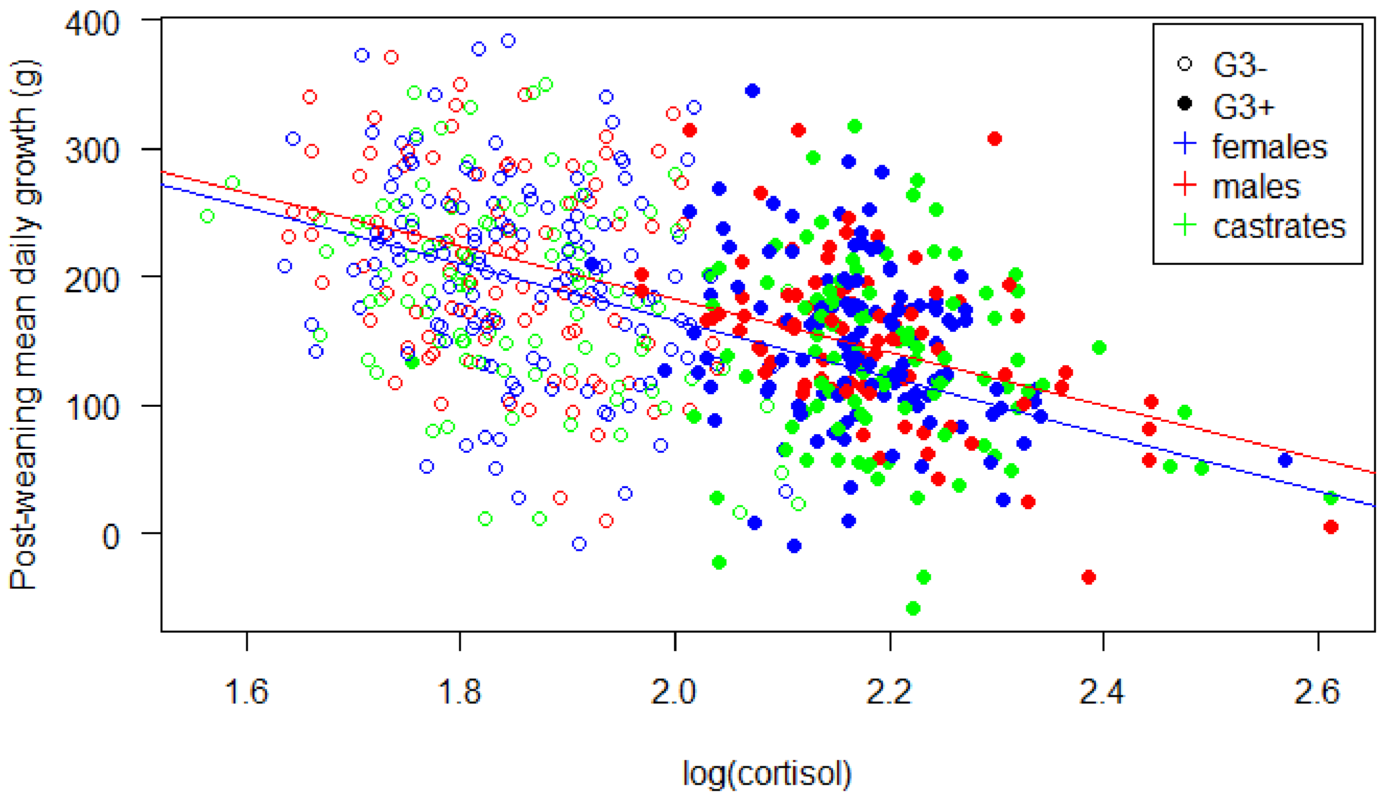
Selected lines G3 – mean daily growth



G3+ - G3- = -11 g/day, n.s.
Within groups, - 35 g/day per unit log(cortisol), n.s.

NO effect of selection
NO influence of cortisol

Selected lines G3 – post-weaning growth



G3+ - G3- = -55 g, P < 2.10^{-16}

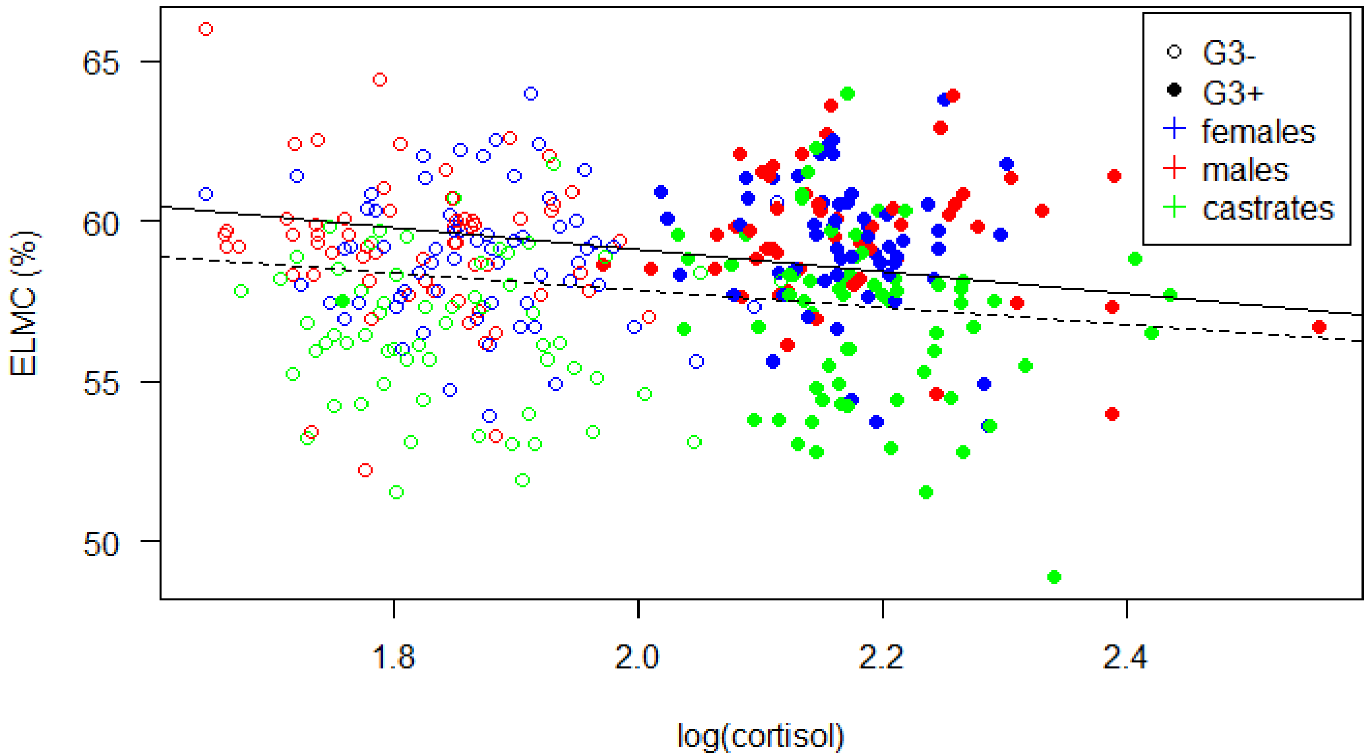
Within groups, -214 g per unit log(cortisol), P < 2.10^{-15}

At the same level of cortisol, G3+ - G3- = +16 g; n.s.

Strong effect of selection

Related to cortisol levels

Selected lines G3 – meat content



G3+ - G3- = 0.49%, P < 0.05
Within groups: ELMC -4.12% per unit log(cortisol), P < 0.002
At the same level of cortisol, G3+ - G3- = 1.90%, P < 0.0005

Small effect of selection
Unrelated to cortisol levels

Sources of genetic variability in the HPA axis

CENTRAL DRIVE
- Inputs to the HPA axis
- CRH, vasopressin and receptors

CORTICOSTEROID HORMONE PRODUCTION
- Response of the adrenal cortex to ACTH

BIOAVAILABILITY
- Corticosteroid-binding globulin (CBG)
- 11β-hydroxysteroid dehydrogenases

ACTION ON TARGET TISSUES
- Receptors or transduction pathways

FUNCTIONAL CONSEQUENCES

Production / Meat quality / Robustness

SYSTEMS GENETICS

FUNDING

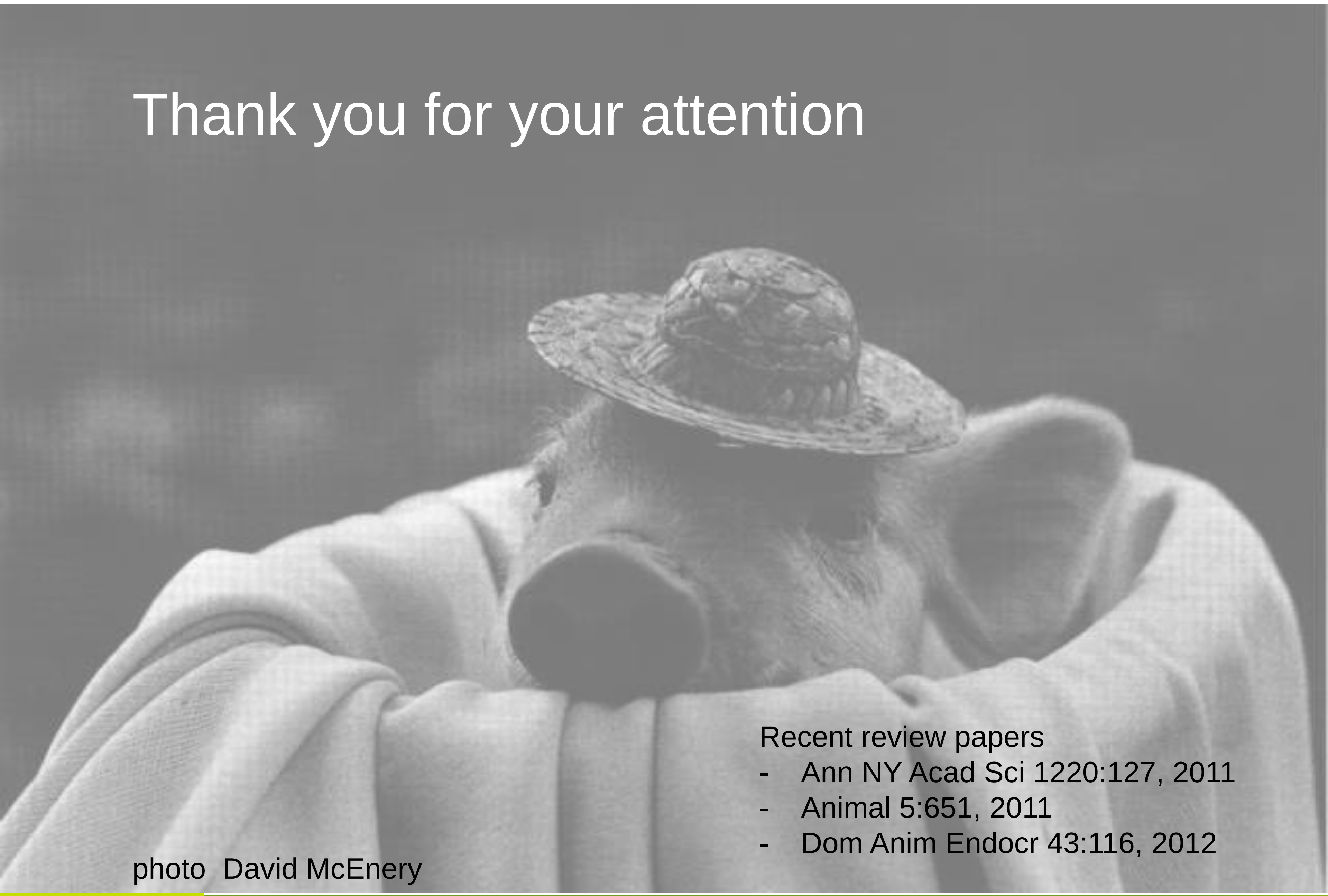
INRA, Animal Genetics Division

ANR, Agence Nationale de la Recherche

- INRA, Genetics, Physiology and Breeding Systems, Toulouse
  - Elena Terenina, Catherine Larzul, Nathalie Villa-Vialaneix
  - Valérie Sautron (thesis)

- INRA, Genesi, Le Magneraud (experimental unit)
  - Yvon Billon

Thank you for your attention



Recent review papers
- Ann NY Acad Sci 1220:127, 2011
- Animal 5:651, 2011
- Dom Anim Endocr 43:116, 2012

photo David McEnery