69th Annual Meeting of the European Federation of Animal Science,
27.08. – 31.08.2018, Dubrovnik, Croatia
Session 38: Sustainable control of production diseases in pigs and poultry,
with emphasis on early survival

Genomic analysis of immune traits for two maternal pig lines

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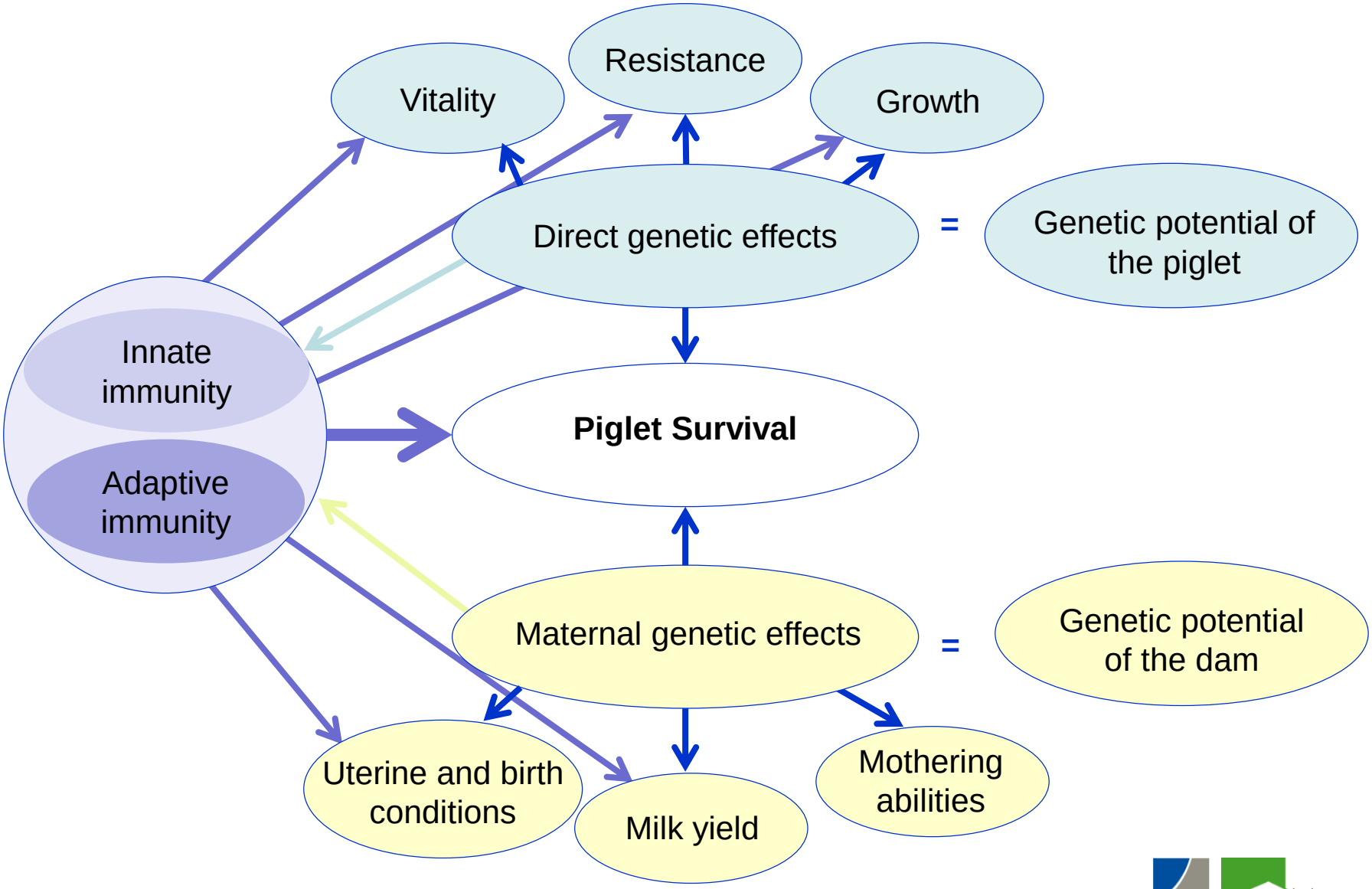
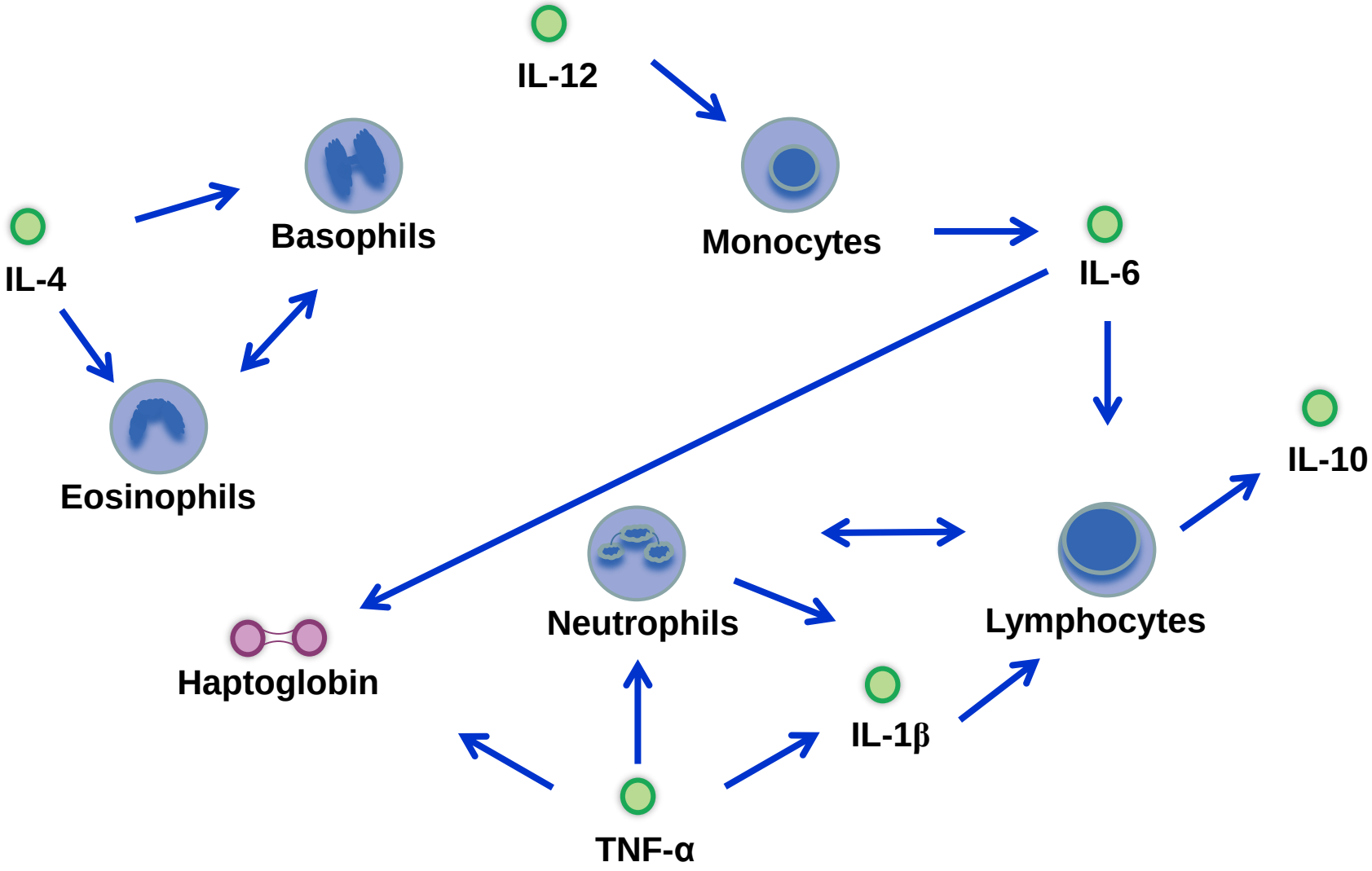
Genetic Effects on Piglet Survival



Modified according to Roehe et al. 2010

Interactions of Immune Parameters



Modified according to: Schindler et al. 1990; Seutter 1995; Thorn 2000; Flori et al. 2011; Tizard 2013

Hypothesis and Objectives

Research gap:

- Genetic improved resistance for factor diseases and infections
- Genetic variability and close relationships of immune parameters

?

Aim of pigFit Project:

Breeding-based improvement of health traits and survival of piglets and growing pigs through immune profiling and genomic selection

Objectives of the study:

- Variance component analysis
- Genomewide Associaton Study (GWAS)

Experimental Set-up

Landrace

2 x 250 **Dams**

Large White

2 x 500 **Piglets** (♂/♀)

Blood samples (N=1500):

Blood → Immune parameters, Haptoglobin, Cytokines

BS Dams: (ø 7d p.p.)
BS Piglets: (ø 35d p.p.)

Genotyping (N=1500):

Illumina PorcineSNP60v2 Bead Chip

BS=Blood samples; Pictures: BHZP GmbH

Mixed Model – Variance Components

$$\boldsymbol{y} = \boldsymbol{X\beta} + \boldsymbol{Za} + \boldsymbol{Zm} + \boldsymbol{e}$$

$\boldsymbol{y}$: observations immune traits for each individual

$\boldsymbol{\beta}$: fixed effects vector parity and herd-year-season-sex, covariate age at the time of sample collection

$\boldsymbol{a}$: random additive genetic effect

$\boldsymbol{m}$: random maternal genetic effect

$\boldsymbol{e}$: environmental residual effects

$\boldsymbol{X}$, $\boldsymbol{Z}$: incidence matrices

Gilmour, A. R. et al. ASReml User Guide Release 4.1. Available at www.vsni.co.uk (2015).

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$\boldsymbol{y}$: observations immune traits for each individual

$\boldsymbol{\beta}$: fixed effects vector parity and herd-year-season-sex, covariate age at the time of sample collection and **principal components of dams immune parameters**

$\boldsymbol{a}$: random additive genetic effect

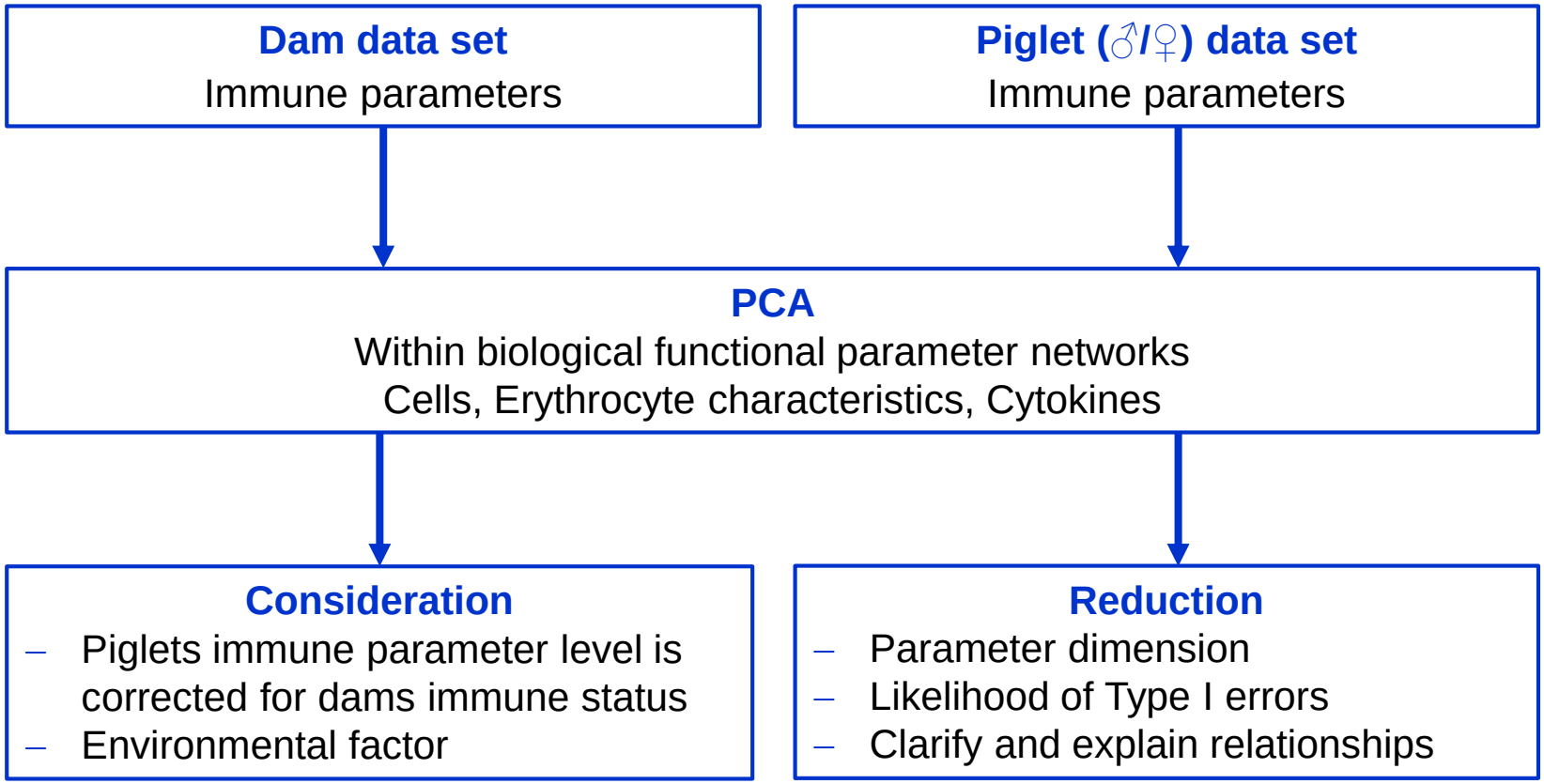
$\boldsymbol{m}$: random maternal genetic effect

$\boldsymbol{e}$: environmental residual effects

$\boldsymbol{X}$, $\boldsymbol{Z}$: incidence matrices

Gilmour, A. R. et al. ASReml User Guide Release 4.1. Available at www.vsni.co.uk (2015).

Principal Component Analysis - PCA



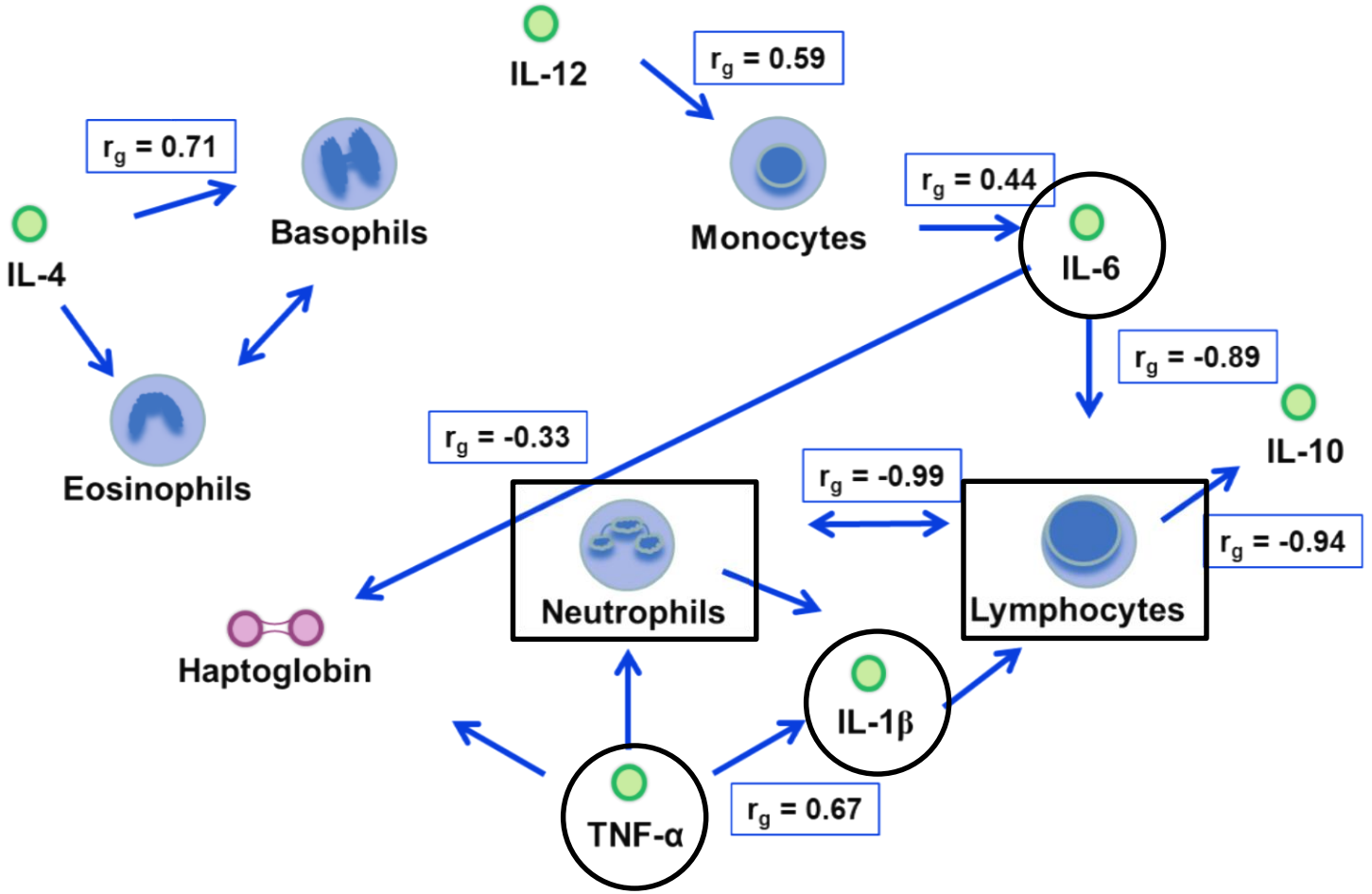
PC Inclusion - h^2 Results

Trait	h^2		m^2	
	Landrace	Large White	Landrace	Large White
Erythrocytes	**0.44**	**0.41**	-	-
Hematocrit	**0.51**	0.20	-	-
MCV	**0.63**	**0.80**	-	-
MCH	0.23	**0.69**	0.06	-
Lymphocytes	**0.48**	0.13	-	0.23
IL-10	0.27	0.29	**0.55**	**0.49**
IL-4	0.21	0.19	**0.49**	**0.45**
IL-6	0.29	0.19	**0.54**	**0.55**
TNF-α	**0.61**	0.18	0.18	0.18

Landrace: n=494, Large White: n=407

MCV=Mean Corpuscular Volume, MCH=Mean Cellular Hemoglobin, MCHC=Mean Corpuscular Hemoglobin Concentration, IFN-γ = Interferon-γ, IL= Interleukin, TNFα=Tumor Necrosis Factor α, SE=Standard Error, m^2=maternal genetic effect, bold font indicates h^2 or m^2 ≥0.4, standard errors reach from 0.00-0.20.

Results – Genetic Correlations r_g



Landrace: n=494, Large White: n=407

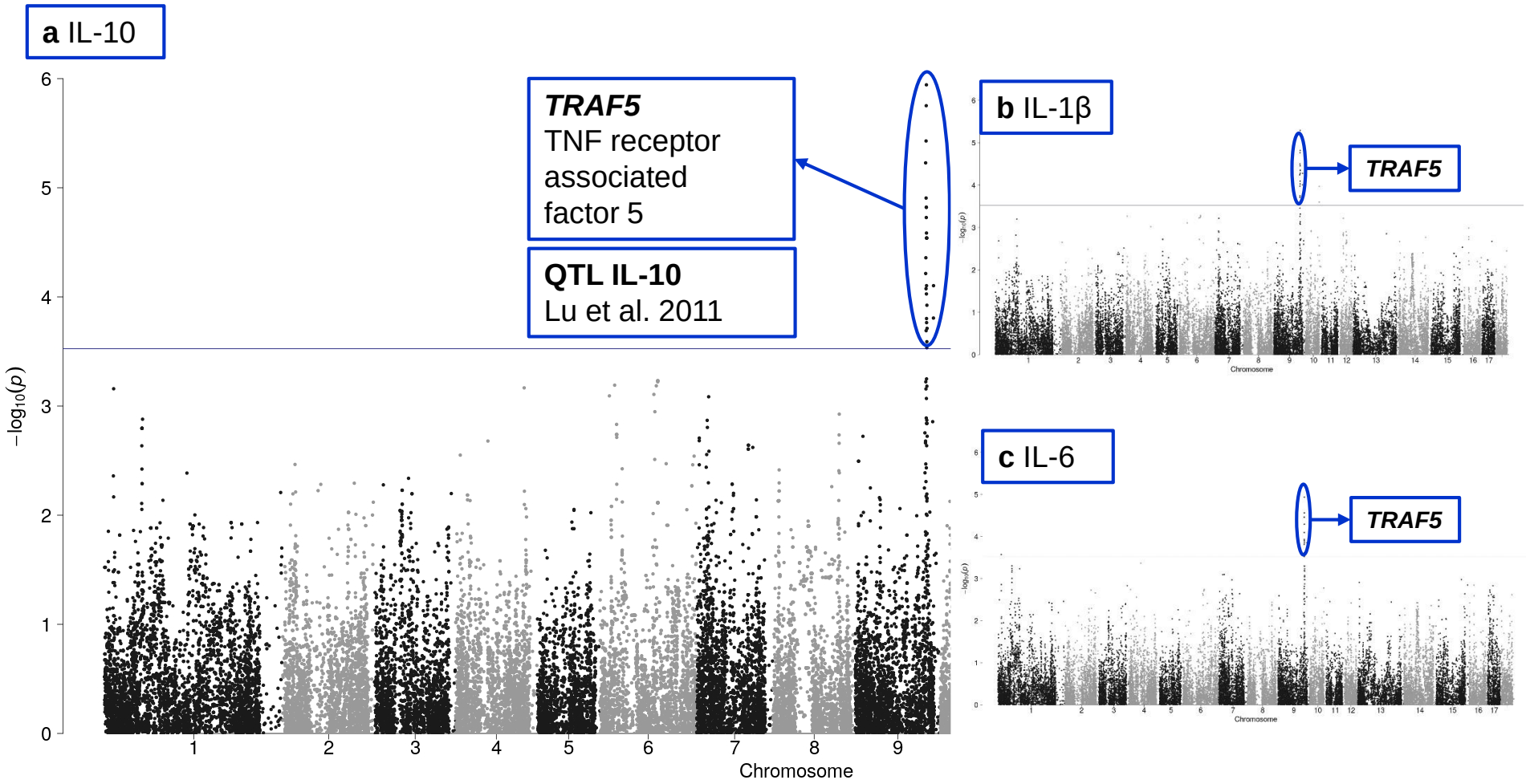
Modified according to: Schindler et al. 1990; Seutter 1995; Thorn 2000; Tizard 2013

PCA – h^2 and r_g

		Cells	Erythrocytes		Cytokines	Breed
		PC1	PC1	PC2	PC1	
Cells	**PC1**	0.08 0.30	0.03 -0.15	-0.33 **-**0.41	-0.18 0.83	LR LW
Erythrocytes	**PC1**	0.07 0.09	0.50 0.43	-	0.41 0.39	LR LW
	PC2	-0.04 0.02	-	0.77 0.28	0.03 0.27	LR LW
Cytokines	**PC1**	0.18 -0.10	0.19 0.10	0.01 0.01	0.42 0.38	LR LW

Heritabilities (h^2±SE) on the diagonal, phenotypic correlations (r_p) under the diagonal and genetic correlations (r_g) above the diagonal; The analyses were conducted with ASReml 4.0 (Gilmour et al. 2015); PC=Principal Component, LR=Landrace, LW=Large White, bold font indicates values ≥|0.4|, standard error ranged from 0.14 to 1.20

Results – GWAS LR



LR: n=531

p-values mapped before FDR. Blue line indicates chromosome-wide significance level, (q<0.05).

Conclusion

Research results:

- Moderate to high heritabilities and strong genetic correlations
- Maternal impact as environmental and genetic effects
- Breed differences

Ongoing:

- Correlation with economically important traits
- Condense information:
  - → PCA, Linear Discriminant Analysis, MV GWAS, post-hoc uv summaries

Pictures: BHZP GmbH

Thank you for your attention!

This study was performed within the ‘pigFit’ project.
The project is supported by funds of German Government’s Special Purpose Fund held at Landwirtschaftliche Rentenbank (FKZ28-RZ-3-72.038).

Conclusion

Research results:

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Pictures: BHZP GmbH

Piglet Survival

Production system:

- Management[1]
- Infection pressure[1]

Piglet survival

Politics and society:

- Animal welfare[2]
- Health status[5]
- Public acceptance[6]

adisagroblog.wordpress.com

Breeding:

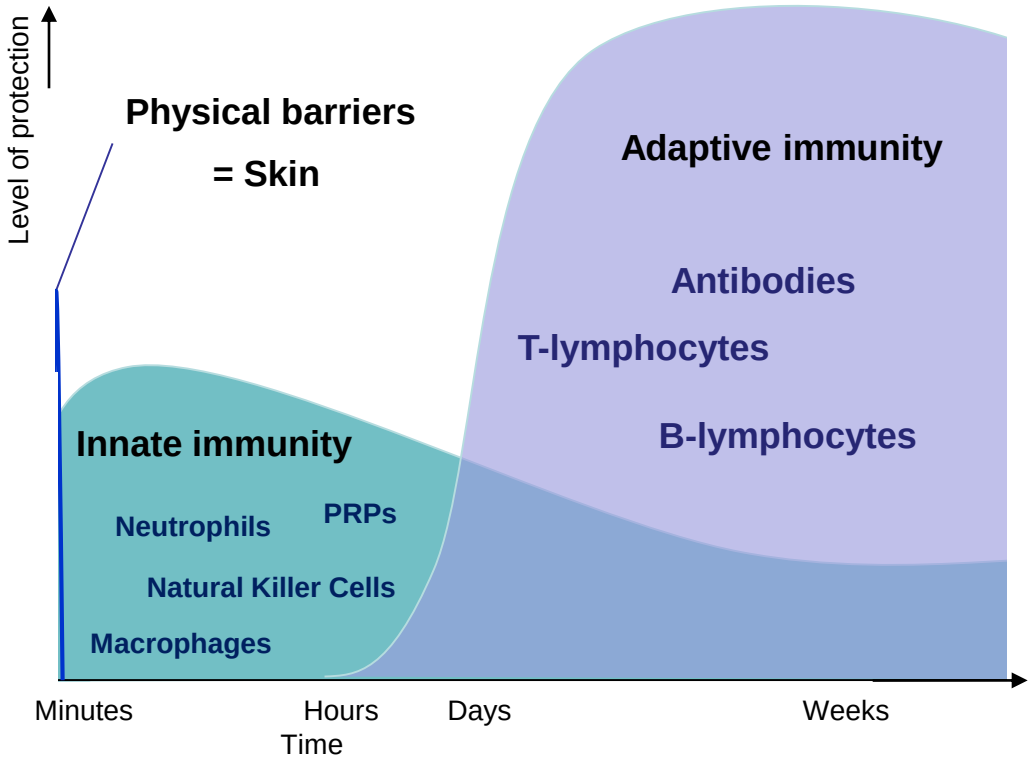
→ Litter size[2]
→ Birth weight[3]
→ Variance of birth weights[4]

Farmer:

- Economic viability[7]
- Public acceptance[5]

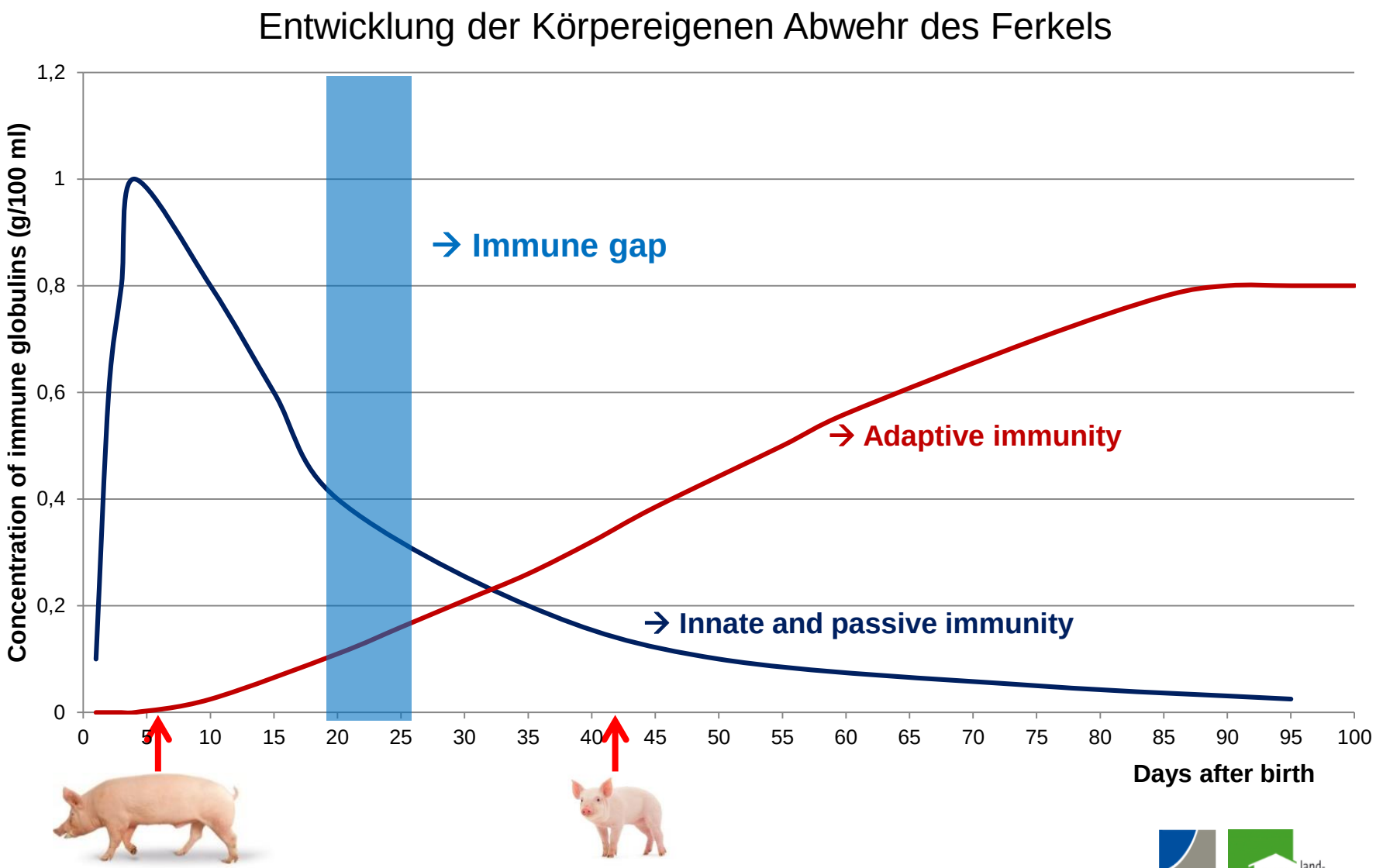
[1] Edwards and Baxter, 2015; [2] Rutherford, 2013; [3] Roehe und Kalm, 2000; [4] Milligan et al., 2002 [5] FLI, 2015; [6] Kapell et al., 2008; [7] BLE, 2015

Immune System – Overview



Modified according to Tizard (2013)

WP II - Experimental Set-up



Modifiied according to Kreutzmann und Schneider (2012);
Pictures: BHZP GmbH, landberichte.de

Phenotyping of Immune-Associated Traits

SYNLAB

GeneControl

Serum → Haptoglobin (N=1586)

Plasma → Complete blood count (N=1586)

ADVIA 2120® hematology system: flow cytometry-based analysis of blood

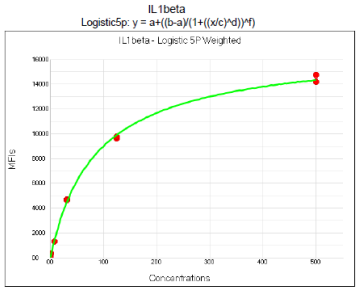
Tissue and blood samples → Genotyping (N=2000)

Illumina PorcineSNP60 BeadChip®

➡ Challenges: clotting and reference values

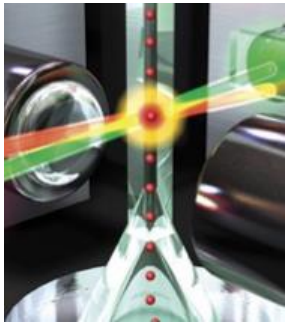
➡ Challenge: data assignment

Phenotyping of Immune-Associated Traits



Immunology Multiplex Assay:

IL-1β, IL-6, IL-4, IL-8, IL-12, IL-10, TNF-α, and IFN-γ (N=1500)



Milliplex® Porcine Cytokine Assay (Merk Millipore)
Luminex® 200™ System and Xponent® Software

➡ Challenges: plate design and reference values

Results - Heritabilities h^2

Trait	h^2				m^2			
	Landrace		Large White		Landrace		Large White	
	-	PC included	-	PC included	-	PC included	-	PC included
Erythrocytes	0.38	**0.44**	**0.49**	**0.41**	0.02	-	0.03	-
Hemoglobin	**0.47**	**0.45**	**0.45**	0.33	-	-	-	-
Hematocrit	**0.52**	**0.51**	0.26	0.20	-	-	-	-
MCV	**0.62**	**0.63**	**0.87**	**0.80**	-	-	0.02	-
MCH	0.32	0.23	**0.91**	**0.69**	0.05	0.06	0.01	-
MCHC	0.06	0.01	0.30	0.40	0.03	0.05	0.07	0.02
Lymphocytes	**0.52**	**0.48**	0.07	0.13	-	-	0.34	0.23
IFN-γ	0.03	0.11	0.22	0.25	0.62	**0.56**	0.60	**0.59**
IL-10	0.27	0.27	0.27	0.29	0.55	**0.55**	0.58	**0.49**
IL-4	0.17	0.21	0.08	0.19	0.57	**0.49**	0.59	**0.45**
IL-6	0.25	0.29	0.25	0.19	0.55	**0.54**	0.58	**0.55**
TNF-α	**0.61**	**0.61**	0.17	0.18	0.20	0.18	0.26	0.18

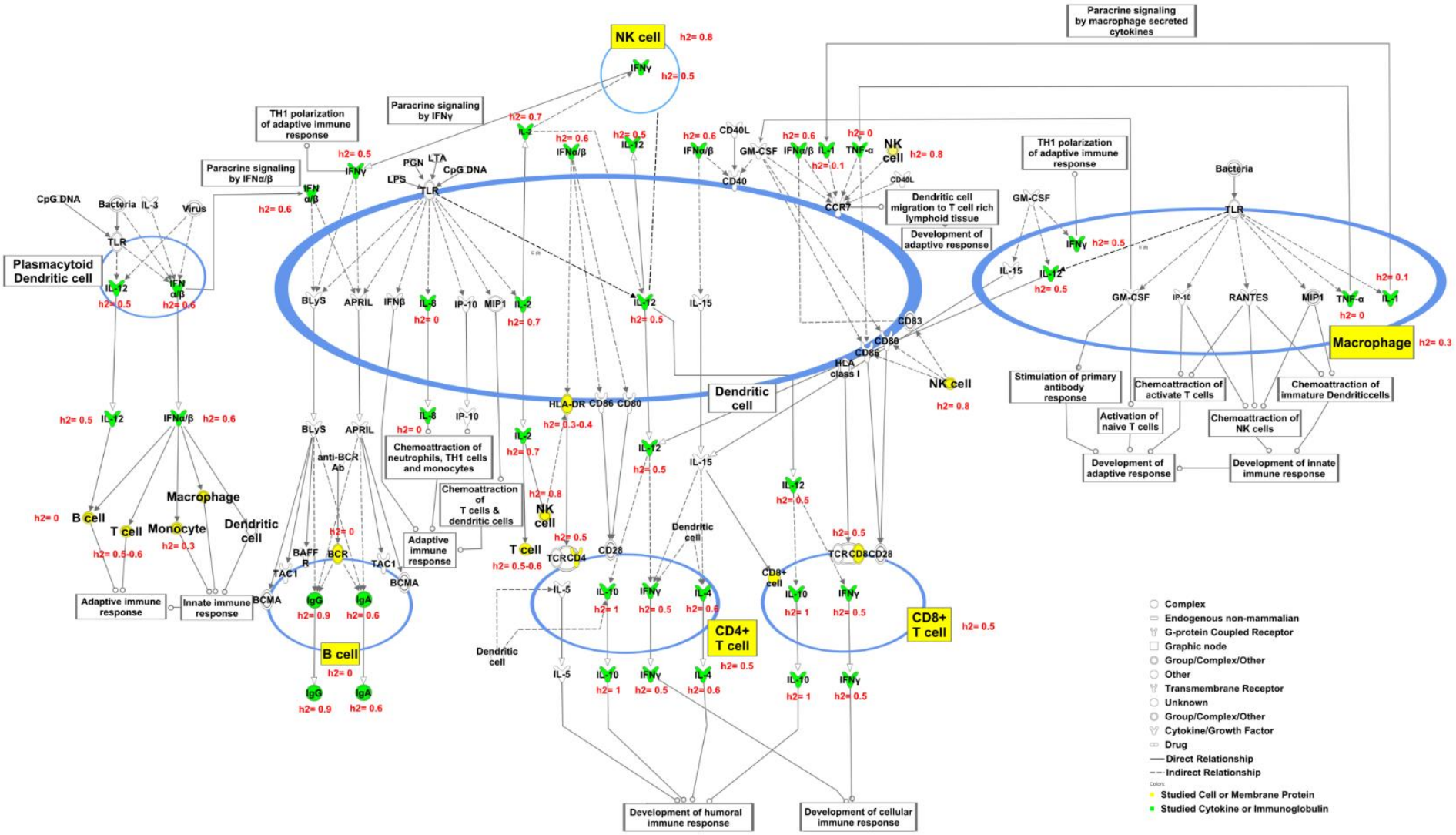
Landrace: n=494, Large White: n=407

MCV=Mean Corpuscular Volume, MCH=mean cellular hemoglobin, MCHC=mean corpuscular hemoglobin concentration, IFN-γ = Interferon-γ, IL= Interleukin, TNFα=Tumor necrosis factor α, SE=Standard error, m2=maternal genetic effect, bold font indicates h^2 or m^2 ≥0.4, standard error reaches from 0.00-0.20.

Heritabilities for Immunity Traits in Pigs

Parameters	Edfors-Lilja et al. (1994)	Henryon et al. (2006)	Clapperton et al. (2008)	Clapperton et al. (2009)	Flori et al. (2011)	Mpetile et al. (2015)	Ponsuksili et al. (2016)		
n	220	4204	500	606	443	518	591	494	407
Breed	Swedish Yorkshire	Duroc, Landrace, Yorkshire	Large White	Large White, Landrace	Large White	Yorkshire	Landrace	Landrace	Large White
Leukocytes	0.44 (0.29)	0.25 (0.05)	0.24 (0.15)	0.28 (0.11)	0.73 (0.20)	0.23 (0.19)	0.23	0.32 (0.11)	0.34 (0.19)
Neutrophils		0.22 (0.04)			0.61 (0.20)	0.31 (0.21)		0.31 (0.11)	0.38 (0.14)
Lymphocytes	0.24 (0.21)	0.24 (0.05)			0.72 (0.21)	0.15 (0.19)	0.49	0.48 (0.13)	0.13 (0.16)
Monocytes		0.22 (0.04)	0.52 (0.17)	0.26 (0.13)	0.38 (0.20)	0.36 (0.20)		0.28 (0.11)	0.06 (0.16)
Eosinophils		0.30 (0.05)			0.80 (0.21)	0.58 (0.12)		0.27 (0.16)	0.15 (0.11)
Basophils						0.12 (0.19)		0.22 (0.11)	0 (0)
Thrombocytes					0.56 (0.19)	0.11 (0.23)	0.39	0.26 (0.16)	0.06 (0.12)
Erythrocytes					0.43 (0.20)	0.62 (0.25)	0.41	0.44 (0.13)	0.41 (0.21)
Haemoglobin						0.56 (0.13)	0.40	0.45 (0.13)	0.33 (0.15)
Hematocrit					0.57 (0.03)	0.06 (0.14)	0.34	0.51 (0.13)	0.20 (0.19)
MCV						0.47 (0.24)	0.69	0.63 (0.12)	0.80 (0.15)
HBE						0.37 (0.24)	0.67	0.23 (0.14)	0.69 (0.20)
MCHC						0.04 (0.16)	0.67	0.01 (0.09)	0.40 (0.21)
IFN-γ					0.00 (0.17)			0.11 (0.14)	0.26 (0.19)
IL-10					0.35 (0.19)			0.27 (0.19)	0.29 (0.21)
IL-12					0.51 (0.20)			0 (0)	0.33 (0.22)
IL-1β					0.12 (0.19)			0.37 (0.21)	0.22 (0.19)
IL-4					0.15 (0.18)			0.22 (0.19)	0.19 (0.20)
IL-6					0.11 (0.19)			0.29 (0.20)	0.19 (0.20)
IL-8					0.00 (0.17)			0.61 (0.20)	0.31 (0.24)
TNF-α					0.00 (0.19)			0.27 (0.19)	0.18 (0.18)
Haptoglobin		0.14 (0.07)		0.20 (0.11)	0.55 (0.21)			0.08 (0.08)	0 (0)

Heritabilities for Immunity Traits in Pigs



Flori et al. (2011)

Genomic Analyses of Immune Traits

Lu et al. (2013):

- Landrace, Yorkshire, Songolia Black (N=562)
- Challenge study → classical swine fever (CSF)
- GWAS: IFN-γ, IL-10, IFN-γ-IL-10 ratio, IgG (CSF)

→ Moderate number of SNPs and QTL associated with immune traits

Zhang et al. (2014):

- Chinese Sutai (N=495)
- GWAS: Leucocytes, erythrocytes and thrombocytes
- QTL found for Leukocytes

Ponsuksili et al. (2016):

- German Landrace (N=591)
- GWAS : Leucocytes, lymphocytes, erythrocytes, haemoglobin, a.o.

→ Moderate number of QTL regions for haematological traits

Innate and adaptive immune response:

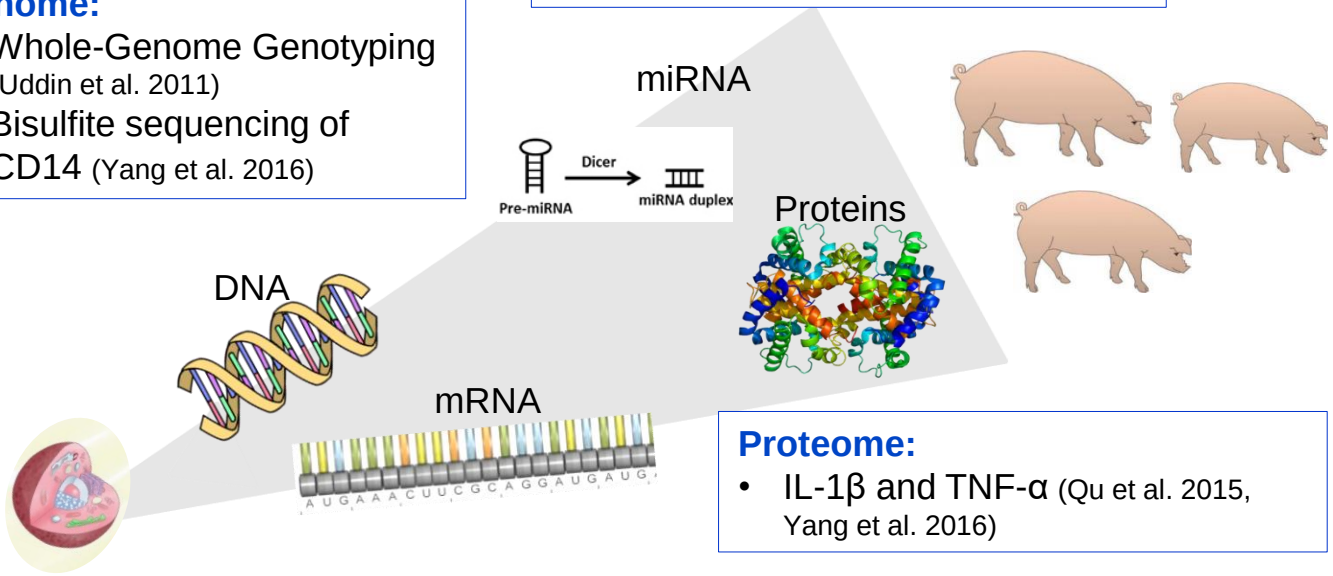
- Porcine reproductive and respiratory syndrome virus (PRRSV)
- Lipopolysaccharide (LPS)

miRNAome:

- Global miRNA profiling of PBMCs (Islam et al. unpublished)

Genome:

- Whole-Genome Genotyping (Uddin et al. 2011)
- Bisulfite sequencing of CD14 (Yang et al. 2016)



Proteome:

- IL-1β and TNF-α (Qu et al. 2015, Yang et al. 2016)

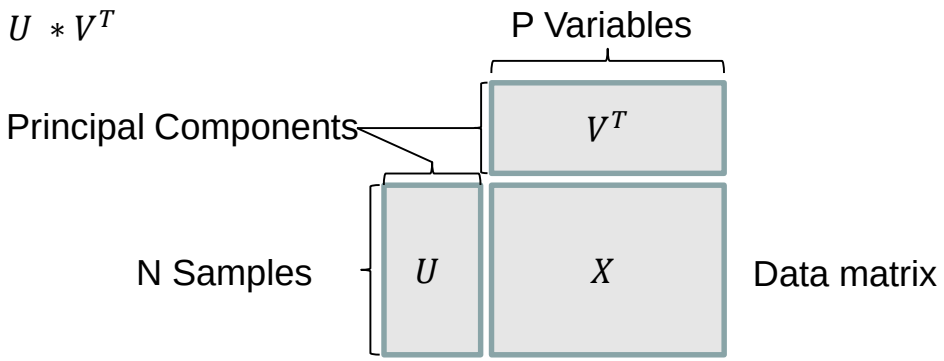
Transcriptome:

- RNA-Seq of lung DCs (Pröll et al. unpublished)
- Microarray analyses of PBMCs (Islam et al. 2017)

Principal Component Analysis - PCA

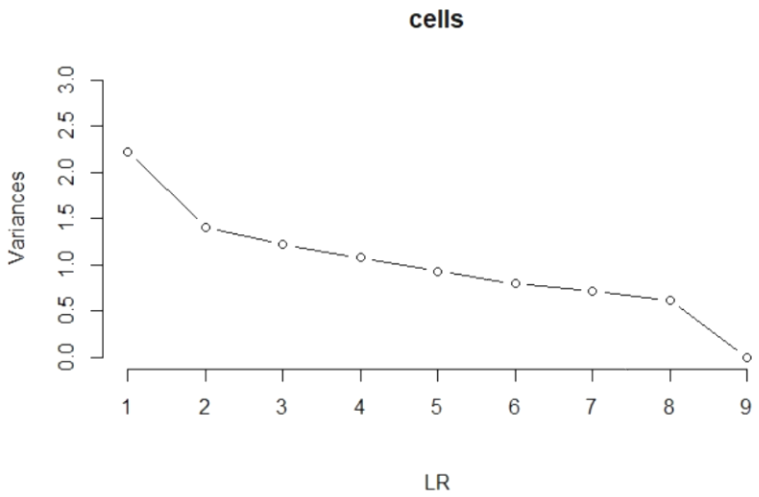
- dimension-reduction tool
- still contains most of the information

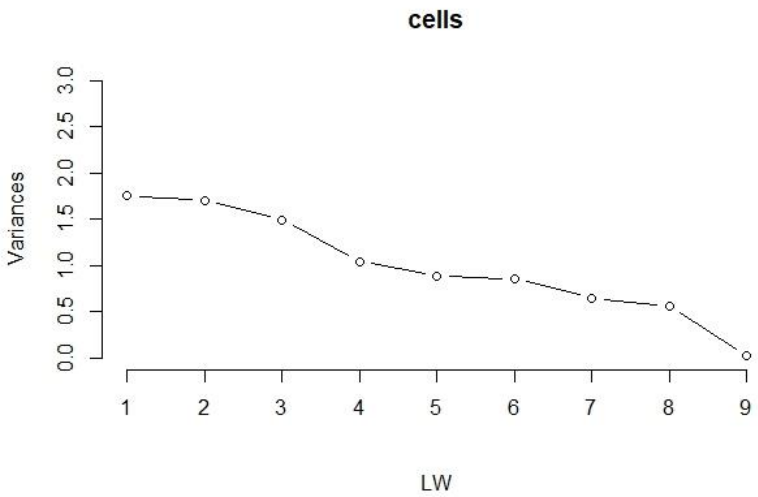
$$X = U * V^T$$



- Loadings and Scores

PCA Screeplot & Variance - Cells



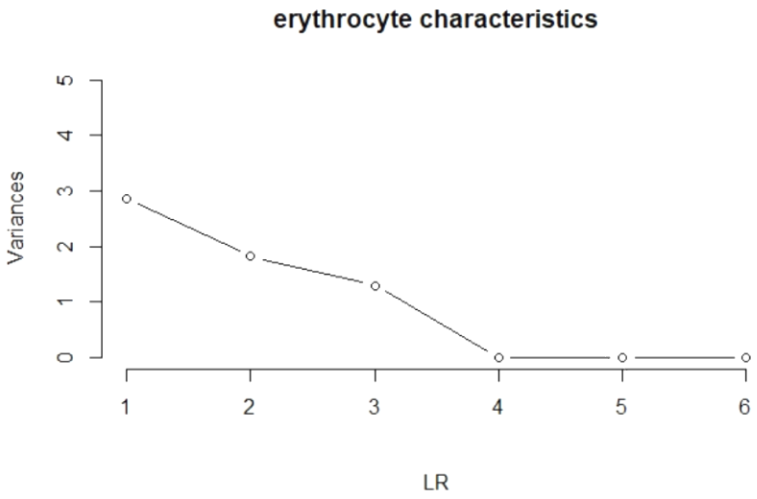


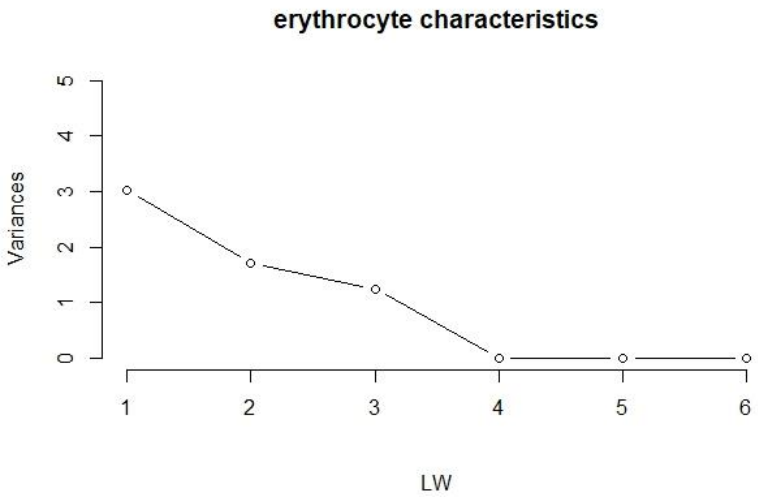
	Line	PC1	PC2	PC3	PC4	PC5
Standard deviation[2]	**LR**	2.18	1.30	1.20	1.02	0.92
	LW	1.76	1.70	1.50	1.05	0.89
Proportion of variance	**LR**	0.27	0.16	0.15	0.12	0.10
	LW	0.20	0.19	0.12	0.10	0.09

PCA Loadings - Cells

Trait	PC1		PC2		PC3		PC4	
	LR	LW	LR	LW	LR	LW	LR	LW
Leukocytes	0.37	-0.24	-0.29	0.08	0.36	**-0.65**	-0.25	0.01
Neutrophils	**0.62**	0.01	0.16	**0.71**	-0.21	-0.04	-0.10	0.18
Band cells	**-0.65**	**-0.65**	-0.28	-0.31	**0.50**	0.18	**0.75**	-0.07
Lymphocytes	0.03	**0.62**	-0.23	-0.30	-0.06	-0.15	-0.25	0.13
Monocytes	**-0.61**	0.27	**0.42**	-0.26	**0.51**	-0.16	-0.21	**0.56**
Eosinophils	-0.13	0.25	**0.60**	0.36	-0.25	0.37	**0.41**	**-0.41**
Basophils	-0.01	0.02	**0.46**	-0.08	0.39	**-0.48**	-0.10	**-0.65**
Haptoglobin	-0.17	-0.03	0.05	0.32	0.30	-0.37	-0.25	0.17

PCA Screeplot & Variance – Erythrocytes



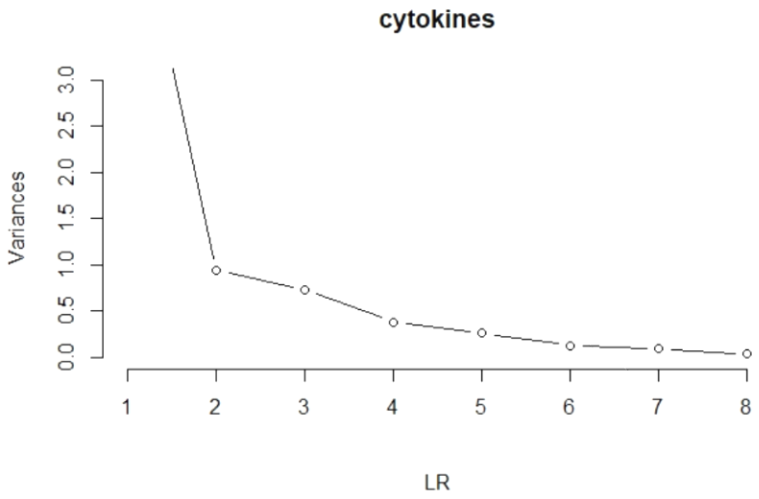


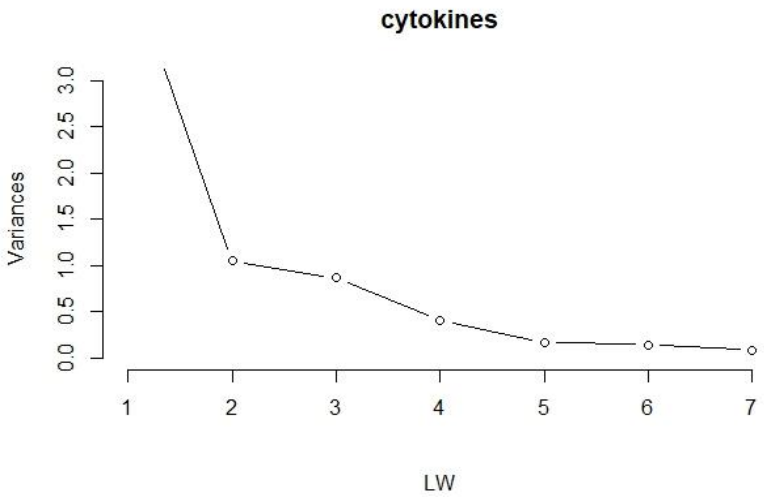
	Line	PC1	PC2	PC3	PC4
Standard deviation²	**LR**	2.87	1.82	1.30	0.01
	LW	3.02	1.72	1.25	0.00
Proportion of variance	**LR**	0.48	0.30	0.22	0.00
	LW	0.50	0.29	0.21	0.00

PCA Loadings – Erythrocytes

Trait	PC1		PC2		PC3		PC4	
	LR	LW	LR	LW	LR	LW	LR	LW
Erythrocytes	**-0.47**	**-0.47**	0.39	**-0.41**	-0.24	0.02	0.02	-0.12
Hemoglobin	**-0.56**	**-0.56**	0.01	-0.11	-0.21	-0.06	-0.08	0.00
Hematocrit	**-0.56**	**-0.54**	0.18	-0.12	0.08	0.18	-0.05	-0.08
MCV	-0.28	-0.23	-0.36	**0.61**	**0.63**	0.38	-0.15	0.09
MCH	-0.24	-0.30	**-0.67**	**0.60**	0.00	-0.20	-0.18	0.23
MCHC	0.01	-0.11	**-0.44**	0.03	**-0.70**	-0.85	-0.06	0.21
Thrombocytes	0.15	0.02	0.20	-0.28	-0.04	0.22	**-0.97**	**0.93**

PCA Screeplot & Variance – Cytokines



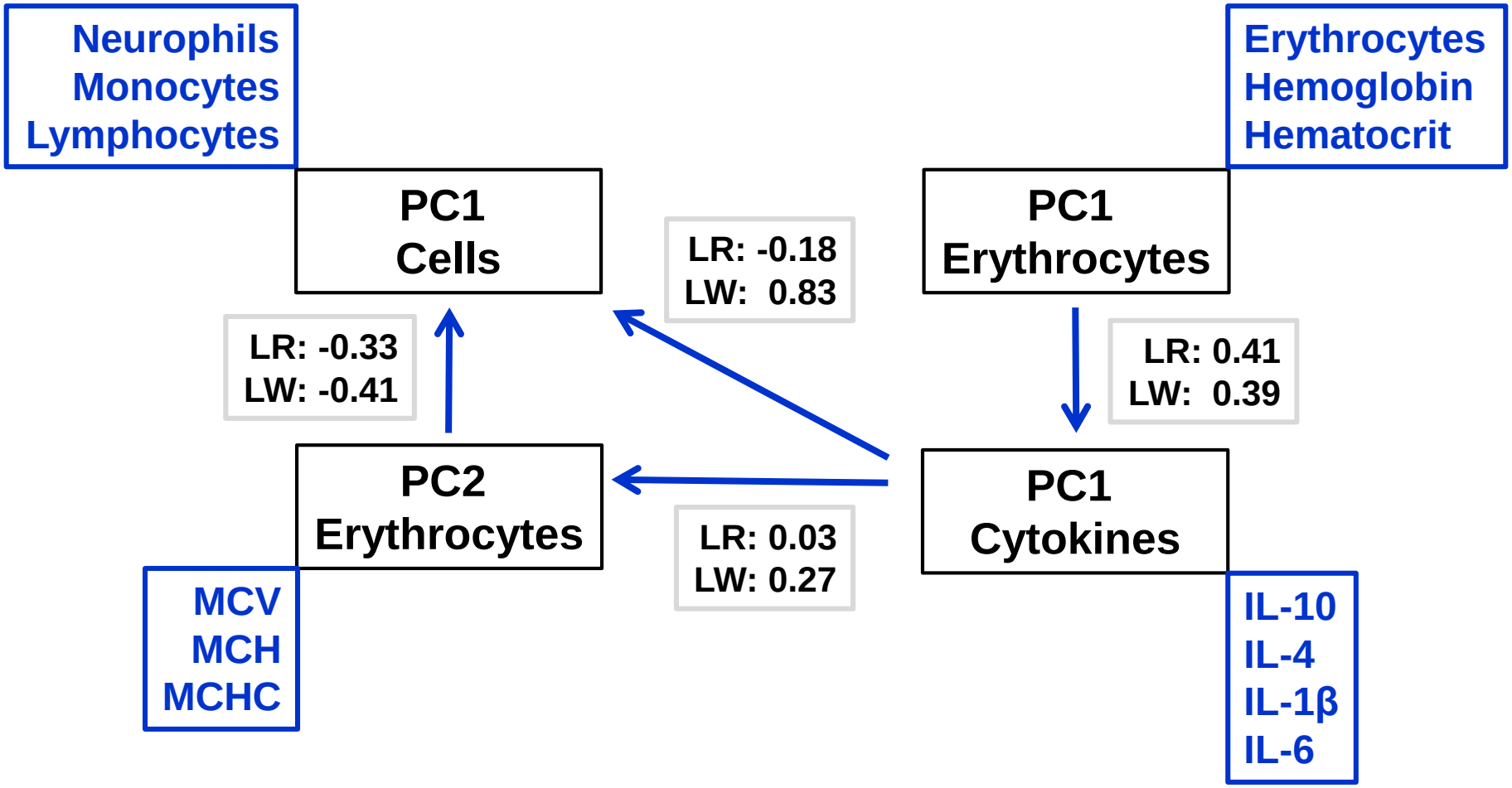


	Line	PC1	PC2	PC3
Standard deviation[2]	**LR**	5.42	0.94	0.73
	LW	4.75	1.05	0.87
Proportion of variance	**LR**	0.68	0.12	0.09
	LW	0.60	0.13	0.11

PCA Loadings – Cytokines

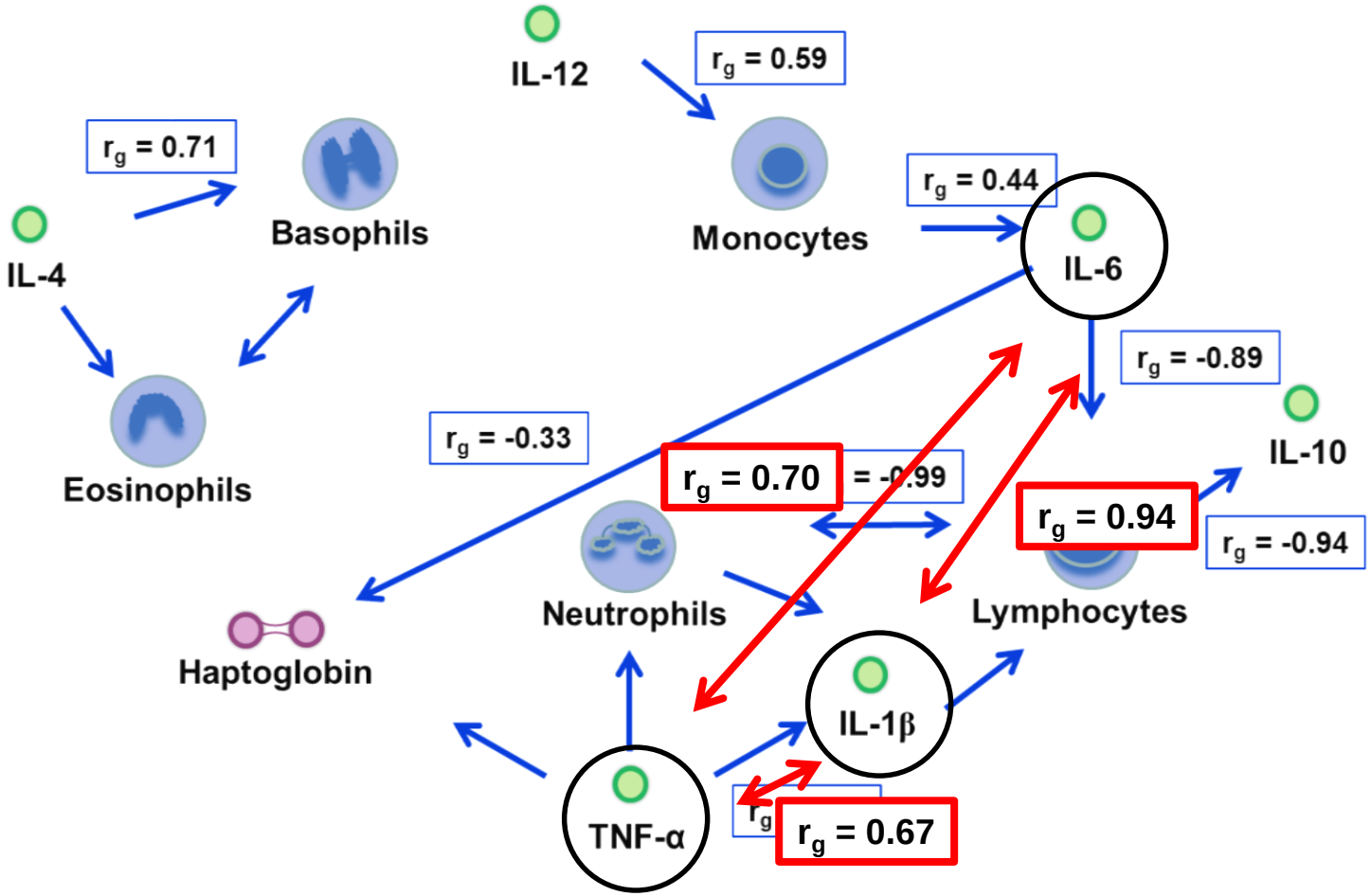
Trait	PC1		PC2	
	LR	LW	LR	LW
IFNγ	-0.35	-0.33	-0.16	-0.22
IL10	**-0.42**	**-0.42**	-0.11	0.08
IL12	-0.26	-0.32	0.27	0.07
IL1β	-0.39	**-0.41**	0.04	0.07
IL4	**-0.41**	**-0.41**	-0.11	0.09
IL6	**-0.41**	**-0.41**	-0.14	0.10
IL8	-0.15	-0.01	**0.92**	**-0.92**
TNFα	-0.35	-0.30	-0.07	-0.27

Interactions of Immune Parameters



PC=Principal Component, MCV=Mean Corpuscular Volume, MCH=Mean Cellular Hemoglobin, MCHC=Mean Corpuscular Hemoglobin Concentration, IL= Interleukin

Results – Genetic Correlations r_g



Landrace: n=494, Large White: n=407

Modified according to: Schindler et al. (1990); Seutter (1995); Thorn (2000); Tizard (2013)