CiBreed
Center for Integrated Breeding Research

Göttingen Minipigs Genetics: Breed history, breeding strategies and genome analyses

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Contents

- History
- Breeding Program of the GMP
- World wide breeding structures
- Population Genetics
- Next Generation Sequencing (NGS)
- Assessing breed integrity of Göttingen Minipigs

History

Brief overview

The GMP today

First years

1960 bis 1969

Growing demand for animal model species

- Physiologically close to humans
- small
- But sufficiently large for surgeries
- uniform
- Moderate temperament
- Easy to house and handle

Prof. Fritz Haring
(1907 – 1990)

First years

1960: Import of first animals

- 3♂ und 2♀ Minnesota Minipigs (Hormel Institute, Austin, USA)
- 3♂ und 4♀ Vietnamese potbellied pigs (Wilhelma Zoo, Stuttgart)
  → heterogeneous coat colours

1965: Import of additional animals

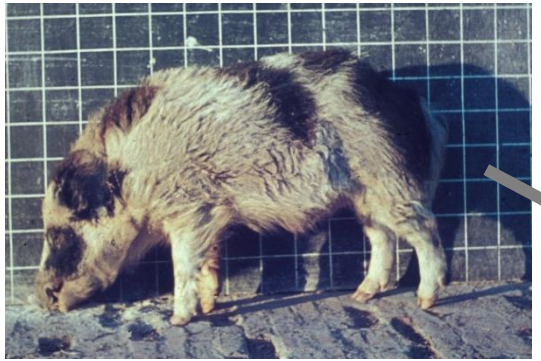
- 4♀ Vietnamese potbellied pigs (Zoo Friedrichsfelde, East Berlin)
  → reduced size and white coat colour

1965 – 1969: Development of pure white-coated lines

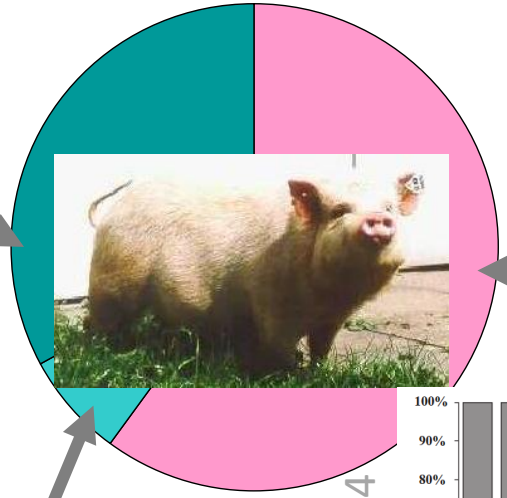
- Artificial insemination with landrace
  → dominant white
  → lean
  → BUT larger

At Friedland

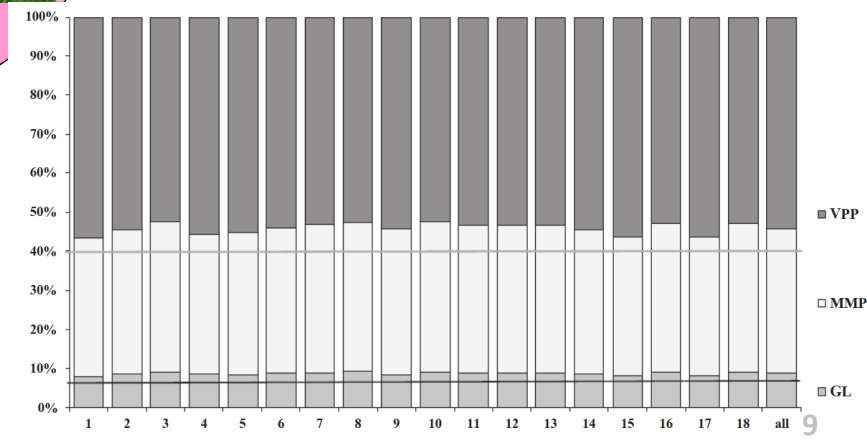
Minnesota Minipig (33%)



Vietnamese Potbellied pig (60%)



German Landrace (7%)



Gaerke et al. 2014

At Friedland





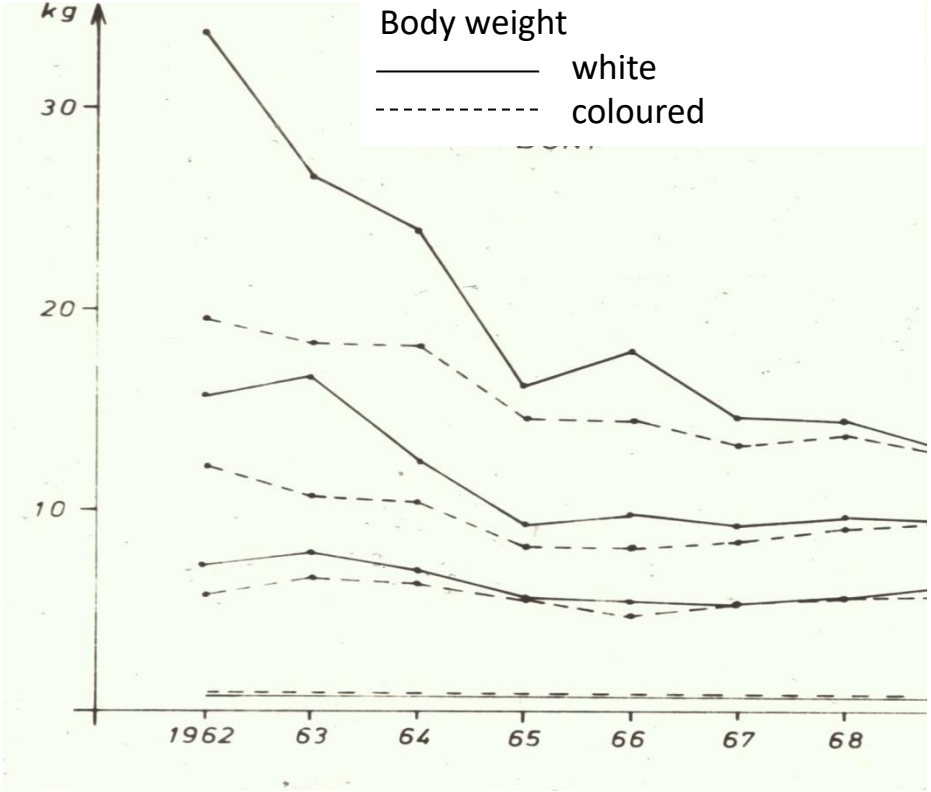

F_1-sow (Landrasse x Vietnamese) x Minnesota boar

At Friedland

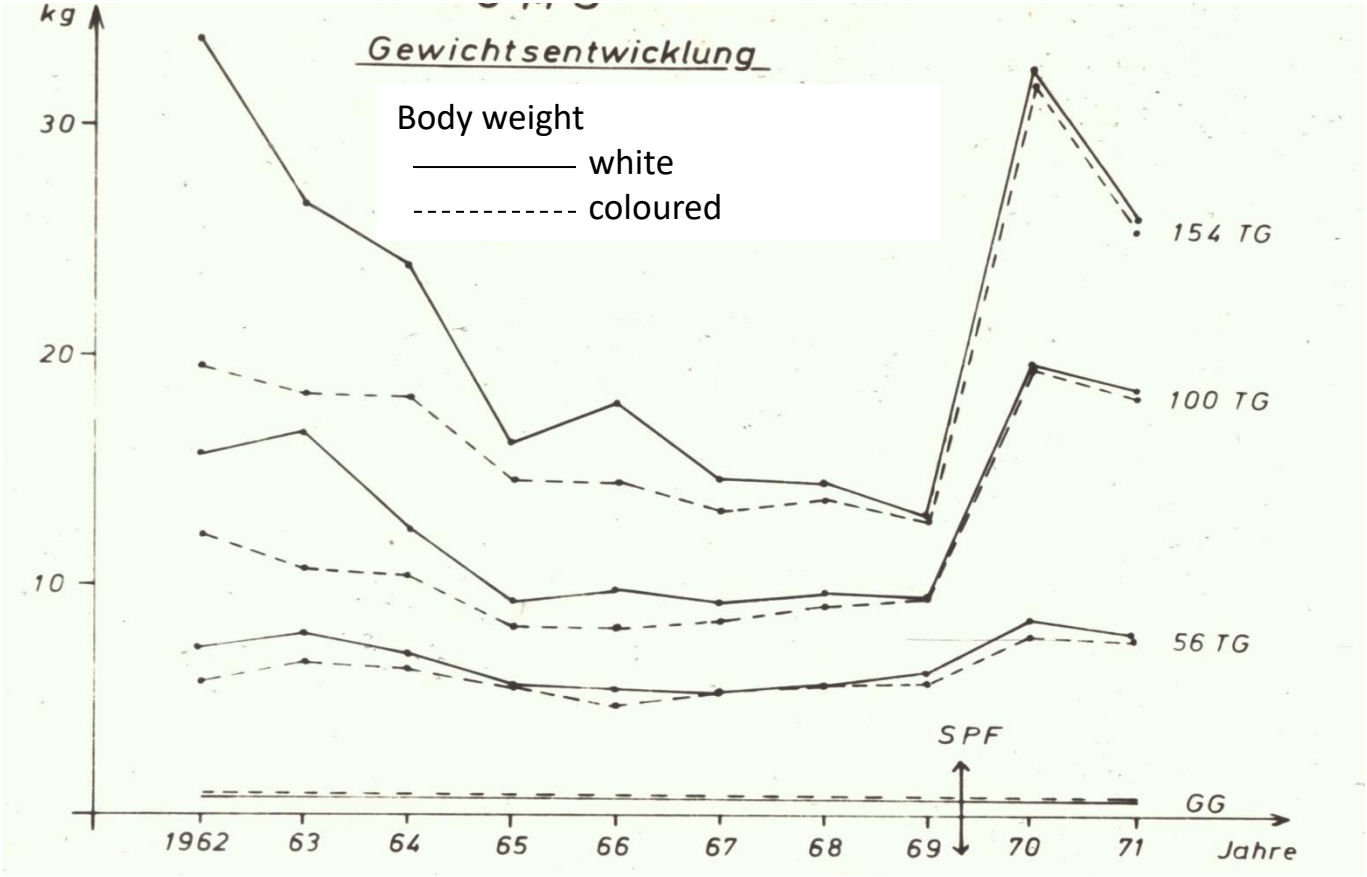
Low-input farming at Friedland let to

- high losses
- growth reduction due to hygienic problems
- lack of recording of phenotypes (5-month weight)

From Friedland to Relliehausen

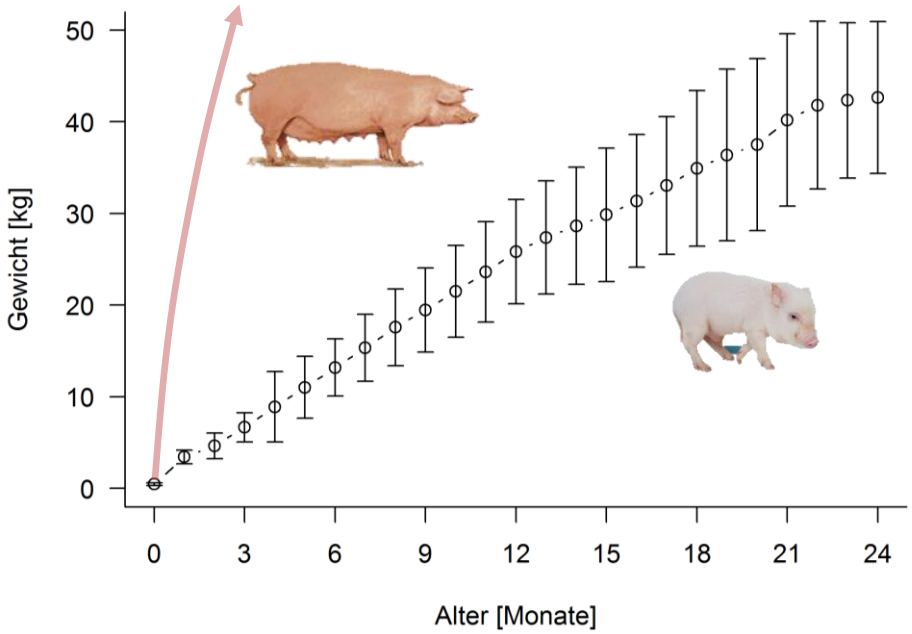


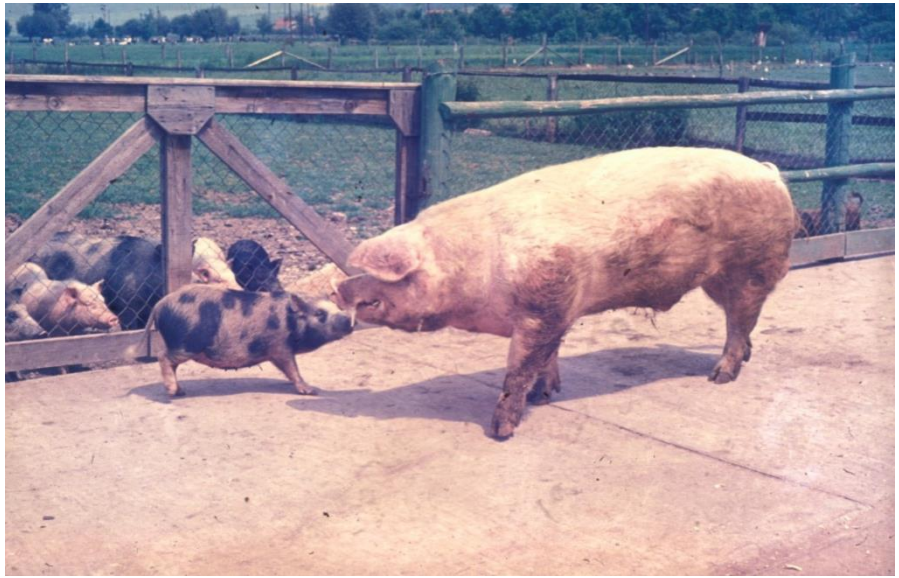
From Friedland to Relliehausen



Breeding Program of the GMP

Growth of the GMP





Breeding scheme

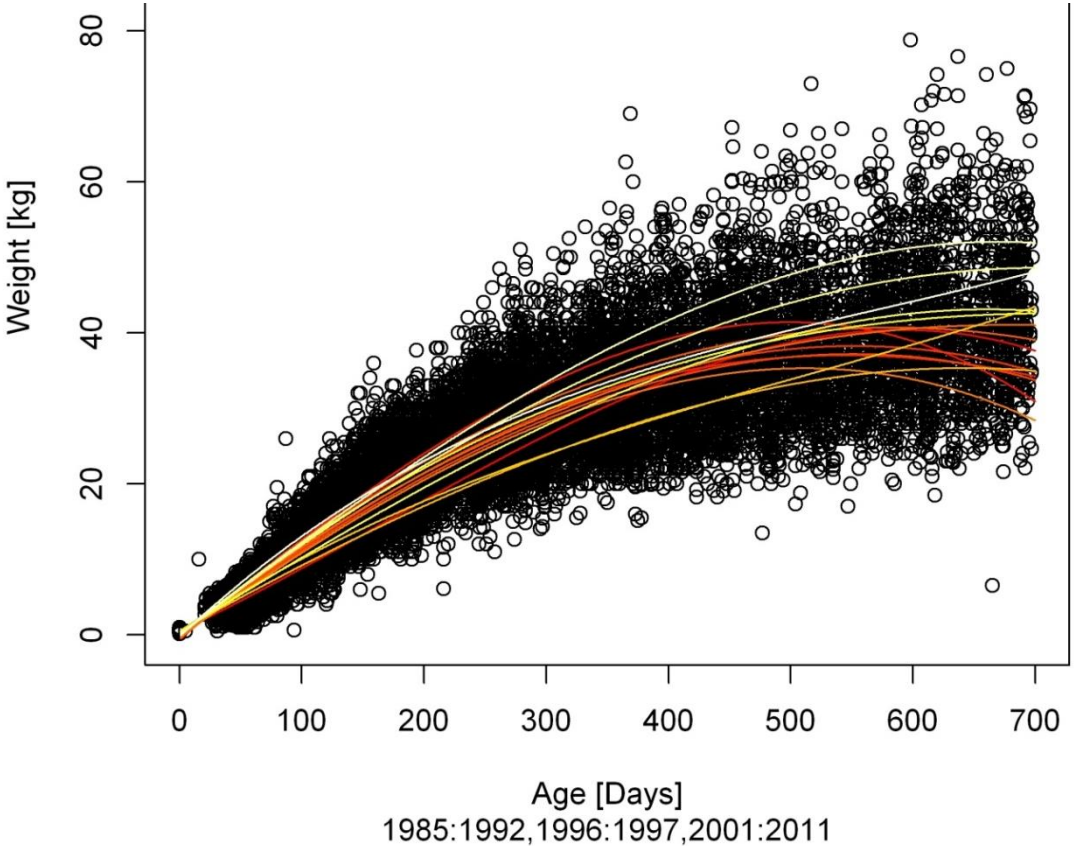
- Breeding goals
  - Weight
    - Relative Weight Reduction
  - Reproduction
    - Piglets born alive

Breeding scheme

- Pedigree based breeding value estimation
- postgreSQL database
- ongoing phenotyping
  - Weight
  - Piglets born alive and weaned
  - No. Of teats
  - Anomalies
- Optimum Genetic Contribution

Relative weight reduction

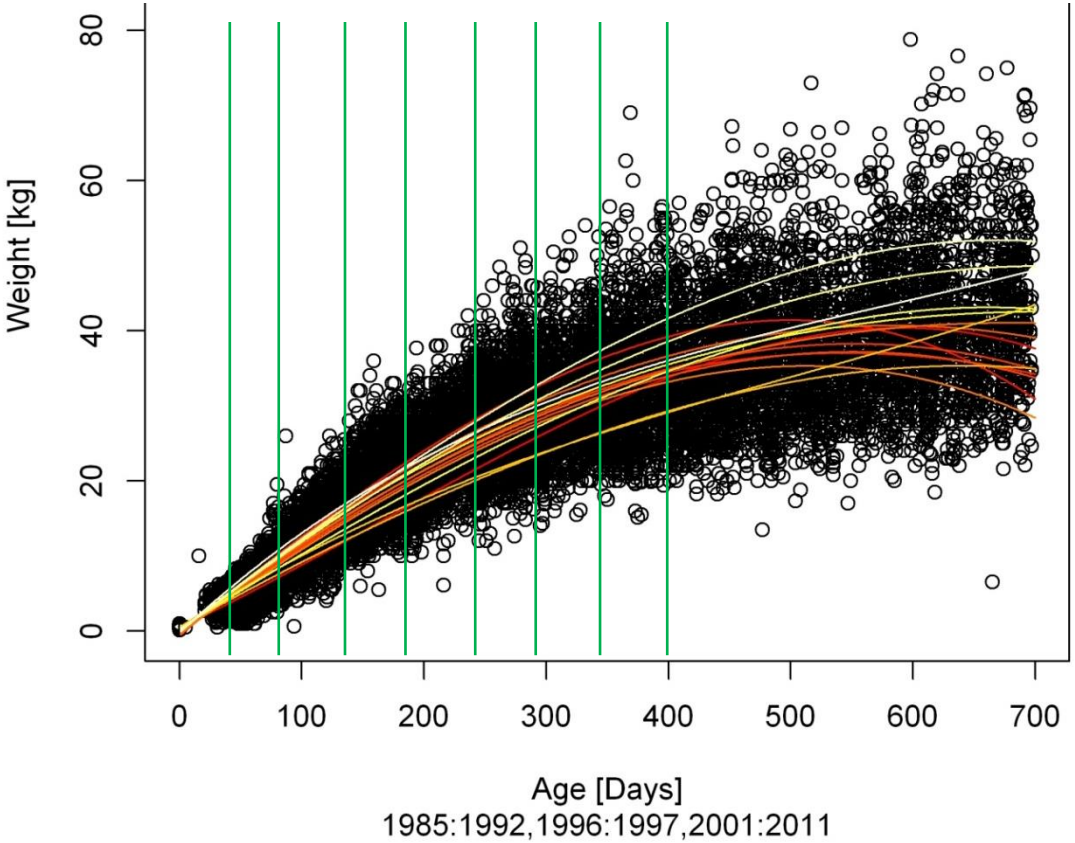
1. step: estimation of a growth curve



$$\text{weight} = \text{age} + \text{age}^2 + \text{age}^3 + \text{age}^4$$

Relative weight reduction

1. Comparison of 8 timepoints



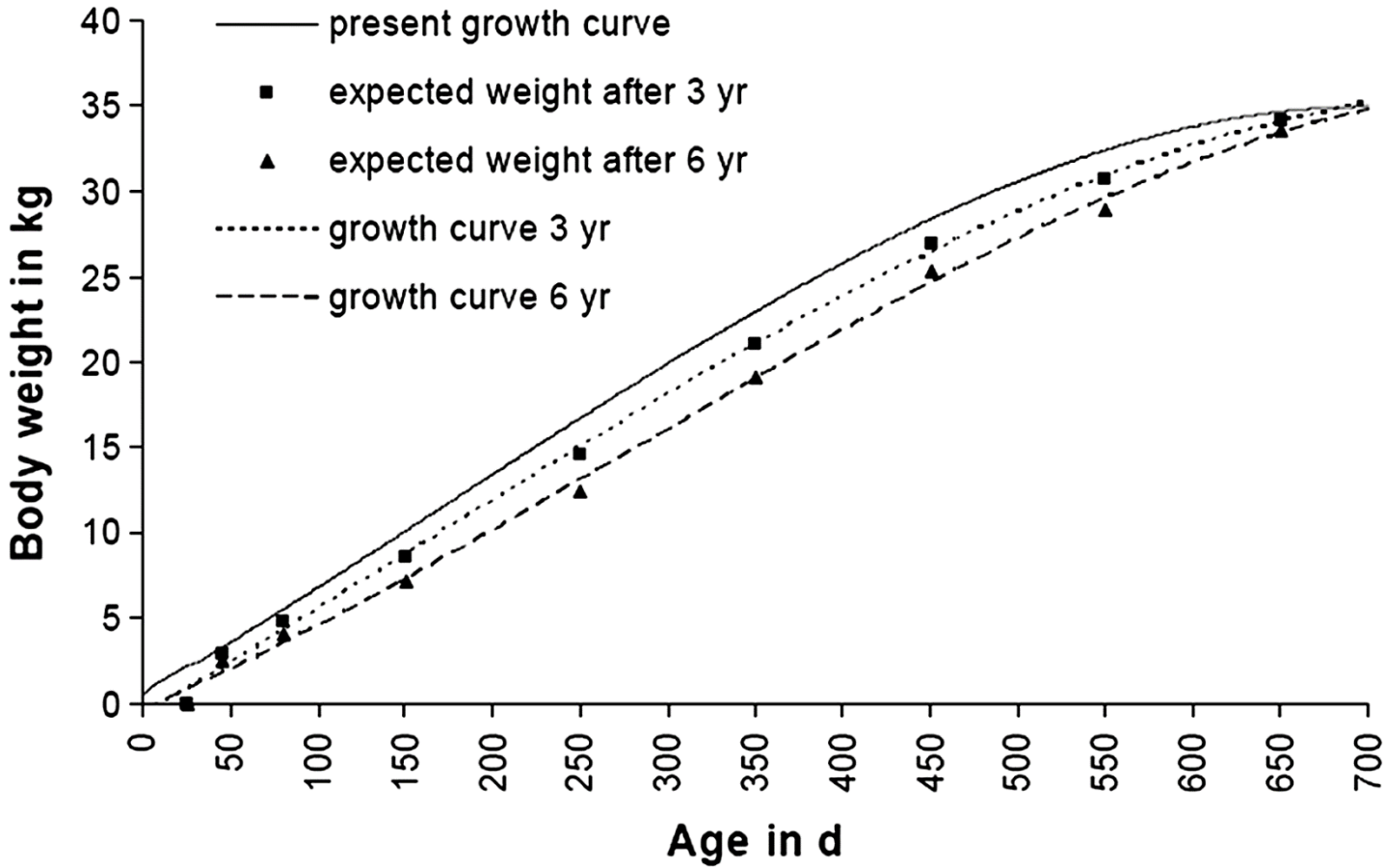
$$\Delta = E(gew) - gew$$

Relative weight reduction

- weighting of the differences to an index breeding value

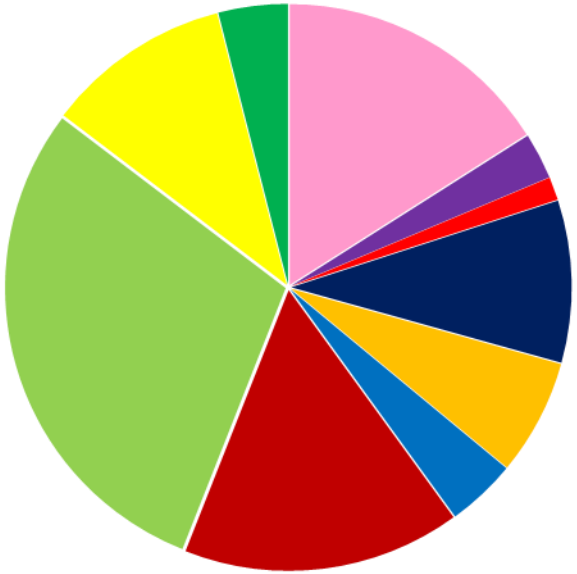
	weighting factor	age class [days]
rwr1	0.00	less than 61
rwr2	0.31	61 – 100
rwr3	3.09	101 – 150
rwr4	2.62	151 – 201
rwr5	1.12	201 – 250
rwr6	0.73	251 – 300
rwr7	0.31	301 – 350
rwr8	0.20	351 – 400

Relative weight reduction



Optimum genetic contribution

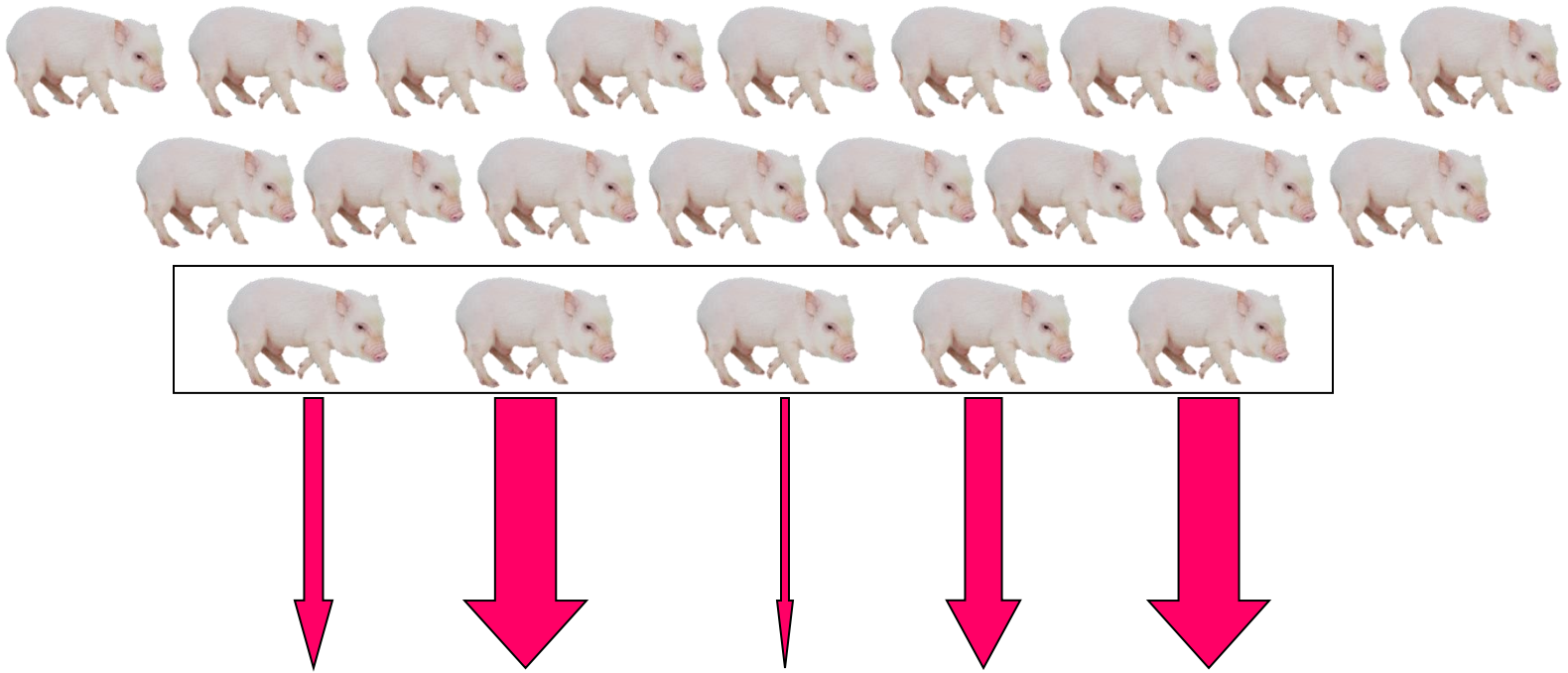
10 Founder



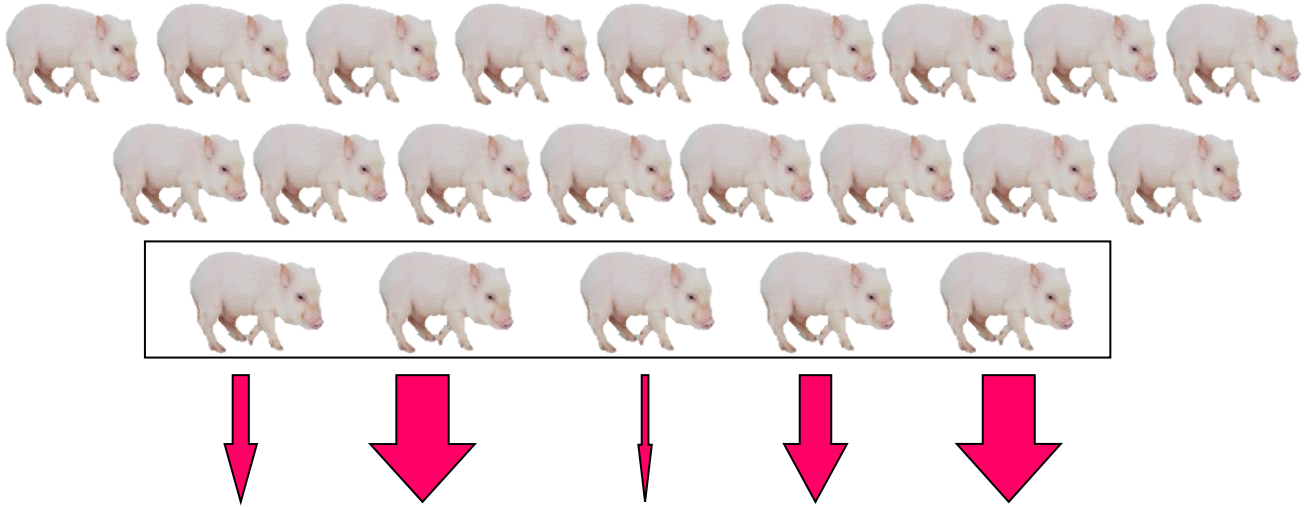
Optimal contribution (minimum inbreeeding)

real contributions (more than unavoidable inbreeding)

Optimum genetic contribution



Optimum genetic contribution

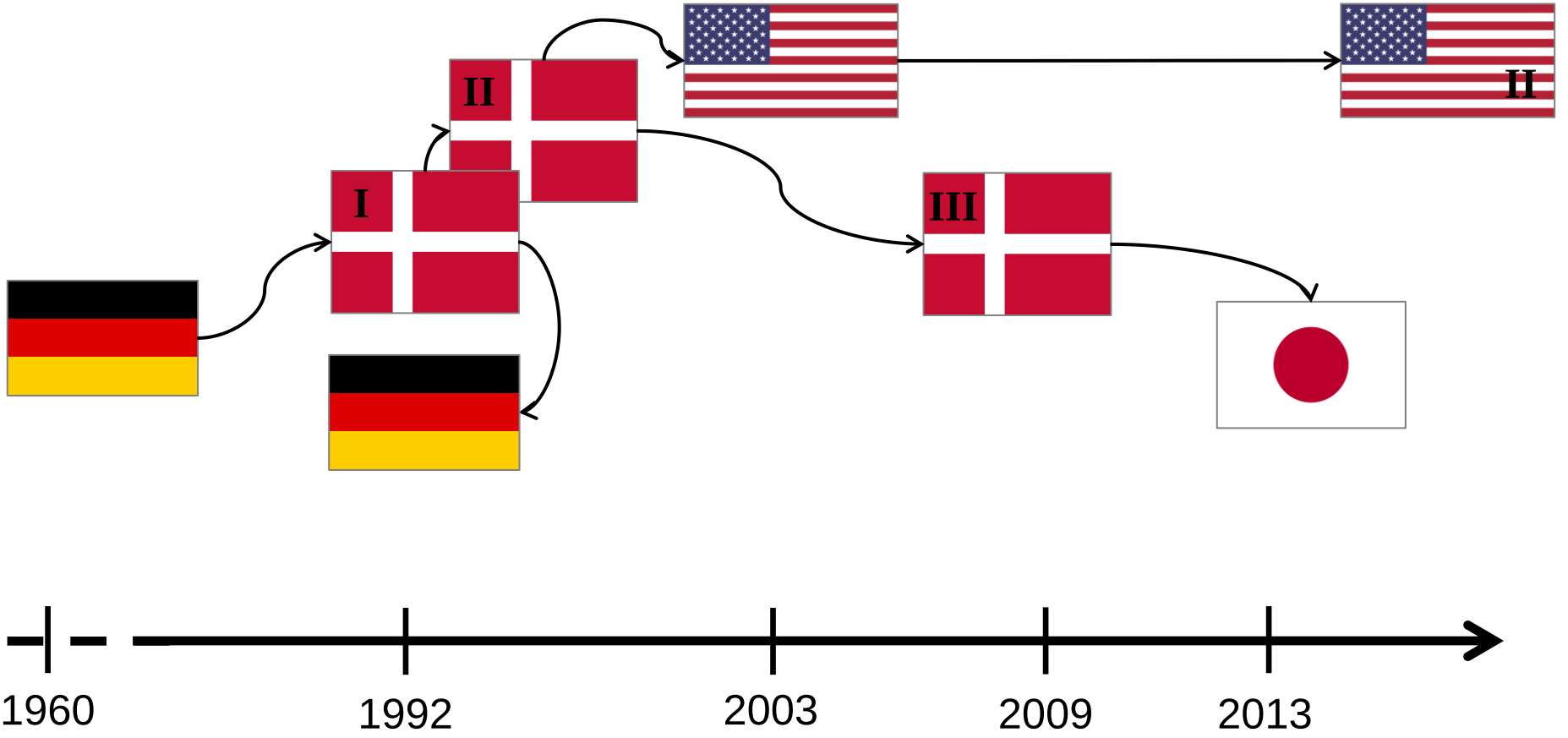


Current Population

Optimum Contributions

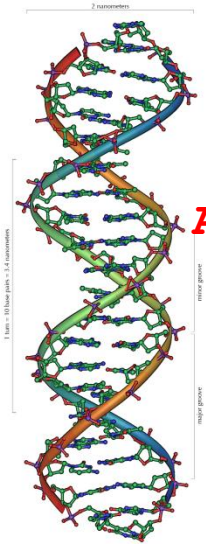
World wide breeding structures

History of colonies

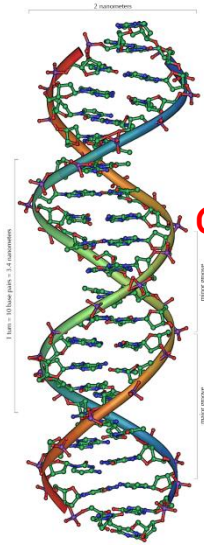


Population Genetics

Allele (Gene) frequency



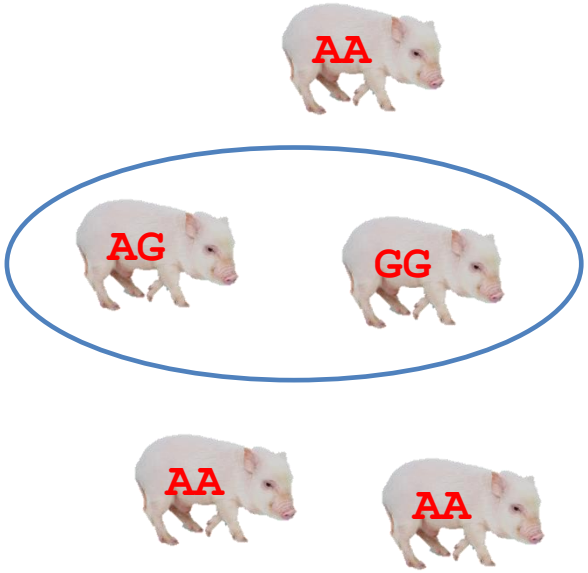
A Major allele in GMP



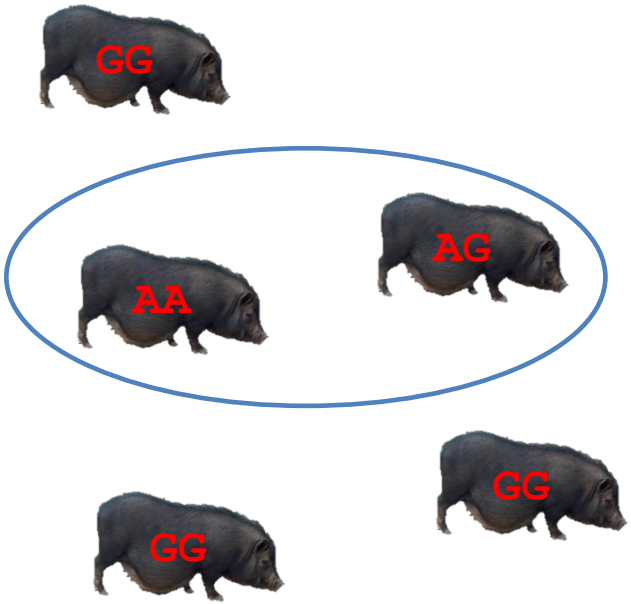
G Major allele in VPP

SNP [`Snipp´] = Single nucleotide polymorphism

Allele (Gene) frequency



$$p(A) = \frac{7}{10} = 0.7$$

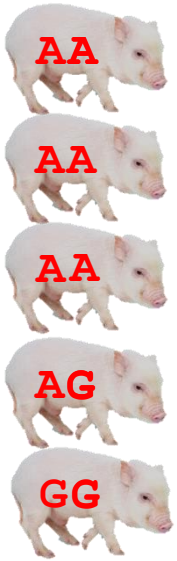


$$p(A) = \frac{3}{10} = 0.3$$

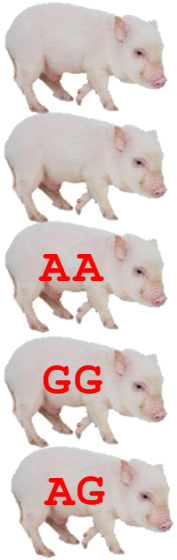
Allele frequency changes

- Genetic Drift — **Random transition of alleles to the next generation**
- Selection — **„Fitness“ of an allele determines probability of transition**
- Mutation — **Ancestral alleles mutate into alternative state with low probability**
- Migration — **Allele exchange by introduction of new animals into population**

Drift



Drift is a process of random sampling with a binomial probability (in biallelic loci)



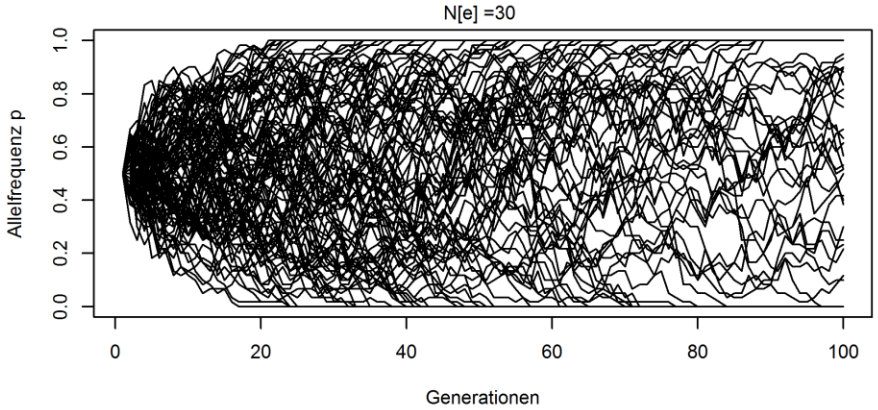
Generation t

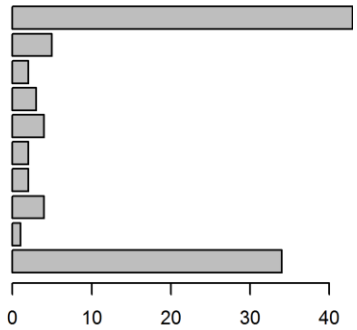
$$p(A) = \frac{7}{10} = 0.7$$

Generation $t + 1$

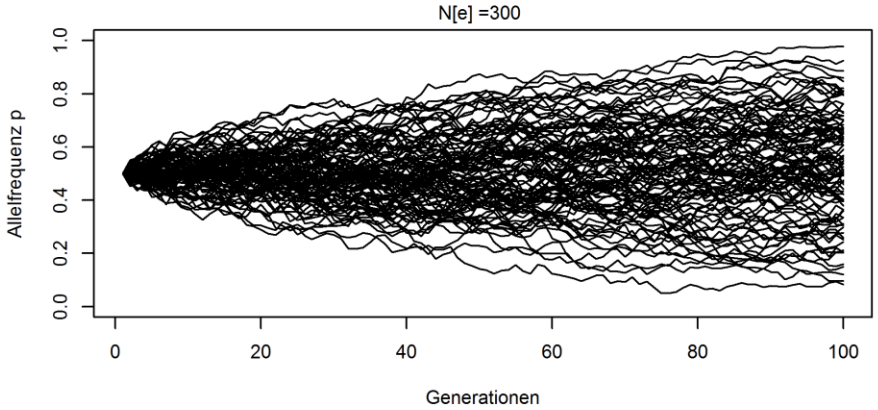
$$p(A) = \frac{4}{10} = 0.4$$

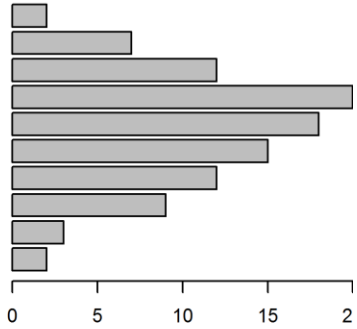
Drift





$$p(A) = 0.5$$
$$Ne = 30$$





$$p(A) = 0.5$$
$$Ne = 300$$

Selection

1

$1 - \frac{1}{2}S$

$1 - S$

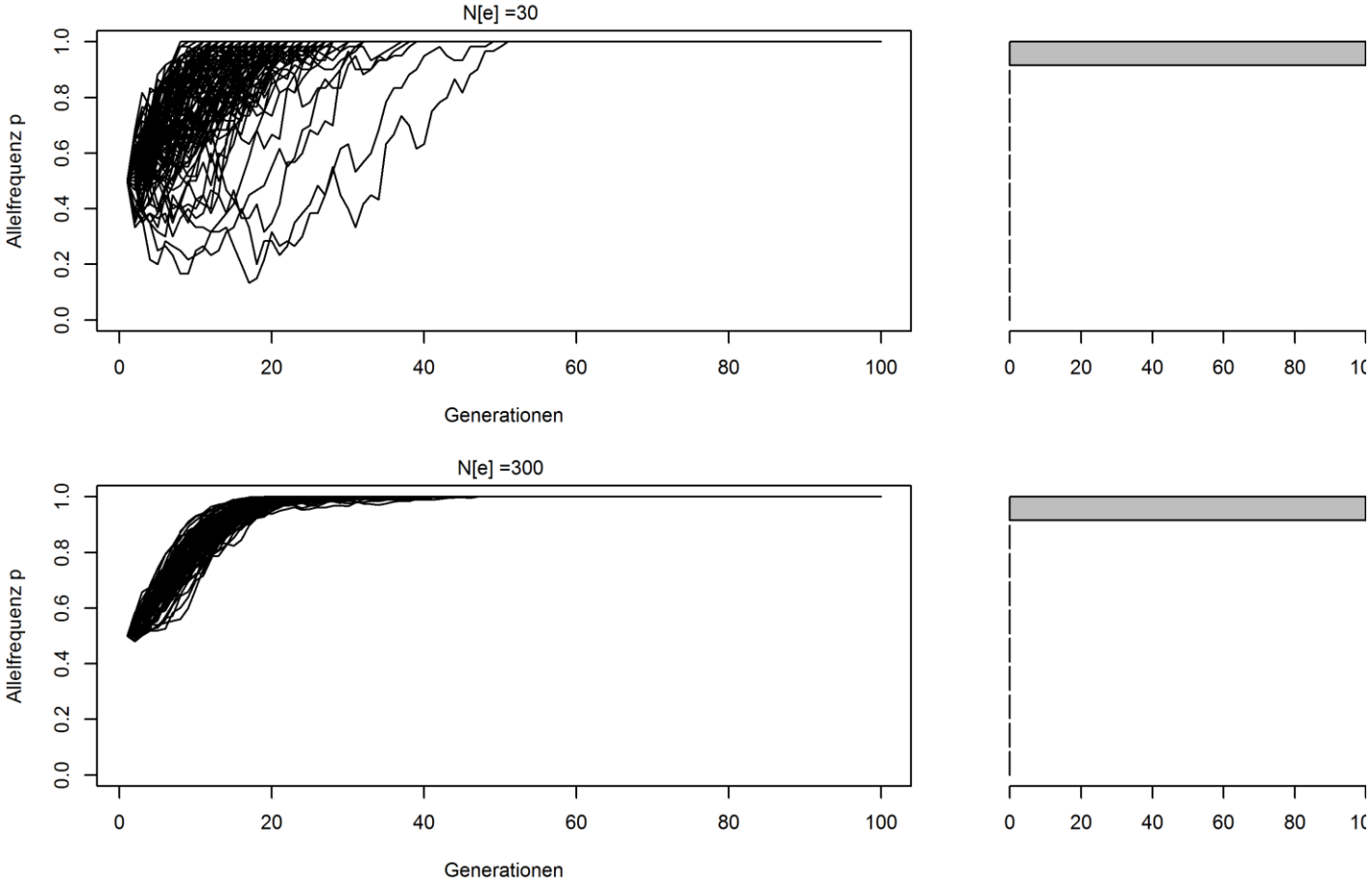
$$p_{A1} = \frac{11}{18} = 0.611$$

$$Selection\ coefficient$$
$$S = 0.5$$

$$p_{A0} = 0.5$$

Gametic contribution
(without dominance)

Selection

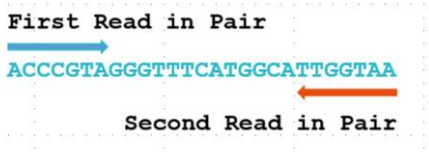

N[e] =30
Allelfrequenz p
0.0
0.2
0.4
0.6
0.8
1.0
0
20
40
60
80
100
Generationen
N[e] =300
Allelfrequenz p
0.0
0.2
0.4
0.6
0.8
1.0
0
20
40
60
80
100
Generationen

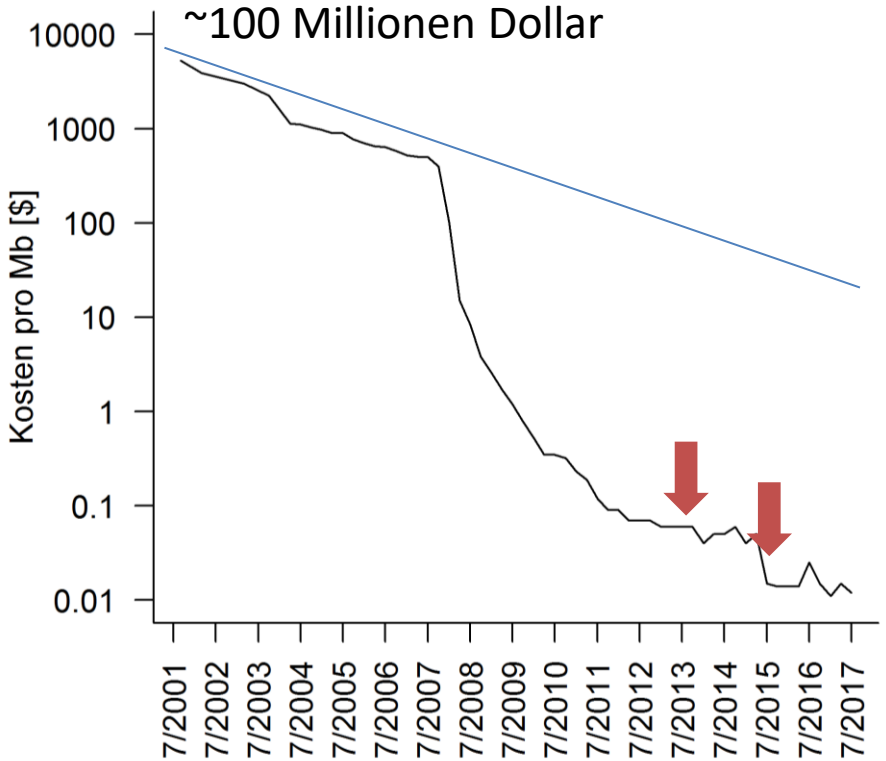
Next Generation Sequencing (NGS)

Whole-genome Shot-Gun Sequencing (WGS)

Next-Generation-Sequencing

- Whole genome represented
- Marker density to a multiple higher than für arrays
- Causal varition (theoretically) contained
- No array ascertainment bias
- Discovery of unknown variation





Reference genome

http://www.nature.com

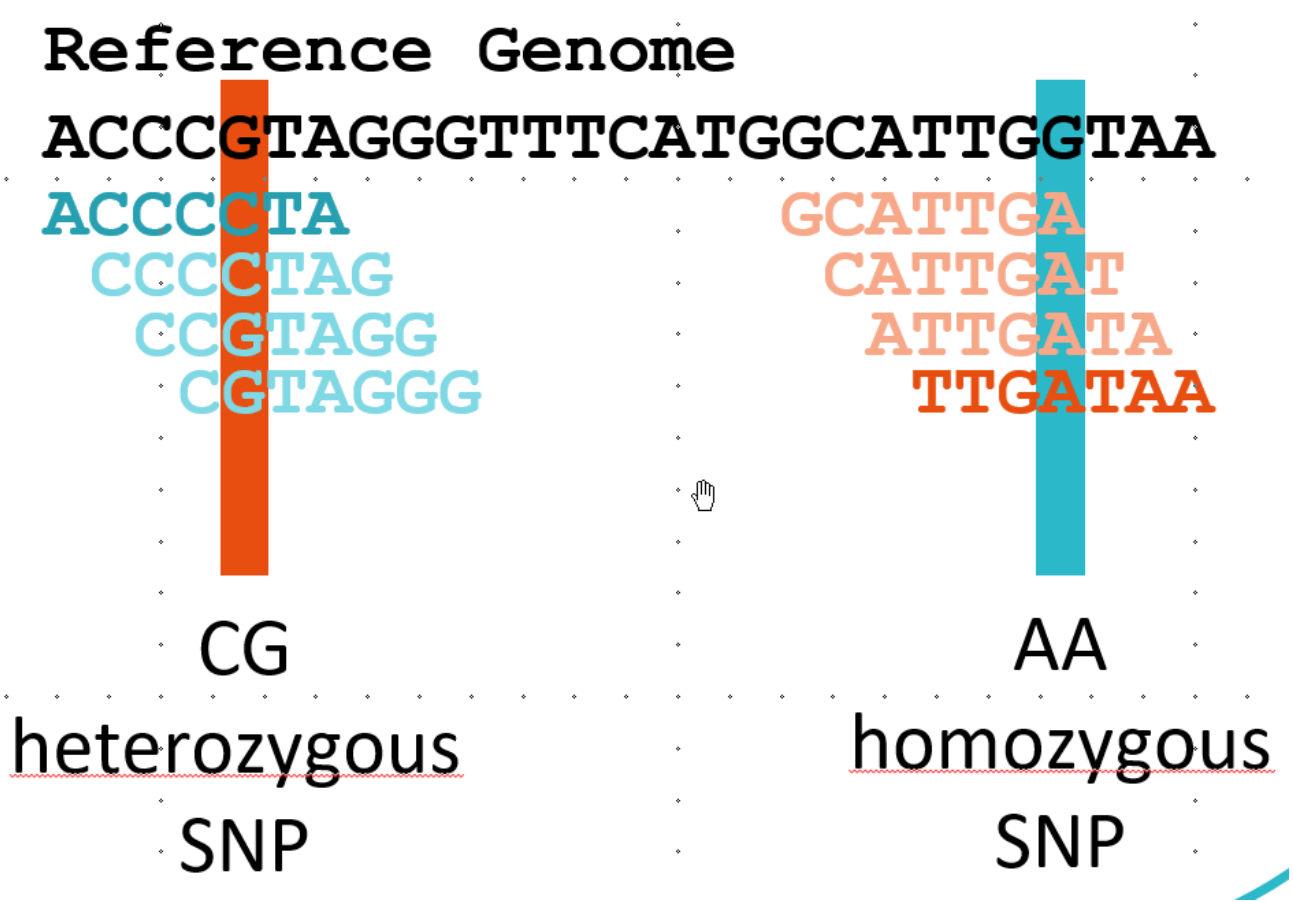
1. Burrows-Wheeler-Alignment (BWA, Li und Durbin, 2009)

Reference genome

1. Sorting by chromosomal postion with Samtools (Li et al., 2009)
2. Marking duplicates with Picard (Picard, 2009)
3. Indexing

➔ **SAM/ BAM (Sequence Alignement/ Map - Format)**

➔ **BAI Bam-Index**


Reference Genome
ACCCGTAGGGTTTCATGGCATTGGTAA
ACCCCTA
CCCCTAG
CCGTAGG
CGTAGGG
GCATTGA
CATTGAT
ATTGATA
TTGATAA
CG
AA
heterozygous
SNP
homozygous
SNP

Integrity

Reimer *et al. BMC Genomics* (2020) 21:308
https://doi.org/10.1186/s12864-020-6590-4

BMC Genomics

RESEARCH ARTICLE Open Access

Assessing breed integrity of Göttingen Minipigs

Christian Reimer[1,2*], Ngoc-Thuy Ha[1,2], Ahmad Reza Sharifi[1,2], Johannes Geibel[1,2], Lars Friis Mikkelsen[3], Martin Schlather[2,4], Steffen Weigend[2,5] and Henner Simianer[1,2]

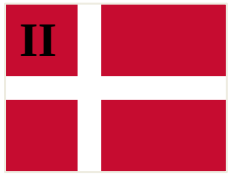
Aim

- GMP colonies are genetically isolated, up to ~30 years
- Are they drifting apart from each other?
- Does breeding ensure selection for the same traits in all colonies?
- How much unique diversity is contained in each colony?
  - Risk of loosing a colony due to pests/ inbreeding/ fire?

Materials & Methods - Sampling

DA2 1

DA2 2



NR 1

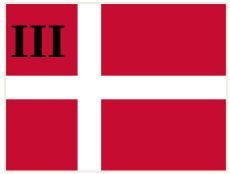
NR 2

NI 1

NI 2

RE 1

RE 2

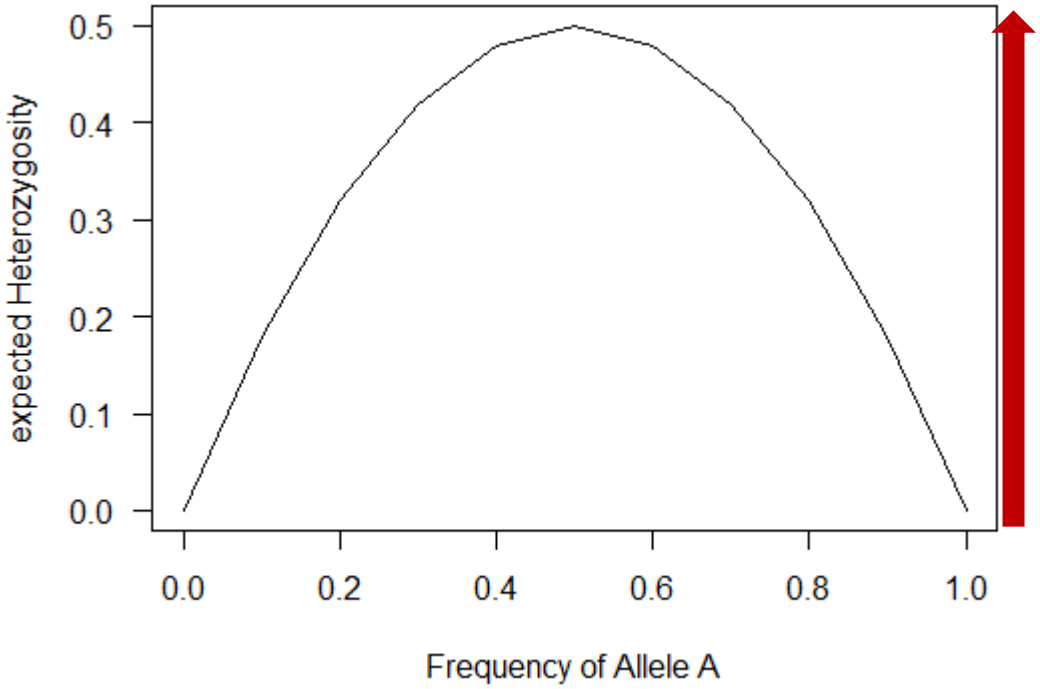


DA3 1

DA3 2

Expected Heterozygosity

- A measure of genetic diversity

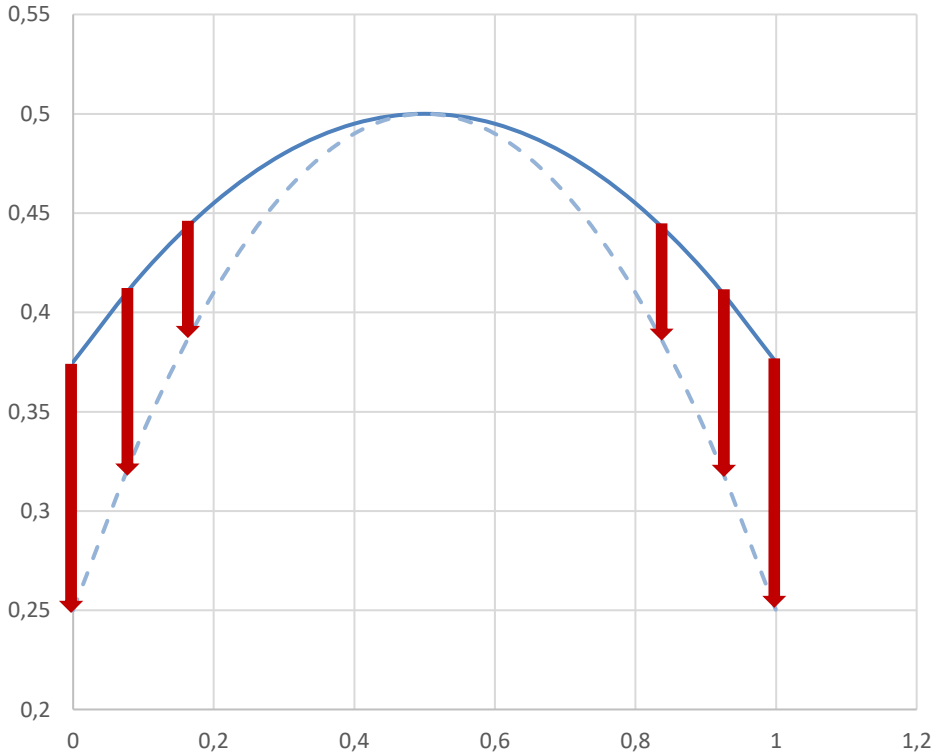


	$p(A)$	$p(a)$
$p(A)$	$p(A)^2$	$p(A)*p(a)$
$p(a)$	$p(A)*p(a)$	$p(a)^2$

Diversity

$$expHet = 2 * p(A) * (1 - p(A))$$

Wahlund Effect



$p(A)_{pop1} = 0.5$

$p(A)_{pop2} = x$

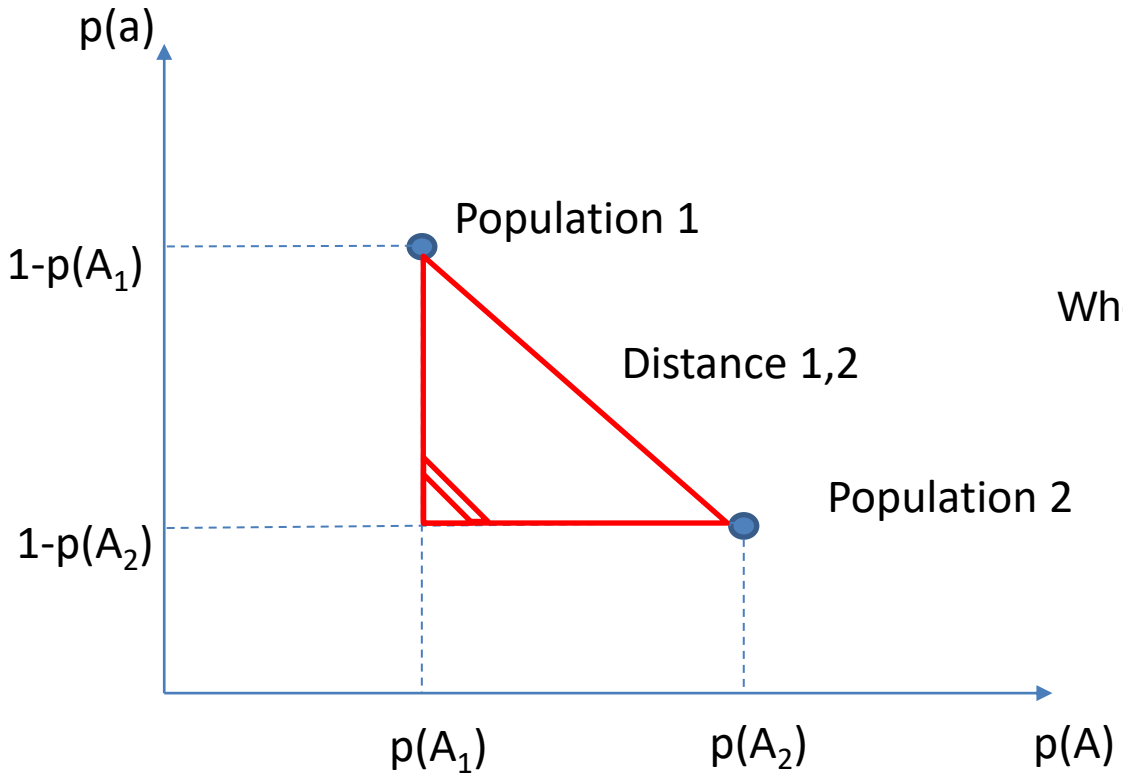
$expHet(\overline{p(A)})$

$\frac{expHet(p(A)_{pop1}) + expHet(p(A)_{pop2})}{2}$

Decrease in Heterozygosity due to stratification

$\rightarrow F_{ST}$

Genetic distance



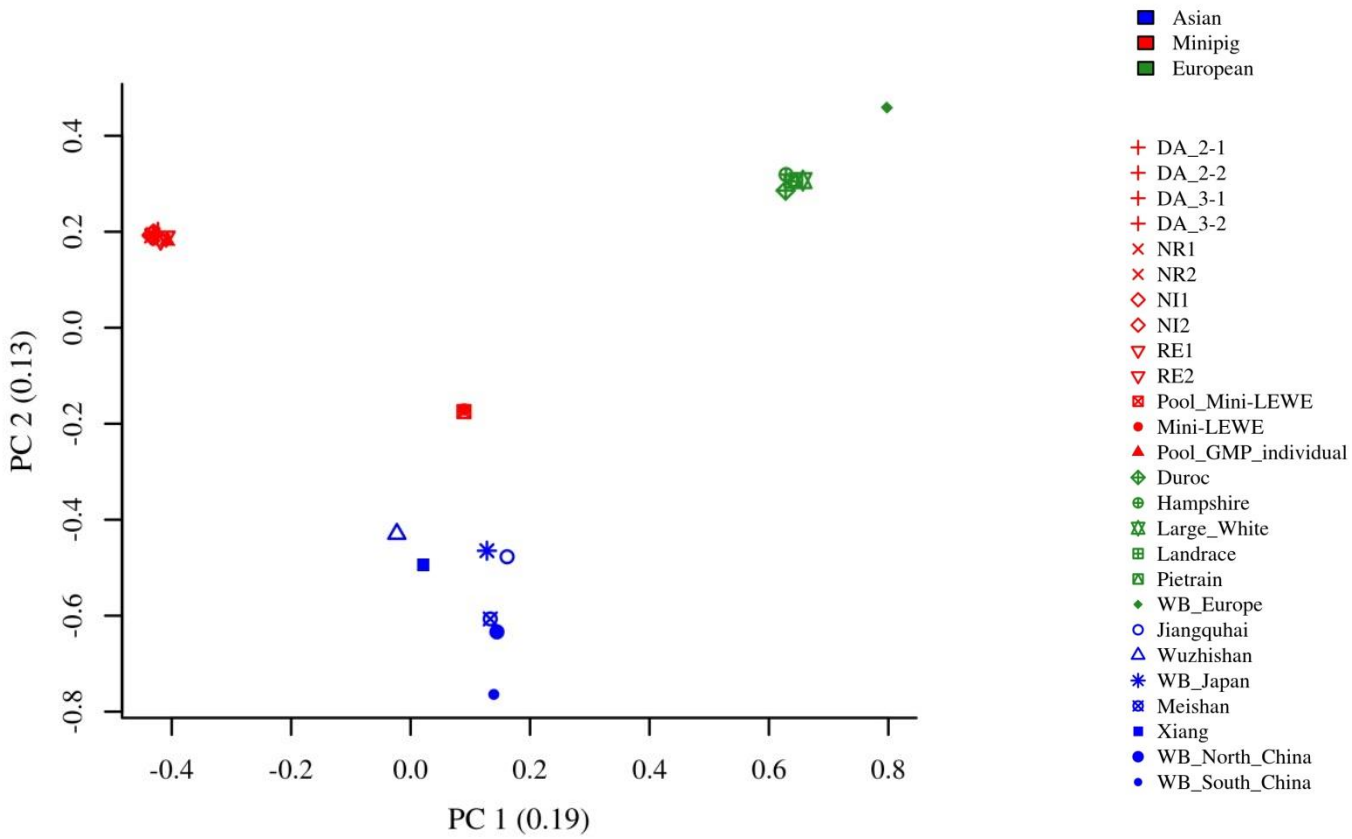
$$D = \sqrt{\sum_{i=1}^{k} (x_i - y_i)^2}$$

Where k – number of loci;
x_i and y_i allele frequencies at locus k of pop. i and j

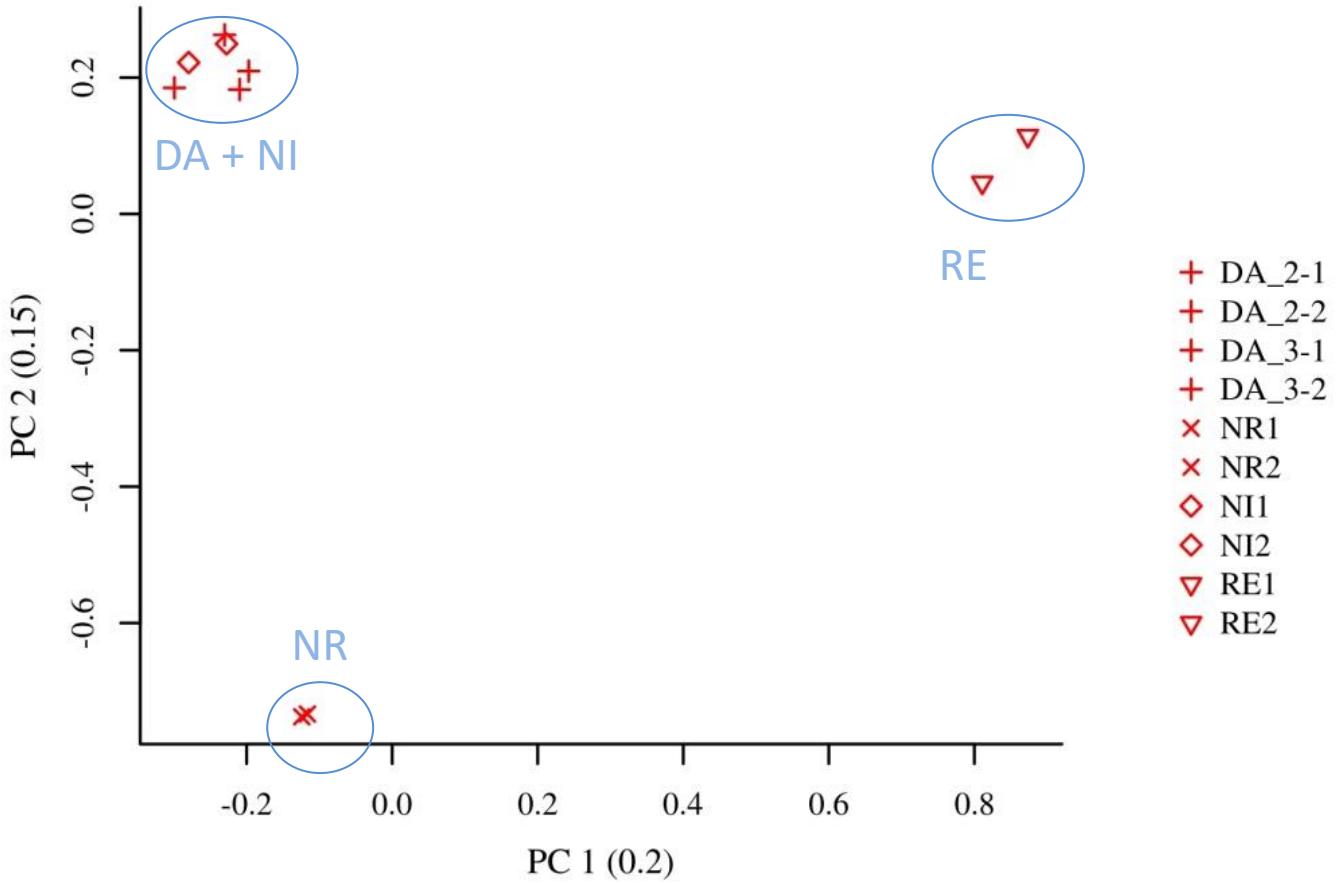
Average Fst and Distances

		GMP	Asian	European
	GMP	0.066	0.262	0.307
FST	Asian		0.271	0.327
	European			0.163
	GMP	0.113	0.364	0.408
D_R	Asian		0.372	0.428
	European			0.246

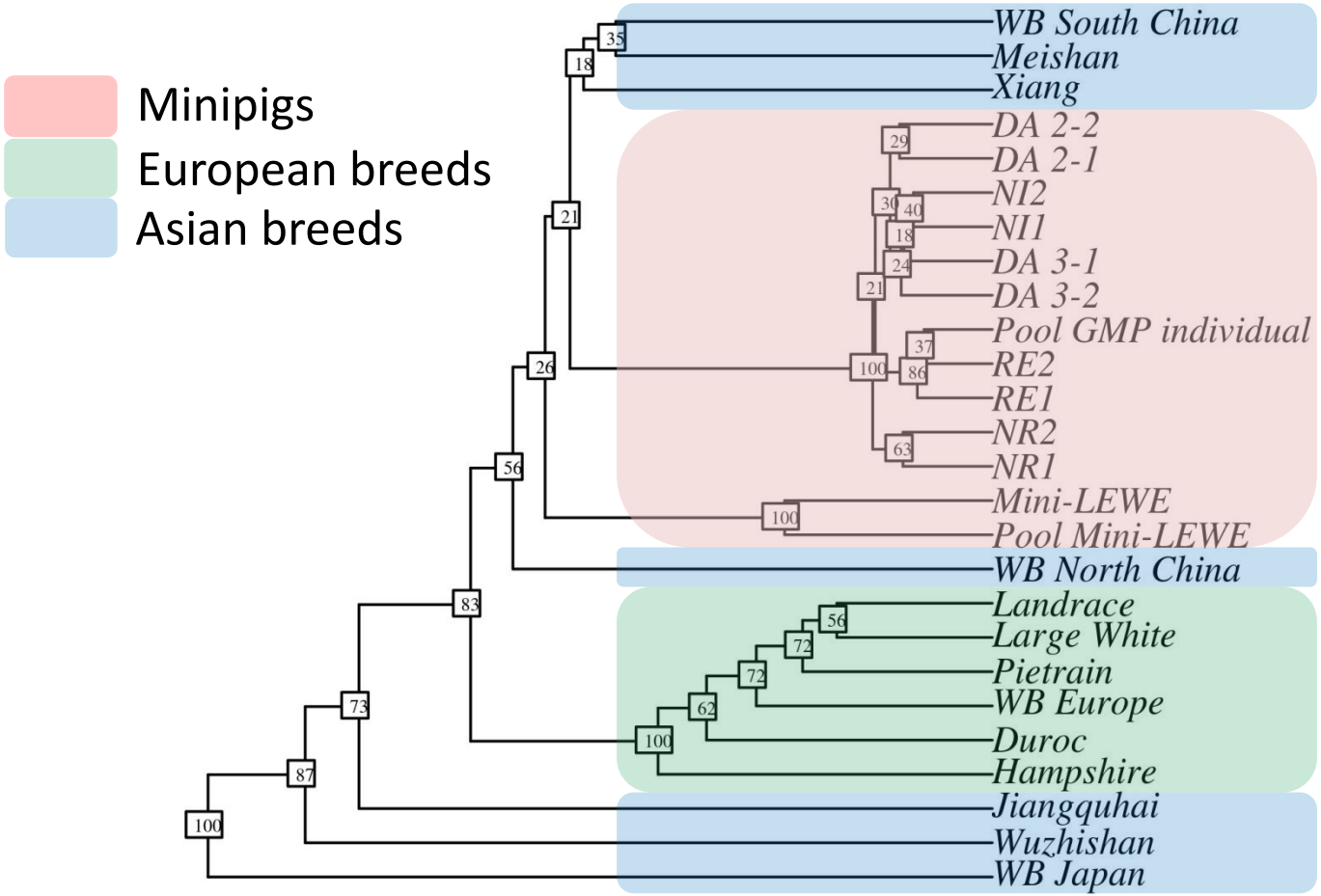
Principal component analysis



Principal component analysis



Phylogenie

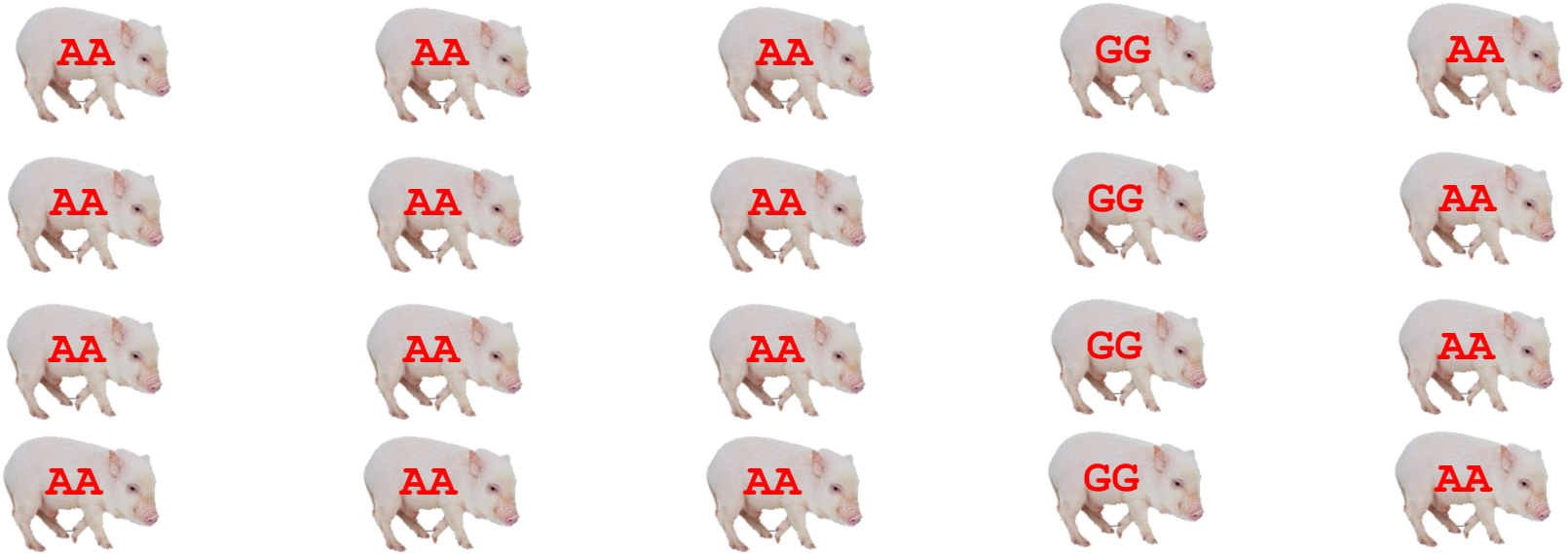

Minipigs
European breeds
Asian breeds
WB South China
Meishan
Xiang
DA 2-2
DA 2-1
NI2
NI1
DA 3-1
DA 3-2
Pool GMP individual
RE2
RE1
NR2
NR1
Mini-LEWE
Pool Mini-LEWE
WB North China
Landrace
Large White
Pietrain
WB Europe
Duroc
Hampshire
Jiangquhai
Wuzhishan
WB Japan

Diversity within colonies

Expected Heterozygosity estimated from the virtual union of both unit pools

	RE	DA2	DA3	NR	NI
H_{exp}	0.298	0.292	0.294	0.285	0.295
SD	0.175	0.175	0.178	0.181	0.176
Nloci [M]	16.498	16.498	16.498	16.499	16.499
NNA	260	452	346	441	231

Private alleles

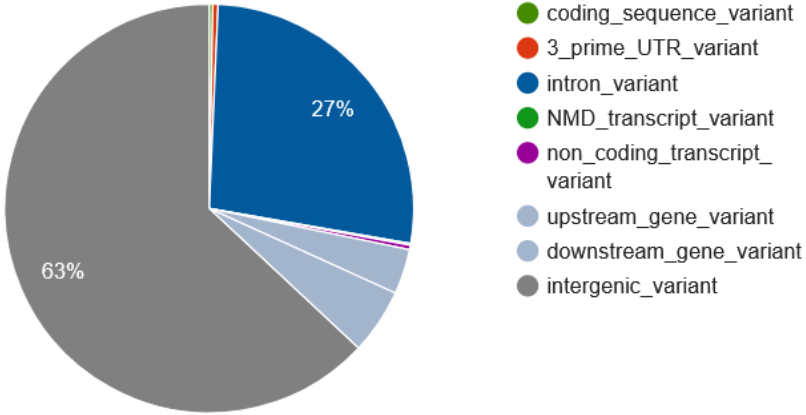


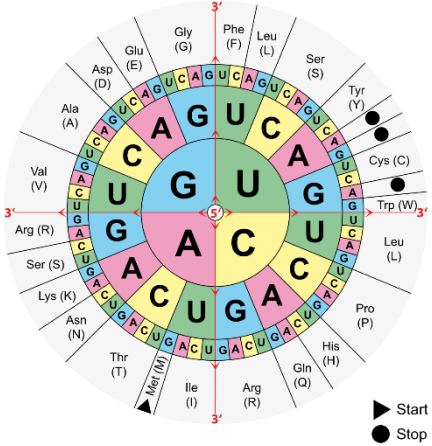
Allele G is a private allele in the USA stock

Private alleles

	No of private loci
RE	555‘591
DA2	163‘853
DA3	134‘158
NR	192‘896
NI	156‘502

Ensembl variant effect predictor

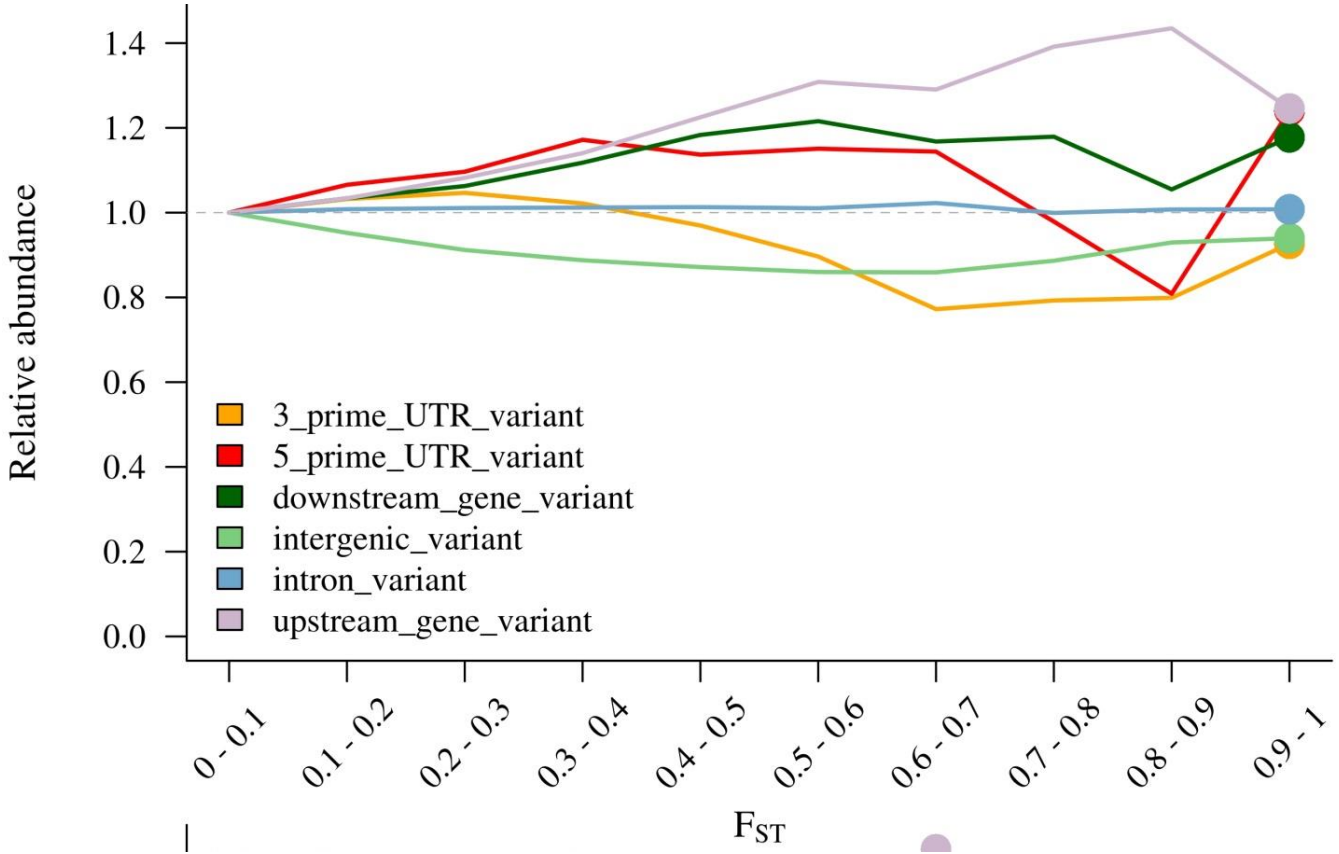




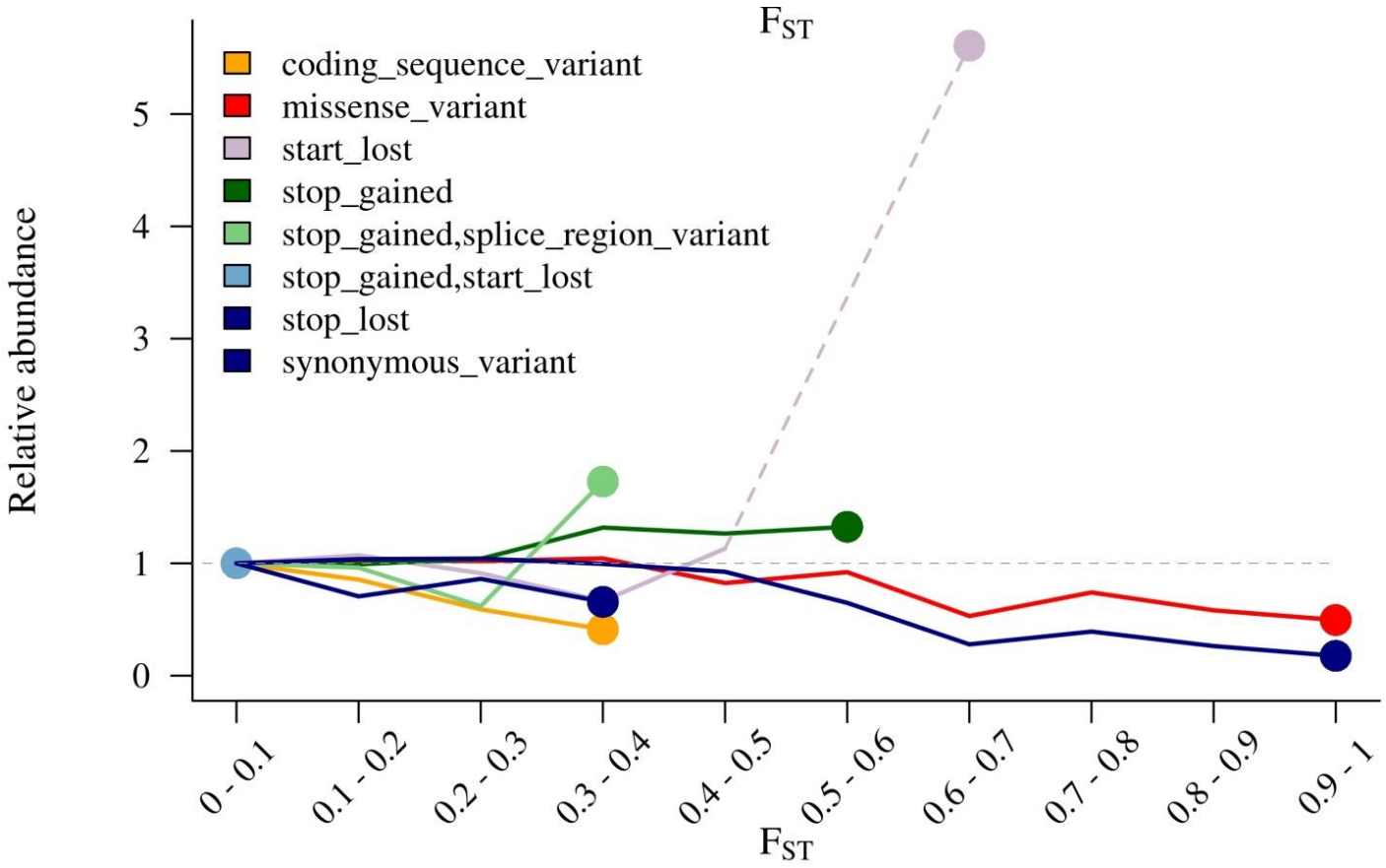
https://commons.wikimedia.org/w/index.php?curid=5986132

Consequence type	Count
coding_sequence_variant	4
3_prime_UTR_variant	6
intron_variant	387
NMD_transcript_variant	2
non_coding_transcript_variant	5
upstream_gene_variant	51
downstream_gene_variant	74
intergenic_variant	902

Functional annotation



Functional annotation



Final remarks

- The GMP has a long and stringent breeding history, which resulted in a well-established animal model

- World-wide collaboration of various parties ensures sufficient population size, market access and knowledge gain

- Centralized breeding organization guarantees uniformity of all Göttingen Minipigs, while efficiently maintaining genetic diversity

Thank you!

ELLEGAARD

GÖTTINGEN MINIPIGS

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