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Genetic analysis of feather pecking in divergent selected lines and in a F2 cross

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Feather pecking leads to:

1. Reduced feather cover
2. Increased mortality
3. Increased feed consumption
4. Reduced egg production

What influences feather pecking?

- Genotype
- Physical factors:
  - Environmental complexity
    - Physical composition of litter
    - Enrichment, string, styropor
  - Stocking density
  - Light
    - Intensity
    - Colour
- Social factors
  - Group size
  - Group composition
  - Social learning
- Nutritional factors
  - Feed quantity
  - Feed quality
    - Fibre fraction
    - Essential aminoacids
    - Roughages
- Stress factors
  - Handling
  - Crating
  - Transport
  - Temperature
  - (Un)Predictability

Heritability estimates

Trait	h^2
Egg production to 72 weeks	0.10
Feather pecking at 6, 38 and 69 weeks	0.06-0.33
Egg weight at 32 weeks	0.50
Body weight at 32 weeks	0.55
Cannibalism, group selection, Muir, Purdue	0.65
Cannibalism, calculation, Bijma, Wagen.	0.06-0.19
Cannibalism, group sel., floor, realised	0.00?

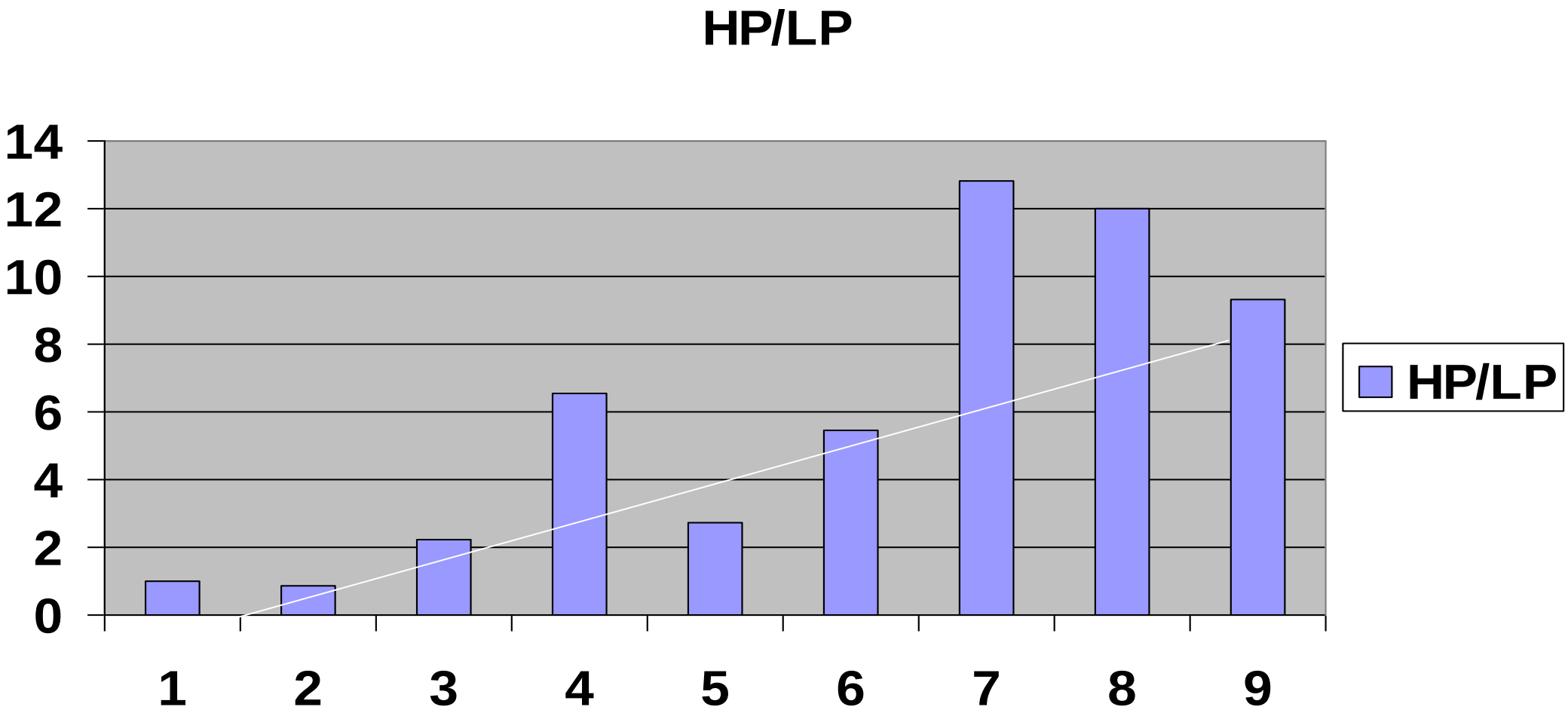
Selection experiment

Control: White Leghorn line random bred
LP line: Selected for low feather pecking
HP line: Selected for high feather pecking

Trait: Individual feather pecking, only

- Generations 0-5: Danish Institute of Animal Sciences, Foulum, Denmark (Kjaer et al., 2001)

- Generations 6-11: Institute of Animal Science, University Hohenheim, Germany.


HP/LP
14
12
10
8
6
4
2
0
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HP/LP

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1. Signatures of selection in two laying hen pure lines divergently selected for feather pecking behaviour

2. Structural Equation Models (SEM) on F2-crosses

3. Genome-wide association study for feather pecking in F2-crosses

Signatures of selection

- Selection leads to a reduced nucleotide diversity of target loci and also of loci in high linkage disequilibrium (LD) with the target loci (genetic hitch-hiking) leading to selection signatures

- Selection signatures are defined as regions of the genome that harbor functionally important sequence variants and have been under selection (Qanbari and Simianer, 2014)

Data

- Chickens of the pure lines of the White Leghorn feather pecking selection lines divergently selected for high and low feather pecking for 11 generations

  - FP was recorded at the age of 25 to 37 weeks
  - Group size: 40 hens
  - Groups consisted of equal numbers of HFP and LFP hens
  - Each observer observed each pen during a session of 20 min over three consecutive days

Genotyping

- 41 HFP and 34 LFP from generation 11 using the Illumina 60K chicken Infinium iSelect chip
- The following markers were excluded:
  - SNPs located on one of the sex chromosomes W or Z or on linkage group LGE22C19W28_E50C23 or LGE64
  - SNPs not allocated to a particular chromosome or linkage group
  - monomorphic loci (MAF = 0.0)
  - SNPs with call frequencies below 0.95 were filtered out

Statistical analysis

- **The population differentiation index F_{ST}**

$$F_{ST} = \frac{\sigma_p^2}{\bar{p}(1-\bar{p})}$$

$\bar{p}$	is the mean of the allele frequency of the two lines
σ_p^2	is the variance of the allele frequency across the two lines: $\sigma_p^2 = \left(\overline{p^2}\right) - \left(\bar{p}^2\right)$, where $\overline{p^2}$ is the mean of the squared allele frequencies in the two lines.

Weir and Cockerham (1984, eq 8)

- **Sliding windows**
- Selection sweeps will affect the FST of consecutive SNPs due to the LD between them. Therefore, we calculated FST values also for sliding windows. Each window consisted of 25 SNPs.

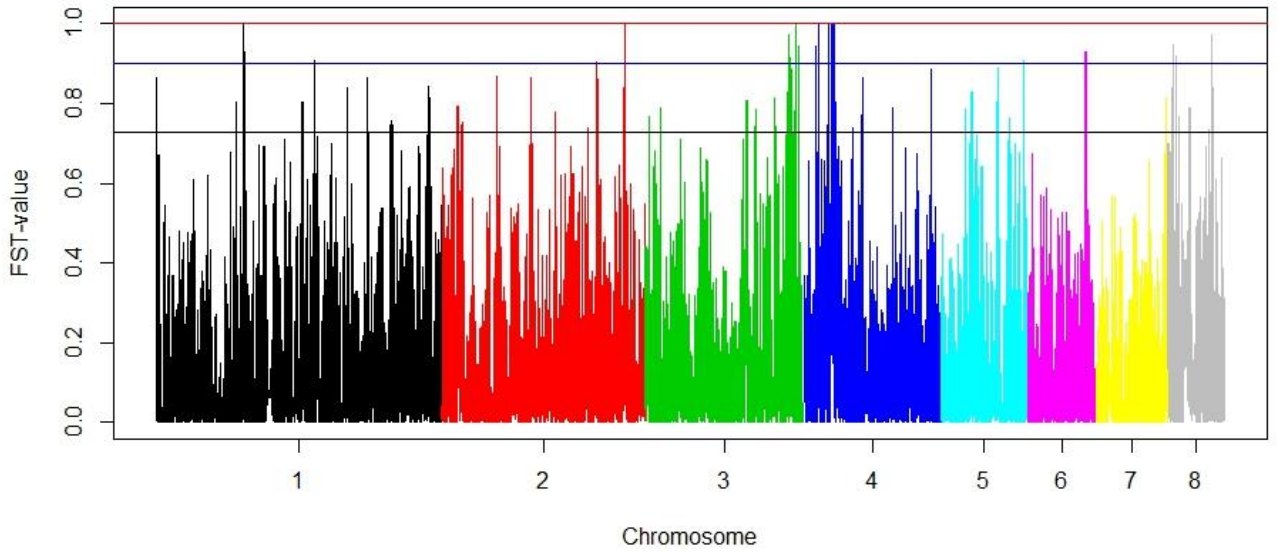
- **Clustering**
- It is likely that selection will have resulted in increased F_{ST} values of a series of consecutive SNPs.
- Therefore, we identified clusters of SNPs, which would provide stronger evidence of selection sweeps, compared to single F_{ST} values.
- A cluster contained a minimum of two significant SNPs ($p_{nominal} \leq 5x10^{-5}$) with a maximum distance of 3 Mb.

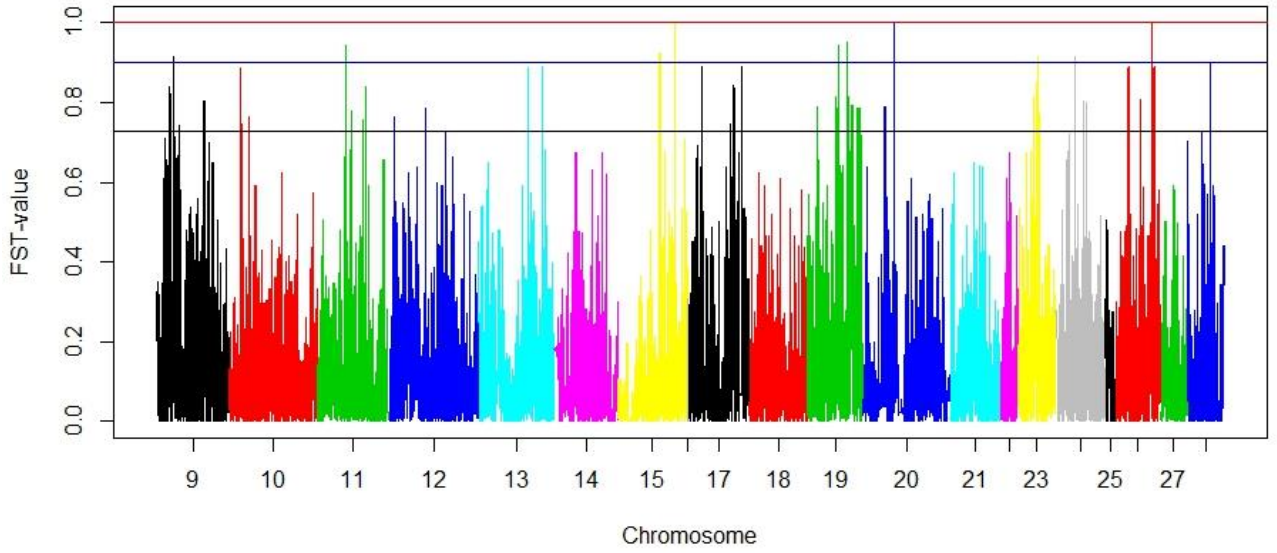
Results

Number of significant SNPs, threshold F_{ST}-value and FDR of significant SNPs for three significance level.

Significance level		Number of SNPs	F_{ST}-value	FDR
$p_{genome\ wide}$	< 0.05	17	1.000	< 0.001
$p_{nominal}$	$\leq 5 \times 10^{-5}$	49	0.901	≤ 0.015
$p_{nominal}$	$\leq 5 \times 10^{-4}$	276	0.730	≤ 0.034

Results





$p_{genomewide} \leq 0.05$

$p_{nominal} \leq 0.00005$

$p_{nominal} \leq 0.0005$

Results- Window approach

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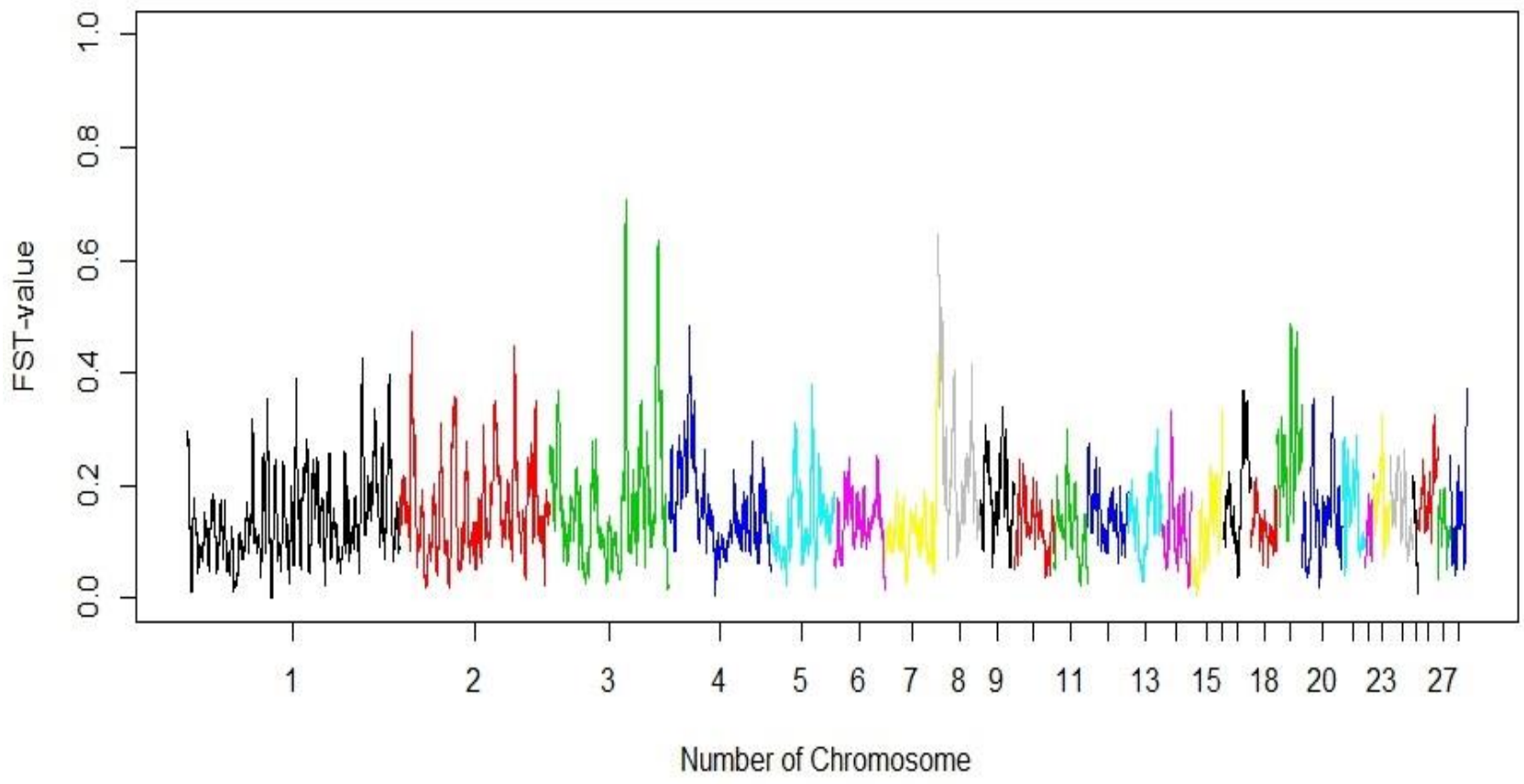
Revealed 5 distinct peaks, i.e. two on chromosome 3, one on chromosome 4, 8, and 19



Results - Cluster

- 12 Clusters
- Chromosome: 1, 3, 4, 6, 8, 11, 15, 19
- Most clusters were small in size and included only a few significant SNPs
- 12 genomewide significant SNPs were located within the clusters

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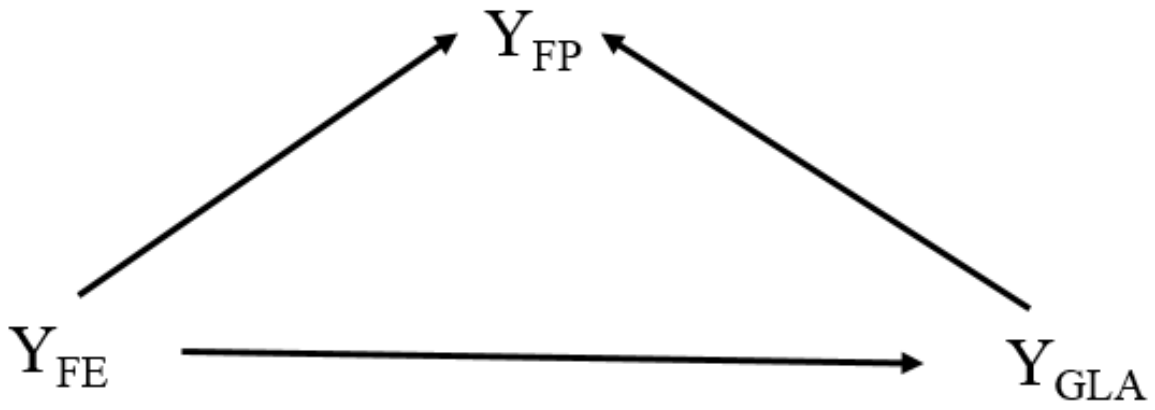
Structural Equation Models (SEM) for describing relationships between Feather Pecking, Feather Eating and General Locomotor Activity

Hypotheses:

1. Feather pecking is triggered by the motivation of eating feathers (Bessei and Kjaer, 2014; McKeegan and Savory, 2001; Harlander-Matauschek and Bessei, 2005)
2. and/or is caused by hyperactivity disorder (Kjaer, 2009)
3. Feather eating leads to a higher activity of the hens



Structural Equation Models (SEM) versus Standard Multi Trait Models (MTM)

1. SEM are an extension of MTM
2. SEM handles situations where recurrent or simultaneous effects occur in multivariate systems
3. They can show causal relationships (path analysis)
4. SEM estimates the rate of change in trait 1 with respect to the level of trait 2 (structural coefficient λ_{12})

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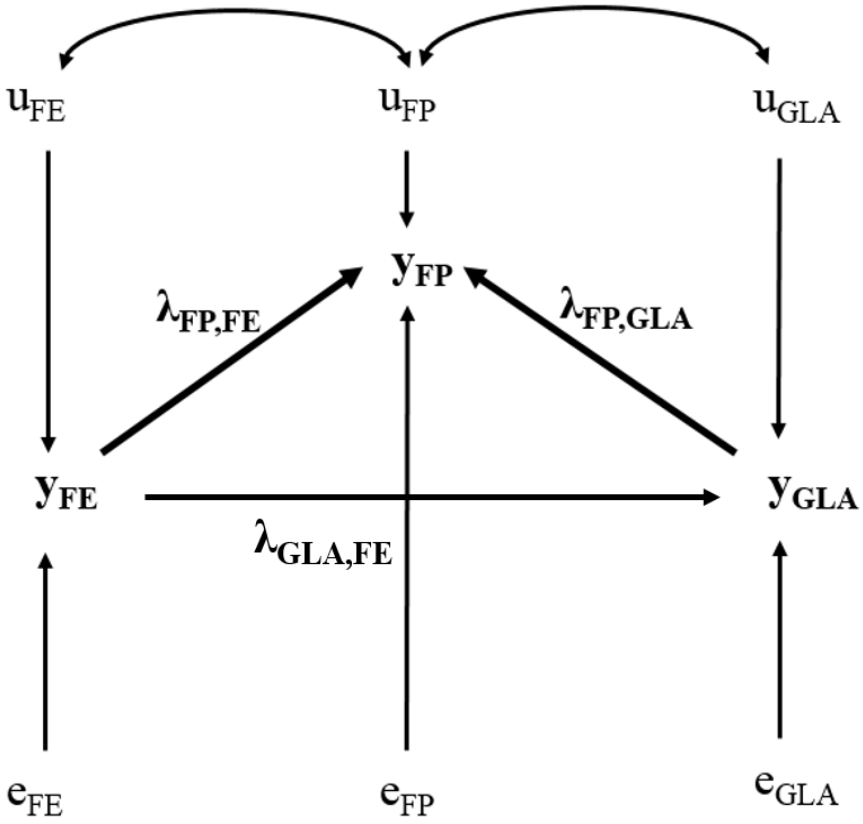
Statistical analysis

Using notation from Gianola and Sorensen, 2004 and Rosa et al., 2011:

$$y = (\Lambda \otimes I_n)y + X\beta + Zu + e$$

y	vector with the phenotypic records
Λ	matrix of structural coefficients
I	Identity matrix
⊗	Kronecker product
β	vector of fixed hatch effects
u	vectors of the additive genetic effects and
e	the model residuals
X und Z	incidence matrices



$$\Lambda = \begin{bmatrix} 0 & 0 & 0 \\ \lambda_{GLA,FE} & 0 & 0 \\ \lambda_{FP,FE} & \lambda_{FP,GLA} & 0 \end{bmatrix}$$

Data

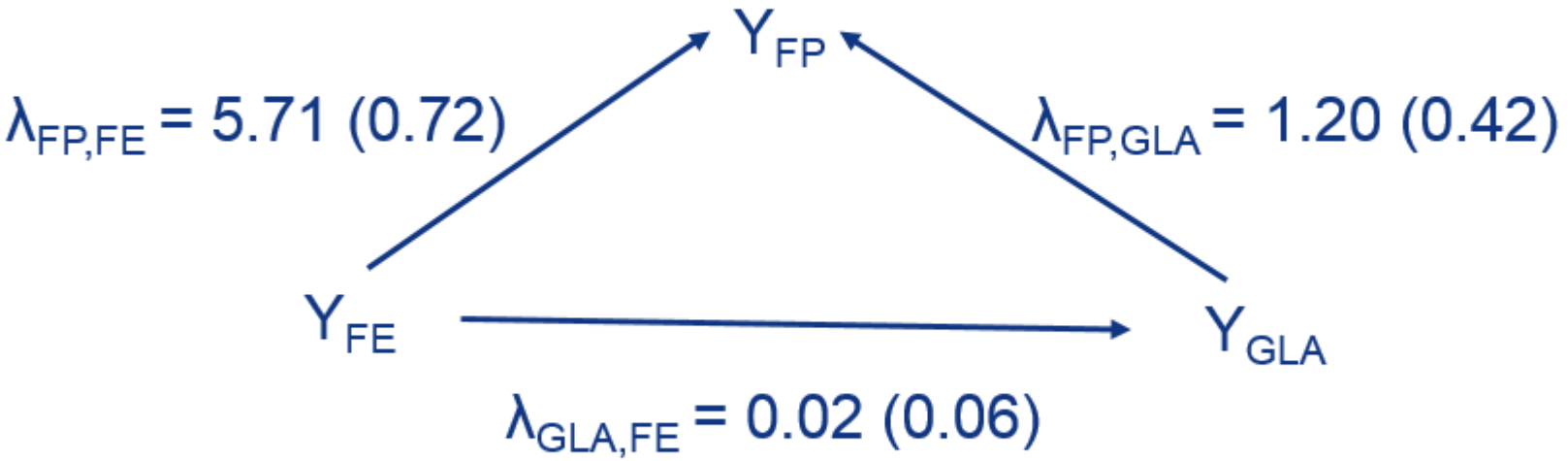
- **A total of 897 F2-hens**, set up from two lines divergently selected for high and low feather pecking
- The analyses were performed using the ASReml software (Gilmour et al. 2006)

- **Feather pecking (FP):**
  - At the age of 27 weeks
  - Group size: 36 – 42 hens
  - Recording: Each pen was visually observed for 20 minutes per observer for three consecutive days. Up to 7 observers.
- **Feather eating (FE):**
  - At the age of 20 weeks
  - individual cages
  - Over a period of 10 days, 10 feathers per day
  - were fixed next to the feeder
  - → maximum consumption of feathers was 100 per hen

Data

- **General locomotor activity (GLA):**
  - At the age of 18 weeks
  - Group size: 185 - 275 hens
  - Electronic transponders and antennas in the litter
  - Recording period: 12 hours per day for 9 consecutive days

Results – recursive effects



Trait	Abbr.	N	mean	SD	min	max
Feather pecking	FP	897	13.82	26.13	0	198.33
Feather eating	FE	897	62.32	34.06	0	100.00
General locomotor activity	GLA	897	49.27	31.20	0	210.25

Heritability estimates and correlations

(genetic above, phenotypic below diagonal)

SEM	FP	FE	GLA
FP	0.14 (0.08)	0.13 (0.27)	0.12 (0.30)
FE	0.20 (0.04)	0.37 (0.18)	0.48 (0.14)
GLA	0.09 (0.06)	0.16 (0.04)	0.29 (0.10)

MTM	FP	FE	GLA
FP	0.14 (0.06)	0.13 (0.27)	0.12 (0.27)
FE	0.20 (0.04)	0.37 (0.09)	0.48 (0.19)
GLA	0.09 (0.04)	0.16 (0.04)	0.29 (0.08)

Conclusions

- FP was clearly affected by FE and GLA
- FE had a larger effect than GLA
- FE had very little effect on GLA

- These hypotheses should be further investigated:
  - Feather eating leads to feather pecking
  - Feather pecking is caused by a high general activity

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Genome-wide association study for feather pecking in the F2-cross

(preliminary data)

Genotyping

- **817 F2-hens**, set up from two lines divergently selected for high and low feather pecking

- **The following markers were excluded:**
  - **SNPs located on one of the sex chromosomes W or Z or on linkage group LGE22C19W28_E50C23 or LGE64**
  - **SNPs not allocated to a particular chromosome or linkage group**
  - **monomorphic loci (MAF = 0.0)**
  - **SNPs with call frequencies below 0.95 were filtered out**

- **28401 SNPs remained for the statistical analysis**

Statistical analysis

$$y_i = SNP_{ij} * b_j + g + e_i$$

y	vector with the phenotypic records
SNP_{ij}	SNP *j* of animal *i*
b_j	is the additive effect (fixed effect) of the candidate SNP *j* to be tested for association
g	is the polygenic effect (random effect) i.e. the accumulated effect of all SNPs except those on the chromosome where the candidate SNP is located
e	is the residuals

- GWAS was performed using GCTA Software (Yang et al., 2014)
- To judge how many false positives were among the significant associations, we applied the false discovery rate (FDR) technique, using the software QVALUE
- The Bonferroni correction was applied

Results

Significance level		Number of SNPs	FDR	Chromosome
$p_{genome\ wide}$	< 0.05	8	0.0003	6, 7
$p_{nominal}$	$\leq 5x10^{-5}$	12	0.03	6, 7, 11
$p_{nominal}$	$\leq 5x10^{-4}$	31	0.40	4, 6, 7, 9, 11, 12, 13, 19

Conclusions

- Selection on feather pecking has been successful and has left multiple signatures in the genome
- FP is heritable
- Feather eating triggers FP
- Activity triggers FP
- Trait-associated SNPs were detected → FP is a polygenic trait

Discussion

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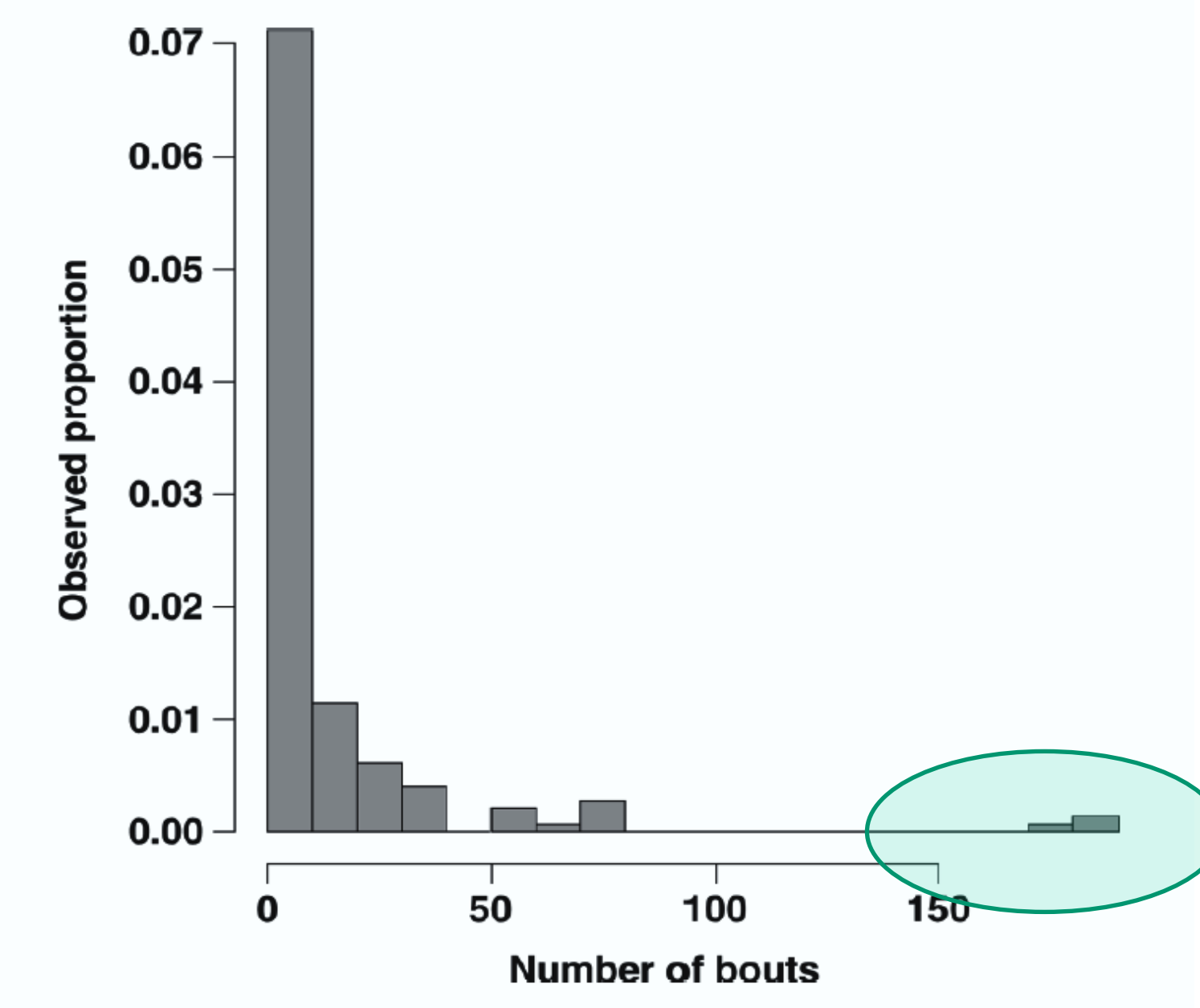
- QTL-analysis:
  - One significant QTL for severe FP (Buitenhuis et al., 2003)

- Gene expression:
  - Superpeckers have an expression profile different to peckers
  - One or a few major genes involved? (Labouriau et al. 2009)


0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
Observed proportion
0
50
100
150
Number of bouts

Discussion

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- QTL-analysis:
  - One significant QTL for severe FP (Buitenhuis et al., 2003)

- Gene expression:
  - Superpeckers have an expression profile different to peckers
  - One or a few major genes involved? (Labouriau et al. 2009)

  - Eight genes differed between feather peckers and controls

(Brunberg et al., 2011)

Discussion

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- Six genes showed differential expression between HFP and LFP:
  - GLUL, TSPO, HTR1B, SIP1, PSEN1, MAOA (monoamine oxidase A), a gene involved in the dopamine pathway

  (Wysocki et al., 2013, using the feather pecking selection lines)

- One SNP in MAOA was reported as associated with feather damage

  (Biscarini et al., 2010)

So, what to do now?

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- Behavioural observations on individual birds in breeding stocks (pure and crosses)
- Get rid of the ‚Superpeckers‘
- Feather pecking to be included in the selection index
- Pair behavioural and genomic data to find haplotypes for genomic selection

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