Behav Genet (2010) 40:715–727
DOI 10.1007/s10519-010-9370-0

ORIGINAL RESEARCH

Across-Line SNP Association Study for Direct and Associative Effects on Feather Damage in Laying Hens

F. Biscarini · H. Bovenhuis · J. van der Poel · T. B. Rodenburg · A. P. Jungerius · J. A. M. van Arendonk

Received: 27 January 2010 / Accepted: 7 May 2010 / Published online: 23 May 2010

Abstract An association study between SNP markers and feather condition score on the back, rump and belly of laying hens was performed. Feather condition score is a measure of feather damage, which has been shown to be closely related to feather pecking behaviour in hens housed in groups. A population of 662 hens was genotyped for 1536 SNPs of which 1022 could be used for the association study. The analysis was conducted across 9 different lines of White Leghorn and Rhode Island Red origin. Across lines linkage disequilibrium is conserved at shorter distances than within lines; therefore, SNPs significantly associated with feather condition score across lines are expected to be closer to the functional mutations. The SNPs that had a significant across-line effect but did not show significant SNP-by-line interaction were identified, to test that the association was consistent across lines. Both the direct effect of the individual's genotype on its plumage condition, and the associative effect of the genotype of the cage mates on the individual's plumage condition were analysed. The direct genetic effect can be considered as the susceptibility to be pecked at, whereas the associative genetic effect can be interpreted as the propensity to perform feather pecking. Finally, 11 significant associations between SNPs and behavioural traits were detected in the direct model, and 81 in the associative model. A role of the gene for the serotonin receptor 2C (HTR2C) on chromosome 4 was found. This supports existing evidence of a prominent involvement of the serotonergic system in the modulation of this behavioural disorder in laying hens. The genes for IL9, IL4, CCL4 and NFKB were found to be associated to plumage condition, revealing relationships between the immune system and behaviour.

Keywords Feather pecking · Plumage condition · Association study · SNPs · Laying hens · Direct and associative effects

Introduction

Feather pecking (FP) is one of the most serious behavioural disorders of laying hens. Severe FP, the type of pecking that causes most feather damage, consists of the forceful pecking and pulling of feathers of other birds (Savory 1995). FP is a multifactorial problem caused by both genetic and environmental factors. There is evidence of line differences in FP (Kjaer et al. 2001; Uitdehaag et al. 2008), and it has been demonstrated that FP is influenced by group size, light intensity, diet and type of litter (Hughes and Duncan 1972; Blokhuis and Arkes 1984; Savory 1995). As for its aetiology, FP has been considered to be redirected ground pecking (Blokhuis 1986), abnormal dustbathing behaviour (Vestergaard and Lisborg 1993), or the consequence of a more general hyperactivity disorder (Kjaer 2009). Most of the evidence point at the redirected ground pecking theory (Huber-Eicher and Wechsler 1997), with active or even hyperactive birds having the highest risk of developing FP (Newberry et al. 2007; Kjaer 2009). The serotonergic system has been shown to play an important role in the modulation of FP (van Hierden et al. 2002, 2004a, b; Buitenhuis et al.

Edited by Stephen Clark Maxson.

F. Biscarini (✉) · H. Bovenhuis · J. van der Poel · T. B. Rodenburg · J. A. M. van Arendonk
Animal Breeding and Genomics Centre, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands
e-mail: filippo.biscarini@wur.nl

A. P. Jungerius
Hendrix Genetics, Boxmeer, The Netherlands

2006). Especially gentle FP is viewed as a stereotyped behaviour, which shows similarities with obsessive compulsive disorders in other species, in which the serotonergic system plays a comparable role (Pigott 1996). Feather pecking, which can lead to feather damage and cannibalism, thereby causing mortality and economic losses for the laying industry, has been traditionally controlled through the husbandry practice of beak-trimming. However, the EU laying hens directive 1999/74/EC is causing member states to move from conventional cages to larger groups (furnished cages, non-cage systems) which will make the problem more difficult to control; and in some countries beak trimming, as preventive measure, is or will be prohibited (Jendral and Robinson 2004). Selection of more sociable animals with a less pronounced tendency to peck each other might therefore be highly beneficial to the farming of layers. Feather pecking has already been shown to be heritable (Kjaer and Sørensen 1997; Rodenburg et al. 2003), and it has been demonstrated that individual selection against FP is feasible (Kjaer et al. 2001). Social interactions have been revealed to play a role in survival related to FP and cannibalism in laying hens (Ellen et al. 2008). This associative effect due to the genotypes of group mates can contribute substantially to the total heritable variation (Bijma et al. 2007a, b). However, measuring feather pecking requires direct observations which are time consuming and expensive. A convenient indirect way of measuring FP is looking at plumage condition: Bilčik and Keeling (1999) showed that feather condition scores used to assess plumage condition are related to feather pecking activity. Few genetic studies on plumage condition have been carried out until now (Jensen et al. 2005). Previous studies detected microsatellite markers located in chromosomal regions involved in feather pecking (Buitenhuis et al. 2003a, b).

The aim of this study was to detect associations between mutations in the genome and feather damage across lines of laying hens, focusing both on the feather peckers and on the victims of feather pecking. We performed an association study between SNP markers and feather condition score across 9 lines of layers of White Leghorn (white feathered) and Rhode Island Red (brown feathered) origin, looking at the interaction between the SNP and line effects (Saccone et al. 2008; Biscarini et al. 2010). SNPs showing a significant across line association and no SNP-by-line interaction were considered to be consistently associated with the phenotype. Both the direct genetic effect of the individual and the associative genetic effect of cage mates on feather condition scores were analysed. The direct effect can be considered the susceptibility to receive feather pecking, while the associative effect reflects the propensity to express a pecking behaviour. To our knowledge this is the first time that the associative genetic effect is analysed in an association study.

Material and methods

Experimental population

The animal population used in this study consisted of 662 laying hens randomly chosen from 9 genetic lines, 4 of Rhode Island Red type (RIR, brown feathered) and 5 of White Leghorn type (WL, white feathered). The number of hens per line is reported in Table 1. The birds originated from mating 175 roosters with 401 dams. Every rooster was mated on average with 2.3 females while each hen was mated with only one male. Four generations of ancestors were extracted from the pedigree file for the calculation of the additive genetic relationships among the birds.

All hens were housed in battery cages (44 cm height × 46 cm depth × 39 cm width) within the same stable; cages comprised 4 hens from the same line, either full-sibs or randomly mixed. Hens arrived at the laying facility at 17 wk of age and remained in the stable for the entire laying period of 52 wk.

The hens had intact beaks and received routine vaccinations against Marek's disease (d 1), New Castle disease (wk 2, 6, 12, 15), infectious bronchitis (d1, wk 2, 10, 12, 15), infectious bursal disease (wk 3, 15), fowl pox (wk 15) and avian encephalomyelitis (wk 15).

During the experiment, feed and water were available ad libitum. From the beginning of the experiment (at 19 wk of age) until 42 wk of age hens were fed a standard commercial phase 1 diet (159 g/kg crude protein, 43 g/kg crude

Table 1 Number of hens per line, average feather scores for the single lines, and mean and standard deviations of the traits in the overall population

Breed	Line	*n*		Individual feather scores			
				BR51	Belly51	BR69	Belly69
Rhode Island Red	B1	81		2.28	0.34	4.42	2.09
	B2	76		1.09	0.04	3.88	1.68
	B3	75		2.79	0.45	4.83	2.23
	BB	66		2.02	0.88	4.70	2.53
White Leghorn	W1	68		5.53	2.09	6.63	3.24
	WA	77		4.93	1.56	5.60	3.16
	WB	77		4.18	1.41	4.61	2.96
	WC	63		5.86	2.67	6.65	3.51
	WF	79		2.23	1.87	3.92	2.03
Total		662	*n*	662	662	655	655
			Mean	3.37	1.22	4.96	2.57
			sd	2.49	1.35	1.93	1.13

BR51, BR69 = sum of the individual feather scores for the back and rump regions at 51 and 69 wk of age (scale 0–10); Belly51, Belly69 = individual feather scores for the belly region at 51 and 69 wk of age (scale 0–5)

fibre, and 11.17 MJ ME/kg); from 42 wk onwards, until the end of the experiment, a standard commercial phase 2 diet (152 g/kg crude protein, 47.0 g/kg crude fibre, and 11.01 MJ ME/kg) was given. Wing bands allowed individual identification of the hens. After arrival in the stable, hens were kept on a 9L:15D light scheme (light from 7.00 until 16.00), where L stands for light and D for darkness. After 1 wk, the light period was increased by 30 min, starting at 6.30. Thereafter, the light period was increased approximately 10 min per day. From 30 wk onwards hens received light from 00.00 until 16.00 (16L:8D). This is a standard light regime.

Phenotypes

Feather damage was assessed at two ages (51 and 69 wk) by assigning a score to plumage condition on the back, rump and belly of the hens. Damage to these regions is unlikely due to abrasion and these regions are a frequent target of feather pecking (Bilčik and Keeling 1999). The classification of Bilčik and Keeling (1999) was followed, with a range going from 0 to 5 (higher scores indicate more severe damage). Damage to the rump and back area was combined into a single score: back and rump feather scores were summed to give a backrump (BR) score ranging from 0 to 10, as previously described in Uitdehaag et al. (2008). Feather condition scores measured at 51 and 69 wk of age were used in the analysis (Belly51, BR51, Belly69 and BR69). The number of available observations ranged from 655 at 69 wk to 662 at 51 wk (see Table 1). Part of the phenotypes used in this study were previously analysed by Uitdehaag et al. (2008).

Genotypes

Genotyping was done in a 1536-plex format using the GoldenGate assay (Illumina, San Diego) by a commercial genotyping facility (ServiceXS, Leiden, NL). As part of a bigger experiment, SNPs were selected to cover QTL regions for behavioural and immune traits identified in previous mapping studies catalogued in the QTL database (http://www.genome.iastate.edu/cgi-bin/QTLdb/GG/index, accessed May 2006). In addition, specific candidate genes, which from literature (mouse/human) have a known or expected effect on immune or behavioural traits (e.g. genes for interleukins or serotonin receptors), were considered for the choice of the SNPs. Per selected gene 2–4 SNPs were chosen; for a QTL region SNPs equally spread over the QTL region were chosen. Twenty-four of the 39 chromosomes of the chicken genome were partly covered (Table 2). The SNPs that did not show three distinct clusters in the allelic discrimination plot as provided by the Illumina Beadstudio software (194 SNPs) and the SNPs with a minor allele frequency $\leq$0.05 (320 SNPs) were excluded from the analysis. Thus, 1022 SNPs were used in the association study. Details on the SNP editing procedure are described by Biscarini et al. (2010).

Data analysis

The direct genetic effect of the individual's SNP genotype and the associative genetic effect of the SNP genotypes of cage mates on feather condition scores were analysed. A two-step procedure was used for data analysis. First, an association study was performed without accounting for relationships between animals. Potentially interesting SNPs from the first step were then analyzed in more detail using a mixed model thus taking additive genetic relationships among animals into account.

The association between genotypes and phenotypes was tested across lines using a single SNP approach. The across-line analysis picks up only markers that are in LD with the QTL across lines.

The direct genetic effect refers to the association of the SNP genotype of a hen and its plumage condition score. In the first step analyses were performed across lines using the following statistical model:

$$y_{ijk} = \mu + SNP_i + line_j + (line \times SNP)_{ij} + e_{ijk} \quad (1)$$

where y_{ijk} represents the feather damage score of animal k, with SNP genotype i, in line j; μ is the overall mean; SNP_i is the effect of the SNP genotype; $line_j$ is the line effect (nine classes); $(line \times SNP)_{ij}$ is the interaction between SNP genotype and line; and e_{ijk} are the residuals.

Model [1] was run twice, once without the line-by-SNP interaction term to obtain *p*-values for the SNP effect and once with the line-by-SNP term to determine the significance of the interaction. Adapting the approach of Saccone et al. (2008), we looked for SNPs that had an across-line significant effect in model [1] ($p \leq 0.05$) and did not show a significant line-by-SNP interaction ($p > 0.05$), to test that the association was consistent in all lines.

The false discovery rate (FDR, Benjamini and Hochberg 1995) was calculated for all the SNPs tested in the association study. SNP-phenotype associations from model [1] with a FDR $\leq$ 0.15 were selected for the second step of the association study in which a polygenic effect was added to the model to account for family relationships among animals. The following mixed model was used:

$$y_{ijk} = \mu + SNP_i + line_j + a_k + e_{ijk} \quad (2)$$

where all the terms are as specified in Eq. 1 except a_k, which is the random genetic effect of the kth animal. $Var(\mathbf{a}) = \mathbf{A}\sigma_a^2$, with $\mathbf{A}$ being the additive relationship matrix and $Var(\mathbf{e}) = I\sigma_e^2$. The ratio between residual and genetic variances was fixed using heritabilities for feather

Table 2 Number of SNPs per chromosome along the chicken genome, number of SNPs deviating from Hardy–Weinberg equilibrium, and homozygosity in the nine genotyped lines of layers

chr	Size (Mbp)	SNPs		FL (across lines)	Within-line values																	
		All	Used		B1		B2		B3		BB		W1		WA		WB		WC		WF	
					HW	FL	HW	FL	HW	FL	HW	FL	HW	FL	HW	FL	HW	FL	HW	FL	HW	FL
1	201	68	61	6	4	19	1	17	1	12	3	16	0	24	8	21	0	20	0	15	1	23
2	155	24	22	2	1	6	0	7	1	3	1	5	0	10	2	8	1	10	1	9	0	9
3	114	140	121	12	3	31	0	35	2	28	7	22	0	41	9	40	0	34	0	27	0	37
4	94	421	371	65	9	102	1	126	6	120	13	105	2	141	24	142	11	117	8	126	16	126
5	62	285	265	25	9	48	6	56	7	46	22	41	3	78	23	58	4	64	20	62	8	76
6	37	27	22	1	2	3	2	3	0	3	3	2	0	4	3	2	2	4	1	3	1	3
7	38	175	149	18	5	33	1	35	5	30	9	35	3	48	14	42	5	43	10	39	8	43
8	31	6	6	0	2	1	1	0	0	1	2	0	0	3	2	2	2	2	0	0	0	0
9	26	12	10	0	0	2	0	2	0	2	0	5	1	4	0	1	0	3	0	3	0	6
10	22.6	4	3	0	0	1	0	1	2	1	0	2	1	0	0	0	1	0	0	1	0	1
11	21.9	8	6	3	0	3	0	3	0	4	0	4	0	4	1	4	1	4	0	4	0	4
12	20.5	7	7	2	0	4	0	3	0	4	0	3	1	3	0	5	0	4	2	3	0	3
13	18.9	37	32	3	2	6	0	9	0	9	3	5	0	9	9	7	1	8	4	7	3	7
14	15.8	7	6	0	0	0	0	1	0	0	0	1	1	1	0	0	2	0	0	1	0	1
15	13	7	5	1	0	2	0	2	0	2	0	1	0	4	0	4	1	2	1	2	0	1
16	0.43	17	12	3	0	3	2	3	3	3	0	3	1	4	1	4	2	4	1	3	0	4
17	11.2	7	7	0	0	1	0	1	0	1	1	1	0	1	2	1	0	0	1	1	0	1
19	9.9	27	20	4	2	6	2	6	2	5	2	6	1	9	2	6	1	7	2	8	0	6
21	7	4	4	1	0	1	0	1	0	2	0	1	0	2	1	3	2	2	1	2	0	2
22	3.9	3	2	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	1	0
23	6	4	2	1	0	1	0	1	0	1	0	1	0	1	1	1	0	1	0	1	0	1
24	6.4	16	15	3	0	6	0	4	0	4	1	5	2	3	2	5	1	5	0	4	1	4
26	5.1	60	50	4	6	12	2	12	2	12	3	11	2	16	8	15	1	12	4	17	0	11
Z	75	160	138	21	0	47	0	49	0	47	0	50	0	76	0	61	0	60	0	63	0	62
–		8	6	0	1	2	1	2	0	2	1	2	1	2	0	1	1	2	1	1	0	1
Total		1534	1342	175	46	340	19	379	31	342	71	327	20	488	113	433	40	408	57	402	39	432

The symbol "–" refers to SNPs that were not assigned to any chromosome. Size refers to the size of the whole chromosome as derived from the NCBI chicken genome databass

HW loci not in Hardy–Weinberg equilibrium, *FL* fixed loci

condition score in laying hens estimated by van der Winkel on 17009 White Leghorn hens (unpublished results), averaged over the three lines (6324, 7018 and 3667 hens, respectively) used in that study: 0.03 for BR51, 0.08 for Belly51, 0.17 for BR69 and 0.20 for Belly69. The SNPs that still showed a significant effect ($p \leq 0.05$) on the traits from model [2] were reported.

The associative genetic effect refers to the effect of the SNP genotypes of cage mates on plumage condition of individual hens. In the analysis, individual feather condition scores were regressed on the allele frequency of cage mates. Note that the SNP genotype of the animal itself is not included in the analysis. Cages with 3 or 4 hens (hence 2 or 3 cage mates) were considered in the analysis. The following model was used:

$$y_{ijk} = \mu + \beta_1 p_k + line_j + \beta_{kj}(line \times p)_{kj} + e_{ijk} \quad (3)$$

where y_{ijk} represents the feather condition score of animal i of line j in cage k; μ is the overall mean; p_k is SNP allele frequency of the cage mates of animal i; $line_j$ is the line effect (nine classes); $(line \times p)_{kj}$ is the interaction between SNP allele frequency of cage mates and line; and e_{ijk} are the residuals, weighted for the number of group mates present in each cage (either 2 or 3). β_1 and β_{kj} are the regression coefficients. Equation 3 was also run twice, with and without the interaction term. SNPs with an across-line significant associative genetic effect ($p \leq 0.05$) and no significant line-by-allele frequency interaction ($p > 0.05$) were considered to be consistently associated with the phenotypes in all lines. Also in the case of the associative genetic effect, SNP-phenotype associations from model [3] with a FDR ≤ 0.15 were selected for the second step of the association study in which family relationships among animals were accounted for. The following mixed model was used:

$$y_{ijk} = \mu + \beta_1 p_k + line_j + a_i + e_{ijk} \quad (4)$$

where all the terms are as specified in Eq. 3 except a_i, which is the random genetic effect of the ith animal. The variance structure is as specified for model [2].

Data editing, analyses with models [1] and [3], and all other statistical analyses were performed using the open source statistical package R. The polygenic effects, and SNP effects as described in models [2] and [4] were estimated with a REML procedure using the Asreml software package (Gilmour et al. 2006).

Results

Descriptive statistics

Descriptive statistics of individual feather condition scores are summarized in Table 1. The damage to the plumage due to feather pecking cumulates over time; therefore feather condition scores at older ages (BR69, Belly69) are about 1.5–2 times higher than feather condition scores at younger ages (BR51, Belly51). There was more feather damage on the back-rump region than on the belly area (feather damage score of 1.84 vs. 1.22 at 51 wk, and of 2.71 vs. 2.57 at 69 wk, after correcting for the different scale). The overall across-line coefficient of variation ranged from 39% for BR69 to 111% for Belly51, indicating considerable variability in the feather condition scores of hens. Also within lines there was substantial variability: the coefficient of variation ranged from 25% for BR69 in line B3 to 532% for Belly51 in line B2, with an average of 75%. There were ample differences in feather condition scores between lines: e.g., from 1.09 (line B2) to 5.86 (WC) for BR51, and from 1.68 (line B2) to 5.66 (line W1) for Belly69. Brown layers showed less feather damage than white layers, for both regions and ages (on average more than 2 times lower), as described by Uitdehaag et al. (2008). Phenotypic correlations (results not shown) among the traits were weak, ranging from 0.14 between BR69 and Belly51, to 0.53 between both BR51 and Belly51, and BR69 and Belly69.

SNPs

The SNPs used in this study were located on 24 of the 39 chromosomes of the chicken genome. Positions of the SNPs were derived from the NCBI database (Galgal2.1 build 128). The average interval between SNPs varied from 25.29 kbps ($\sim$0.06 cM) on chromosome 16–5740.74 kbps ($\sim$14.35 cM) on chromosome 2. On average, the proportion of SNPs that deviated significantly (at the Bonferroni-corrected 0.05 level) from HW equilibrium within lines was 7.1% (Table 2), with the lowest percentages in lines W1 (2.7%) and B3 (5.7%) and the highest percentages in lines WA (13.1%) and BB (9.7%). Table 2 also reports the number of monomorphic (fixed) loci for the various chromosomes in the different lines. There were more fixed loci in the white layers (28.2%) than in the brown layers (22.6%). This is compatible with White Leghorn hens' longer history of artificial selection, which is expected to result in higher homozygosity and lower genetic polymorphism (Hillel et al. 2003).

Association study

In the direct analysis where individual SNP genotypes were associated with individual feather condition scores, 321 significant across-line SNP-trait associations were detected at the 5% significance level: of these 275 showed no significant genotype-by-line interaction. Among these 275, there were 11 SNPs with a FDR lower than 0.15. After

Table 3 SNPs significantly associated with feather scores (FS) in the analysis of direct genetic effects

chr	SNP	kbps	cM[a]	Individual FS			
				BR51	Belly51	BR69	Belly69
chr 1	rs15385785 (*MAOA*)[b]	114912046	287.3	3.15 (0.10)		2.76 (0.12)	
chr 3	rs13773912	5595342	14.0			3.20 (0.07)	
chr 4	rs13640917 (*HTR2C*)[b]	2798627	7.0	3.25 (0.12)			
	rs13788969	20659524	51.6	3.56 (0.10)			
	rs13517693	43865427	109.7		3.07 (0.17)		
	rs13522023	54013816	135.0		4.12 (0.08)		
chr 5	rs15692150	30998335	77.5			2.10 (0.10)	
	rs15707740	42231488	105.6			3.23 (0.10)	
chr 6	rs16558389	Unmapped				3.25 (0.10)	
chr 13	rs14999300 (*IL9*)[b]	15574216	38.9	3.43 (0.10)			

$-\log_{10}$ of the *p*-values are reported in the columns, with corresponding FDR in brackets. BR51, BR69 = sum of the individual feather scores for the back and rump regions at 51 and 69 wk of age (scale 0–10); Belly51, Belly69 = individual feather scores for the belly region at 51 and 69 wk of age (scale 0–5)

[a] Assuming 1 cM = 4×10^5 bps

[b] *MAOA* = gene for the mono-amino oxidase A; *HTR2C* = gene for the serotonin receptor 2C; *IL9* = gene for the interleukin 9

accounting for relationships among animals in the model, all the 11 SNPs still showed significant associations. The results of the analysis are shown in Table 3. The reported *p*-values come from model [2].

In the associative analysis where the allele frequency of the cage mates is related to the individual feather condition score, 478 significant across-line SNP-trait associations were detected ($p \leq 0.05$) in the first step of the association study: of these 357 showed no significant genotype-by-line interaction. Eighty-one (81) of these had a FDR ≤ 0.15. After accounting for relationships between animals, 57 of these SNP showed significant associations ($p \leq 0.05$) and are given in Table 4. The reported *p*-values come from model [4].

In the analysis of direct genetic effects, the detected associations comprised 4 SNPs for BR51, 2 for Belly51 and 5 for BR69. All SNPs reported in Table 3 had effect on one trait, with the exception of SNP rs15385785 on chromosome 1, which had an effect on the plumage condition of the back-rump region both at 51 and 69 wk. In many cases, neighbouring SNPs also showed effects, but had a FDR > 0.15 and are therefore not reported in Table 3.

In the associative analysis there were 27 SNPs associated to BR51, 19 to Belly51, 15 to BR69 and 7 to Belly69. Forty-seven of the SNPs reported in Table 4 were associated with only one trait, 9 with two traits and 1 SNP was associated with three different traits.

Two SNPs proved to be significantly associated with feather condition score both in the direct and associative model: SNPs rs13640917 on chromosome 4 and rs14999300 on chromosome 13. They were both associated with plumage condition on the back and rump regions at 51 wk. The same allele of SNP rs13640917, in the sequence of the serotonin receptor 2C (HTR2C), was associated with more feather damage on the back and rump at 51 wk in the direct as well as in the associative analysis, with an effect of approximately $0.5\sigma_p$ in both analyses. SNP rs16340917 is a SNP at position 2798627 on chromosome 4 of the chicken genome: it is an intronic SNP within the HTR2C gene. As for SNP rs14999300, alternative alleles were associated with greater damage on the back and rump at 51 wk in the direct and associative analysis, with an effect of about $0.75\sigma_p$ in the direct analysis and $0.6\sigma_p$ in the associative analysis.

The strongest associations from the direct analysis were found for Belly51 ($-\log_{10}(p\_value) = 4.12$) and BR51 ($-\log_{10}(p\_value) = 3.56$) on chromosome 4. With the associative model the strongest associations were with BR51 on chromosome 3 ($-\log_{10}(p\_value) = 6.70$) and on chromosome 5 ($-\log_{10}(p\_value) = 4.27$), and with BR69 on chromosome 5 ($-\log_{10}(p\_value) = 4.67$).

Based on this association study, some genomic regions of interest for feather pecking behaviour in laying hens have been identified. From the associative model, the SNPs rs13717237, rs13717379, rs13717382, rs13717441 and rs13717447 on chromosome 3, all lying in less than 1 cM, were associated with the traits BR51 and Belly51. The average across lines LD between these SNPs, measured by r^2, was 0.45. Again on chromosome 3, the SNPs rs13717686, rs13717773 and rs13717778, all in a range of 0.5 cM and with an r^2 of 0.15 and 0.34,respectively, were found to have an effect on feather condition of the back and rump region at 51 and 69 wk. These two regions on chromosome 3 were not in LD: the average r^2 between

Table 4 Significance of SNP allele frequency of cage mates on the feather score of individual hens

chr	SNP	Map	cM[a]	Social effects			
				BR51s	Belly51s	BR69s	Belly69s
chr Z	rs16101283	20655488	51.6	3.15 (0.06)			
	rs16101484	20904552	52.3	2.93 (0.07)			
	rs16105159	23468761	58.7	2.20 (0.10)	2.91 (0.06)		1.90 (0.07)
	rs16104871 (*GFM2*)[b]	23690970	59.2		2.35 (0.10)		
	rs16106976	33770169	84.4			1.67 (0.10)	
	rs13762897 (*TRPM3*)[b]	34827204	87.1	2.57 (0.11)			
chr 1	rs14810117	36931526	92.3				2.63 (0.06)
chr 3	rs13503459	9757137	24.4	1.85 (0.12)			
	rs13503401	9873492	24.7	2.94 (0.09)			
	rs13503220	10297909	25.7	2.28 (0.12)		1.95 (0.11)	
	rs13717237	18943533	47.4	2.89 (0.06)			
	rs13717379	19142921	47.9		2.92 (0.10)		
	rs13717382	19146715	47.9		2.74 (0.14)		
	rs13717441	19232318	48.1		2.51 (0.14)		
	rs13717447	19243861	48.1		3.13 (0.06)		
	rs13717645 (*PFN3*)[b]	19755775	49.4	3.05 (0.07)		2.05 (0.14)	
	rs13717686	19841213	49.6	2.40 (0.07)			
	rs13717773	20014822	50.0			1.79 (0.13)	
	rs13717778	20015177	50.0	6.70 (0.00)		3.25 (0.06)	
	rs13717881	20357976	50.9			1.61 (0.13)	
chr 4	rs13640917 (*HTR2C*)[b]	2798627	7.0	3.39 (0.03)		2.30 (0.01)	
	rs13512983	30896422	77.2			2.74 (0.11)	
	rs13514279	33836739	84.6			2.34 (0.08)	
	rs13515243 (*PPP2CB*)[b]	35781204	89.5	2.70 (0.10)			
	rs13517937 (*GALNT7*)[b]	44712609	111.8	2.69 (0.13)			
	rs13521963	53833480	134.6		2.51 (0.06)		
	rs13522188	54500061	136.3		2.90 (0.06)		
	rs13522598 (*TRPC3*)[b]	55438724	138.6		2.40 (0.08)		1.96 (0.07)
	rs13522688 (*TRPC3*)[b]	55714579	139.3	2.21 (0.08)			
	rs13523367	57888982	144.7	2.28 (0.10)		1.48 (0.14)	
	rs16422070	62651897	156.6		2.03 (0.12)		
chr 5	rs15661619	11950758	29.9		2.09 (0.08)		
	rs13758305	16664011	41.7			1.43 (0.03)	
	rs13794185	23057797	57.6	2.44 (0.12)	3.16 (0.06)		
	rs15681243 (*CKAP5*)[b]	25554863	63.9		2.48 (0.09)		
	rs13756469 (*RGS6*)[b]	28876011	72.2	4.27 (0.01)			
	rs13585105	32180230	80.5		3.39 (0.06)		
	rs13585316	33526797	83.8		2.62 (0.10)		
	rs13585357	33758339	84.4	3.82 (0.03)		2.08 (0.10)	
	rs13585704 (*NPAS3*)[b]	37788082	94.5	2.72 (0.11)			
	rs13585761 (*EGLN3*)[b]	38086335	95.2	2.66 (0.14)			
	rs13586409	39786663	99.5		2.37 (0.12)		
	rs13587250	42038263	105.1			1.90 (0.14)	
	rs15707740	42231488	105.6			4.67 (0.01)	
chr 6	rs13562501	5575686	13.9	2.18 (0.10)			
	rs16548180 (*NFKB2*)[b]	18021166	45.1	2.95 (0.06)		2.14 (0.03)	

Table 4 continued

chr	SNP	Map	cM[a]	Social effects			
				BR51s	Belly51s	BR69s	Belly69s
chr 7	rs13781704	23592646	59.0				1.67 (0.06)
	rs13596168	25491840	63.7				1.92 (0.13)
	rs13598049 (*SNX4*)[b]	29698020	74.2				1.76 (0.08)
	rs13598125 (*PARP14*)[b]	29835983	74.6	1.56 (0.11)			
	rs13598160	29915809	74.8	2.52 (0.06)			
	rs13601268	37224680	93.1		2.00 (0.14)		
chr 13	rs14999300 (*IL9*)[b]	15574216	38.9	2.27 (0.07)			
	rs15709659 (*IL4*)[b]	17534793	43.8		3.16 (0.06)		
chr 19	rs13573020 (*CCL4*)[b]	376195	0.9				1.30 (0.08)
	rs14119838 (*HSPB1*)[b]	4216458	10.5	3.16 (0.10)			
chr 24	rs15209193 (*TIRAP*)[b]	430067	1.1		1.82 (0.14)		

$-\log_{10}$ of the *p*-values are reported in the columns, with corresponding FDR in brackets. BR51, BR69 = sum of the individual feather scores for the back and rump regions at 51 and 69 wk of age (scale 0–10); Belly51, Belly69 = individual feather scores for the belly region at 51 and 69 wk of age (scale 0–5)

[a] 1 cM = 4×10^5 bps

[b] *GFM2* = gene encoding elongation factor G2; *TRPM3* = gene encoding transient receptor potential cation channel, subfamily M, member 3; *PFN3* = gene encoding profilin; *HTR2C* = gene encoding serotonin receptor 2C; *PPP2CB* = gene encoding protein phosphatase 2; *GALNT7* = gene encoding GalNAc transferase 7; *TRPC3* = gene encoding ransient receptor potential cation channel, subfamily C, member 3; *CKAP5* = gene encoding cytoskeleton associated protein 5; *RGS6* = gene encoding regulator of G-protein signaling 6; *NPAS3* = gene encoding Neuronal PAS domain protein 3; *EGLN3* = gene encoding Egl nine homolog 3; *NFKB2* = gene encoding Nuclear factor NF-kappa-B p100 subunit; *SNX4* = gene encoding sorting nexin-4; *PARP14* = gene encoding poly(ADP-ribose) polymerase family, member 14; *IL9* = gene encoding interleukin 9; *IL4* = gene encoding interleukin 4; *CCL4* = gene encoding chemokine CCL4; *HSPB1* = gene encoding heat shock 27 kDa protein 1; *TIRAP* = gene encoding toll-interleukin 1 receptor

them was lower than 0.01. This suggests that these are two distinct regions with different genes influencing feather pecking behaviour. On chromosome 7 a region with effect on BR51 and Belly69 was identified at SNPs rs13598049, rs13598125 and rs13598160, spanning for 0.5 cM (see Table 4). The LD between these two pairs of SNPs was 0.11 and 0.18, as measured by r^2.

In the direct model, the percentage of the phenotypic variance explained by the SNPs reported in Table 3 ranged between 1 and 4%, with an average of 1.65%. The SNPs explaining the highest proportion of the phenotypic variance were rs13773912 on chromosome 3 (2.93% of σ_p^2) and rs13640917 on chromosome 4 (4% of σ_p^2).

For the SNP effects of Table 4 (associative model), the regression coefficients of allele frequency on feather condition score were estimated (results not shown): these had on average a magnitude of $0.6\sigma_p$, with a maximum of $1.22\sigma_p$ and a minimum of $0.22\sigma_p$. For example, the SNP rs13640917 in the gene for the serotonin receptor 2C (HTR2C) had an effect of $0.54\sigma_p$ on BR51, and the SNP rs13717447 on chromosome 3 had an effect of $1.22\sigma_p$ on Belly51. Allelic frequencies for cage mates vary from 0 to 1, therefore the estimated regression coefficients reflect the maximum values when all cage mates are homozygous for the same allele.

Discussion

In the present study we looked at genetic marker effects on plumage condition from two different perspectives: the direct effect of the genotype of the individual on its feather condition, and the associative effect of the allele frequency of its cage mates on the individual's feather condition. They reflect the genetic influence of, respectively, the individual hen and the cage mates on individual feather condition. Since feather damage on the back, rump and belly regions can be attributed almost exclusively to feather pecking (Bilčik and Keeling 1999), the genetic direct and associative effects as described in this paper can be interpreted as the propensity to either receive or perform feather pecking. However, interactions among cage mates may result in a feather condition score different than expected, as for instance in cages where all hens are peckers.

With the associative model more significant results than with the direct model were found and significance levels from the associative model were generally higher than those from the direct model. This can be explained in part by the fact that performing feather pecking is more heritable than receiving it: heritability estimates for performing and receiving FP ranged from 0.12 to 0.56, and from 0.00 to 0.15, respectively (Kjaer and Sørensen 1997; Rodenburg

et al. 2003). This suggests a larger basis for associative rather than direct genetic effects. Furthermore, associative effects can contribute substantially to the total heritable variation, and have been shown to be often bigger in magnitude than direct genetic effects (Bijma et al. 2007a, b; Ellen et al. 2008).

In our experiment mortality was not high: blood samples for genotyping were collected at 40 wk of age, and only 13 of the genotyped hens died before having a feather condition score or between recording it at 51 and 69 wk. We looked at their SNP allelic frequencies and noticed that they generally had higher frequencies of the unfavourable alleles for feather damage, compared to the hens with both genotype and phenotypic observation (survivors). For SNP rs13640917 (HTR2C) on chromosome 4, for instance, the unfavourable allele frequency was 0.92 in the 13 dead hens and 0.62 in all females. This might imply that in those cases feather pecking led to cannibalism and death.

Methodology

In this study we simultaneously analysed multiple lines using a two-step procedure. In the across-line analysis, the SNPs detected were expected to be closer to the genes for behavioural traits due to the reduced extent of LD conserved across lines. Building on the work by Saccone et al. (2008), we tested for the SNP-by-line interaction to ensure consistency of the association across lines. The method is visually illustrated in Fig. 1, where a region of chromosome 1 is reported. Histogram 1A reports the results of the analysis when all the lines are included. Histograms 1B and 1C report the results for the white and brown layers. These constitute two subpopulations of different origin (White Leghorn and Rhode Island Red, respectively), both comprising different lines. White and brown layers form two distinct phylogenetic clusters (Biscarini et al. 2010), implying that LD patterns are better conserved within than across them (Andreescu et al. 2007). When analysing the two subpopulations separately many SNPs result to be significantly associated with the phenotype (BR51): 3 in the white layers and 8 in the brown layers. This reflects the larger extent of LD conserved within homogenous populations in which fewer recombinations have occurred and more surrounding SNPs are linked to the QTL. When combining the two subpopulations in the across-lines analysis, most of those SNP association signals are lost, due to either lower significance of association or significant SNP-by-line interaction. Finally, only 1 SNP (rs15385785) is consistently associated with the phenotype in all lines, and due to the lower extent of LD conserved across all lines, this is likely to be closer to the QTL for the trait. The same procedure was applied by Biscarini et al. (2010) in an association study of immune response in laying hens.

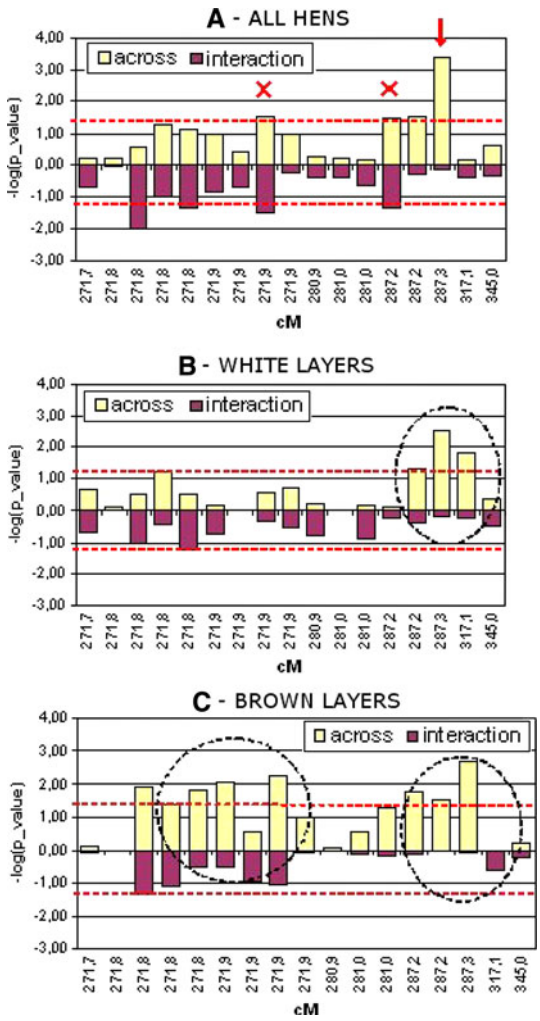

Fig. 1 significance of SNP (−log(*p* value), above 0) and of the SNP-by-line interaction (log(*p* value), below 0) for the genomic region surrounding SNP rs15385785 on chromosome 1. The combined analysis for all lines and the separate analyses for five white and four brown layers lines are presented. The dashed lines are the threshold of significance (−log(0.01) ∼ 1.3 and log(0.01) ∼ −1.3)

A possible source of false positive associations due to population stratification was avoided by including a line effect in the model. Consequently, SNPs explaining part of the between-line variation could not be detected in this approach. Family relationships within lines could be another source of false positive associations which was dealt with by including a polygenic effect in the model that accounted for the effects of all other genes on the trait. After taking into account family relationships, the significance levels as measured by the opposite of the logarithm of *p* values, decreased on average by about 10% in the direct model and by about 20% in the associative model. The analysis proved to be robust to variations in heritability: heritabilities were varied with limited impact on the significance of the results. This agrees with the results of Hassen et al. (2009).

Detected associations

Some of the results of the present work confirmed findings from previous QTL mapping studies. Buitenhuis et al. (2003a, b) detected a QTL for receiving FP on chromosome 5, and QTLs for performing FP on chromosomes 1, 4, 13 and 24. QTLs for fear related behaviours were found on chromosome 1 by Schütz et al. (2004) and Buitenhuis et al. (2004), and on chromosomes 3 and 4 by Buitenhuis et al. (2004). A relation between fearfulness and FP and its consequences has been revealed by several studies (Jones et al. 1995; Rodenburg et al. 2004; Uitdehaag et al. 2008).

Several of the associations detected in the present study were in the sequences of candidate genes that could play a role in behaviour. These include the genes for the monoamine oxidase of type A (MAO-A) and the serotonin receptor (HTR2C) from the direct model, and the genes for the cation channels (TRPM3, TRPC3), the neuronal transcription factor NPAS3 and again the serotonin receptor (HTR2C) from the associative model. In the case of SNP rs13640917, in the sequence of the serotonin receptor 2C (HTR2C), the same allele was associated with more feather damage on the back and rump at 51 wk in the direct as well as in the associative analysis. Theoretically it is expected that the opposite SNP alleles will be associated with greater feather damage in the direct analysis (receiving FP) and in the associative analysis (performing FP). That the same allele of SNP rs13640917 is associated with feather damage both in the associative and direct analysis is therefore against expectations. If one allele is associated with more feather damage in the direct analysis (feather pecking received) it should be associated with lower feather damage in the associative analysis (FP performed). Unless, in the case of a gene that leads to higher FP behaviour, all animals in a cage have the "positive" allele (more FP). Then there will be more feather pecking in the cage, and also peckers (and not only receivers) will show higher feather damage. It is not uncommon that feather peckers get pecked themselves. If there are 4 peckers in a cage this may even be likely.

Associations with feather condition score have been found also for the genes of the interleukins 4 and 9 (IL4, IL9), of the nuclear factor KB (NFKB) and of the CCL4 chemokine: these are cytokines involved in the mediation of the immune response. Relations between behaviour and immunity have been suggested also in other works. Biscarini et al. (2010) detected associations between the serotonin receptors HTR2C and HTR2A and, respectively, complement activity and antibody titres. Combining their results with the ones of the present study, we see that in the case of HTR2C higher feather damage corresponds to lower complement activity. Buitenhuis et al. (2006) found higher IgG titres and lower leukocyte concentration, CD4+ lymphocytes percentage and MHC I expression in high FP compared to low FP lines. Parmentier et al. (2009) also found a relationship between immunity and feather pecking: birds challenged with the antigen human serum albumin (HuSA) at young age were more likely to develop feather damage at a later age, compared to unchallenged birds. This points to complex and interesting links between behaviour and immune system.

From the analysis of associative effects, some SNPs on the sex chromosome Z have been associated to feather condition score on the back-rump and belly regions. These findings on the sex chromosome may relate to previous observations that feather pecking is affected by gonadal hormones and is more common in females than in males (Hughes 1973; Jensen et al. 2005).

When looking at gene effects, we consistently observed a higher frequency of the alleles linked to more feather damage in hens of White Leghorn origin as compared to Rhode Island Red origin (results not shown). For SNP rs15385785 on chromosome 1 (MAOA), for instance, the frequency of the allele associated with greater feather damage was 0.79 in brown layers and 0.94 in white layers. For SNP rs13640917 on chromosome 4 (HTR2C), it was 0.35 in brown layers and 0.84 in white layers. This is consistent with reports of more feather damage on the back and rump of white layers in comparison with brown layers (Uitdehaag et al. 2008), which indicates higher incidence of feather pecking behaviour in White Leghorns. Differences in fearfulness and in the metabolism of the neurotransmitters serotonin and dopamine between white and brown layers have also been observed (Uitdehaag, personal communication).

Social interactions

Feather pecking is a trait in which social interactions between group mates play an important role (Ellen et al. 2008). In our study we looked separately at the direct genetic effects (receiving FP) and at the associative effects (performing FP). The existence of social interaction raises the question of group composition. Groups of closely related animals (e.g. full sibs) on one hand tend to have less negative social interactions, but on the other hand pose statistical challenges. In laying hens, for instance, feather pecking and cannibalism are reduced in cages of full-sibs (Bijma et al. 2007a, b), but from such data associative effects can not be estimated (Bijma et al. 2007a, b) and variance components might be more difficult to estimate (Biscarini et al. 2008). Depending on the case, groups of animals with similar or different personality traits might perform better (Rodenburg et al. 2010). We took the SNPs for the MAOA and HTR2C and looked at the unadjusted feather condition scores. We saw that from a mere

phenotypic point of view having more heterozygous hens in a cage leads to increased feather damage. In both cases one allele is associated to increased feather damage and the other to lower feather damage. However, the effect of the number of heterozygous hens per cage was not significant when added to the statistical model. Probably the relation between feather damage and cage composition is too weak to be revealed by this dataset size and experimental design. Higher feather damage can reflect either propensity to peck or docility. Hens that tend to peck can in fact be involved in numerous fights damaging their own plumage. Other hens might be so docile that they do not respond to the pecking insult and might be preferred target for peckers. So genes linked to high or low feather damage may reflect both types of situation. Homozygous hens for the mentioned SNPs might therefore be either active peckers or docile animals. Heterozygotes will be somewhere in the middle. When only docile hens are in a cage, not much is likely to happen. When only active peckers are in a cage, they might be afraid of each other and refrain to fight. Intermediate hens might on the contrary give rise to fights resulting in higher feather damage in those cages.

The inclusion of the associative effects in the model, for traits influenced by social interactions such as FP in laying hens, can lead to a considerable increase in the genetic response to selection, thanks to the additional heritable genetic variation of the associative effects (Ellen et al. 2007).

Bijma et al. (2007b) showed that non-genetic covariance among group mates can bias the estimates of genetic associative effects. Although we modelled social interactions differently, we fitted a random group effect to asses the magnitude of such non-genetic covariance in our analysis (see Bergsma et al. 2008). The significance of the estimates of the SNPs effects from models [3] and [4] decreased only fractionally and did not affect the presented results at all.

Serotonin

Substantial scientific evidence of the role of the serotonergic system in the development and modulation of feather pecking behaviour in laying hens has accrued over the last years (van Hierden et al. 2002, 2004a, b; Bolhuis et al. 2009). These studies related the occurrence of feather pecking with serotonin concentration and activity either in the brain or peripherally (circulatory system or peripheral nervous system), predominantly suggesting that lower levels of serotonin are associated with predisposition to perform feather pecking (van Hierden et al. 2004a, b; Bolhuis et al. 2009). We detected an association between the gene for the serotonin receptor HTR2C and feather condition score in the back-rump region both in the direct (receiving FP) and associative (performing FP) analyses. Flisikowski et al. (2008) associated the gene for a regulatory factor of the serotonergic system (DEAF1) on chromosome 5 with feather pecking behaviour. The same authors postulated that finding the same association in populations of Rhode Island Red and White Leghorn origin indicates that the origin of the allele predisposing to feather pecking predates the breeding activity of at least the last 50 years. They suggest that detecting the same genetic markers in different populations implies that they are close to the functional mutation, which agrees with the theory of across-line association studies (Biscarini et al. 2010). Therefore feather pecking in laying hens can seemingly be controlled by modulating their serotonergic system, by means of genetic selection or husbandry practices, either pharmacologically or dietary (van Hierden et al. 2004a). Interestingly, the recent study by Bolhuis et al. (2009) shows that genetic selection for low mortality, using the social models, leads to changes in the serotonergic system, already in the second generation of selection.

The results of this work contribute to a better understanding of the genetic background of feather pecking behaviour in laying hens. The analysis of both direct and associative genetic effects confirmed that social interactions play an important role in the emergence of feather pecking, and is therefore a valuable tool for the investigation of this behavioural characteristic of birds. To our knowledge this was the first time that the associative effect was addressed in an association study in laying hens. The gene for the serotonin receptor (HTR2C) was found to be associated with feather damage, which adds to existing evidence of the role of the serotonergic system in the modulation of feather pecking. The involvement of the genes for interleukins (IL4, IL9) and chemokines (CCL4) points at fascinating relationships between behaviour and immunity.

Acknowledgements We would like to thank ISA B.V., the layer breeding division of Hendrix Genetics, for providing animals and facilities. We would also like to thank the staff at the ISA test facility 'Stevensbeek' for taking excellent care of the laying hens. Further, we would like to thank Esther Ellen, Laura Star, Koen Uitdehaag and Patrick Wissink for conduction the plumage condition scoring. This work was conducted as part of the SABRETRAIN Project, funded by the Marie Curie Host Fellowships for Early Stage Research Training, as part of the 6th Framework Programme of the European Commission.

References

Andreescu C, Avendano S, Brown SR, Hassen A, Lamont SJ, Dekkers JCM (2007) Linkage disequilibrium in related breeding lines of chicken. Genetics 177:2161–2169

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc B 57:289–300

Bergsma R, Kanis E, Knol EF, Bijma P (2008) The contribution of social effects to heritable variation in finishing traits of domestic pigs (*Sus scrofa*). Genetics 178:1559–1570

Bijma P, Muir WM, Van Arendonk JAM (2007a) Multilevel selection 1: quantitative genetics of inheritance and response to selection. Genetics 175:277–288

Bijma P, Muir WM, Ellen ED, Wolf JB, Van Arendonk JAM (2007b) Multilevel selection 2: estimating the genetic parameters determining inheritance and response to selection. Genetics 175:289–299

Bilčik B, Keeling LJ (1999) Changes in feather condition in relation to feather pecking and aggressive behaviour in laying hens. Br Poult Sci 40:444–451

Biscarini F, Bovenhuis H, van Arendonk JAM (2008) Estimation of variance components and prediction of breeding values using pooled data. J Anim Sci 86:2845–2852

Biscarini F, Bovenhuis H, van Arendonk JAM, Parmentier HK, Jungerius AP, van der Poel JJ (2010) Across-line SNP association study of innate and adaptive immune response in laying hens. Anim Gen 41:26–38

Blokhuis HJ (1986) Feather-pecking in poultry: its relation with ground-pecking. Appl Anim Behav Sci 16:63–67

Blokhuis HJ, Arkes JG (1984) Some observations on the development of feather-pecking in poultry. Appl Anim Behav Sci 12:145–157

Bolhuis JE, Ellen ED, Van Reenen CG, De Groot J, Ten Napel J, Koopmanschap RE, De Vries Reilingh G, Uitdehaag KA, Kemp B, Rodenburg TB (2009) Effects of genetic group selection against mortality on behaviour and peripheral serotonin in domestic laying hens with trimmed and intact beaks. Physiol Behav 97:470–475

Buitenhuis AJ, Rodenburg TB, van Hierden YM, Siwek M, Cornelissen SJB, Nieuwland MGB, Crooijmans RPMA, Groenen MAM, Koene P, Korte SM, Bovenhuis H, van der Poel JJ (2003a) Mapping quantitative trait loci affecting feather pecking behavior and stress response in laying hens. Poult Sci 82:1215–1222

Buitenhuis AJ, Rodenburg TB, Siwek M, Cornelissen SJB, Nieuwland MGB, Crooijmans RPMA, Groenen MAM, Koene P, Bovenhuis H, van der Poel JJ (2003b) Identification of quantitative trait loci for receiving pecks in young and adult laying hens. Poult Sci 82:1661–1667

Buitenhuis AJ, Rodenburg TB, Siwek M, Cornelissen SJB, Nieuwland MGB, Crooijmans RPMA, Groenen MAM, Koene P, Bovenhuis H, van der Poel JJ (2004) Identification of QTLs involved in open-field behaviour in young and adult laying hens. Behav Genet 34:325–333

Buitenhuis AJ, Kjaer JB, Labouriau R, Juul-Madsen HR (2006) Altered circulating levels of serotonin and immunological changes in laying hens divergently selected for feather pecking behaviour. Poult Sci 85:1722–1728

Ellen ED, Muir WM, Teuscher F, Bijma P (2007) Genetic improvement of traits affected by interactions among individuals: sib selection schemes. Genetics 176:489–499

Ellen ED, Visscher J, van Arendonk JAM, Bijma P (2008) Survival of laying hens: genetic parameters for direct and associative effects in three purebred layer lines. Poult Sci 87:233–239

Flisikowski K, Schwarzenbacher H, Wysocki M, Weigend S, Preisinger R, Kjaer JB, Fries R (2008) Variation in neighbouring genes of the dopaminergic and serotonergic systems affects feather pecking behaviour of laying hens. Anim Gen 40:192–199

Gilmour AR, Gogel BJ, Cullis BR, Thompson R (2006) ASReml user guide release 2.0. VSN International Ltd, Hemel Hempstead, UK

Hassen A, Avendano S, Hill WG, Fernando RL, Lamont SJ, Dekkers JCM (2009) The effect of heritability estimates on high-density single nucleotide polymorphism analyses with related animals. J Anim Sci 87:868–875

Hillel J, Groenen MAM, Tixier-Boichard M et al (2003) Biodiversity of 52 chicken populations assessed by microsatellite typing of DNA pools. Genet Sel Evol 35:533–557

Huber-Eicher B, Wechsler B (1997) Feather pecking domestic chicks: its relation to dustbathing and foraging. Anim Behav 54:757–768

Hughes BO (1973) The effect of implanted gonadal hormones on feather pecking and cannibalism in pullets. Br Poult Sci 14:341–348

Hughes BO, Duncan IJH (1972) The influence of strain and environmental factors upon feather pecking and cannibalism in fowls. Br Poult Sci 13:525–547

Jendral MJ, Robinson FE (2004) Beak trimming in chickens: historical, economical, physiological and welfare implications, and alternatives for preventing feather pecking and cannibalistic activity. Avian Poult Biol Rev 15:9–23

Jensen P, Keeling L, Schutz K, Andersson L, Mormede P, Brandstrom H, Forkman B, Kerje S, Fredriksson R, Ohlsson C, Larsson S, Mallmin H, Kindmark A (2005) Feather pecking in chickens is genetically related to behavioural and developmental traits. Physiol Behav 86:52–60

Jones RB, Blokhuis HJ, Beuving G (1995) Open-field and tonic immobility responses in domestic chicks of two genetic lines differing in their propensity to feather peck. Br Poult Sci 36:525–530

Kjaer JB (2009) Feather pecking in domestic fowl is genetically related to locomotor activity levels: implications for a hyperactivity disorder model of feather pecking. Behav Genet 39:564–570

Kjaer J, Sørensen P (1997) Feather pecking behaviour in White Leghorns, a genetic study. Br Poult Sci 38:335–343

Kjaer JB, Sørensen P, Su G (2001) Divergent selection on feather pecking behaviour in laying hens (*Gallus gallus domesticus*). Appl Anim Beahv Sci. 71:229–239

Newberry RC, Keeling LJ, Estevez I, Bilcik B (2007) Behaviour when young as a predictor of severe feather pecking in adult laying hens: the redirected foraging hypothesis revisited. Appl Anim Behav Sci 107:262–274

Parmentier HK, Rodenburg TB, De Vries Reilingh G, Beerda B, Kemp B (2009) Does enhancement of specific immune responses predispose laying hens for feather pecking? Poult Sci 88:536–542

Pigott TA (1996) OCD: where the serotonin selectivity story begins. J Clin Psychiatry 57:11–20

Rodenburg TB, Buitenhuis AJ, Ask B, Uitdehaag KA, Koene P, van der Poel JJ, Bovenhuis H (2003) Heritability of feather pecking and open-field response of laying hens at two different ages. Poult Sci 82:861–867

Rodenburg TB, Buitenhuis AJ, Ask B, Uitdehaag KA, Koene P, van der Poel JJ, van Arendonk JAM, Bovenhuis H (2004) Genetic and phenotypic correlations between feather pecking and open-field response in laying hens at two different ages. Behav Genet 34:407–415

Rodenburg TB, Bijma P, Ellen ED, Bergsma R, de Vries S, Bolhuis JE, Kemp B, van Arendonk JAM (2010) Breeding amiable animals? Improving farm animal welfare by including social effects in breeding programmes. Anim Welf 19(Suppl 1):77–82

Saccone NL, Saccone SF, Goate AM, Gruzca RA, Hinrichs AL, Rice JP, Bierut LJ (2008) In search of causal variants: refining disease association signals using cross-population contrasts. BMC Genet 9:58

Savory CJ (1995) Feather pecking and cannibalism. Worlds Poult Sci J 51:215–219

Schütz KE, Kerje S, Jacobsson L, Forkman B, Carlborg Ö, Andersson L, Jensen P (2004) Major growth QTLs in fowl are related to fearful behavior: possible genetic links between fear response and production traits in a Red Junglefowl × White Leghorn intercross. Behav Genet 34:121–130

Uitdehaag KA, Komen H, Rodenburg TB, Kemp B, van Arendonk JAM (2008) The novel object test as predictor of feather damage in cage-housed Rhode Island Red and White Leghorn laying hens. Appl Anim Behav Sci 109:292–305

Van Hierden YM, Korte SM, Ruesink EW, van Reenen CG, Engel B, Korte-Bouws GAH, Koolhaas JM, Blokhuis HJ (2002) Adrenocortical reactivity and central serotonin and dopamine turnover in young chick from a high and low feather pecking line of laying hens. Physiol Behav 75:653–659

Van Hierden YM, Koolhaas JM, Korte SM (2004a) Chronic increase of dietary L-tryptophan decreases gentle feather pecking behavior. App Anim Behav Sci 89:71–84

Van Hierden YM, de Boer SF, Koolhaas JM, Korte SM (2004b) The control of feather pecking by serotonin. Behav Neurosci 118:575–583

Vestergaard KS, Lisborg L (1993) A model for feather pecking development which relates to dustbathing in the fowl. Behaviour 126:291–308