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Feather pecking in chickens is genetically related to behavioural and developmental traits

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Abstract

Feather pecking (FP) is a detrimental behaviour in chickens, which is performed by only some individuals in a flock. FP was studied in 54 red junglefowl (ancestor of domestic chickens), 36 White Leghorn laying hens, and 762 birds from an F_2-intercross between these two lines. From all F_2-birds, growth and feed consumption were measured. Age at sexual maturity and egg production in females, and corticosterone levels in males were also measured. From 333 F_2-birds of both sexes, and 20 parental birds, body composition with respect to bone mineral content, muscle and fat was obtained by post-mortem examinations using Dual X-Ray Absorptiometry (DXA). In femurs of the same birds, the bone density and structure were analysed using DXA and Peripheral Quantitative Computerized Tomography (pQCT), and a biomechanical analysis of bone strength was performed. Furthermore, plumage condition was determined in all birds as a measure of being exposed to feather pecking. Using 105 DNA-markers in all F_2-birds, a genome-wide scan for Quantitative Trait Loci (QTL), associated with the behaviour in the F_2-generation was performed. FP was at least as frequent in the red junglefowl as in the White Leghorn strain studied here, and significantly more common among females both in the parental strains and in the F_2-generation. In the F_2-birds, FP was phenotypically linked to early sexual maturation, fast growth, weak bones, and, in males, also high fat accumulation, indicating that feather peckers have a different resource allocation pattern. Behaviourally, F_2 feather peckers were more active in an open field test, in a novel food/novel object test, and in a restraint test, indicating that feather pecking might be genetically linked to a proactive coping strategy. Only one suggestive QTL with a low explanatory value was found on chromosome 3, showing that many genes, each with a small effect, are probably involved in the causation of feather pecking. There were significant effects of sire and dam on the risk of being a victim of feather pecking, and victims grew faster pre- and post-hatching, had lower corticosterone levels and were less active in a restraint test. Hence, a wide array of behavioural and developmental traits were genetically linked to FP.

Keywords: Chicken; Feather-pecking; Behaviour; Resource-allocation; Growth; QTL

1. Introduction

Feather pecking in fowl is a detrimental behaviour in which a bird pecks at and pulls out the feathers of a

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recipient, sometimes followed by eating the removed feathers [1]. Although the behaviour can be performed with different degrees of severity, in its most severe form, it may cause naked body areas, mainly on the back and rump of the victims, and may develop into cannibalism [2]. It is therefore considered a serious welfare problem. The evolution of such behaviour is largely unexplained, and it is not known to what extent the behaviour occurs in the ancestor of domestic chickens.

The background and developmental causes of the behaviour are not fully known. Several authors consider it a redirected pecking behaviour, either derived from food or ground pecking [3] or from dustbathing [4]. It has also been suggested that the behaviour develops from a stage of stereotyped gentle feather pecking at an early age, perhaps caused by redirection of ground pecking, to severe feather pecking at a later age, possibly mediated by stimuli from damaged feathers in potential victims [5]. In an F_2 intercross between red jungle fowl and White Leghorn layers, we recently found that victims are partly predisposed by the genotype on *PMEL17*, one of the loci controlling plumage pigmentation; pigmented (wild-type) birds ran a higher risk of being feather-pecked [6].

Not all individuals in a group will develop the behaviour, a fact which has drawn research attention to the question of individual differences among birds as a possible causative factor. Various attempts have been done to use different tests in order to predict the individual propensity to develop feather pecking, and whereas some researchers have found some predictive value of certain tests [7,8], others have failed to find consistent predictions [9]. Hence, it is not likely that feather pecking is the result of a fixed individual developmental pathway. Individual differences may rather reflect different individual predispositions to react in a certain way to specific environmental and social challenges.

In line with this idea, some research has approached the possibility that feather pecking develops differentially between individuals adopting different coping strategies. A coping strategy is an individual specific and consistent physiological and behavioural reaction pattern to different challenges. Some studies have indicated that proactive coping may be associated with a larger tendency to develop feather pecking [10,11]. Since proactive coping and feather pecking are both associated with high activity levels, proactive birds may be expected to reallocate resources from fat deposition and reproduction (egg laying) to muscles, in line with the resource allocation theory [12].

The studies linking feather pecking to coping strategies are largely based on experiments on selection lines differing in feather pecking propensity [10,11], and feather pecking has a relatively high heritability (estimated h of 0.22–0.54; [13,14]). A problem with this is that the difference obtained in feather pecking may reflect a side-effect of other traits, which may inadvertently been included in the selection. Hence, the fact that feather peckers tended to adopt a more proactive coping response may be a secondary relationship to some other, unknown factor.

We used a different genetic approach, where we crossed red junglefowl, the ancestor of modern poultry [15] with a specific White Leghorn line and produced an F_2-intercross. Segregation and recombination of alleles will therefore cause traits, which are genetically linked (either because they are controlled by the same genes, or because of linked loci), to be phenotypically correlated in the F_2-generation. This allowed us to conduct two types of analyses: Firstly, by analysing the phenotypic connections between feather pecking and other traits in this generation, we may be able to find behaviours which are genetically linked to feather pecking. Secondly, by analysing a suitable set of DNA-markers, well distributed in the genome, we could search for specific chromosomal regions associated with feather-pecking behaviour, so called Quantitative Trait Loci (QTLs) [16]. We have earlier reported a number of QTLs for fear related behaviour and production traits in the same animal material [17]. However, earlier QTL-analyses based on the previously mentioned selection lines, have failed to identify any QTLs with strong effects on feather pecking [18], indicating that many genes, each with small effects, may be involved, or that the environmental component to the behaviour is strong.

The aim of the present study was to explore the phenotypic relationships between feather pecking and behaviour in stress related situations, and between feather pecking and resource allocation in an F_2 intercross between red junglefowl and White Leghorn layers, and to compare this with the differences between the parental strains. Furthermore, the aim was to search for QTLs for feather pecking in the same intercross.

2. Material and methods

2.1. Parental lines

Fifty-four red junglefowl (34 females and 20 males) and 36 White Leghorns (17 females and 19 males) were included in the study. The red junglefowl originated from a Swedish zoo population [19]. The White Leghorn line used (SLU13) has been selected for egg mass since about 30 years. It originates from a Scandinavian selection and crossbreeding experiment [20] and is maintained at the Swedish University of Agricultural Sciences. The animals were hatched and reared under identical conditions to those used for the F_2-birds (see below).

The animals were characterised for feather pecking behaviour, using the methods described below. Data for the other behavioural traits recorded in this study have been reported earlier [21]. Developmental and production related data were recorded as described below, and again some of these data have been reported earlier in other contexts [21]; a few of them are reported again here for comparison.

2.2. F_2-Birds

One male red junglefowl and three female White Leghorns were used as P_0-animals in a cross-breeding experiment. The approach to use so few founders is a common design to increase the power of QTL-detection [16]. The eggs from the P_0 were hatched and the F_1-chickens were raised under identical conditions indoors with wood-shavings as substrate and with free access to perches, food and water. Four F_1-males and 36 F_1-females were used to generate the F_2-generation (eight to ten females were assigned to each male and the groups were kept separate under the same housing conditions). The F_2-birds were hatched in six batches. In each batch the animals were kept in mixed sex groups of about 40 animals, in pens (3 × 3 m) with the same housing conditions as described above. The birds were kept on a 12 h light, 12 h dark schedule with constant room temperature (17–19 °C). At day 200 (about 29 weeks of age) the birds were transferred to single cages (81.5 cm length × 47.5 cm width, height between 37.5 and 42.5 cm) with ad libitum access to food and water, for individual measurements of food consumption and egg production. All birds were individually marked with neck marks (light plastic tags attached with a thin plastic thread through the neck skin) from hatching.

We used 762 F_2 birds in total for this study. For different measurements and comparisons, we used different subsets of the animal material, as described in connection with each specific analysis. Hence, all animals did not contribute to all data sets used in this study.

2.3. Recording of developmental traits and body composition

2.3.1. Growth, sexual maturity, food intake and egg production

All birds were weighed at days 1, 8, 46, 112 and 200 in order to obtain growth rates, using a Sartorius 1501 scale containing a built-in weighing program with an accuracy of ±0.1 g. Sexual maturity, as determined by the increased distance between *Tuber ischii* characterizing the onset of egg laying, was determined in the females using methods detailed in Ref. [21]. Individual food intake was measured during one week, starting when the birds were 28 weeks old, as detailed in Ref. [21]. Residual food intake (RFI) was calculated as the difference between actual and expected feed intake. Expected feed intake was estimated within each batch and sex using a multiple regression equation [22] including total egg weight, metabolic body weight (body weight$^{0.75}$) and plumage condition (for scoring methodology, see Ref. [2] and below). The number of eggs produced and egg weight (mean and total) was recorded during the same period of time.

2.3.2. Plumage condition

We measured the risk of being the victim of feather pecking by scoring plumage damage due to feather-pecking at between 200 and 210 days of age on three parts of the body (belly, rump, tail), known to be specific target areas for feather pecking [2]. On each of these body parts, the condition was quantified on a 6-degree scale, where 0 signified perfect condition, and 6 totally naked. The sum of these figures was used as a plumage condition score, ranging from 0 to 18. Since scoring was based on areas known to be primary targets of feather pecking [2], and there were no signs that, for example, abrasions or parasites could have caused the damages we observed, we considered poor plumage condition to reflect exposure to feather pecking.

2.3.3. Measures of body composition and bone strength

A sample of 333 F_2 birds, and 20 parental birds, were killed at an age of 230 days for measures of body composition. The bodies were defeathered and frozen immediately after killing and stored in −20 °C until analysis. At the time for analysis each bird was thawed and BMD, lean mass and fat mass of the total body were measured using a DXA (Dual X-Ray Absorptiometry) scanner (Prodigy, Lunar Co, Madison, USA) using the small animal mode for highest precision. Total body weight of the carcasses was measured prior to DXA analysis. After DXA analysis, both femurs were dissected out and analysed as specified below.

Measurement of Bone Mineral Content (BMC) and areal Bone Mineral Density (aBMD) of the femur ex vivo was performed with the Norland pDEXA Sabre (Norland, Fort Atkinson, WI, USA) and the Sabre Research software (v3.6), as described previously [23]. Peripheral Quantitative Computerized Tomography (pQCT) was performed with the Stratec pQCT XCT Research M (Norland; v5.4B) operating at a resolution of 70 μm as described previously [23]. Trabecular BMD was determined ex vivo, with a metaphyseal pQCT scan of the distal femur, and the trabecular bone was defined by setting an inner threshold to 400 mg/cm^3. Cortical bone parameters were determined ex vivo with a mid-diaphyseal pQCT scan of the femur.

The right femora, which had previously been measured with DXA and pQCT, were tested to failure in three-point bending on an electromechanical testing machine (Avalon technologies, Rochester, MN, USA). The bones were placed in such a way that the load was applied with 1 mm/s, 6 mm distal from the mid part of the femoral diaphysis with an anterio–posterior direction. An axial load cell with the range 0–500 N was used (Sensotec Inc., Columbus, OH, USA). In addition, the left femora were tested until failure in torsion on an electromechanical testing machine (Avalon technologies, Rochester, MN, USA) at a rate of 4°/s, with no axial load during the testing. A torsional load cell with the range 0–4 N m was used (RTS load cell, Transducer Techniques Inc., Temecula, CA, USA).

From the whole body DXA analysis we obtained areal densities (g/cm^2), whereafter the percentage of the body weight made up of fat, muscle, and bone mineral was

calculated. The femoral pQCT analysis provided data on cortical bone mineral content (BMC) (mg/mm), thickness of cortical bone (mm), and BMC of metaphyseal bone (mg/mm). DXA provided the BMC of the femur (g). From the biomechanical strength tests we obtained four different measures of stiffness and of the force needed to break the bone.

The eight different measures of bone strength and content—BMC, thickness of cortical bone, BMC of metaphyseal bone, BMC of femur, four measures of stiffness and breaking force—were entered into a principal component analysis, which produced one principal component with an eigenvalue over 1, and no absolute loadings lower than 0.72. This component explained 77.3% of the variation, and the factor scores were then used as a measure of "bone weakness" (higher factor score means weaker bones).

2.4. Behavioural recordings

2.4.1. Feather pecking

When the birds were about 190 days old, they were taken out of their home pens and placed in groups of 8–12 familiar birds of both sexes in a pen similar to their home pens, measuring 3×3 m with access to perches and litter on the floor. During a sample period of 30 min, all occurrences of severe feather pecking (where the performer pecked, held and pulled at a feather) were recorded together with the identity of the performer. Subsequently, each bird was classified as a feather pecker if it had performed two or more feather pecks during the observations (we disregarded all observations of only one feather peck to decrease the risk of misclassification due to occasional, uncertain recordings). Mean number of feather pecks was 14.7 (SEM = 2.4) for the birds classified as feather peckers ($n = 87$) and 0.14 (SEM = 0.02) for the rest ($n = 675$).

2.4.2. Other behavioural tests

The birds were exposed to a series of behavioural tests, providing a number of measures of reaction patterns in mildly to severely stressful situations. All birds were singly tested in an open-field (OF) test at the age of 29 weeks, before they were transferred to individual cages, with methods detailed earlier [21]. From automatically obtained video tracks, we measured total distance moved (cm) for each bird. Behavioural response to novel food and to a novel object was tested when the birds were 29 weeks of age, 1–2 weeks after the OF test, during the period when they were kept individually in cages, using methods detailed earlier [21]. The variables obtained were the number of seconds until examination of the novel object or the novel food. All birds were tested for tonic immobility (TI) response, when they were 29–30 weeks of age, during the time they were confined in individual cages, using methods detailed earlier [21]. The number of induction attempts and time until righting were recorded. All birds were exposed to a restraint test (RT), where one leg was restrained for 5 min, when they were 29–30 weeks old, using methods detailed earlier [21]. The following variables were recorded; *time to first activity:* latency in seconds until first step, jump or pull made by the bird; *time spent lying:* in seconds; *jump:* number of jumps in which both legs of the bird left the floor; *defecation:* number of defecations.

2.5. Corticosterone measurement

Blood samples were taken from males immediately before and after the open-field test (for practical reasons, it was not possible to obtain blood samples from all the animals, so it was decided to concentrate this part of the study on males only). The birds were captured in their home cages and 1.5 ml blood was taken from the right wing vein within 5 min of capturing. After testing, the same amount of blood was taken from the left wing vein. The samples were cooled in an ice-cold water bath and centrifuged for 10 min. After centrifugation 2×400 μl plasma was stored at −20 °C pending analysis. Corticosterone levels in blood were then measured at the Laboratory for Neurogenetics and Stress, Institute F. Magendie, Bordeaux, France, using a competitive protein-binding assay. The inter-assay variation was $7.7 \pm 5.4\%$, and intra-assay variation was $4.6 \pm 3.1\%$.

2.6. Treatment and analysis of phenotypic data

Males and females were mostly analyzed separately, as there was a significant effect of sex on almost all variables. Differences in proportions of feather peckers between breeds and between sexes were tested with χ^2-analysis.

Since the variables were sufficiently normally distributed, we used *t*-test to determine the differences between feather peckers and non-feather peckers for all the measured variables. Regression analysis was used to test the relations between plumage condition (as a measure of victimization) and other phenotypic traits.

Effects of sires and dams (F_1-parents) were estimated with GLM, using sire and dam as independent variables.

2.7. QTL-analysis

Blood samples were collected from all F_2-individuals, their parents (F_1) and grandparents (F_0) and all animals were genotyped for 105 genetic markers evenly distributed in the genome as previously described [24]. The sex-averaged map spanned 2750 cM and the average marker spacing was 25.7 cM.

QTL-analysis was performed on the 24 autosomal linkage groups covered using the line cross least squares based method described by Ref. [25] (for details, see Ref. [21]). These probabilities were used to calculate additive and dominance coefficients for a putative QTL at each position under the assumption that the QTL was fixed for alternative alleles in the two breeds. The trait values were

then regressed onto these coefficients in intervals of 1 cM. Detection of QTLs was based on an F-statistic that was computed from the sums of squares explained by the additive and dominance coefficients for the QTLs. The significance threshold values for genome-wide significance were derived for each trait separately by randomization tests using 1000 random permutations of the data [26]. The randomization tests were performed using a QTL mapping software implemented for parallel computing on distributed memory platforms [27]. The least squares regression model used for QTL-analysis included the fixed effects of sex and batch along with additive and dominance coefficients for the putative QTLs.

3. Results

3.1. Feather pecking in red junglefowl and White Leghorn

In red junglefowl, nine out of 34 females and none out of 20 males were classified as feather peckers (χ^2-test, $p<0.01$). In the Leghorns, the corresponding figures were two feather peckers out of 17 females, and one out of 19 males (n.s.). There was no statistically significant breed difference.

3.2. Other traits in red junglefowl and White Leghorn

Growth and production data red junglefowl and White Leghorn birds have been reported earlier, and only a few of those are repeated here for the sake of comparison [28]. In addition, a number of variables not previously described for parental strain birds are presented in Tables 1 and 2. There were no significant differences in the total bone mass or bone mineral content in relation to body weight between the breeds. Leghorn female bodies had a significantly higher fat content, whereas leghorn males had a significantly lower fat content. No meaningful factor scores for bone weakness could be calculated due to the small sample size.

Table 1
Non-behavioural traits of female red junglefowl and White Leghorns, and the statistical significance levels of the differences (t-test; NS=not significant)

Variable	Red junglefowl	White Leghorn	p-value
Weight at hatch (g)[a]	25.4±0.4	37.8±1.1	$p<0.01$
Weight at 200 days (g)[a]	799.5±22.4	1629.3±26.9	$p<0.01$
Growth 8–46 days (g)	272.5±8.6	463.5±9.73	$p<0.01$
Growth 46–112 days (g)	301.6±6.3	613.2±21.8	$p<0.01$
Growth 112–200 days (g)	167.8±9.4	466.7±19.6	$p<0.01$
Fat (% of body weight)	11.6±0.6	29.6±2.0	$p<0.01$
Bone mineral (% of body weight)	4.20±0.09	5.2±0.29	NS
Total egg weight (g)[a]	97.3±16.6	367.1±26.7	$p<0.01$
Sexual maturity (weeks)[a]	24.9±0.5	19.9±0.4	$p<0.01$

All data are given as means with standard errors. Red junglefowl $n=34$, White Leghorn $n=17$.
[a]: means reproduced from Ref. [28].

Table 2
Non-behavioural traits of male red junglefowl and White Leghorns, and the statistical significance levels of the differences (t-test; NS=not significant)

Variable	Red junglefowl	White Leghorn	p-value
Weight at hatch (g)[a]	27.9±0.5	38.4±0.9	$p<0.01$
Weight at 200 days (g)[a]	1119.1±30.9	2107.2±34.4	$p<0.01$
Growth 8–46 days (g)	370.0±12.5	571.8±15.5	$p<0.01$
Growth 46–112 days (g)	552.8±13.6	1104.2±15.5	$p<0.01$
Growth 112–200 days (g)	122.3±16.6	340.3±22.1	$p<0.01$
Baseline corticosterone (ng/ml)	1.5±0.1	2.3±0.3	NS
Post-test corticosterone (ng/ml)	2.0±0.4	3.4±0.6	NS
Fat (percent of body weight)	18.1±1.4	9.3±0.9	$p<0.01$
Bone content (percent of body weight)	4.60±0.09	4.6±0.2	NS

All data are given as means with standard errors. Red junglefowl $n=20$, White Leghorn $n=19$.
[a]: means reproduced from Ref. [28].

3.3. Feather pecking and non-behavioural traits in F_2-birds

Feather pecking individuals were significantly more common among females than males (18% vs 7.9% of the birds; $p<0.001$, χ^2-test).

As shown in Tables 3 and 4, there were several connections between traits associated with development and resource allocation, and feather pecking. In females, feather peckers became sexually mature earlier, and grew faster up to 112 days of age, although there were no differences in initial or final body weight. Feather pecking females had significantly lower bone mineral content and significantly weaker bones. Male feather peckers had a body composition with a higher proportion of fat, and, like the females, had weaker bones. There were no differences in growth pattern between male feather peckers and non-feather peckers.

There were no differences in corticosterone levels between feather peckers and other birds.

Table 3
Non-behavioural traits of female non-feather peckers and feather peckers in the F_2-generation, and the statistical significance levels of the differences (t-test; NS=not significant)

Variable	F_2 non-feather peckers	F_2 feather peckers	p-value (number of tested birds)
Weight at hatch (g)	37.0±0.2	37.5±0.45	NS (379)
Weight at 200 days (g)	1073.6±8.3	1099.8±16.9	NS (379)
Growth 1–8 days (g)	10.0±0.27	11.4±0.63	0.04 (379)
Growth 8–46 days (g)	247.2±2.35	257.7±4.92	0.07 (379)
Growth 46–112 days (g)	486.9±4.5	516.2±10.2	0.009 (379)
Growth 112–200 days(g)	292.3±5.1	276.9±11.37	NS (379)
Fat (percent of body weight)	18.5±0.6	17.9±1.2	NS (148)
Bone mineral (percent of body weight)	4.00±0.05	3.70±0.09	0.004 (148)
Bone weakness (factor score)	0.84±0.06	1.15±0.08	0.03 (110)
Total egg weight (g)	221.1±4.4	222.3±9.9	NS (322)
Sexual maturity (weeks)	23.3±0.1	22.1±0.3	0.004 (322)

All data are given as means with standard errors.

Table 4
Non-behavioural traits of male non-feather peckers and feather peckers, and the statistical significance levels of the differences (*t*-test; NS=not significant)

Variable	F_2 non-feather peckers	F_2 feather peckers	*p*-value (number of tested birds)
Weight at hatch (g)	37.3±0.2	37.4±0.9	NS (380)
Weight at 200 days (g)	1487.0±10.4	1507.0±33.7	NS (380)
Growth 1–8 days (g)	10.5±0.3	11.0±1.1	NS (380)
Growth 8–46 days (g)	290.3±2.9	276.6±8.0	NS (380)
Growth 46–112 days (g)	727.8±6.1	733.1±21.2	NS (380)
Growth 112–200 days (g)	420.7±6.1	446.2±18.1	NS (380)
Baseline corticosterone (ng/ml)	3.1±0.09	3.4±0.4	NS (350)
Post-test corticosterone (ng/ml)	3.9±0.09	4.4±0.6	NS (344)
Fat (percent of body weight)	14.7±0.4	18.9±2.2	0.03 (154)
Bone content (percent of body weight)	4.4±0.04	4.4±0.1	NS (154)
Bone weakness (factor score)	−0.78±0.05	−0.38±0.15	0.04 (121)

All data are given as means with standard errors.

3.4. Feather pecking in relation to other behavioural traits in F_2-birds

Taking the data from both males and females into account (Table 5), feather peckers were faster at approaching both novel food and a novel object, and they moved longer distances in the OF test (not significant for any of the two sexes when analysed separately, although the tendency was the same in both). Feather-peckers were also more active in the restraint test, measured as numbers of jumps but there were no differences between the categories of birds in the tonic immobility test. In addition, male feather peckers, but not females, had a significantly higher frequency of defecation in the restraint test (1.8±1.4 vs 1.2±1.3 defecations per test; $p=0.03$, $df=379$).

3.5. Behavioural and non-behavioural traits of victimized F_2-birds

There was no significant correlation between plumage condition and the propensity to feather peck. In both sexes there were significant relationships (regression analysis, $p<0.05$) between a poor plumage condition, indicating higher exposure to feather pecking, and higher hatch weight, higher growth rates, and higher adult body weight. Furthermore, males with poorer plumage condition had a lower fat content (regression analysis, $p<0.05$).

Both in males and females, there were significant relationships between poor plumage and high feed intake (regression analysis, $p<0.01$), and in males (but not females) also a higher residual feed intake (regression analysis, $p<0.05$). In males (not measured in females), poorer plumage condition was significantly related to lower corticosterone levels ($p<0.05$), both before and after the OF test.

Behaviourally, there were few significant relationships to poor plumage, but birds with poorer feather condition had a longer latency till first activity in the restraint test, and males with poorer plumage defecated less during the restraint test (regression analysis, $p<0.05$ for both variables).

3.6. Genetic analysis

There were no significant effects of either sire or dam on the frequency of feather peckers in the F_2-generation. However, there was a significant effect of both on feather condition (sire: $p<0.001$; dam: $p<0.01$), and this was true for both sexes.

There was also a significant effect of sire ($p<0.03$) on corticosterone levels before the OF-test, but not of dam. There was a tendency for a sire-effect also on corticosterone levels after the OF-test ($p=0.07$), but again, no similar effect of dam.

One suggestive QTL for feather pecking was detected on chromosome 3, position 128 cM (flanking markers: HUJ006 and LEI161). The *F*-value was 7.1 (threshold for $p<0.2$ is 6.0, and for $p<0.05$, 8.0). The additive effect (a) of this QTL was −1.45 (SE=0.5) and the dominance effect (d) was −2.37 (SE=0.6). The negative values of the coefficients indicate that the White Leghorn alleles at this locus gave higher trait values. The residual variance explained by the QTL was 0.7%. A significant QTL, and its causative gene, for feather condition has been reported earlier [6].

There was also a significant QTL for corticosterone basal levels (before OF-test) on chromosome 1, position 298 (Flanking markers: LEI088 and LEI139; $F=7.2$; $a=-0.34$, $d=-0.1$; for corticosterone variables, $p<0.2$ threshold is $F=5.0$, and $p<0.05$ threshold is $F=7.0$), and a suggestive QTL on chromosome 7 position 54 (Flanking markers: MCW236 and MCW133; $F=5.7$, $a=0.4$, $d=-0.6$). The first QTL also affected the difference in corticosterone levels over the OF-test ("corticosterone reactivity") ($F=5.31$, $a=0.6$, $d=0.6$), and there was yet one QTL for corticosterone reactivity on chromosome Z, position 38 (Flanking markers: MCW055 and ADL272; $F=5.4$, no a

Table 5
Behavioural traits of F_2 feather peckers and non-feather peckers, as measured in four behavioural tests, and the statistical significance levels of the differences (*t*-test, NS=not significant), and the number of birds included in the analysis

Variable	Non-feather peckers	Feather peckers	*p*-value (number of tested birds)
Novel food approach (s)	95.5±1.6	83.2±4.9	0.009 (759)
Novel object approach (s)	149.0±2.14	132.1±6.6	0.008 (759)
Distance moved in OF (cm)	636.8±34.4	855.2±105.3	0.01 (757)
TI attempts (number)	1.67±0.03	1.73±0.09	NS (675)
TI duration (s)	103.9±5.11	96.2±15.4	NS (673)
Restraint, first activity (s)	44.8±2.1	48.4±6.4	NS (757)
Restraint, time lying (s)	53.5±5.5	80.0±18.6	NS (758)
Restraint, jump (number)	0.76±0.03	1.1±0.1	0.003 (758)
Restraint, defecation (number)	0.83±0.04	0.9±0.1	NS (778)

All data are given as means with standard errors.

and *d* values calculated on sex chromosome). For behavioural, reproductive and growth variables, a number of QTLs have been reported earlier from the same animal material [17,21,24,29].

4. Discussion

Our results show that feather pecking, both in the parental strains and in the F_2-generation, was more common in females. Furthermore, feather peckers were more investigative in the novel food and novel object test, moved more in the open field test, and performed more active escape attempts in the restraint test. This indicates that feather peckers had a higher activity in stressful situations. The results also showed that female feather peckers tended to grow faster and start laying earlier, and male feather peckers tended to accumulate more fat. Feather peckers had weaker bones, indicating less allocation of bone minerals to the skeleton. We also found that a suggestive QTL on chromosome 3 was associated with the performance of the behaviour, but the explanatory power of the QTL was small. Victims were more common among birds with higher growth rates, both pre- and post-hatching, and they had lower corticosterone levels.

Although not significant (probably due to low number of animals), feather pecking seemed more frequent among junglefowl than among the Leghorns. It is well known that feather pecking differs widely among strains of domestic chickens [9,14,30]. Although our observations are limited to one population under a restrictive observation schedule, the results strongly indicate that feather pecking has not emerged during domestication and selection, but is expressed also in the ancestor, when the animals are kept in captivity.

Feather pecking was more common among females, which is consistent with other studies [1]. Hormonal effects have been implicated [31], and it is also possible that it derives from the fact that the nutritional requirements of females may be higher due to egg production. This could theoretically lead to a higher general pecking tendency in females, or to specific hunger for nutrients available in feathers.

We found a number of connections between the tendency to feather peck and other behavioural measures. In general, feather peckers were more active and investigative (perhaps reflecting less fearfulness in novel situations) in several of the test variables, but we found no connection between feather pecking and behaviour in a tonic immobility test. The high activity in the tests performed may indicate that feather peckers adopted a more proactive coping pattern, as has previously been suggested [10]. However, the lack of difference in tonic immobility and in corticosterone levels, which both ought to differ between proactive and reactive copers, do not fit this explanation (but previous data on this have been inconsistent; for example, see Ref. [32]). The fact that this reaction pattern was correlated to feather pecking in F_2-birds indicates that there is a common genetic ground for coping and the propensity to develop feather pecking. In earlier research, based on selection lines, conflicting results have been found in several of these respects. For example, Jones et al. [33] found that birds from a high feather pecking line were less active in an open-field, whereas Rodenburg et al. [8] found the opposite in the same lines of birds.

Feather peckers differed from non-feather peckers in the way in which they allocated resources to growth, body composition and reproduction. Female feather peckers grew faster and started laying earlier, and male feather peckers accumulated more fat. Both male and female feather peckers had weaker bones, possibly as a result of a prioritization of resources for growth and egg production. These are traits, which have been targets of selection in poultry breeding, and the present results suggest that they may be genetically linked to feather pecking. However, feather pecking was not more common in White Leghorns than in red junglefowl, which indicates that other selection forces may have counteracted the behaviour.

Victims appeared not to be afflicted at random, since birds exposed to feather pecking had a faster growth already pre-hatching, and a larger adult body weight. These findings support other results, showing that victim traits are important in the development of feather pecking [6]. The mechanisms are not clear, although fluctuating asymmetry may be a mediating effect—birds with high growth rates may be more asymmetric, and asymmetry has been shown to be important for the risk of being victimized [34]. Behaviourally and physiologically, there were few indices of specific victim traits. A somewhat more passive reaction pattern in the restraint test and lower corticosterone levels may perhaps indicate that victim birds were less active and agitated. The higher feed consumption is most likely related to the fact that the poor plumage condition may have required the birds to increase their feed intake in order to maintain body temperature.

No sire- or dam-effects for feather pecking were observed, whilst there were significant effects of both on plumage condition, which again demonstrated the importance of the victim traits. This may have been partly mediated by the plumage pigmentation, which has been shown to be crucial for the risk of being victimized in this population of birds [6].

In spite of a QTL search based on a high marker density and a powerful cross, which has revealed many QTLs for other traits previously [17,21,24,29], we found only one suggestive QTL for feather pecking. Furthermore, the QTL was not located close to any QTL, which we have found earlier. It had a low explanatory value, and a low significance level. The leghorn alleles at this locus were associated with higher frequencies of feather pecking. A

possible explanation for the scarcity of QTLs is that this behaviour is controlled by many small QTLs, which may have escaped detection with our relatively conservative analysis methods. Buitenhuis et al. [18] found one significant QTL for severe feather pecking, in that case on chromosome 2, in a cross between a high and a low feather pecking Leghorn line. However, like in our study, that QTL had a limited explanatory value. This supports the idea that the behaviour is controlled by a large number of loci, each with small effects, or that the behaviour is strongly affected by environmental effects.

We also did not find any QTLs with large effects on corticosterone levels, and those which were identified were not closely associated with QTLs for either feather pecking or plumage condition. A QTL affecting tonic immobility has earlier been found on chromosome 1, position 236 [17], which is about 60 cM distant from the corticosterone QTL reported here. The distance makes it unlikely that the loci should be identical.

The present study mainly aims at increasing the basic knowledge about the background of feather pecking behaviour. However, it also suggests some future practical implications. For example, since many genes obviously exert small effects, selection against the behaviour in commercial lines will probably have to be based on the behaviour itself (selection of phenotypes), rather than on, i.e., marker assisted selection. Furthermore, it is unlikely that selection against feather pecking will have negative effects on the most important production related traits, i.e., egg production, although a correlated negative effect may be expected on growth. In addition, our results suggest that selection against feather pecking may have a correlated improvement effect on bone weakness in layers, which is a serious welfare problem in some lines of hens [35].

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