

QTL Analysis of a Red Junglefowl × White Leghorn Intercross Reveals Trade-Off in Resource Allocation between Behavior and Production Traits

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Received 7 Dec. 2001—Final 22 May 2002

Behaviors with high energetic costs may decrease in frequency in domestic animals as a response to selection for increased production. The aim of this study was to quantify production traits, foraging behavior, and social motivation in F₂ progeny from a White Leghorn × red junglefowl intercross (n = 751–1046) and to perform QTL analyses on the behavioral traits. A foraging-social maze was used for behavioral testing, which consisted of four identical arms and a central box. In two arms there was *ad libitum* access to the birds' usual food, and in the other two there was novel food (sunflower seeds) mixed with cat litter. In one arm with each of the two food sources, social stimuli were simulated by the presence of a mirror. Each bird could therefore feed on novel or well known food either alone or in the perceived company of a conspecific. Egg production, sexual maturity (females), food intake, and growth were measured individually, and residual food intake and metabolic body weight were estimated using standard methods. A genome scan using 104 microsatellite markers was carried out to identify QTLs affecting behavioral traits. Phenotypic growth rates at different ages showed weak associations in both sexes. Sexual maturity and egg weight were not strongly correlated to growth, indicating that these traits are not genetically linked. Time spent in each arm and in the central part of the maze was analyzed using principal component analyses. Four principal components (PC) were extracted, each reflecting a pattern of behavior in the maze. Females with early onset of sexual maturity scored higher on the PC1 reflecting preference for free food without social stimuli, and females with higher egg production scored higher on the PC2 reflecting exploration. Males with an overall higher growth rate and higher residual food intake scored higher on the PC3, which possibly reflected fear of the test situation, and tended to score higher on the PC4 reflecting low contrafreeloading. Significant QTLs were found for PC1 and PC4 scores on chromosomes 27 and 7, respectively. The location of the QTLs coincided with known QTLs for growth rate and body weight. The results suggest a trade-off between energy-demanding behavior and high production and that some of this may be caused by genetic linkage or pleiotropic gene effects.

KEY WORDS: QTL analysis; fowl; resource allocation; behavior; production traits.

INTRODUCTION

Artificial selection for high production, i.e., high feed conversion efficiency, has been a major goal in animal breeding during the past decades. Average production levels in farm animals have increased by more than 80% during the past 50 years (Rauw *et al.*, 1998) and the selection for high production has led to a number of correlated and undesired responses in farm

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animals. Disturbances in behavior, physiology, and immune functions have been observed in, for example, broilers, pigs, and cattle (reviewed by Rauw *et al.*, 1998). Many of these problems are assumed to be linked to genetic selection for high production. With modern molecular techniques it is possible to study the genetic background of complex traits such as behavior. This may provide suggestions for improved selection programs, where animal welfare is given a major importance.

With respect to behavior in laying hens, selection for high feed conversion efficiency (low residual food intake) has been found to be correlated with lower levels of activity compared to less efficient animals (Braastad and Katle, 1989). The White Leghorn is a breed that has been selected for commercial egg production and high feed conversion efficiency. Compared with its wild ancestor, the red junglefowl (*Gallus gallus*, West and Zhou, 1989), the behavior of the Leghorn has been modified in many ways. For example, when given a free choice between food sites that require different amounts of energy to obtain the food, junglefowl prefer to feed from the site that requires more effort, whereas the Leghorn obtain a higher proportion of food from the site that requires less effort (Schütz and Jensen, 2001; Schütz *et al.*, 2001). This behavior, to search for food even though food can otherwise be easily obtained, is referred to as contrafreeloading and has been shown in many species, such as gerbils (Forkman, 1993), rats (Carder and Berkowitz, 1970), starlings (Inglis and Ferguson, 1986), and pigeons (Neuringer, 1969). Contrafreeloading is suggested to be of adaptive importance in a natural environment, since it might be beneficial for a wild animal to invest a certain proportion of energy into searching for possible future food sites (Inglis *et al.*, 1997). The behavior has also been shown to exist to some extent in the domestic hen (Duncan and Hughes, 1972). Compared with red junglefowl, Leghorns ingest a higher proportion of food (about 65% compared with about 30%) from a food site that requires a minimum of effort (Schütz and Jensen, 2001; Schütz *et al.*, 2001; Lindqvist *et al.*, submitted).

Furthermore, higher frequencies of general activity and social behavior (aggressive, nonaggressive, and affiliative behavior) have been found in immature junglefowl and a Swedish bantam breed (a Swedish breed of chicken that has not been selected for production traits) compared with White Leghorns. A more active response in fearful situations in junglefowls also indicates a genetically induced difference in behavioral strategies in the Leghorn (Schütz *et al.*, 2001).

Modifications toward less costly behaviors can be explained by the theory of resource allocation (Beilharz *et al.*, 1993; Beilharz, 1998; Beilharz and Nitter, 1998). According to that theory, an individual has a limited amount of resources that is allocated to all biological activities, such as growth, reproduction, immune functions, and different behaviors. Resources can be limited due to environmental causes, such as lack of mating partners or food access, or by morphological or metabolic limits, such as feed intake capacity and feed conversion efficiency. An individual's fitness depends on the number of progeny that survives to reproductive age and each individual will therefore tend to optimize behavioral strategies in order to maximize its fitness. An equilibrium will be established in every environment and, if conditions change, a new equilibrium with modified behaviors will be established. If, for example, the amount of available resources increases, the fitness of the animal increases and new optimal behaviors and a new equilibrium will be established. Behaviors differ in their energetic costs (Deerenberg and Overkamp, 1999), and it is therefore important for the animal not to spend resources on behaviors that fail to improve its fitness. It is likely that during the domestication process, animals were able to increase their reproductive fitness when natural selection was modified through human influences. When selection pressure for high production increased, animals were genetically selected to invest a higher proportion of resources into production, such as fast growth, high milk, or high egg production. Also the housing conditions of animals were considerably changed, and it is very likely that environmental and physiological cues again cause resources to be limited. This is likely to be reflected in the animals' behavioral repertoire. Energetically costly behaviors can therefore be expected to decrease in frequency in animals selected for high production, especially if certain behaviors are not directly linked to the fitness of the animal in the environment where the selection is performed. The genetic mechanism for such correlated responses is not clear. Several possibilities exist, for example, genetic linkage or common regulation of several traits by a few genes. To start investigating such mechanisms, we decided to analyze both phenotypic correlations and the genetic bases of the important traits.

Behaviors are examples of complex quantitative traits and are likely to be influenced by multiple genes as well as by multiple environmental factors. QTL analyses (quantitative trait locus) can be used to scan the genome for linkage between DNA markers and genes that are involved in the regulation and control of behavior (Andersson, 2001). By using DNA markers throughout

the genome, it is possible to identify chromosomal regions controlling variation in the behaviors studied. One strategy for QTL analysis is to use F_2 intercrosses between two lines or breeds that differ considerably in phenotypic traits. Each F_2 animal has a unique combination of alleles from the parental lines. The techniques in molecular genetics are progressing very rapidly and QTLs affecting behaviors have been found, for example, in honeybees (Hunt *et al.*, 1998; Page *et al.*, 2000), mice (Flint *et al.*, 1995; Wehner *et al.*, 1997; Talbot *et al.*, 1999; Turri *et al.*, 2001a, 2001b), rats (Ramos *et al.*, 1999), and in cattle (Schmutz *et al.*, 2001).

One aim of this study was to investigate phenotypic correlations in an F_2 intercross between red junglefowl and White Leghorn with respect to different foraging strategies and social behaviors. Production data were recorded to study phenotypic correlations to the behavior of the animals in line with the resource allocation theory. A further aim was to scan the genome for possible QTLs associated with the behavioral traits. We hypothesized that animals showing resource demanding traits like rapid growth, high egg production, or low residual food intake would display more energy-conserving behaviors, i.e., that they would feed on easily obtainable food and show less motivation to engage in social interactions, compared to animals that do not invest a high proportion of energy into production. We also hypothesized that, genetically, there would be distinct QTLs for behavioral traits that differ clearly between White Leghorns and junglefowl and that behaviors that are phenotypically related to different production traits would be associated with QTLs for the corresponding production traits.

MATERIALS AND METHODS

Parental Lines

Twenty red junglefowl and 31 White Leghorns were hatched and bred using the same housing conditions as the F_2 birds (see later) during 2001. These birds were treated and tested in the same way as the F_2 birds, except for not being tested in the foraging-social maze (see later), as data already have been published with respect to the behavior in the maze (Schütz *et al.*, 2001).

F_2 Birds

A total of 1046 F_2 progeny from a White Leghorn × red junglefowl intercross were hatched in six batches during May–December 1999. Some animals died during rearing, some lost their individual markings, and

others were excluded from different tests for various practical reasons. The number of animals for which data were obtained on all phenotypic traits and all DNA markers was 751. The number of animals included in different analyses varied, depending on the subset of variables being analyzed.

One male red junglefowl and three female White Leghorn were used as F_0 animals. The technique to use so few founders is a common method to maximize the power of QTL detection in the F_2 intercross (Weller, 2001). The red junglefowl male originated from zoo birds and was obtained from Tovetorp research station (Stockholm University). The White Leghorn line (SLU13) has a long history of selection for egg mass. SLU13 originates from the Scandinavian selection and crossbreeding experiment (Liljedahl *et al.*, 1979) and is maintained at the Swedish University of Agricultural Sciences. The eggs from the F_0 were hatched and the F_1 chickens were raised under similar conditions at Tovetorp research station. They were transferred to research facilities outside Skara at around 10 weeks of age and kept 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 (8 to 10 females were assigned to each male and the groups were kept separate under the same housing conditions). The females were placed in single cages during 2 weeks for egg collection. The eggs were kept in a refrigerator (8°C) and turned twice per day until the start of incubation, at a maximum of 14 days after laying. The F_2 birds were hatched in six batches for practical reasons. In each batch the animals were kept in mixed sex groups of about 40 animals, in pens (3 × 3 m) with wood-shavings as substrate and free access to perches, food, and water, to standardize housing conditions. The birds were kept on a 12-h light 12-h dark schedule with constant room temperature (17–19°C). At day 200 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. All birds were individually marked with neck marks (light plastic tags attached with a thin plastic thread through the neck skin) from hatching.

Recording of Phenotypic Traits

Growth

All birds were weighed at day 1, 8, 46, 112, and 200 to obtain growth rates. A scale with an animal weighing program was used (Sartorius 1501) ranging from 0 to 11,000 g with an accuracy of 0.1 g.

Sexual Maturity

The cloacal region of all females was palpated once every week starting from the day when the first egg was found in the pen. The females were considered to be sexually mature when the distance between left and right *Tuber ischii* was approximately 2.5 cm (Gunnarsson, 2000). It was not possible to measure sexual maturity in the males.

Residual Food Intake and Egg Production

Food intake was measured at 29 weeks of age during 1 week. The birds were individually housed in cages as described above with *ad libitum* access to food and water. A ration of 1000 ± 0.2 g commercial chicken food for each animal was weighed at the beginning of the week. The food was kept in a waterproof box marked with the individual code of the animal for which it was intended. At day 1, about 200 g of feed was placed in a food trough in front of the bird outside the cage. The food trough was made of metal with high walls to minimize food spillage. Every day additional food from the food box was placed in the trough, depending on the amount of food the bird ingested, so the animals always had *ad libitum* access to food. At the end of the week, the remaining food was weighed in the trough and in the food box.

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 including egg weight, metabolic body weight ($\text{body weight}^{0.75}$) and plumage condition (Luiting and Urff, 1991). The number of eggs produced and egg weight (mean and total) was recorded during the same period of time.

Behavior in the Foraging-Social Maze

All birds were tested in a specially constructed foraging-social maze at 28 weeks of age. Four birds were tested simultaneously in four identical mazes, all of which were located in the same, separate test room. Each arena consisted of four arms (0.8 m length \times 0.5 m width \times 0.5 m height, each) and one central box (0.5 \times 0.5 \times 0.5 m) arranged as in a plus-maze. The mazes were made of 5-mm-thick hardboard siding with solid sides. In two of the arms, sunflower seeds mixed with cat litter were placed, thus providing novel food, which the birds had to spend energy to obtain. The other two arms were provided with freely available commercial laying hen food, which the birds were familiar to. At the end of two of the arms, one with each type

of food, a mirror was placed at an angle of about 20° outside the arms. The mirror provided a simulated social stimulus and the angle prevented the bird from seeing this until it was close to the mirror. Hence, the “companion bird” in the mirror could be viewed when the bird was located in the arm but not from the central box. Hens have good visual acuity and it has been shown that slides of other hens are perceived as unknown conspecifics (Bradshaw, 1992); we therefore assumed that the same would apply to a mirror image.

Wire mesh was used as a roof and as a barrier between the mirror and the arms. The level of illumination in each arm was between 70 and 75 lux during tests. Outside the maze were two boxes (0.5 \times 0.5 \times 0.5 m) with two sides of wire mesh, each containing a companion bird. In the central part of each arm of the maze, there was one opening on one side (0.4 \times 0.4 m) facing towards one of the two companion boxes. This means that the focal bird could view a familiar bird when it was in the central box, but not when it went into the arms. This was meant to prevent the birds experiencing social isolation that might disturb the test results.

The birds were randomly divided and tested in trios that were familiar to each other by being housed together in the same pen. When one bird in the trio was being tested, the other two acted as companion birds. Four birds were tested simultaneously in the four arenas for 20 min before changing to the next bird in the trio, followed by the third individual. The animals were introduced into the arenas in complete darkness, which prevented them from any activity before the simultaneous start of the test sessions. The tests were started by turning on the light in the test room.

The percent of time spent in each arm was recorded using the Ethovision software package (version 2.1, Noldus Technology). A video image of each of the four arenas was used to record the movements of the hen, and the time spent in the different arms in the maze. The animal was detected by the subtraction method, which means that the Ethovision detected all objects that differed from the background image obtained when the arena was empty (sample rate: 3.57 samples/sec).

Treatment and Analysis of Phenotypic Data

The duration of time spent in each arm and the central box were subjected to a principal component analysis (Minitab version 12.21), which extracted four components with an eigenvalue > 1 . The effects of the different production traits on the principal component

scores of each individual were analyzed using analysis of covariance (GLM, Minitab version 12.21). In the model we included the effect of batch, test arena (1–4), test order within trio (1–3), time of day, RFI, and growth (grams and percent of body weight) with RFI and growth as covariates. No systematic effects of any particular batch, arena, time of day, or position in test order were found (using Tukey's post-hoc test), so these variables were not considered further. However, they were retained in the models. Because growth values in grams and percent were correlated, two identical models were used with the difference that one contained the growth in grams (model a) and the second contained the growth in percent of body weight (model b). Males and females were analyzed separately because there was a significant effect of sex on all PC scores except for PC 1. The variables sexual maturity, mean egg weight, and total egg weight were included in the model for females.

Patterns in the correlations between production variables in each sex were analyzed using a principal component analysis (Minitab version 12.21), which extracted two components for males and three components for females with an eigenvalue > 1. Based on the shape of the scree plot for females only, the two first components were further analyzed for both sexes.

Effects of breed on the production data of the parental lines were analyzed using analysis of covariance (GLM, Minitab version 12.21) for body weight at 1 and 46 days of age, and for residual food intake. For body weights at 10, 112, and 200 days of age, metabolic body weight, sexual maturity, egg number, egg weight, and food consumption, the nonparametric Kruskal-Wallis test was used (Minitab version 12.21) because data were found not to be normally distributed (Anderson-Darling's test for normality).

OTL Analysis

DNA Isolation and Genetic Marker Analysis

Blood samples were collected from all F₂ individuals, their parents (F₁) and grandparents (F₀), and DNA was isolated using the DNeasy 96 Tissue Kit for mouse tails (Qiagen) with some modifications. All animals were genotyped for 104 genetic markers evenly distributed in the genome as described in detail by Kerje *et al.* (submitted). Linkage maps for 24 autosomal linkage groups were computed with the CRI-MAP software (Green *et al.*, 1990). The sex-averaged map spanned 2750 cM, and the average marker spacing was 25.7 cM.

QTL Mapping

QTL analysis was performed on the 24 autosomal linkage groups covered using the line cross least-squares-based method described by Haley *et al.* (1994). Marker genotypes were used to estimate the probabilities of breed origin of each gamete at 1-cM intervals throughout the genome for each F₂ individual. 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 at 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 QTL. The significance threshold values for genome-wide significance were derived for each trait separately by randomization tests using 1000 random permutations of the data (Churchill and Doerge, 1994). The randomization tests were performed using a QTL mapping software implemented for parallel computing on distributed memory platforms (Carlborg *et al.*, in press).

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 QTL for all traits. For the traits collected from the foraging-social maze, the arena was added as a fixed effect together with co-variables for the weight from 46 to 112 days and the residual food intake.

The study was approved by the local ethical committee of The Swedish National Board for Laboratory Animals. The committee assessed the welfare of the animals in relation to the purpose of the study and the possibility for the problem to be solved without the use of experimental animals and ascertained that the experiment was not an unnecessary repetition of previous experiments.

RESULTS

Phenotypic Analysis

Table I shows data for production traits for red junglefowl, White Leghorn, and the F₂ progeny (mean ± SD). Female White Leghorn had higher body weight at all ages compared with the female junglefowl, and this was also the case for males at all ages except at 1 day of age. Consequently, Leghorns had higher metabolic body weight compared with the junglefowl. Leghorns consumed a higher amount of food compared

Table I. Means and Standard Deviations of Production Traits in F₂ Progeny (*n* = 793–1046) and the Parental Lines, Red Junglefowl (*n* = 20), and White Leghorn (*n* = 31)

	Red Junglefowl (mean ± SD)	White Leghorn (mean ± SD)	F ₂ Progeny (mean ± SD)
Production trait males			
Body weight 1 day (g)	27.9 ± 2.4	38.4 ± 3.8	37.2 ± 3.8
Body weight 8 days (g)	(10 days) 74 ± 9.5 ^a	(10 days) 87.6 ± 7.6 ^b	48 ± 6.1
Body weight 46 days (g)	444 ± 62.2 ^a	659.4 ± 50.1 ^b	340.6 ± 57.1
Body weight 112 days (g)	996.8 ± 112.5 ^a	1766.8 ± 102.7 ^b	1067.0 ± 145.8
Body weight 200 days (g)	1119.1 ± 136.3 ^a	2107.2 ± 148.3 ^b	1486.5 ± 196.3
MBW	193.2 ± 17.7 ^a	310.9 ± 16.4 ^b	239.0 ± 23.7
Food consumption (g/wk)	437.5 ± 96 ^a	592 ± 144.4 ^b	558.9 ± 116.6
Excess food intake (g/g MBW)	2.3 ± 0.4	1.9 ± 0.5	2.3 ± 0.5
Production trait females			
Body weight 1 day (g)	25.4 ± 2.4 ^a	37.8 ± 4.7 ^b	37 ± 3.8
Body weight 8 days (g)	(10 days) 57.6 ± 10.4 ^a	(10 days) 81.9 ± 9.3 ^b	47.4 ± 6.1
Body weight 46 days (g)	330.1 ± 58.3 ^a	545.4 ± 43.8 ^b	298.7 ± 45.3
Body weight 112 days (g)	631.7 ± 90.4 ^a	1158.7 ± 101.8 ^b	787.2 ± 109.7
Body weight 200 days (g)	799.5 ± 130.1 ^a	1629.3 ± 110.4 ^b	1076.0 ± 146.2
MBW	150 ± 18.5 ^a	256.3 ± 13 ^b	187.7 ± 19.3
Food consumption (g/week)	358.3 ± 118.1 ^a	835.8 ± 95.7 ^b	585.1 ± 108.3
Excess food intake (g/g MBW)	1.6 ± 0.6	1.4 ± 0.3	1.5 ± 0.4
Sexual maturity (wk)	24.9 ± 2.7 ^a	19.9 ± 1.5 ^b	23 ± 2.8
Egg number (no./wk)	2.6 ± 2.3 ^a	6.0 ± 1.7 ^b	4.8 ± 1.6
Mean egg weight (g)	23 ± 19.8 ^a	57.5 ± 15.2 ^b	43.2 ± 11.1
Total egg weight (g/wk)	97.3 ± 96.6 ^a	367.1 ± 109.6 ^b	221.9 ± 77.8

MBW = metabolic body weight. Different letters (*a* and *b*) indicate statistically significant differences between red junglefowl and White Leghorn (*p* < .05, GLM or Kruskal-Wallis test).

with junglefowl, whereas there was no significant difference in excess food intake (food consumption/[metabolic body weight + egg weight]). Female Leghorns also had an earlier onset of sexual maturity than female junglefowl and laid more and heavier eggs. For all production traits, except for body weight at 8 and 46 days, the F₂ progeny showed intermediate values (Table I).

Results of the principal component analysis for production trait associations are shown for males in Figure 1a and for females in Figure 1b. In males, different growth rates between 1 and 112 days were associated with each other, with low loadings on PC1 and PC2. Growth rate between 112 and 200 days was not closely associated with other growth rates. Among females, the different growth rates were associated with each other and, as in males, the loadings were low on PC1 and PC2. The different growth rates were more closely associated with each other than with egg weight and sexual maturity. Food intake in females tended to be more associated with egg weight than with growth rates (Fig. 1).

Results of the principal component analysis of time spent (percent) in each arm and in the central box are shown in Table II. PC1 was mainly associated with

high loadings of time spent in the arm containing free food and with low loadings of time spent in the arm containing free food and social stimuli. PC2 was associated with high loadings of time spent in the arm with mixed food and with low loadings of time spent in the arm containing mixed food and social stimuli and in the central box. PC3 was associated with high loadings of time spent in the central box and with low loadings of time spent in the arm containing mixed food and social stimuli. PC4 was associated with high loadings of time spent in the arm containing free food and with low loadings of time spent in the arm containing only the mixed food (Table II).

Effects of production data on PC scores are shown for both sexes in Table III. Males with a high PC1 score had a lower growth rate (GR) in percent of body weight (BW) between the age of 8 and 46 days and a tendency for lower GR in percent of BW between 46 and 112 days. Animals with high PC2 scores showed a tendency of having lower GR in grams between 1 and 8 days of age. F₂ males with high PC3 scores had a higher GR in grams between 8 and 46 days but lower from 46 to 112 days of age. Considering GR in percent of BW, an-

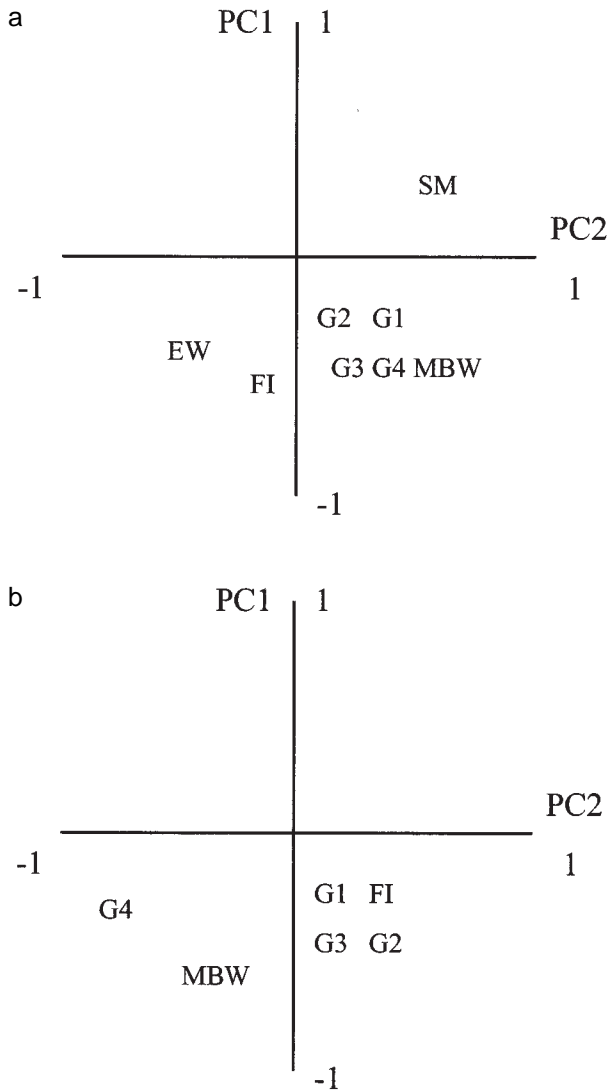


Fig. 1. Results from a principal component analysis for production traits in 383 male (a) and 390 female (b) F₂ progeny. MBW, metabolic body weight; FI, food intake; G1, growth rate between 1 and 8 days; G2, growth rate between 8 and 46 days; G3, growth rate between 46 and 112 days; G4, growth rate between 112 and 200 days; SM, sexual maturity; EG, mean and total egg weight.

imals with a high PC3 score tended to grow slower between 46 and 112 days but grew significantly faster between 112 and 200 days. There was also a significant effect of RFI on PC3 scores. Animals with a high RFI had higher PC3 scores. Males with a high PC4 score tended to grow faster in percent of BW between 46 and 112 days and 112 and 200 days and in grams between 112 and 200 days (Table III).

Among females, sexual maturity had a significant effect on PC1 scores. Females that had an early onset

Table II. Results of Multivariate Principal Component Analysis of Correlation Between Time Spent in Each Arm and in the Central Box, in the Foraging-Social Maze for F₂ Progeny (n = 790)

	PC1	PC2	PC3	PC4
Percent Time Spent in Arms				
Central box	0.077	-0.499	0.732	-0.248
Mixed novel food and social stimuli	0.357	-0.470	-0.651	-0.175
Free food and social stimuli	-0.763	0.069	-0.117	0.207
Mixed novel food	0.101	0.620	0.008	-0.714
Free food	0.523	0.375	0.164	0.596
Proportion of variance	0.321	0.243	0.234	0.202

of sexual maturity had higher PC1 scores. Females that had high PC2 scores had higher total egg weight. There was a tendency for low mean egg weight to have an effect on PC2 scores. GR between 1 and 8 days of age had a significant effect on PC3 scores, females having lower GR in grams having higher PC3 scores. Females with late onset of sexual maturity tended to have higher PC3 scores (Table III).

QTL-Analysis

The QTL graphs, representing plots of the F statistic across chromosomes are shown in Figure 2 for chromosomes with QTLs significant at the 5% genome-wide level. Estimates for the QTL effects are given in Table IV. Significant QTLs were found for PC1 score and PC4 score. The PC4 score QTL was located on chromosome 7. Animals with a homozygous junglefowl (JF/JF) locus on the PC4 score QTL had a lower score than animals with a homozygous Leghorn (L/L) locus. For the PC1 score a significant QTL was found on chromosome 27. Animals with a homozygous JF/JF had a lower PC1 score than L/L, but the most prominent effect was that the heterozygote (L/JF) had a significantly lower score than the two homozygotes.

DISCUSSION

The present study is an attempt to map QTLs for behavioral traits in fowl. A large intercross between two divergent chicken populations, red junglefowl and a White Leghorn line selected for egg production, was generated. Previous studies (Schütz *et al.*, 2001) on the parental lines under the same environmental conditions demonstrated marked differences for a number of the

Table III. *P*-Values for Effects of Production Data on PC Scores of Male ($n = 372$) and Female ($n = 374$) F_2 Progeny, Where $p < .10$

	PC1	PC2	PC3	PC4
Variable males				
Growth rate 1–8 days		0.05 ^a		
Growth rate 8–46 days	0.005 ^b		0.02 ^a	
Growth rate 46–112 days	0.09 ^b		0.04 ^a /0.07 ^b	0.10 ^b
Growth rate 112–200 days			0.03 ^b	0.08 ^a /0.09 ^b
Residual food intake			0.01 ^a /0.01 ^b	
Variable females				
Growth rate 1–8 days			0.07 ^a	
Sexual maturity	0.03 ^{a,b}		0.09 ^a	
Mean egg weight		0.09 ^b		
Total egg weight		0.002 ^a /0.003 ^b		

Two models were used including growth rate in grams (model a) and in percent of body weight (model b), and the model used for obtaining the p value is indicated at each value (ANOVA, GLM, Tukey's post hoc).

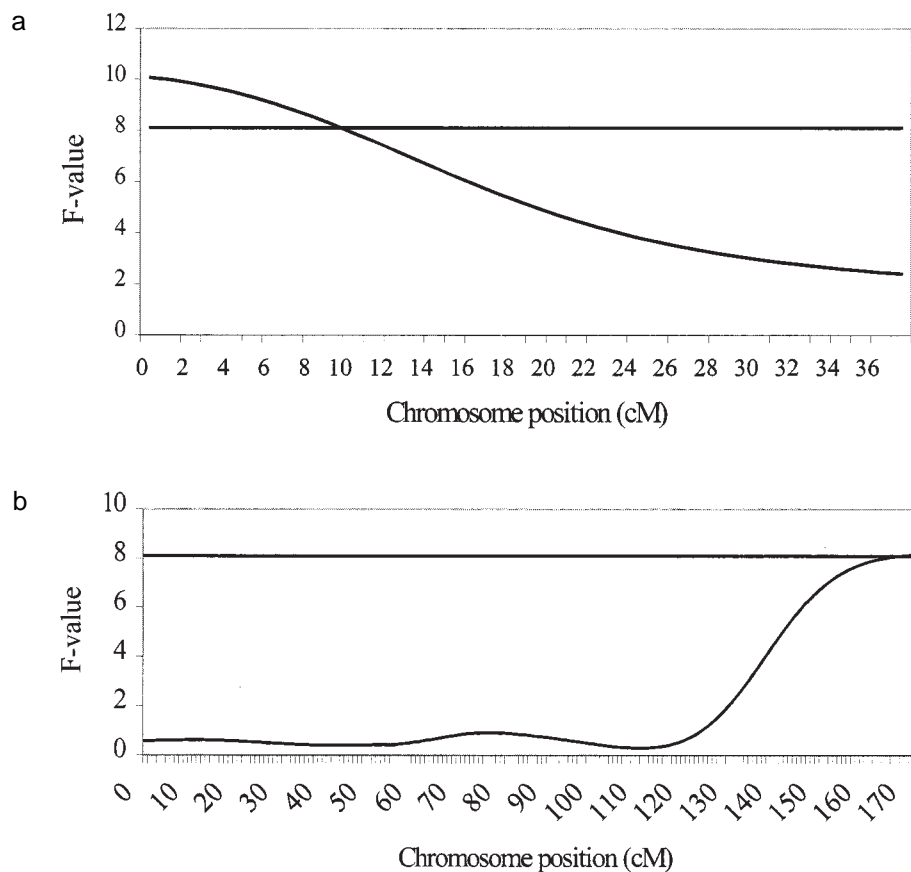


Fig. 2. Genome-wide F values for PC1 score on chromosome 27 (a) and for PC4 on chromosome 7 (b). The horizontal line refers to the 5% genome-wide significance threshold established by a permutation test.

Table IV. Evidence for QTL Significant at the 5% Genome-Wide Level for Traits Collected in the Maze Experiments, by Chromosome

Trait	Chr	Post (cM)	F	Thresh	A	D	RE (%)
PC4 score	7	174	8.2	8	-0.25 ± 0.07	0.1 ± 0.1	2.2
PC1 score	27	0	10.1	8.1	-0.2 ± 0.1	-0.8 ± 0.2	2.7

Estimated location on chromosome (*Chr*), position in cM (*Pos.*), the value of the F-statistics at the estimated location (*F*), significance threshold values at 5% genome level (*Thresh*), additive (*A*) and dominance (*D*) gene effects (\pm standard errors) ^a and the percentage of the residual error (*RE*) variance explained by the QTL.

The additive effect was estimated as half the difference between the phenotypic values for homozygotes for the junglefowl and Leghorn alleles at the QTL while the dominance effect was estimated as the deviation of the heterozygote from the mean of the two homozygotes.

traits analyzed here. These findings strongly suggest an important genetic component for these traits. Moreover, behavioral tests were designed in this study to allow the testing of more than 700 F₂ progeny. Despite this ambitious attempt only two QTLs reached the genome-wide significance threshold, and the QTLs only explained 2–3% of the residual phenotypic variance for the traits. As a comparison, the number of QTLs for growth significant at the genome-wide level approached 10 in total where the individual QTL explained 2–20% of the residual variance (Kerje *et al.*, submitted). The results show, as expected, that behavioral traits have a more complex genetic background than classical production traits like growth and that each QTL explains a smaller part of the phenotypic variance. Another possibility for the difference in explained variance between production and behavior may be that the Leghorns were selected specifically for production traits, which may have decreased the genetic variation for these traits.

Significant QTLs were found for PC1 scores on chromosome 27 and for PC4 on chromosome 7. The QTL for PC1 coincided with QTLs found in a study on the same animals by Kerje *et al.* (submitted) for growth rate between 46 and 112 days and for body weight at 112 days and at 200 days. The results most likely reflect pleiotropic effects of a single QTL, although we cannot exclude the possibility of multiple, closely linked QTLs. A single locus with pleiotropic effect would be consistent with the generally observed trade-off between resource demanding production traits and behavior. A QTL affecting PC4, possibly reflecting contrafreeloading tendency, was found on chromosome 7, showing that this trait is partly under genetic control. This QTL coincided with a QTL for body weight at 112 days of age in the same animals (Kerje *et al.*, submitted) indicating that contrafreeloading may be genetically linked to production traits.

The phenotypic associations were not as clear-cut as hypothesized but, in general, several of the phenotypic associations support the prediction from resource allocation theory (Beilharz *et al.*, 1993). Production traits, and hence resource allocation, varied largely between Leghorns and junglefowl, and most traits had an intermediate average in the F₂ generation. A QTL analysis of the production traits will be presented elsewhere (Kerje *et al.*, submitted).

Among both males and females, there was an association between metabolic body weight and growth rates at different ages. The growth rates at different ages were not as strongly correlated to each other as one might expect, which could be an effect of crossing two lines that might have two separate genetic patterns for growth. High metabolic body weight in females was associated with growth rates at different ages and, to some extent, food consumption. Sexual maturity and high egg weight were not closely associated with body weight. This indicates that these different aspects of resource allocation may not be genetically correlated. Selection in SLU13 has been done mainly on egg weight, and it is generally assumed that this automatically selects also for early sexual maturity and large body weight (Liljedahl *et al.*, 1979). The present results indicate that it would be possible to select separately for each of these traits.

The results from the principal component analysis can be interpreted in terms of uncorrelated behavior patterns in the maze test. PC1 appears associated with social motivation in animals that prefer to feed from freely available food, because PC1 separated time spent in the arm with only the freely available food and time spent in the arm containing freely available food and social stimuli. Animals with high PC1 scores therefore were more inclined to feed in arms with free food and without social stimuli. We interpret PC2 as being

involved in exploration because PC2 separated time spent in the arm containing the mixed food and time in the arm with mixed food and social stimuli and in the central box. Animals with high loadings on PC2 therefore were more inclined to feed from mixed food without social stimuli and less inclined to stay in the central box, which may be interpreted as exploration for novel food. PC3 was associated with high loadings of time spent in the central box and with low loadings of time spent in the arm containing mixed food and social stimuli. It is therefore possible that PC3 is associated with fearfulness, indicated by a resistance to leave the central start box. PC4 was associated with high loading of time spent in the arm containing free food and with low loading of time spent in the arm containing only the mixed food. A high PC4 score therefore indicates a low degree of contrafreeloading (Inglis *et al.*, 1997). PC4 probably reflects the differences found between red junglefowl and White Leghorn in foraging strategy, where the Leghorn behaves more in an energy-conserving fashion. We will therefore tentatively label PC1 as “Social tendency,” PC2 as “Exploration,” PC3 as “Fear,” and PC4 as “Contrafreeloading.”

Males that grew slower between 8 and 46 days in percent of body weight, and females that had an early onset of sexual maturity were found to have higher “Social tendency” scores. Domestication and selection for production traits will generally lead to an earlier onset of sexual maturity (e.g., Price, 1999). Female Leghorns matured sexually earlier compared with female junglefowl, and it is likely that this was the case also in males, although that could not be measured. During the ontogeny of chickens, there will be at least two occasions that will cause a disturbance to the social hierarchy that will in turn lead to increased competition and agonistic behaviors. Wood-Gush (1955) reported that competition among chickens does not occur before 3 days of age and agonistic interactions to establish social hierarchy starts at around 2 weeks of age. In small groups of hens a social hierarchy will be established no later than at 8 weeks of age for males and at 10 weeks of age for females (Guhl, 1962), and probably much earlier than that. The slower weight gain in percent body weight in young males that as adults prefer to feed from freely available food could indicate that these males allocated more resources to social behavior (in a wide sense) than to growth. The other occasion that is likely to cause an increase in social behaviors and a rearrangement of the social hierarchy is at the age of sexual maturity. Females that mature early may therefore prefer the freely available food and allocate more resources to reproduction than to social behavior.

Females with high total egg weight had higher “Exploration” scores. This indicates that exploration decreased with increased egg weight. Because exploration is energy demanding (Schütz and Jensen, 2001), this result is in line with the predictions of resource allocation theory.

Among males, a high “Fear” score was associated with high RFI and higher growth rate in grams between 8 and 46 days and with high growth rate as percent body weight between 112 and 200 days, but low growth rate in grams between 46 and 112. Among females, a high “Fear” score tended to be associated with lower growth in grams between 1 and 8 days and late onset of sexual maturity. This association may possibly indicate that males with overall higher growth were more fearful. In females, the effect of growth on “Fear” score is difficult to interpret.

Males with a fast growth rate in percent of body weight between 46 and 200 days tended to have a lower degree of contrafreeloading (i.e., a higher score on PC4). This is in accordance with the general prediction from resource allocation theory that individuals which grow fast prefer food that requires a minimum of effort. This has previously been shown as a difference in foraging strategy between red junglefowl and White Leghorns (Schütz and Jensen, 2001; Schütz *et al.*, 2001; Lindqvist and Jensen, in prep.). In a natural environment it is thought to be favorable to invest energy in looking for possible future food sources and, in that sense, fast growth may be correlated to a more domesticated feeding strategy. Hence, contrafreeloading is likely to decrease as a result of selection for increased production. In females, no such effects were detected.

Growth, age at sexual maturity, egg production, and food consumption all differed significantly between junglefowl and White Leghorn laying hens. F₂ progeny from junglefowl × Leghorn intercrosses showed intermediate values of most of the traits, as expected. Several of the production traits showed highly significant correlations to behavior of the F₂ progeny in a foraging-social maze test, in a direction indicating some trade-off between resource-demanding production traits and energy-demanding behavior. Significant QTL were found for two behavioral traits, of which one coincided with QTLs for growth and body weight at a certain age, indicating a close genetic linkage or a pleiotropic gene effect.

ACKNOWLEDGMENTS

Sincere thanks are due to Gunilla Jacobsson, Siw Johansson, and Anette Wichman for excellent technical assistance. This work was part of a Swedish research

program, FOOD 21, which was funded by the MISTRA Foundation. Financial support was also received from the AgriFunGen programme at the Swedish University of Agricultural Sciences, Swedish Research Council for Forestry and Agriculture, Wallenberg Consortium North and the KSLA foundation. The National Supercomputing Central provided computer resources for QTL mapping. Örjan Carlborg was partly funded by the National Graduate School in Scientific Computing.

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