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Mapping of quantitative trait loci affecting organ weights and blood variables in a broiler layer cross

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Abstract 1. A genome scan was performed to locate genomic regions associated with traits that are known to vary in birds (most commonly broilers) suffering from heart, lung or muscular dysfunction and for weight of the dressed carcass and some internal organs.
2. The F_2 population studied was derived from a cross between a broiler and a layer line and consisted of over 460 birds that were genotyped for 101 markers.
3. There was strong support for segregation of quantitative trait loci (QTL) for carcass and organ weights and blood variables. We identified 11 genome-wide significant QTL (most of them for dressed carcass weight) and several genome-wide suggestive QTL.
4. The results point to some genome regions that may be associated with health-related traits and merit further study, with the final aim of identifying linked genetic markers that could be used in commercial breeding programmes to decrease the incidence of muscular and metabolic disorders in broiler populations.

INTRODUCTION

In recent years the development of molecular techniques has allowed the construction of detailed linkage maps for a wide variety of species, including the chicken (Groenen *et al*., 2000; Schmid *et al*., 2000). Several studies have used these maps to identify marker-trait associations, for characters of economic importance, mainly growth-related (Van Kaam *et al*., 1998, 1999; Ikeobi *et al*., 2002, 2003; McElroy *et al*., 2002; Sewalem *et al*., 2002; De Koning *et al*., 2003) but also health-related (Yonash *et al*., 1999), in different mapping populations. These associations could be exploited in breeding programmes to achieve more rapid improvement, by either marker-assisted selection (MAS) or marker-assisted introgression (MAI).

In this study, we used an F_2 population derived from the cross of a male broiler line and a layer line from which phenotypes were available for a series of traits that are known to vary in birds that suffer from heart, lung or muscular dysfunction. Although broilers and layers have both been heavily selected for decades, selection has been done on different traits. Broilers have been heavily selected to improve on growth, feed efficiency and meat yield while layers have mainly been selected on egg-production traits. As a result, broilers and layers differ substantially in size, muscling and reproductive fitness, but they also differ in traits for which selection has not been consciously made, such as susceptibility to heart and lung disorders (like ascites and sudden death) and skeletal muscle abnormalities, more commonly suffered by broilers. The purpose of this study was to investigate if quantitative trait loci (QTL) that affect the birds' susceptibility to these pathologies were segregating in this F_2 population. In addition to traits related to cardiopulmonary or muscular disorders, we analysed some unrelated traits, to use as controls.

Hocking *et al*. (1985) and Deeb and Lamont (2002) observed that differences in live weight between broilers and layers were generally reflected in all measures of weight and size, but broilers have relatively longer intestines and the relative weight of other organs is higher in layers. Emmans and Kyriazakis (2000) postulated that, in fast growing birds, the development of the heart and other supply organs is penalised

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due to the energetic needs of the growing muscle and that this is at the origin of metabolic disorders and tissue and organ dysfunction in fast growing chickens. Mitchell and Sandercock (1995) showed that the concentration of creatinine kinase in avian blood can be used as an indicator of skeletal muscle dysfunction or damage and Sandercock *et al*. (2001) pointed out that there are differences in creatinine kinase activity between broilers and layers that cannot be explained only by differences in live weight. Maxwell *et al*. (1994) observed that ascitic broilers exhibit higher levels of troponin T (which is an indicator of early myocardial damage) than healthy birds. Ascitic birds also show raised packed cell volume (PCV) and red and white blood cell count, as well as increased (although not different at a 5% significance level) mean cell volume (MCV) (Maxwell *et al*., 1986). Furthermore, Maxwell *et al*. (1990) found that broilers have higher PCV and more red blood cells than layers, but they did not observe significant differences in MCV between strains.

We carried out a genome scan looking for one or two QTL per linkage group using the least squares method developed by Haley *et al*. (1994) to analyse data from F_2 populations derived from crosses between outbred lines.

MATERIALS AND METHODS

Mapping population and traits

The F_2 population was derived from the cross of two males and two females from both a large broiler line and a small egg-laying line. The F_1 consisted of eight males and 32 females that were crossed to produce an F_2 of over 460 birds (for more details on the mapping population see Sewalem *et al*. (2002)).

Concentrations of creatinine kinase and troponin T, total blood cell count (TBCC), PCV, weights, dressed carcass, organ (liver, heart, spleen and gizzard) weights and intestine length were recorded. All traits were recorded at 9 weeks except creatinine kinase that was recorded at 6 weeks. Phenotypes of between 461 and 314 F_2 birds were available depending on the trait (see Table 1). Pedigree, phenotypic and marker information was stored in http://www.resspecies.org (Law and Archibald, 2000).

Genotyping and linkage map

For details on genotyping see Sewalem *et al*. (2002). The linkage map was constructed using Cri-map (Green *et al*., 1990) (X. Yu, 2001, Roslin Institute, Roslin, UK, personal communication). It consisted of 101 markers scattered across 26 linkage groups. The total map length was 2503 cM, which corresponds to a coverage of about 80%, assuming that last markers in each linkage group are 20 cM away from the end of the linkage group. Map distances were assumed to be equal for males and females in the analyses. Further details of the linkage map can be found at Sewalem *et al*. (2002). The mean distance between consecutive markers on a linkage group was 42·5 cM and it ranged from 0·2 to 100 cM.

Statistical analyses

Basic least squares model

The analyses for creatinine kinase and troponin T concentration were carried out on the natural logarithm of the original observations because the trait distributions on the transformed scale were closer to a normal distribution than untransformed data. For all traits analysed, the fixed effects of sex, F_2 family and pen were fitted. Except for creatinine kinase, for which we fitted live weight at 6 weeks as a covariate, all the other traits were analysed including dressed carcass weight in the basic model. Troponin T

Table 1. *Acronyms, number of records, means and residual standard deviations (F_2sd) for the traits analysed*

Trait	Acronym	Number of F_2 records	Mean	F_2sd
Creatinine kinase concentration (IU/l)	–	451	208·78	131·96
(Ln-transformed trait analyzed)	LNCREAT	451	5·18	0·39
Troponin T concentration (ng/ml)	–	445	0·04	0·20
(Ln-transformed trait analyzed)	LNTROP	445	−3·47	0·41
Packed cell volume (%)	PCV	313	28·80	1·70
Total blood cell count (10^6/mm^3)	TBCC	314	2·35	0·16
Mean cell volume (μm^3)	MCV	312	123·27	7·15
Heart weight (g)	HEART	461	10·79	1·59
Dressed carcass weight (g)	CARCASS	461	1350·10	159·78
Liver weight (g)	LIVER	461	39·95	3·92
Spleen weight (g)	SPLEEN	461	4·25	0·80
Gizzard weight (g)	GIZZARD	461	29·00	4·51
Intestine length (cm)	INTESTINE	461	162·60	11·46

was adjusted for assay tube and assay number effects. Observations for which the standardised residuals exceeded 4 after correction for these fixed effects were removed from the data-set. The maximum number of birds removed from the data-set was 6 (1·3% of data), and that was for the transformed troponin T concentration data. The statistics presented in Table 1 were obtained after removing these observations.

QTL analyses and confidence intervals for QTL locations

QTL analyses were conducted using a least squares framework, following the method developed by Haley *et al.* (1994) for F_2 populations. This method assumes that the grandparental lines used to derive the F_2 are fixed for alternative QTL alleles (*Q* and *q*), but may be segregating at marker locations. The analyses are carried out in two steps. First, the probabilities of each F_2 individual being each of the 4 possible QTL genotypes (*QQ*, *Qq*, *qQ* and *qq*, where the first allele is inherited from the male parent and the second allele from the female parent) are computed for each location in the genome using multiple marker genotypes. Secondly, for each location, trait values are regressed on linear combinations of these probabilities to estimate the additive (*a*), dominance (*d*) and parent of origin (*i*) effects for a putative QTL at each location. *a* is the effect of *QQ* and *qq* has an effect of $-a$. For further details on the parameterisation see Knott *et al.* (1998). Markers on chromosome Z appeared to belong to a region that did not recombine with the W chromosome (they were outside any pseudo-autosomal region) and this was taken into account for the computation of QTL genotype probabilities for this sex chromosome. Males carry two copies of any putative QTL on chromosome Z, so all possible QTL genotypes (*QQ*, *Qq*, *qQ* and *qq*) can be present in the F_2 population, therefore a model with additive and dominance effects can be fitted. Females carry only one copy of the putative QTL and only the effect of being *QW vs qW* (where *W* could originate from either broilers or layers) can be estimated.

In a first stage, each linkage group was searched for a single QTL with *a* and *d* effects and a single QTL with *a*, *d* and *i* effects, firstly assuming sex-equal and then assuming sex-different effects (that is, with the QTL having the same effect in males and females or not). The model including a QTL was compared with a model without a QTL using an F ratio. For each linkage group, the location showing the highest F ratio was considered the most likely location for a QTL on this linkage group. If the test statistic at this 'best location' exceeded the (model-dependent) genome-wide threshold for suggestive linkage (see below) for only one of the QTL models fitted, this model was chosen for further analyses (that is, searches including background genetic effects). If the test statistic for more than one model exceeded the suggestive linkage threshold and the best location for the QTL was (roughly) the same, because the models are nested, we could test which of them fitted the data best. If the best location for different significant models could not be considered to be the same we tested the models against each other at both best locations and, generally, we used the model with fewer parameters in further analyses. Searches for two QTL simultaneously per linkage group were also carried out.

In a second stage, the searches were repeated for all linkage groups that showed suggestive or significant linkage (see below), including in the basic model the QTL identified in other linkage groups that affected the trait being analysed. The inclusion of unlinked QTL would take account of unlinked genetic variation and reduce the residual variance, potentially increasing power and removing biases in QTL parameter estimates (Jansen, 1993; Zeng, 1993). Tests statistics and estimates of QTL locations and effects are presented for analyses including unlinked QTL, except for analyses for which the QTL was allowed to have different effects across families (see below).

Finally, confidence intervals for QTL locations were obtained by bootstrapping (Visscher *et al.*, 1996). A thousand resamples were used and the 95% confidence intervals were the regions for which the 950 less extreme samples were obtained.

We carried out additional searches using a model for which we allowed the QTL to have different effects (*a* and *d*) across F_2 families. This could be observed if one or both grandparental lines were segregating at the QTL.

Chromosome 1, a very long chromosome with regions of low information content, was analysed in two overlapping segments covering 342 cM (from position 0 cM to position 342 cM) and 346 cM (from position 199 cM to position 555 cM), respectively. This chromosome contained a larger number of adjacent partially informative markers that can be handled by the software used and splitting the linkage group into overlapping segments made its analysis feasible.

The module F_2 QTL analysis from QTL Express (Seaton *et al.*, 2002) implements the method developed by Haley *et al.* (1994) and was used to perform genome scans (excluding sex

chromosomes) looking for a single or two QTL per linkage group and tests for linkage at single positions, as well as to obtain the confidence intervals for QTL locations. FORTRAN programs were used for analyses of sex chromosomes.

Significance thresholds

Genome-wide significance thresholds (assuming a QTL with *a* and *d* effects, that is, for a model with two degrees of freedom (Df) for the numerator, ∞ for the denominator) were obtained by permutation using a simulated data-set in a previous study (Sewalem *et al.*, 2002). The genome-wide threshold for suggestive linkage (where we expect to obtain, by chance, one significant result per genome scan) is 5·0 and the 5% and 1% genome-wide significance thresholds are 8·2 and 10·0, respectively (see Lander and Kruglyak, 1995).

Approximate significance thresholds for alternative single QTL models (with sex interaction and/or parent of origin effect) can be obtained from a standard F distribution table. The F ratio threshold obtained by simulation for the model with an additive and dominance component (2 Df for the numerator and ∞Df for the denominator) corresponds to a tabulated probability (α) under a standard F distribution (2 Df/∞Df). The tabulated critical value for a standard F distribution with x Df/∞ Df for α can be used as an approximate significance threshold for a QTL model where x QTL components are estimated. Nested single QTL models were compared using the nominal point-wise significance.

No thresholds were obtained empirically to test for the presence of two *vs* no QTL. Instead, we used genome-wide suggestive thresholds obtained for single QTL searches adjusted for Df as described above. To test for the presence of two *vs* one QTL we used again this threshold as suggested by Spelman *et al.* (1996) and empirically validated for a particular data-set by De Koning (2001).

RESULTS

Descriptive statistics of traits and significance thresholds

Table 1 shows the total number of F_2 birds with phenotypic records, means and residual standard deviations of all traits, after removal of outliers. The significance thresholds used are presented in Table 2.

Table 2. *Point-wise and genome-wide significance thresholds*

	Point-wise	Genome-wide		
	5% significance	Suggestive linkage $P<0{\cdot}007$	5% significance $P<0{\cdot}0003$	1% significance $P<0{\cdot}0001$
1 Df/∞Df	3·9	7·3	13·3	15·4
2 Df/∞Df	3·0	5·0	8·2	10·0
3 Df/∞Df	2·6	4·1	6·4	7·2
4 Df/∞Df	2·4	3·6	5·4	6·3
6 Df/∞Df	2·1	3·0	4·3	4·8
8 Df/∞Df	2·0	2·7	3·7	4·1
58 Df/∞Df	1·4	1·6	1·9	2·0

Single QTL analyses

Table 3 shows the location with the highest test statistic and the estimates of the QTL effects at this location for linkage groups with genome-wide suggestive or significant results for models with sex-equal effects. If a model with sex-different effects fitted the data best, no results are presented at this table. Test statistics, 95% confidence intervals, marker brackets for QTL locations and the percentage of the variance accounted for by the QTL are also presented. A positive additive estimate means that the QTL allele coming from the broiler line increases the trait value relative to that from the layer line and a positive parent of origin effect estimate means that inheriting the broiler QTL allele through the male parent (and the layer allele through the female) increases the trait value relative to inheriting it through the female parent.

QTL with sex-equal effects

We identified two QTL significant at a genome-wide level for health-related traits (LNTROP on chromosome 11 and TBCC on chromosome 2). In addition, 6 genome regions located on chromosomes 1, 3, 4, 8, 13 and 27 showed evidence of significant linkage at a genome-wide level for dressed carcass weight and one in chromosome 1 was significant for spleen weight. Several regions showed suggestive evidence of linkage for LNCREAT, LNTROP, PCV, TBCC, MCV, heart, carcass, liver and gizzard weight and for intestine length.

QTL detected with this model acted mainly in an additive fashion. For TBCC and carcass weight the broiler allele always increased the trait value whereas for the rest of the traits there was not such a clear pattern. The dominance component was only statistically different from zero (5% significance) for the QTL identified for LNCREAT on chromosomes 9 and 11, LNTROP on chromosomes 2 and 11, TBCC (chromosomes 1, 2 and 6), MCV on chromosome 2 and one

Table 3. *Results of searches of single QTL with sex-equal effects. The location with the highest test statistic (L) and the estimates of the QTL effects (and SE) at this location for linkage groups (LG) with genome-wide suggestive or significant results are shown, together with test statistics (F), 95% confidence intervals (CI) and marker brackets for QTL locations and percentage of variance accounted for by the QTL (%VE)*

Trait	LG	F	[1]	L (cM)	CI	QTL effects (SE)			%VE[2]	Flanking markers	
						a	*d*	*i*			
LNCREAT	4	4·6	+	63	0–201	−0·13 (0·05)	−0·07 (0·11)	−0·1 (0·05)	2·7	ROS0015	ADL0266
LNCREAT	9	4·6	+	0	0–152	0·04 (0·03)	0·10 (0·04)	−0·08 (0·04)	2·7	ROS0078	MCW0135
LNCREAT	11	5·5	+	0	0–70	−0·08 (0·03)	0·08 (0·04)	–	2·2	MCW0097	LEI0110
LNTROP	2	7·2	+	244	84–341	−0·06 (0·04)	0·27 (0·08)	–	3·6	ADL0196	LEI0127
LNTROP	5	5·8	+	127	0–153	0·23 (0·07)	−0·36 (0·26)	–	2·8	ROS0084	ADL0298
LNTROP	11	8·7	*	22	3–61	−0·07 (0·03)	0·16 (0·04)	–	4·4	ROS0111	ADL0308
PCV	14	4·3	+	0	–	−0·18 (0·20)	0·35 (0·38)	1·06 (0·30)	3·7	–	–
TBCC	1	4·7	+	1	0–348	0·03 (0·02)	0·06 (0·02)	0·04 (0·02)	4·3	MCW0168	ADL0160
TBCC	2	10·1	**	114	67–273	0·08 (0·02)	0·07 (0·03)	–	6·6	ADL0176	ROS0018
TBCC	6	7·1	+	88	17–88	0·05 (0·01)	0·04 (0·02)	–	4·5	ADL0142	ADL0323
MCV	2	6·8	+	115	100–298	−2·67 (0·86)	−2·99 (1·38)	–	4·0	ADL0176	ROS0018
MCV	14	4·8	+	0	–	0·64 (0·84)	1·16 (1·58)	4·26 (1·26)	4·2	–	–
HEART	1	5·0	+	101	0–322	0·51 (0·22)	1·30 (0·65)	–	2·0	MCW0010	ADL0180
HEART	9	6·2	+	152	27–152	−0·38 (0·11)	0·08 (0·16)	–	2·6	ROS0030	MCW0134
CARCASS	1	7·4	+	191	35–202	45·29 (11·91)	22·89 (21·11)	–	3·1	LEI0146	MCW0018
CARCASS	1	13·8	**	429	403–479	53·47 (11·27)	46·07 (18·02)	–	6·0	LEI0106	ADL0183
CARCASS	2	7·3	+	268	65–321	36·87 (9·63)	−1·17 (13·66)	–	3·1	LEI0127	LEI0147
CARCASS	3	8·7	*	181	72–204	61·67 (14·81)	1·30 (35·17)	–	3·7	MCW0187	ADL0306
CARCASS	4	29·9	**	147	135–161	171·18 (22·15)	2·55 (70·69)	–	12·7	ADL0266	LEI00733
CARCASS	8	9·1	*	46	0–94	113·32 (26·57)	−72·42 (98·63)	–	3·9	ADL0179	ROS0075
CARCASS	13	10·9	**	60	36–76	58·62 (14·13)	41·95 (27·98)	–	4·7	ADL0147	ADL0225
CARCASS	27	18·3	**	0	–	67·64 (11·23)	−17·87 (15·49)	–	8·0	–	–
LIVER	1	6·3	+	417	360–541	1·06 (0·31)	0·45 (0·45)	–	2·6	LEI0106	MCW0036
LIVER	4	5·4	+	102	0–139	0·66 (0·32)	0·81 (0·57)	1·12 (0·36)	3·2	ADL0266	LEI0073
LIVER	15	5·5	+	39	10–45	−1·31 (0·44)	−1·19 (0·93)	–	2·2	LEI0083	MCW0080
LIVER	24	7·1	+	0	–	−1·37 (0·39)	−1·17 (1·00)	–	3·0	–	–
SPLEEN	1	8·7	*	189	147–238	−0·29 (0·07)	−0·08 (0·12)	–	3·6	LEI0146	MCW0018
GIZZARD	1	5·4	+	201	8–338	−1·11 (0·37)	−0·98 (0·60)	–	2·2	LEI0146	MCW0018
INTESTINE	11	4·6	+	40	3–70	−2·43 (0·98)	1·19 (1·79)	3·02 (1·11)	2·7	ROS0111	ADL0308
INTESTINE	14	7·8	+	0	–	3·82 (1·06)	−3·56 (1·90)	–	3·4	–	–

[1]+ Indicates significance at the genome-wide suggestive level and * and ** at the 5% and 1% genome-wide level, respectively.
[2]Variance explained by individual QTL obtained as the per cent reduction in residual mean squares after fitting the relevant fixed effects, covariates and cofactors.

of the two QTL identified on chromosome 1 for carcass weight. For all except LNTROP, the broiler allele was dominant.

The first scans revealed a series of suggestive or significant locations for which a model that included a parent of origin effect was the only model for which the test statistic exceeded the genome-wide suggestive threshold (LNCREAT on chromosomes 1 and 9, PCV on chromosomes 1 and 14, MCV on chromosome 14, HEART on chromosomes 1 and 17, CARCASS on chromosome 9 and LIVER on chromosome 4) or fitted the data best (TBCC on chromosome 1, GIZZARD on chromosomes 2 and 6 and INTESTINE on chromosome 11). After fitting the relevant background genetic effects, for 7 of these locations the test statistic still exceeded the suggestive genome-wide threshold (Tables 2 and 3), the model that included a parent of origin effect fitted the data best, and the parent of origin component was statistically different from zero (5% level). Except for the QTL for LNCREAT, the broiler allele coming through the male parent increased the trait value.

QTL with sex-different effects

Table 4 shows the same estimates and statistics as Table 3 for models with sex-different effects, when this was the best resulting model. Some locations showed suggestive linkage in the first stage of the study only when the model allowed the QTL to have different effects across sexes.

We identified two significant and 9 suggestive QTL with different effects in males and females (and in some cases mode of action) for both health-related traits and anatomical measures. As an example, a significant QTL on chromosome 13 for HEART acted in an overdominant fashion in males but only additively in females, with a smaller effect. For this QTL location the test statistic also exceeded the 5% significance threshold for a model with no sex interaction, but the fit of this model was significantly worse (5% level). This was also true

Table 4. *Results of searches of single QTL with sex-different effect. The location with the highest test statistic (L) and the estimates of the QTL effects for males (M) and females (F) (and SE) at this location for linkage groups (LG) with genome-wide suggestive or significant results are shown, together with test statistics (F), 95% confidence intervals (CI) and marker brackets for QTL locations and percentage of variance accounted for by the QTL (%VE)*

Trait	LG	F	[1]	L (cM)	CI		QTL effects (SE)			%VE[2]	Flanking markers	
							a	*d*	*i*			
LNCREAT	1	3·6	+	255	208–555	M	−0·11 (0·05)	0·18 (0·09)	−0·01 (0·05)	3·8	ADL0319	LEI0101
						F	0·08 (0·05)	0·07 (0·09)	0·16 (0·05)			
LNTROP	17	4·4	+	0	–	M	−0·16 (0·06)	−0·33 (0·12)	–	4·0	–	–
						F	0·01 (0·06)	0·18 (0·11)	–			
PCV	1	4·8	+	340	163–516	M	0·17 (0·22)	−0·87 (0·32)	–	5·4	LEI0088	ROS0081
						F	0·67 (0·22)	0·34 (0·34)	–			
PCV	2	3·7	+	114	0–397	M	0·77 (0·28)	0·47 (0·45)	−0·85 (0·25)	5·8	ADL0176	ROS0018
						F	0·14 (0·28)	−0·04 (0·46)	0·49 (0·25)			
TBCC	11	3·8	+	52	0–70	M	−0·03 (0·02)	0·05 (0·03)	–	4·3	ROS00111	ROS0112
						F	0·02 (0·02)	−0·10 (0·03)	–			
HEART	1	4·6	+	489	213–555	M	−0·98 (0·23)	−0·03 (0·43)	–	3·6	LEI0079	ROS0025
						F	−0·05 (0·21)	0·83 (0·43)	–			
HEART	13	5·5	*	67	0–76	M	−0·51 (0·20)	−1·25 (0·38)	–	4·5	ADL0147	ADL0225
						F	−0·22 (0·21)	0·03 (0·37)	–			
LIVER	Z	6·2	+	36	0–106	M	0·18 (0·82)	2·18 (1·03)	–	3·8	ROS0072	LEI0111
						F	2·12 (0·75)	–	–			
GIZZARD	2	7·7	**	114	43–243	M	−2·94 (0·59)	0·63 (0·97)	–	6·3	ADL0176	R0S0018
						F	−0·24 (0·59)	1·90 (0·95)	–			
GIZZARD	5	3·8	+	95	0–149	M	−2·40 (0·63)	−0·50 (0·97)	–	2·7	ROS0084	ADL0298
						F	−0·21 (0·62)	0·70 (1·28)	–			
INTESTINE	Z	6·2	+	108	3–127	M	5·15 (1·71)	−4·64 (2·06)	–	3·8	LEI0111	LEI0075
						F	1·47 (1·62)	–	–			

[1]+ Indicates significance at the genome-wide suggestive level and * and ** at the 5% and 1% genome-wide level, respectively.
[2]Variance explained by individual QTL obtained as the per cent reduction in residual mean squares after fitting the relevant fixed effects, covariates and cofactors.

Table 5. *Results for a two-QTL model with no sex interaction. Best locations (L1 and L2) from two-dimensional searches of linkage groups (LG) with more than two markers are presented together with test statistics (F 4 Df tests two QTL* vs *no QTL and F 2 Df tests two QTL* vs *one QTL), estimates of QTL effects for both QTL (QTL1 and QTL2) and SE at these locations, marker brackets for QTL locations and percentage of variance accounted for by the QTL (%VE)*

Trait	LG	F 4 Df	F 2 Df	[1]	L1 (cM)	L2 (cM)	QTL1 effects (SE)		QTL2 effects (SE)		%VE[2]
							a	*d*	*a*	*d*	
CARCASS	1	11·2	8·0	+	190	429	46·55 (11·60)	10·89 (21·51)	55·00 (11·44)	49·81 (18·30)	9·3
Flanking markers							LEI0146	MCW0018	LEI0106	ADL0183	

[1]+ Indicates significance at the genome-wide suggestive level.
[2]Variance explained jointly by two linked QTL obtained as the per cent reduction in residual mean squares after fitting the relevant fixed effects, covariates and cofactors.

for the QTL identified for LNTROP and GIZZARD.

For two of the suggestive QTL (LNCREAT on chromosome 1 and PCV on chromosome 2), including a parent of origin component significantly improved the fit of the model. For both traits the estimate of the parent of origin effect had opposite signs in males and females.

Two locations on sex chromosome Z showed suggestive linkage for LIVER and INTESTINE. In both cases the broiler allele increased the trait value, but to a larger extent in females than in males for LIVER and the other way round for INTESTINE. The dominance component was different from zero for both traits, which could be interpreted as a sign of interaction of the QTL alleles with the background of unrecombined broiler or layer Z chromosomes for males.

Two-QTL analyses

QTL with sex-equal effects

Two-dimensional searches were carried out for all linkage groups with more than two markers. Single QTL were identified for CARCASS on both overlapping segments of chromosome 1. The length of segment 2 was modified so as to contain the QTL identified on segment 1 for the two-dimensional search. Results are shown in Table 5.

Table 6. *Results for a two-QTL model with sex-different effects. Best locations (L1 and L2) from two-dimensional searches of linkage groups (LG) with more than two markers are presented together with test statistics (F 4 Df tests two QTL* vs *no QTL and F 2 Df tests two QTL* vs *one QTL), estimates of QTL effects of both QTL (QTL1 and QTL2) and SE at these locations for males (M) and females (F), marker brackets for QTL locations and percentage of variance accounted for by the QTL (%VE)*

Trait	LG	F 8 Df	F 4 Df	[1]	L1 (Cm)	L2 (cM)		QTL1 effects (SE)		QTL2 effects (SE)		%VE[2]
								a	*d*	*a*	*d*	
HEART	1	3·6	3·7	+	268	486	M	−0·14 (0·24)	2·01 (0·54)	−0·94 (0·24)	0·06 (0·48)	4·9
							F	0·01 (0·24)	−0·16 (0·52)	−0·03 (0·22)	−0·16 (0·48)	
Flanking markers								LEI0101	LEI0108	LEI0079	ROS0025	

[1]+ Indicates significance at the genome-wide suggestive level.
[2]Variance explained jointly by two linked QTL obtained as the per cent reduction in residual mean squares after fitting the relevant fixed effects, covariates and cofactors.

Table 7. *Total trait difference explained by suggestive and significant QTL for males, females and the mean of both sexes expressed in trait units (Units) and in F_2 residual standard deviations (F_2sd)*

Trait	Males		Females		Mean	
	Units	F_2sd	Units	F_2sd	Units	F_2sd
LNCREAT (units)	−0·56	−1·44	−0·18	−0·46	−0·37	−0·95
LNTROP (units)	−0·18	−0·44	0·16	0·39	−0·01	−0·02
PCV (%)	1·52	0·89	1·26	0·74	1·39	0·82
TBCC (10^6/mm^3)	0·26	1·63	0·36	2·25	0·31	1·94
MCV (μm^3)	−4·06	−0·57	−4·06	−0·57	−4·06	−0·57
HEART (g)	−4·88	−3·07	−0·32	−0·20	−2·60	−1·64
CARCASS (g)	1221·70	7·65	1221·70	7·65	1221·70	7·65
LIVER (g)	−1·56	−0·40	2·32	0·59	0·38	0·10
SPLEEN (g)	−0·58	−0·73	−0·58	−0·73	−0·58	−0·73
GIZZARD (g)	−12·9	−2·86	−3·12	−0·69	−8·01	−1·78
INTESTINE (cm)	13·08	1·14	5·72	0·50	9·40	0·82

After fitting unlinked QTL as cofactors, only for CARCASS on chromosome 1 did both the test statistic for two *vs* no QTL and two *vs* one QTL exceed the proposed thresholds for suggestive genome-wide linkage. The best locations corresponded to the QTL identified in the single QTL searches of the two segments in which chromosome 1 was originally divided to facilitate its analysis.

QTL with sex-different effects

Given that several QTL with different effects across sexes were identified in the single QTL searches, two-QTL analyses were repeated including a sex interaction. Results are shown in Table 6. A two-QTL model best explained the data for HEART on the second segment of chromosome 1. One of the locations corresponded to the QTL with sex-different effects identified when searching for a single QTL with sex-different effects.

QTL effects

The proportion of the phenotypic variance explained by the individual suggestive or significant QTL ranged from 2·0 (HEART) to 12·7% (CARCASS). By adding twice the additive effects estimated for all the suggestive or significant QTL, we estimated the overall effect of these QTL (namely, the difference in trait values between broilers and layers accounted for by these QTL). Table 7 shows the overall effect for males, females and the mean of both expressed in absolute units and as a proportion of the F_2 population trait distribution residual standard deviation (F_2sd). As previously, a positive effect means that the broiler allele increases the trait value. The sign of the overall effects was the same for males and females, except for LIVER and LNTROP. Overall standardised effects (absolute values) ranged in males from 0·40 to 7·65 F_2sd for LIVER and CARCASS, respectively, and from 0·20 to 7·65 F_2sd for HEART and CARCASS in females. The overall effect for males expressed as a proportion of the effect for females ranged from −1·13 for LNTROP to 15·25 for HEART.

Confidence intervals

Ninety-five per cent confidence intervals for QTL locations are presented in Tables 3 and 4. These were generally large even for QTL that reached 5 or 1% genome-wide significance.

Table 8. *Location (L) with the highest test statistic for searches of QTL with different effects across F_2 families for each linkage group (LG) with genome-wide suggestive or significant results are shown, together the with test statistics (F), a comparison of fit of the model with F_2 family interaction with a model with no interaction (Yes/No and test statistic (Fit)) and the test statistic of the model with no QTL × F_2 family interaction at this location (F $a+d$)*

Trait	LG	F	[1]	L (cM)	Fits best?	Fit	F $a+d$
LNCREAT	1	1·6	+	335	Yes	1·7	0·4
PCV	1	1·6	+	335	Yes	1·5	5·8
PCV	5	1·6	+	66	Yes	1·6	1·0
PCV	9	1·7	+	5	Yes	1·7	0·8
TBCC	1	1·9	*	338	Yes	1·7	4·6
TBCC	2	1·6	+	348	No	1·3	10·6
TBCC	5	1·6	+	62	Yes	1·5	1·6
HEART	3	1·7	+	150	Yes	1·7	2·2
HEART	4	1·8	+	200	Yes	1·8	1·5
CARCASS	4	1·6	+	150	No	0·8	25·9
LIVER	6	1·6	+	19	Yes	1·6	2·9
SPLEEN	1	1·6	+	244	Yes	1·5	3·4
GIZZARD	2	1·6	+	146	No	1·3	12·1
GIZZARD	4	1·7	+	35	Yes	1·7	0·8
GIZZARD	9	1·6	+	95	Yes	1·6	1·0

[1] + Indicates significance at the genome-wide suggestive level and * at the 5% genome-wide level.

In some cases they covered a large proportion (or all) of the length of the linkage group. Confidence intervals for QTL on chromosome 1 have been obtained for each segment, and could therefore be biased downwards.

QTL with F_2 family interaction

Only portions of linkage groups 1, 2, 3, 4, 5, 6, 9, 11, 13 and 28 could be scanned because, at other genomic regions, markers were not informative within families. Table 8 shows the location with the highest test statistic for linkage groups with genome-wide suggestive or significant results for test of QTL (with a and d differing over families) *vs* no QTL, together with a comparison of the model with F_2 family interaction with a model with no interaction at this location. No estimates for the QTL effects are given, for standard errors were very large because of the small F_2 family sizes (6 to 27 individuals depending on families and traits). The test statistic for a model with F_2 family interaction exceeded the 5% genome-wide significance threshold for TBCC at one location on chromosome 1 and this model fitted the data better at this location than a model with a QTL with the same effect across families. For all other traits except LNTROP, MCV and INTESTINE the test statistic exceeded the genome-wide suggestive threshold in one or more chromosomes. For 12 out of the 15 suggestive or significant locations, the model with family interaction fitted the data significantly better than a model with no interaction.

For locations where the test statistic for other models exceeded the relevant threshold, a model with family interaction was also compared to a model with a QTL with an additive and a dominance effect and no interaction (results not shown). This comparison was not always possible because a model that allowed for different effects across families could not be fitted for large portions of the genome. In these cases the model comparison was carried out a few cM apart or was not carried out when it was not possible (for small linkage groups for example). Using this comparison, a model with a QTL with family-different effects did not fit the data best at any of the locations tested.

DISCUSSION

We have found strong support for QTL segregation for carcass and organ weights and blood variables. Without taking into account the analyses that allowed for the interaction of the QTL with family, we identified 11 genome-wide significant QTL (most of them for CARCASS) and several genome-wide suggestive ones. Ikeobi *et al*. (2003) published analyses of carcass weight adjusted for live weight for the same cross. We present results for the unadjusted trait for comparison with results obtained for organ weights.

We chose to scan the genome for 4 alternative QTL models, which increases the chances of obtaining false positives, compared to a more conservative strategy for which the genome would have been scanned for a QTL with an additive and a dominance component and only the locations for which the test statistic exceeded the suggestive/significant genome-wide threshold would have been tested for alternative QTL models (with parent of origin

effect or sex interaction for example). In contrast, by using this more conservative strategy, one could potentially 'miss' genuine QTL because the model used for analysis does not agree with the true genetic model (for example, a QTL with opposite effects in males and females would not be found). Scanning the genome only with the most complex models could also lead one to 'miss' QTL because of a lack of power (since in many cases we would be unnecessarily fitting extra parameters) (De Koning *et al*., 2002).

All QTL significant at the genome-wide level would have been found by using the most conservative strategy but this would not have been the case for 8 genome-wide suggestive QTL (for LNCREAT on chromosomes 1 and 9, HEART on chromosome 1, LIVER on chromosomes 4, TBCC on chromosome 11 and PCV on chromosomes 1 and 2).

The genome-wide significant QTL identified by the single QTL searches were seldom picked up in the two-dimensional searches. This could be caused by the large number of degrees of freedom used in the tests when carrying out two-dimensional searches.

For 9 of the suggestive QTL there was suggestive evidence of parent of origin effect. Imprinting is an example of biological mechanism that would yield a non-zero estimate for the parent of origin component. By chance, combinations of linked (especially for long chromosomes) or unlinked genetic factors could also yield non-zero estimates for this component and so could the segregation of QTL within the founder lines, as demonstrated by simulation by De Koning *et al*. (2002).

We carried out searches for single QTL with an additive and a dominance effect with F_2 family interaction, but a large proportion of the genome could not be scanned because of a lack of information in a number of families. Although no power study has been done, since the number of parameters that have to be estimated is high when using a model that allows for different QTL effects across families, we can assume that power of this experiment to detect QTL segregating in the F_0 is low. Accordingly, one would expect to potentially 'miss' even genuine QTL. Nonetheless, we observed a relatively high number of locations that showed evidence of suggestive or significant linkage. Some of these locations were close to already detected significant or suggestive QTL and the model with family interaction did not always fit the data significantly better. Some other of the suggestive or significant locations found when searching for QTL with family-different effects were on linkage groups for which we had not previously found any evidence of linkage, or in linkage groups where we had, but at locations reasonably far away from the QTL identified assuming equal effects across families, suggesting that they were different QTL being detected. In these locations, generally, the test statistic for a model with no family interaction was low. For chromosomes where we had not previously detected QTL, we can suggest that these QTL are segregating within the F_0 lines and hence the power to detect them when assuming that the QTL is fixed in the founder lines is low (especially if their effect is small) as demonstrated by simulation by Alfonso and Haley (1998). For linkage groups where other QTL had been identified in previous analyses, a simple explanation would be that there are two QTL on them, one (already detected in searches of single QTL with the same effect across families) that would be fixed in the F_0 lines and a second one that is segregating. This is a plausible explanation since the locations identified with and without family interaction often correspond with the best locations of two-QTL analyses that failed to reach significance (results not shown). Alternatively, this could be an indication of a more complex genetic architecture of the traits: a simple model fails to detect QTL but, as the model becomes more complex, allowing for a difference in QTL effect depending on the parental origin of the allele (either parent of origin effect or different effects across families) it can accommodate some of the 'true' complications (or noise), and some locations become significant. In this respect, it is interesting to notice that—with the exception of chromosome 11—the suggestive imprinted QTL were located in either very long chromosomes (1 and 2) or chromosomes with poor marker coverage (4, 9 and 14). Both these scenarios would make it difficult to separate the effects of several genetic factors influencing a trait. In the latter case, improving this coverage would be beneficial.

The very long 95% confidence intervals obtained for some significant locations support the hypothesis of a complex genetic architecture. Including linked cofactors when estimating confidence intervals should decrease their length. This was indeed observed for confidence intervals on chromosome 1 (results not shown) but, in the cases where there is no clear evidence of the existence of more than one QTL on the linkage group, the choice of the locations to fit as cofactors may not be straightforward and it might be easier to simply refer to the frequency distribution obtained for the location parameter not fitting linked cofactors, because in these cases, inclusion of non-obvious cofactors could lead to biased results.

It is relevant to note that when we searched the genome for QTL with different effects across families, suggestive or significant results arose

more frequently for traits for which the grandparental lines have not been heavily selected for (like organ weights and blood variables) than for carcass weight, highly correlated with body weight, for which broilers have been intensely selected for decades. This long-term selection makes it more likely that grandparental lines are fixed for alternative CARCASS QTL alleles. Nonetheless, De Koning *et al*. (2003) detected QTL for growth in a pedigree of commercial broilers on chromosome 4. This study was the first to demonstrate that QTL identified in crosses of chicken populations were also segregating within a broiler population and the authors suggest that QTL for traits that have been long selected for could still be segregating because they may have pleiotropic effects on fitness traits.

In general, the results of our analyses are not conclusive for non-production traits in the sense that they do not provide a clear picture of the genetic control of the trait, but rather a series of hypotheses, that have not formally been tested against each other (which is not always possible). In any case, our results point at regions of the genome that are worth exploring further.

Several authors (Lande and Thompson, 1990; Utz *et al*., 2000; Göring *et al*., 2001) have noted that the use of the same data-set to estimate QTL location and effect would lead to estimates of effects biased upwards, especially for QTL of small effect (because the bias depends on the power of the study). It must therefore be borne in mind that the QTL effects presented are most likely overestimates of the true effects. This has implications when it comes to replicating experiments or use of findings in breeding programmes because not taking it into account would lead to an increased chance of failing to replicate the experiment or an overestimation of the gains to be accomplished with MAS or MAI programmes.

Although the signs of the estimated effects of some of the suggestive QTL are not in accordance with observed differences between layers and broilers (for example, we have identified QTL for which the broiler allele decreases intestine length and increases relative heart weight), the significant QTL are in accordance with observations and, in general, so are the overall effects with the exception of LNCREAT. Our overall estimates suggest that—taking into account these QTL—broilers should have lower creatinine kinase concentrations than layers. From the analyses of LNCREAT, individual QTL account for differences in creatinine kinase between lines from ~40 to ~10 (IU/l), and both positive and negative estimates of additive effects are obtained. Although we do not have information on line differences for LNTROP, we would expect that, overall, broiler alleles would increase troponin T in blood, because the incidence of cardiomyopathies is higher in broilers. However, only the estimated overall effect for females fits this expectation.

For LNCREAT we have found a suggestive location when searching for QTL with family interaction and the later model fitted the data better than one that assumed equal QTL effects across families. Estimates of QTL effects obtained assuming that the grandparental lines are fixed for alternative alleles are expected to be biased if this is not the case. The estimates obtained from the analyses with family interaction (not shown) are of different sign across families.

Overall QTL effects were different for males and females for some traits. Hocking *et al*. (1985) observed that adult outbred Leghorn females had relatively heavier livers than heavy strain females but this was not the case in males. This observation is not in agreement with our results, but this might be due to the difference in age of the birds from both studies. Maxwell *et al*. (1990) reported higher PCV in males than females and that fits with our findings. It has also been extensively reported that the incidence of ascites is higher in broiler males than in females, and the extremely different effect across sexes for relative heart weight or LNTROP could provide an explanation for this.

Several mapping studies of growth-related traits in chicken have been published recently. Sewalem *et al*. (2002) analysed the same population and found strong evidence of QTL for body weight on 7 macrochromosomes (1, 2, 3, 4, 7, 8 and Z) and two microchromosomes (13 and 27) and suggestive evidence of linkage on chromosomes 5, 6 and 9. We found significant or suggestive QTL for carcass weight on chromosomes 1, 2, 3, 4, 8, 13 and 27. As expected, most of the QTL are located within the same marker bracket in both studies, given the high correlation of both traits. Van Kaam *et al*. (1999) found evidence of QTL for body weight at 48 d of age on chromosomes 1 and 4 in a population derived from a cross of broiler lines and Tatsuda and Fujinaka (2001) identified a QTL for weight at 16 weeks on chromosome 1. Li *et al*. (2002) studied a series of production traits, anatomical and physiological measures in two resource populations and identified associations of TGF-beta2 (located on chromosome 3) with spleen weight, tibia length, bone mineral content and density and blood content of glucagon, insulin, T3 and IGF2 in a resource population derived from a cross of a broiler and a Leghorn line. In the same population they found TGF-beta3 (located on chromosome 5) to be associated with body weight, percentage abdominal

fat, spleen and liver weight, several bone characteristics and blood content of T3 and IGF2. In a different population (broiler × Fayoumi cross) they found associations of TGF-beta3 with similar characters. We did not find evidence of linkage for SPLEEN with any location on chromosomes 3 or 5 or for LIVER or CARCASS on chromosome 5. Several authors (Luger *et al*., 2001) have shown that broilers suffering from ascites also present a malfunction of the thyroidal axis and exhibit lower T3 and T4 levels. We have found evidence of genome-wide suggestive linkage for LNTROP with the region of chromosome 5 harbouring TGF-beta3 and therefore suggest that this region deserves further attention in metabolic disorder studies.

Rabie *et al*. (2002) described results from a whole genome scan for QTL affecting ascites-related traits carried out in a population derived from a cross of broiler lines. They reported three genome-wide significant QTL on chromosomes 2, 4 and 6 and suggestive QTL on chromosomes 1, 2, 3, 5, 8, 10, 13 and 28. This group has produced an F_8 derived from this population to validate and to narrow down the QTL regions. We identified associations of LNTROP, PCV, TBCC, MCV and HEART (which are all ascites-related traits) with locations on chromosomes 1, 2, 5, 6, 9, 11, 13, 14 and 17. The fact that QTL for heart weight (adjusted for carcass weight) have been found at different locations than those for weights of other organs (also adjusted for carcass weight) suggests that heart QTL specifically affect heart weight rather than general differences in weight of organs between broilers and layers.

The number of birds showing clinical signs of ascites was very low in our F_2 population. It could be argued that, ascites incidence being very low in this population, birds considered outliers for LNTROP were indeed ascitic birds, because they all showed high LNTROP values. No data are available for us to verify this hypothesis. Analyses of LNTROP data including all phenotypic records were carried out (results not shown) and no evidence of linkage was found for chromosomes 5 or 11. On the other hand evidence of suggestive QTL on chromosomes 2 and 17 was robust to the inclusion of birds with extreme values in the analyses.

In the current study, we have identified QTL with moderate effects for ascites-related traits and an indicator of skeletal muscle damage and hypothesised that some of these QTL could be segregating within the founder lines. Phenotypic studies (Navarro *et al*., 2001, 2002) and selection experiments (Wideman and French, 1999, 2000; Druyan *et al*., 2001, 2002) suggest that genes with large effect are involved in the control of ascites susceptibility and are segregating within broiler populations. Interestingly, when searching the genome assuming equal QTL effects across F_2 families associations with health-related traits have been found in linkage groups that showed no association with carcass weight (a production trait). If QTL affecting ascites-related traits and muscle dysfunctions were segregating within broiler populations, manipulation of allele frequencies at these loci should enable the improvement of broiler health and well-being without affecting carcass weight. It is difficult to draw any conclusion in this respect for linkage groups that harbour both QTL for carcass weight and health-related traits given the large confidence intervals for the location parameter.

Given the welfare and economic consequences of muscular and metabolic disorders in commercial broiler populations, tools to reduce their incidence in broiler flocks would be welcome both by the industry and the consumer. Our findings are a first step towards the understanding of the genetic architecture of these complex disorders. We have pointed to some genome regions that seem to be associated with health-related traits. Further studies—with greater marker coverage, for instance—could provide the means to reduce the incidence of these disorders through MAS or MAI.

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