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Quantitative trait loci for bone traits segregating independently of those for growth in an F_2 broiler × layer cross

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Abstract. An F_2 broiler–layer cross was phenotyped for 18 skeletal traits at 6, 7 and 9 weeks of age and genotyped with 120 microsatellite markers. Interval mapping identified 61 suggestive and significant QTL on 16 of the 25 linkage groups for 16 traits. Thirty-six additional QTL were identified when the assumption that QTL were fixed in the grandparent lines was relaxed. QTL with large effects on the lengths of the tarsometatarsus, tibia and femur, and the weights of the tibia and femur were identified on GGA4 between 217 and 249 cM. Six QTL for skeletal traits were identified that did not co-locate with genome wide significant QTL for body weight and two body weight QTL did not coincide with skeletal trait QTL. Significant evidence of imprinting was found in ten of the QTL and QTL × sex interactions were identified for 22 traits. Six alleles from the broiler line for weight- and size-related skeletal QTL were positive. Negative alleles for bone quality traits such as tibial dyschondroplasia, leg bowing and tibia twisting generally originated from the layer line suggesting that the allele inherited from the broiler is more protective than the allele originating from the layer.

Skeletal disease is an important welfare issue in broiler chickens that has been linked to genetic selection for rapid growth and accompanying management changes to maximise body weight gain (Webster, 1994). While the weight and processing yield of broilers have increased significantly over the past few decades, the skeletal system has apparently failed to evolve in parallel and may not be strong enough to support the weight of the animals (Bradshaw et al., 2002). The frequency of deleterious genes for skeletal traits such as those for tibial dyschondroplasia (TD) have also increased in broiler populations and specific selection pressure to reduce their prevalence has been required (Nicholson, 1998). Pattison (1993) reported that selection against TD, valgus-varus deformity, twisted leg and spondylolisthesis in broilers has successfully reduced the incidence of these diseases. Selection against these conditions is largely based on subjective assessment and skeletal weakness and disease persist in commercial broiler flocks. Indirect selection for genes that act favourably to improve skeletal traits via Marker Assisted Selection (MAS) would make the goal of eliminating skeletal disease achievable.

An F_2 cross between two divergent lines creates a population that maximises the opportunity of obtaining marker-trait associations and dramatic morphological differences between broiler and layer lines should maximise the number of segregating alleles of large effect in the F_2 population. Using statistical techniques and genetic markers of known

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location, Quantitative Trait Loci (QTL) that significantly influence skeletal traits can be identified. However, for accurate selection, the actual genes determining trait values or DNA markers that are closely associated with them are required (Smith and Smith, 1993). QTL studies therefore provide information on the regions of the genome in which to look for candidate genes or close associations between markers and genes in commercial populations (e.g. de Koning et al., 2003, 2004).

Understanding the genetic architecture underlying bone development facilitates the design of more efficient welfare-friendly breeding strategies. Specifically, it is important to assess the potential of MAS to improve skeletal integrity alongside continued increases in production traits. If the genes for these traits co-locate, selection for both is potentially more difficult than if they are on different regions of the genome. A large number of QTL have been identified in chicken crosses that influence not only weight, muscling and fatness but also disease resistance, behaviour and egg production (Hocking, 2005). The purpose of the present study was to identify QTL associated with skeletal traits in chickens using a broiler × layer cross and to compare these with previously identified QTL in the same population for weight (Sewalem, 2002), relative weights of different muscles (Ikeobi et al., 2004), abdominal fat weight, skin weight and fat distribution (Ikeobi et al., 2002), some organ sizes and a suite of health-related traits (Navarro et al., 2005).

Table 1. Chromosomes, number of markers, map length and first microsatellite marker on each chromosome used for genotyping F_2 broiler × layer cross

Linkage group	No. markers used	Map length (cM)	First marker
1	25	528	MCW0168
2	12	474	LEI0163
3	10	244	ADL0177
4	26	276	ADL0143
5	6	166	LEI0082
6	4	88	ROS0062
7	3	109	LEI0064
8	2	94	ADL0179
9	4	132	ROS0078
10	1	–	ADL0209
11	5	70	MCW0097
12	2	33	ADL0240
13	3	70	MCW0340
14	1	–	MCW0123
15	2	45	LEI0083
17	1	–	ADL0199
18	2	23	ROS0022
23	2	1	LEI0090
24	1	–	ROS113
26	1	–	ADL285
27	1	–	ROS071
28	2	39	ROS0095
E38	1	–	ROS073
W25	1	–	MCW249
Z	2	150	ROS0072

Materials and methods

Grandparent lines

Male and female chicks from a commercial broiler male-line and a line of White Leghorn layer chickens were raised to maturity and crossed as described below. A minimum of 24 chicks from each line and sex were housed in 16 pens (n = 8). The birds were weighed and measurements of chest width, body depth and metatarsal length were made at 6 weeks of age.

Mapping population

A detailed description of the experimental design was presented by Sewalem et al. (2002). Briefly, four F_1 full sib families were derived by crossing two male broilers with two female layers and two male layers with two female broilers. Two males and eight females were selected from each F_1 family. Each male was then bred to two females of the same cross but different family and one female from each of the two families of the reciprocal cross, to produce a total of 32 full-sib F_2 families.

Observations

Chest width, body depth and tarsometatarsal (shank) length were measured at 6 weeks of age using large callipers. Chest width was the widest distance between the two sides of the breast between the wings. Body depth was defined as the longest distance between the keel and the dorsal surface of the bird at right angle to it. Tarsometatarsal length was the external distance between the top of the hock joint after flexing the joint to form a right angle to the tibia and the edge of the right foot measured from the caudal aspect omitting the foot pad.

Scores for bowing and twisting of the legs and curvature of the digits ('crooked toes') were made at seven weeks by a commercially experienced assessor. Leg bowing was scored on a 3-point scale as absent, moderate or severe; leg twisting and twisted digits were scored as present or absent (1, 0).

The birds were slaughtered at 9 weeks of age and the carcasses were portioned according to the recommended WPSA protocol as described by Ikeobi et al. (2004). The thighs and drumsticks were dissected into muscle, skin and bone and the femur and tibia were weighed. The lengths of the tibiotarsi were measured with callipers and X-ray images of the tibiotarsi were scanned into digital form using NIH Image software (http://rsb.info.nih.gov/nih-image/) on a Macintosh Power PC 7600. Tibial diameter, thickness of cortical bone, and density were the mean of three areas at the mid-point and 20% of the distance from both ends of the bone. Tibial marrow diameter was calculated as tibial diameter minus 2× tibial cortical thickness. Bone density was defined by comparing the density of the image with that of a calibrated aluminium wedge as the equivalent density of aluminium of a specified depth. The images were screened visually for evidence of TD and confirmed by standard histological methods after staining with H & E. Subjective TD scores were given on the basis of the approximate size of the lesion as absent (score 0), present (1), <25% of area (2) and >25% area of the section (3).

Tibial plateau angles were measured on X-ray images between a perpendicular line drawn at right angles to the long axis of the bone and a second line drawn perpendicular to the long axis at the point where it bisected the proximal extremity and the cnemial crest across the surface of the condyles as described by Hocking et al. (2002). Torsion measurements (degrees) were calculated by comparing the transverse axes of the proximal and distal particular surfaces of the tibiae as described by Duff and Thorp (1985).

Two derived measures of tibial bone quality were determined. Bone strength is a function of tibial diameter and thickness of the cortex and, assuming the cross sectional area is a perfect circle, is related to the

Table 2. Body weight, tarsometatarsal length, chest width and body depth of male and female broiler and layer lines at 6 weeks of age. ** $P < 0.01$; *** $P < 0.001$

Line	Sex	Body weight[a] (g)	Tarsometatarsal length (mm)	Chest width (mm)	Body depth (mm)
Broiler	Male	7.61 (2,018)	104	69	117
	Female	7.51 (1,826)	96	69	110
Layer	Male	5.81 (334)	68	37	73
	Female	5.63 (279)	60	31	67
SED		0.038	1.6	2.4	2.1
Significance					
Line		***	***	***	***
Sex		***	***	–	**
Line × sex		–	–	–	–

[a] Transformed means with backtransformed values in parentheses.

Table 3. Traits and age of measurement, units, number of records, mean and standard deviation and numbers in each class for scored variables

Age (weeks)	Trait	Score/Units	No.	Mean	SD
6	Tarsometatarsal length	mm	433	94.0	5.4
6	Body depth	mm	433	97.2	5.5
6	Chest width	mm	433	62.4	5.2
6	Body weight	g	433	1,290	191
7	Leg bowing score	None (281), moderate (57), severe (89)	427	0.55	–
7	Leg twisting score	No (413), yes (14)	427	0.33	–
7	Crooked digits	No (247), yes (180)	427	0.42	–
9	Femur weight	g	433	28.0	6.4
9	Tibia weight	g	433	20.4	10.3
9	Tibia length	mm	430	125.9	7.50
9	Tibia diameter	mm	419	6.96	0.87
9	Tibia cortical width	mm	419	0.88	0.24
9	Tibia marrow diameter	mm	419	5.19	0.74
9	Tibia radiodensity	mm Al	432	2.35	0.32
9	Tibia plateau angle	Degrees	430	35.6	3.6
9	Tibia torsion	Degrees	432	9.5	4.8
9	Tibia dyschondroplasia	No (415), present (7), <25% (3), >25% (8)	433	0.09	–
9	Body weight	g	433	2,029	353

second moment of inertia (cross sectional moment of inertia, CSMI) as follows (Hiney et al., 2004; Williams et al., 2004):

$$\text{CSMI} = (\pi/4) \times (\text{tibial external radius}^4 - \text{tibial marrow radius}^4)$$

Tibial radiodensity is affected by the diameter of the bone and radiodensity was also analysed with inertia as a covariate to correct for the diameter of the tibia (CRD) and is a more accurate estimate of the mineralisation of the bone and its inherent strength.

Genotyping and linkage map

Genotyping of all the chickens was carried out as explained in Sewalem et al. (2002). Following genotyping, 433 individuals from 30 families with genotypes on 120 microsatellite markers situated on 25 linkage groups were available for analysis. A linkage map was constructed using CRIMAP (Lander and Green, 1987) and is similar to the 2000 consensus map (Schmid et al., 2000). The number of markers, map length and the first marker for each chromosome are presented in Table 1 and QTL location can be anchored to the consensus map using these data for comparison with other publications. It was assumed that maps were the same for males and females. The total length was 2,701 cM including 20 cM for the chromosomes with a single marker. This represents coverage of up to 90% of the chicken genome, ignoring gaps, and the average interval, omitting the densely genotyped chromosome 4, was 42 cM. Information regarding the pedigree, phenotypic records and genotypes of each individual at each marker was stored in www.resspecies.org (Law and Archibald, 2000).

Statistical analysis

Body weight for the grandparent lines was transformed by taking natural logarithms to normalise residual errors. Scores from traits measured on left and right legs were averaged for statistical analysis. Means and standard deviations for the skeletal traits and body weight at 6 and 9 weeks of age were calculated. Phenotypic correlations were determined for quantitative traits by Pearson's correlation and those involving qualitative traits by Spearman's rank correlation methods.

QTL analysis was performed using the least squares method of Haley et al. (1994) for crosses of out-bred lines. The model assumes that the grandparent lines are fixed for alternative alleles. The analysis is carried out in two stages. Initially, the probability of each F_2 individual being each of the four possible genotypes at all positions along the genome is calculated based upon their marker genotypes. Secondly, for each location, trait values are regressed on linear combinations of these probabilities to estimate the additive (a), dominance (d) and imprinting (i) effects for putative QTL at each location. The additive effect was

modelled as half the difference between the two broiler and layer homozygotes, while dominance was the difference between the heterozygote and the mean of the two homozygotes. The imprinting (parent of origin) effect is defined as the difference between the heterozygous genotypes when the broiler (or layer) allele is inherited from parents of the opposite sex (Knott et al., 1998). For the purpose of our analyses sex, family and pen (hatch was confounded with pen) were designated fixed effects and QTL-marker linkage analyses were performed using QTL Express (Seaton et al., 2002).

A total of eight models were used to search for QTL. Initial analyses of the autosomes were of a model with additive and dominance effects with imprinting, sex and both imprinting and sex effects for each trait. The sex chromosomes were analysed with additive genetic and sex effects only. In a second round of analyses, each QTL was reanalysed with a model that included all other suggestive and significant QTL affecting the trait as co-factors to remove background genetic noise (Jansen, 1993; Zeng, 1993). Finally, the assumption that alleles were fixed in the two lines was evaluated for each linkage group using a model with family × QTL interaction with additive and dominance effects for the autosomes and additive genetic effects only for the sex chromosmes.

The optimum model for each QTL was determined by carrying out standard F tests. The more complicated model was taken as the optimum model only if it provided a significantly better ($P < 0.05$) fit of the data. Previously calculated genome-wide significance thresholds (Navarro et al., 2005) were used to determine whether a QTL was significant under the optimum conditions using criteria suggested by Lander and Kruglyak (1995). In cases where a single QTL was suggestive or significant with a single QTL model, a 2-QTL analysis was carried out using the same set of genetic parameters. Comparisons of the 1-QTL and 2-QTL models were made by comparing the F-statistic obtained with the genome-wide significance thresholds. For cases where a 2-QTL model was the optimum model, the 2-QTL analysis was repeated using fewer genetic parameters and the two models were compared using genome-wide thresholds. For all suggestive and significant QTL 95% confidence intervals were calculated using the 2-LOD drop method (Manichaikul et al., 2006).

Results

The broiler line was six times heavier than the layer at 6 weeks of age (Table 2). Tarsometatarsal length and body depth were 1.5 times larger and chest width over twice as wide in the broiler compared with the layer line (Table 2). These differences between the grandparent lines for these traits are of order of 5 to 6 F_2 standard deviations from Table 3.

The number of F_2 records, trait means and standard deviations of the analysis set after edits, including body weight at 6 and 9 weeks, are presented in Table 3. Phenotypic correlations between these traits were calculated and are presented in Table 4. There were significant ($P < 0.05$) correlations between 6 and 9 week weight and all weight influencing traits, with the exception of tibial marrow diameter and cortical width. Tibial plateau angle, torsion, TD and leg bowing score showed significant positive correlations with a number of the weight influencing traits. TD and tibial torsion were also both positively correlated with tibial plateau angle. Crooked digits were significantly negatively correlated with leg twisting and bowing score; and leg twisting was negatively correlated with bowing score.

A total of 61 suggestive and significant QTL were identified on 16 of the 25 linkage groups using models 1 to 6 and QTL were found for 16 of the 18 traits. No QTL were identi-

Table 4. Phenotypic correlations between traits. Correlations significant at $P < 0.05$ are highlighted in bold.

Trait	Tarsom. length	Body depth	Chest width	Weight 6 week	Leg bow	Tibia twist	Crooked digits	Femur weight	Tibia weight	Tibia length	Tibia diameter	Tibia cortex	Tibia marrow	Tibia density	Tibia plateau	Tibia torsion	Tibia dsch.
Tarsometatarsal length (mm)																	
Body depth (mm)	**0.74**																
Chest width (mm)	**0.48**	**0.34**															
Body weight 6 weeks (g)	**0.84**	**0.73**	**0.71**														
Leg bowing score	**0.14**	**0.14**	0.07	**0.14**													
Tibia twist score	0.02	–0.02	0.02	0.03	**–0.11**												
Crooked digits score	0.03	–0.03	–0.07	–0.08	**–0.19**	**–0.11**											
Femur weight (g)	**0.86**	**0.68**	**0.46**	**0.84**	**0.12**	0.00	0.07										
Tibia weight (g)	**0.84**	**0.66**	**0.44**	**0.82**	**0.13**	–0.01	0.05	**0.97**									
Tibia length (mm)	**0.87**	**0.64**	**0.44**	**0.77**	**0.12**	0.02	0.07	**0.85**	**0.85**								
Tibia diameter (mm)	**0.66**	**0.55**	**0.41**	**0.69**	**0.14**	–0.01	–0.04	**0.69**	**0.73**	**0.62**							
Tibia cortical width (mm)	**0.22**	**0.18**	**0.17**	**0.24**	–0.01	–0.01	0.02	**0.22**	**0.23**	**0.16**	**0.52**						
Tibia marrow diameter (mm)	**0.63**	**0.53**	**0.37**	**0.65**	**0.17**	–0.01	–0.06	**0.67**	**0.70**	**0.62**	**0.84**	–0.04					
Tibia radiodensity (mm AL)	**0.53**	**0.43**	**0.20**	**0.48**	0.07	0.08	0.02	**0.62**	**0.63**	**0.51**	**0.55**	**0.29**	**0.46**				
Tibia plateau angle (°)	**0.16**	**0.16**	**0.20**	**0.26**	0.01	–0.02	–0.01	**0.15**	**0.19**	**0.19**	**0.29**	**0.35**	**0.12**	**0.09**			
Tibia torsion (°)	0.08	0.09	**0.13**	**0.17**	0.02	0.01	–0.04	**0.18**	**0.18**	**0.14**	**0.13**	0.04	**0.13**	**0.14**	**0.14**		
Tibial dyschondroplasia score	**0.11**	–0.01	**0.12**	**0.16**	0.00	–0.03	–0.02	0.10	**0.16**	**0.14**	**0.20**	**0.13**	**0.18**	0.09	**0.24**	0.08	
Body weight 9 weeks (g)	**0.83**	**0.70**	**0.59**	**0.88**	**0.12**	–0.02	–0.01	**0.86**	**0.86**	**0.79**	**0.65**	**0.21**	**0.62**	**0.17**	**0.17**	**0.15**	**0.12**

Table 5. Genome-wide significant and suggestive QTL for skeletal traits in an F_2 broiler × layer cross. Linkage group (LG), F-value, location (LN, cM) and 95% confidence interval (CI); additive, dominance, imprinting effects for male and female offspring; proportion of trait phenotypic variation explained by the genetic model (VP%) and flanking microsatellite markers. + Genome wide suggestive significance (one false QTL in a genome scan); * and ** respectively genome-wide significance level 5% and 1%.

Trait	LG	F		LN	CI	Sex	Additive	Dominance	Imprinting	VP%	Flanking markers
Tarsometatarsal length	1	10.43	**	126	114–137		1.05 (0.25)	–0.34 (0.36)		5.1	LEI0146–MCW0007
(mm)	1	11.41	**	402	394–462		1.28 (0.27)	0.34 (0.42)		5.6	MCW0167–ADL0183
	4	10.95	**	86	41–148		1.35 (0.29)	0.26 (0.43)		5.4	LEI0078–MCW0005
	4	42.94	**	241	223–251		2.79 (0.30)	–1.12 (0.53)		19.3	MCW0180–LEI0062
	W25	8.50	*	0	0–0		1.13 (0.29)	0.31 (0.39)		4.1	MCW249
Body depth (mm)	1	4.90	+	126	114–143	M	1.09 (0.39)	–0.14 (0.60)		4.3	LEI0146–MCW0007
						F	1.06 (0.41)	–1.03 (0.57)			
	4	12.09	**	65	39–124		1.54 (0.31)	–0.14 (0.43)		5.9	ADL0241–LEI0100
	4	17.38	**	228	195–254		1.87 (0.32)	–0.14 (0.50)		8.5	MCW0240–MCW0180
	Z	7.88	+	143	0–150		0.88 (0.31)			1.9	LEI0111–LEI0075
Chest width (mm)	1	5.90	*	136	119–151	M	2.03 (0.47)	1.56 (0.80)		5.5	LEI0146–MCW0007
						F	0.59 (0.46)	–0.98 (0.80)			
	3	8.91	*	59	16–104		1.28 (0.35)	1.35 (0.63)		4.5	HUJ0006–ROS0001
	5	10.48	**	54	47–91		2.56 (0.56)	0.75 (0.62)		5.3	ADL0292–ROS0084
	7	4.60	+	109	102–110	M	–0.09 (0.38)	–0.37 (0.55)		4.1	ADL0180
						F	1.64 (0.39)	–0.11 (0.55)			
	8	5.81	+	44	0–94		2.16 (0.77)	3.62 (2.98)		2.8	ADL0179–ROS0075
	18	4.69	+	22	0–23	M	1.14 (0.45)	0.41 (0.75)		4.2	ROS0022–ROS0027
						F	1.65 (0.50)	0.57 (0.76)			
	Z	9.68	+	150	0–150		0.86 (0.28)			2.5	LEI0075
Leg bowing score	1	3.34	+	355	337–382	M	–0.16 (0.10)	0.12 (0.18)	–0.18 (0.11)	3.8	MCW0036–LEI106
						F	–0.02 (0.10)	–0.51 (0.19)	–0.29 (0.11)		
	9	4.67	+	49	20–90		–0.00 (0.08)	0.42 (0.16)	0.25 (0.09)	3.0	ROS0078–MCW0135
	12	5.43	*	0	0–25	M	–0.23 (0.09)	0.03 (0.13)		4.8	ADL0240
						F	0.31 (0.08)	–0.04 (0.13)			
Leg twisting score	1	4.75	+	85	38–111	M	–0.09 (0.03)	–0.14 (0.05)		4.2	MCW0010–ADL0188
						F	0.01 (0.03)	0.02 (0.06)			
	3	4.30	+	144	110–199	M	–0.10 (0.03)	–0.17 (0.08)		3.6	MCW0187–ADL0306
						F	–0.02 (0.03)	0.09 (0.09)			
	6	4.28	+	88	71–88	M	–0.07 (0.02)	–0.05 (0.03)		3.5	ADL0323
						F	–0.01 (0.02)	–0.05 (0.03)			
	7	5.72	+	108	38–109		0.04 (0.01)	–0.03 (0.02)		2.6	ROS0019–ADL0180
	15	4.14	+	24	0–45	M	–0.08 (0.03)	–0.16 (0.07)		3.6	LEI0083–MCW0080
						F	0.06 (0.03)	–0.03 (0.07)			
Crooked digits score	1	6.93	+	309	285–316		–0.13 (0.04)	0.10 (0.06)		3.3	ROS0081–ADL0148
	5	4.54	+	25	6–42		–0.08 (0.04)	0.02 (0.05)	0.11 (0.04)	2.9	MCW0090
	8	6.89	+	54	0–94		–0.29 (0.09)	–0.31 (0.31)		3.2	ADL0179–ROS0075
Femur weight (g)	1	6.59	**	121	111–136	M	0.86 (0.35)	0.24 (0.57)	–0.37 (0.40)	8.7	LEI0068–LEI0146
						F	1.74 (0.37)	–1.27 (0.56)	0.47 (0.41)		
	4	6.25	*	216	200–228	M	–0.01 (0.45)	1.38 (0.64)		5.6	MCW0191
						F	1.90 (0.47)	0.08 (0.63)			
	4	38.07	**	237	225–246	M	2.56 (0.37)	–0.43 (0.65)		29.7	MCW0180–LEI0062
						F	4.26 (0.41)	–1.43 (0.65)			
	6	5.92	+	13	0–68		1.03 (0.30)	0.19 (0.59)		2.7	ROS0062–ROS0003
	W25	24.92	**	0	0–0		1.95 (0.28)	–0.37 (0.38)		12.0	MCW249
Tibia weight (g)	1	3.98	+	122	109–143	M	0.88 (0.58)	0.38 (0.93)	–1.11 (0.66)	4.8	LEI0068–LEI0146
						F	2.35 (0.62)	–1.18 (0.93)	0.53 (0.69)		
	4	5.17	+	103	51–121	M	1.71 (0.60)	–0.76 (0.90)		4.5	MCW0005–ADL0246
						F	1.61 (0.61)	2.36 (0.96)			
	4	33.9	**	240	226–248	M	4.11 (0.65)	–0.53 (1.18)		27.3	MCW0180–LEI0062
						F	7.13 (0.72)	–1.87 (1.17)			
	8	9.84	*	54	17–94		4.48 (1.03)	–6.18 (3.52)		4.8	ADL0179–ROS0075
Tibia length (mm)	4	40.17	**	238	227–249		3.57 (0.40)	–0.62 (0.66)		18.1	MCW0180–LEI0062
	5	5.11	+	47	3–166		–1.03 (0.38)	–1.20 (0.51)		2.3	ROS0013
	W25	14.39	**	0	0–0		2.03 (0.39)	0.45 (0.53)		7	MCW249
Tibia diameter (mm)	1	6.24	+	18	0–105		0.22 (0.07)	–0.23 (0.13)		2.9	ADL0160–MCW0010
	2	10.39	**	313	295–345		0.27 (0.06)	0.10 (0.10)		5.1	ROS0074–ADL0114
	4	5.40	+	64	11–176		0.18 (0.05)	0.002 (0.07)		2.5	ADL0241–LEI0100
	4	12.01	**	246	208–266		0.32 (0.06)	–0.11 (0.11)		5.9	MCW0180–LEI0062
	8	15.24	**	65	36–94		0.60 (0.11)	–0.55 (0.31)		7.5	ADL0179–ROS0075
	11	5.33	+	25	0–70		0.09 (0.05)	0.03 (0.08)	0.23 (0.07)	3.6	ROS0111–ADL0308
Tibial cortex width (mm)	28	3.16	+	24	0–39	M	0.09 (0.04)	0.16 (0.08)	0.11 (0.04)	3.6	ROS0095–ADL299
						F	–0.04 (0.04)	–0.11 (0.08)	0.02 (0.04)		

Table 5 (continued)

Trait	LG	F		LN	CI	Sex	Additive	Dominance	Imprinting	VP%	Flanking markers
Tibial marrow diameter (mm)	1	4.37	+	77	30–146	M	0.11 (0.10)	0.49 (0.22)		3.7	MCW0010–ADL0188
						F	0.30 (0.09)	0.14 (0.23)			
	4	5.88	+	142	78–230		0.14 (0.05)	–0.19 (0.09)		2.7	ADL0266–ROS0024
	4	12.23	**	249	219–275		0.26 (0.05)	–0.17 (0.09)		6.1	MCW0180–LEI0062
	6	4.88	+	20	0–62	M	0.14 (0.07)	0.38 (0.14)		4.3	ROS0062–ROS0003
						F	0.16 (0.07)	–0.22 (0.13)			
	8	9.79	*	50	12–94		0.50 (0.11)	–0.61 (0.40)		4.8	ADL0179–ROS0075
	11	6.56	*	18	0–47		0.09 (0.042)	0.04 (0.06)	0.2 (0.05)	4.6	LEI0110–ROS0111
Tibia radiodensity (mm Al)	4	6.14	*	217	197–257	M	0.05 (0.03)	0.03 (0.04)		5.4	MCW0191–MCW0240
						F	0.12 (0.03)	0.07 (0.04)			
Plateau angle (°)	1	5.24	+	135	104–170		0.85 (0.31)	0.79 (0.53)	–0.88 (0.33)	3.5	LEI0146–MCW0007
	2	5.05	+	225	128–243		0.42 (0.26)	0.08 (0.37)	–1.06 (0.30)	3.3	ADL0196
	3	3.99	+	206	130–243	M	1.30 (0.42)	0.70 (0.81)		3.3	ADL0306–MCW0040
						F	–0.36 (0.42)	–1.72 (0.80)			
	4	8.52	*	111	38–166		1.13 (0.28)	0.44 (0.44)		4.1	ADL0246–MCW0085
	8	4.44	+	41	11–94	M	2.69 (1.03)	–11.75 (3.95)		3.8	ADL0179–ROS0075
						F	1.64 (1.02)	3.53 (4.29)			
Tibia CSMI[a], (mm^4)	4	8.11	+	75	36–236		12.98 (3.30)	4.37 (4.56)		3.9	LEI0078–MCW0005
	8	13.04	**	78	41–94		25.13 (5.31)	–31.49 (11.77)		6.4	ADL0179–ROS0075
Tibia CRD[b], (mm Al/mm^4)	4	5.31	+	208	153–243		0.08 (0.03)	0.05 (0.06)	0.07 (0.03)	3.4	LEI0076–MCW0191

[a] Cross sectional moment of inertia, a measure of tibia strength (see text).
[b] Radiodensity adjusted for tibia inertia (covariate).

fied for tibial dyschondroplasia or tibial torsion with these models. Details of the QTL, the chromosomes on which they were located, the position on the chromosome, 95% confidence intervals and flanking markers are presented in Table 5. Additive, dominance and imprinting effects, for males and females where the sex-effect was different, and the percentage phenotypic variation explained by each QTL are also shown.

At least one significant QTL for tarsometatarsal length, body depth, femur weight, tibial weight, length, angle, diameter, radiodensity and marrow width were identified on chromosome 4. Furthermore, with the exception of plateau angle, one QTL for all these traits was situated on a similar region of chromosome 4 (217–249) and showed overlapping confidence intervals. Tarsometatarsal length, body depth and femur weight also had a second significant QTL on chromosome 4 at varying positions, and tibial weight, diameter, marrow width, CSMI and CRD displayed a suggestive QTL on chromosome 4. No suggestive or significant QTL were identified on chromosome 4 for tibial cortical thickness, chest width, leg bowing, leg twist or crooked digits.

Significant QTL were found on chromosome 1 for tarsometatarsal length (two QTL), chest width, weight of the femur and length of the tibia; chromosome 2 for tibia diameter; chromosomes 3 and 5 for chest width; chromosome 8 for tibia weight, diameter, marrow width and strength; chromosome 11 for tibia diameter and marrow width; chromosome 12 for leg bowing; and QTL for tarsometatarsal length, tibia length and femur weight occurred on linkage group W25.

Significant evidence of imprinting was found in 12 of the suggestive and significant QTL. Significant evidence of sex-different effects was found in one third of cases (22). Only chromosomes 1 to 7, 9, 11, 13 and Z could be analysed using models 7 and 8 because markers were not informative within families. Suggestive and significant QTL where family effects were significant are presented in Table 6. The chromosomes on which QTL were identified and the position of the QTL on the chromosomes are presented but additive and dominance effects are not shown because they had very large standard errors due to the small size of some of the families. A total of 39 suggestive and significant QTL were identified using models 7 and 8. Five of these QTL were identified using models 1 to 6 (i.e. occurring within the 95% confidence interval), but the remaining 31 had not previously been identified. In no case where a suggestive or significant effect was identified with models 1 to 4 did we find a significant family effect and a non-suggestive or significant QTL. Of the 31 newly identified QTL, 18 were significant at the genome level. Nine of these were for tibial torsion and TD, traits for which QTL were not identified with previous models.

Discussion

The large differences between the parent lines for weight and skeletal size (Table 2) have resulted in the identification of a significant number of QTL for skeletal traits including bone quality and skeletal disease. Specifically, we identified a large number of significant QTL on chromosome 4 for weight related skeletal traits. QTL for weight and length of the main leg bones (femur, tibia and tarsometatarsus) in the interval between MCW0180 and LEI0062 accounted for a

Table 6. Traits with genome-wide suggestive or significant family × QTL effects indicating that QTL alleles were not fixed in the grandparent (broiler and layer) lines. QTL in bold are those that were previously identified using models 1 to 6 (see text). + Genome wide suggestive significance; * and ** respectively genome-wide significance level 5% and 1%.

Trait	Linkage group	F-value		Location (cM)
Tarsometatarsal length (mm)	2	1.64	*	332
Chest width (mm)	2	1.74	*	247
	4	1.71	*	68
Body depth (mm)	9	1.5	+	130
Leg bowing score	5	1.47	+	1
	9	**1.61**	*	**45**
Leg twist score	**1**	**1.45**	+	**107**
	7	**1.43**	+	**100**
	13	1.81	*	32
Tibial weight (g)	5	1.49	+	48
	7	1.44	+	55
	11	1.48	+	49
Tibial length (mm)	1	1.53	+	409
	2	1.8	*	333
Tibial plateau angle (°)	1	1.69	*	0
Tibial radiodensity (mm Al)	7	1.44	+	38
Tibial diameter (mm)	1	1.71	*	401
	2	**1.66**	*	**312**
	7	1.47	+	51
Tibial marrow width (mm)	2	1.56	+	307
	Z	1.4	+	122
Tibial torsion (°)	3	1.7	*	84
	4	1.69	*	216
	5	1.64	*	115
Tibial dyschondroplasia score	1	1.96	*	323
	3	2.51	**	37
	4	2.54	**	216
	5	1.85	*	102
	6	1.53	+	31
	9	3.37	**	80
	11	1.87	+	50
	13	1.83	+	55
	Z	2.23	**	132
Tibia CSMI[a] (mm^4)	2	1.69	*	336
	4	**1.88**	*	**145**
	7	1.50	+	51
	11	1.48	+	42
	Z	1.50	+	140
Tibia CRD[b] (mm Al/mm^4)	7	1.62	*	25

[a] Cross sectional moment of inertia, a measure of tibia strength (see text).
[b] Radiodensity adjusted for tibia inertia (covariate).

substantial proportion (18 to 30%) of the phenotypic variation for these traits (Table 5). Sewalem et al. (2002) reported a QTL for body weight on chromosome 4 situated between the markers ADL0266 and LEI0073 that was subsequently shown to be a composite of two QTL at 100 and 237 cM respectively between markers MCW0005–ADL0246 and MCW0180–LEI0062 (Wong et al., 2004). QTL for body weight at locations around 240 cM have also been reported in experimental and commercial populations (de Koning et al., 2003, 2004; Sasaki et al., 2004). Schreiweis et al. (2005) found QTL for adult body weight, length and cross sectional area of the tibia and humerus and weight of the tibia on chromosome 4 in a confidence interval that overlaps with those in this study. Tibial plateau angle had a significant QTL at 111 cM and tarsometatarsal length and body depth respectively had a second significant QTL at 86 and 65 cM. It is noteworthy that no QTL for breast muscling and no significant QTL for leg muscles have been reported in this chromosome whereas the F-values of QTL are very high for tibia weight, femur weight and tibia and tarsometatarsal length near 240 cM. We conclude that the large QTL for body weight in this region are primarily associated with genes for skeletal growth and overall body size rather than the relative degree of muscling.

There were two regions on chromosome 1 containing QTL for weight- and size-related skeletal QTL that locate in the intervals identified by Sewalem et al. (2002) as containing QTL for live weight at 3, 6 and 9 weeks of age. A similar co-location existed between QTL for body weight on chromosomes 2, 5, 6, 7, 8 and 9 and QTL for skeletal traits. QTL for body weight for which no QTL were identified in this paper occurred on chromosomes 13 and 27. Conversely QTL for bone and skeletal traits were identified on chromosomes 11, 12, 15, 18, 28 and W25 for which Sewalem et al. (2002) reported no QTL for body weight. These results for QTL locations are consistent with the low to moderate phenotypic correlations between the traits in the F_2 reported in Table 4.

Estimates of the additive effect for traits associated with or influenced by weight (tarsometatarsal length, body depth, femoral weight, tibial weight, tibial length, tibial radiodensity, diameter, marrow width, cortical thickness and chest width) were positive in nearly all cases (i.e. the broiler allele increased the trait measurement). For the traits leg bowing, leg twist and crooked digits the additive estimate was negative in the majority of cases suggesting that the allele inherited from the broiler is more protective than the allele originating from the layer. Twisted leg and leg bowing are much more common among broilers than layers and these results suggest that broilers are genetically more resistant to these conditions than layers, and that recent selective breeding programs, by conscious selection for better leg health or indirectly as a necessary consequence of selection for high weight gains, may have been successful in reducing the genetic propensity for skeletal disease. However, due to continued selection for high weight gain in broiler chickens, it is probable that further genetic improvements in skeletal health will be required to maintain and even improve these traits. Greater understanding of the genetic background of these diseases will provide a means to continue to reduce these problems through MAS. Specifically, the identification of further QTL for bone disorders rather than bone weight and size are essential to make the potential of MAS for these important traits feasible in the future. The present results suggest that there are QTL locations that affect leg health that are not related to genes for general growth.

There were few estimates of dominance effects that were statistically significant. The result is similar to that for body weight reported by Sewalem et al. (2002) whereas the proportion of QTL with significant dominance effects for muscling was 58% (Ikeobi et al., 2004) and for relative organ size and estimates of fatness about 40% were significant (Ikeobi et al., 2002; Navarro et al., 2005). It is interesting that significant dominance effects were more frequent in the three analyses of models with a covariate and were uncommon in the analyses of body weight and skeletal measurements. It should be pointed out that the majority of dominance effects were only just significant (greater than two standard errors).

The majority of imprinting effects were also not much larger than twice their standard errors. About one in five QTL showed significant imprinting in the present study whereas none were reported for live weight, muscling or fat traits in this population (Ikeobi et al., 2002, 2004; Sewalem et al., 2002). Navarro et al. (2005) reported significant imprinting effects for organ sizes and blood traits in this population and evidence of imprinting effects has been reported for egg laying traits (Tuiskula-Haavisto et al., 2004) and immune traits (Siwek et al., 2003). These statistical results are not consistent with the parent conflict hypothesis of Moore and Haig (1991) and are comprehensively discussed in the paper by Tuiskula-Haavisto et al. (2004).

De Koning et al. (2003) identified QTL for growth in a nucleus flock of broilers, indicating that alleles for weight gain are not fixed in highly selected commercial lines as previously expected. In this study, we identified a number of QTL when family-effects were allowed to differ. In particular, a large number of QTL were identified for TD and tibial torsion. New QTL were also identified for leg twisting and tibial length, plateau angle and diameter. Tibial plateau angle and diameter are generally not used as selection targets. Similarly, Navarro et al. (2005) reported significant family-effects for traits such as organ weight that were also not primary selection traits. It is not surprising to find that alleles on unselected traits are not fixed in the grandparent lines.

In summary, we have identified a large number of QTL for skeletal size and morphology including some that do not co-locate with QTL for body weight. These results suggest that skeletal size may be altered independently of body weight. We have also identified a few genome significant and rather more suggestive QTL for bone quality traits that are related to resistance to skeletal disease and gait problems. A large number of QTL were found that were apparently not fixed in the two grandparent lines indicating that useful QTL for improved skeletal health are segregating in both of the commercial populations. The identification of beneficial alleles in broiler flocks may lead to effective MAS to improve skeletal health. Paradoxically we found evidence for positive and negative alleles respectively in the broiler and layer lines suggesting that phenotypic selection by poultry breeders has led to the selection of beneficial alleles in the commercial broiler population.

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