Genome-Wide Linkage Analysis to Identify Chromosomal Regions Affecting Phenotypic Traits in the Chicken. III. Skeletal Integrity[1]

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ABSTRACT Two unique chicken F_2 populations generated from a broiler breeder male line and 2 genetically distinct inbred (>99%) chicken lines (Leghorn and Fayoumi) were used for whole genome QTL analysis. Twelve phenotypic skeletal integrity traits (6 absolute and 6 relative traits) were measured or calculated, including bone mineral content, bone mineral density, tibia length, shank length, shank weight, and shank length:shank weight. All traits were also expressed as a percentage of BW at 8 wk of age. Birds were genotyped for 269 microsatellite markers across the entire genome. The QTL affecting bone traits in chickens were detected by the QTL express program. Significance levels were obtained using the permutation test. For the 12 traits, a total of 56 significant QTL were detected at the 5% chromosome-wise significance level, of which 14 and 10 were significant at the 5% genome-wise level for the broiler-Leghorn cross and broiler-Fayoumi cross, respectively. Phenotypic variation for each trait explained by all detected QTL across the genome ranged from 12.0 to 35.6% in the broiler-Leghorn cross and 2.9 to 31.3% in the broiler-Fayoumi cross. Different QTL profiles identified between the 2 related F_2 crosses for most traits suggested that genetic background is an important factor for QTL analysis. Study of associations of biological candidate genes with skeletal integrity traits in chickens will reveal new knowledge of understanding biological process of skeletal homeostasis. The results of the current study have identified markers for bone strength traits, which may be used to genetically improve skeletal integrity in chickens by MAS, and to identify the causal genes for these traits.

Key words: genome scan, quantitative trait loci, skeletal integrity, broiler, inbred line

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INTRODUCTION

Improvement in the growth rate and meat yield in broilers over the last 50 yr has resulted, in part, in selection for skeletal integrity. Although this trait was not routinely directly measured, the ability of the skeleton to support the musculature of the modern broiler has been a correlated result of selection on livability. Without sufficient skeletal integrity, broilers succumb to leg problems including tibial dyschondroplasia, twisted leg, rickets, kinky back, brittle bone disease, and general lameness (Lilburn, 1994). Furthermore, skeletal integrity is of particular importance during processing, in which bones must endure automated handling of the carcass without breakage. The effect of skeletal integrity across poultry production from breeders to processing is far-reaching, and when the skeleton is insufficient, it contributes to significant economic loss. The significance that bone plays in the laying hen as a reservoir for minerals deposited in the shell is obvious, and osteoporosis can contribute to significant economic losses in the egg industry as well (Schreiweis et al., 2005b). Although many skeletal issues can be solved by manipulation of nutrition and management, the focus of most research recently (Whitehead et al., 2004; Whitehead, 2004), it is clear that there is a genetic link to bone development in the chicken and other species (Bishop et al., 2000).

The genetic basis for skeletal development in chickens has been previously studied in both broilers and layers. A survey of commercial and traditional breeds indicated that there was significant variation in bone density and strength (Hocking et al., 2003). Heritability estimates of 0.40 for bone strength in laying hens have been demonstrated, in which selection over 7 generations resulted in a 2-fold increase (Bishop et al., 2000). Similar experiments resulted in improvement of bone strength in broilers (Mandour et al., 1989). A recent study evaluated a broiler × layer cross for genetic associations with skeletal integ-

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rity and did not detect any significant QTL influencing bone mineralization (Schreiweis et al., 2005a). This has not been the case in mammals in which multiple QTL have been found associated with bone mineralization (Christians et al., 2003; Ishimori et al., 2006).

As with laying hens, osteoporosis is a significant health issue in postmenopausal women and has been the subject of many studies to identify the genetic basis of the disease in humans and rodents (Liu et al., 2002). Early studies estimated that as much as 80% of the variation in bone mineralization could be under genetic control (Slemenda et al., 1991; Klein et al., 1998). Several QTL have been identified in mice and humans that are associated with bone mineralization, but none explain more than 10% of the phenotypic variation in the evaluated populations (Beamer et al., 2002). Li et al. (2003, 2005) and Zhou et al. (2005) have observed associations of bone mineralization in previous studies of candidate genes including transforming growth factor-4, insulin-like growth factor-I, and very low-density apolipoprotein-II with similar effects on the phenotypic variation. The aim of the current study was to perform a genome-wide analysis for QTL associated with skeletal traits in a well-characterized resource population to determine both the number of loci and their relative effects on bone integrity traits in chickens.

MATERIALS AND METHODS

Resource Populations

The Iowa Growth and Composition Resource Population was established by crossing sires from a broiler breeder male line with dams from genetically distinct, highly inbred (>99%) chicken lines, the Leghorn G-B2 and Fayoumi M15.2 (Zhou and Lamont, 1999; Deeb and Lamont, 2002). The F_1 birds were intercrossed, within dam line, to produce 2 related F_2 populations. Birds (n = 417 in broiler × Leghorn cross, n = 325 in broiler × Fayoumi cross) of the 2 F_2 populations were analyzed, with each population representing progeny from 1 broiler grandsire and 1 F_1 sire of each cross.

Phenotypic Measurements

The phenotypic measurements included bone mineral content (**BMC**), bone mineral density (**BMD**), tibia length (**TBL**), shank length (**SHL**), shank weight (**SHW**), and SHL:SHW (**SHR**). Tibias were analyzed for bone mineral characteristics using the dual-energy x-ray absorptiometry technique (Haarbo et al., 1991; Slosman et al., 1992; Mitchell et al., 1997), using a total-body dual-energy x-ray absorptiometry scanner (Lunar DPX-L, Lunar Corp., Madison, WI). The differential attenuation of low- (38 keV) and high-energy (70 keV) x-rays were measured using the small animal total body research software package in high resolution scan mode. Image analysis was used to accurately measure the BMC of each tibia and the axial cross-sectional area for determination of BMD (BMD = BMC/area). All traits were also expressed as a percentage of BW at 8 wk of age. Sex was determined by macroscopic inspection of the gonads.

Marker Selection, Genotyping, Linkage Analysis, and QTL Mapping

All birds were genotyped for 269 markers as described by Zhou et al. (2006). The marker linkage analysis and QTL mapping used were described in Zhou et al. (2006). Significance levels at the 5 and 1% chromosome-wise and the 5 and 1% genome-wise levels were estimated by permutation as described by Zhou et al. (2006).

Partial Correlation Analysis

The phenotypic correlations among skeletal traits were obtained using the JMP program (Sall and Lehman, 1996). Each partial correlation coefficient was simultaneously adjusted for all other variables other than the 2 being compared.

RESULTS

Phenotypic Correlations Among Skeletal Integrity Traits

The partial correlation coefficients among skeletal integrity traits in the combined 2 F_2 populations are shown in Table 1. In general, there were high correlations between each pair of traits (0.118 to 0.901), except for the relative low correlation between SHR% and both absolute and relative traits of BMC, BMD, and TBL (0.015 to 0.149). The phenotypic correlations between relative traits of BMD, TBL, and SHL and most of other traits (8 out of 11) were negative.

Significance Thresholds

Individual chromosome significance levels at the 5 and 1% level, as determined by the permutation test, differed slightly by trait within chromosome (Table 2). Average 5% chromosome-wise thresholds ranged from 4.18 to 7.15 in the broiler-Leghorn cross and from 4.31 to 7.20 in the broiler-Fayoumi cross. Average 1% chromosome-wise thresholds ranged from 6.00 to 9.21 in the broiler-Leghorn cross and from 6.21 to 9.23 in the broiler-Fayoumi cross. Average 5 and 1% genome-wise thresholds were 9.51 and 11.88, respectively, in the broiler-Leghorn cross and were 9.72 and 12.18, respectively, in the broiler-Fayoumi cross.

General QTL Mapping Results

Estimates for QTL significant at the 5% chromosome-wise level are presented in Tables 3 and 4. The QTL graphs, representing plots of the *F*-statistic across chromosomes, are presented in Figure 1, panels A and B, for BMC, BMD, TBL, and their relative traits, and Figure 2, panels A through E, for SHR, SHW, SHL, and their rela-

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Table 1. The phenotypic partial correlation coefficients among skeletal integrity traits in the F_2 population ($P < 0.05$)

Trait[1]	BMC%	BMD	BMD%	TBL	TBL%	SHR	SHR%	SW	SW%	SHL	SHL%
BMC	0.863	0.901	–0.567	0.545	–0.730	0.759	–0.015†	0.759	0.381	0.674	–0.754
BMC%	—	0.823	–0.118	0.309	–0.358	0.434	0.044†	0.427	0.248	0.376	–0.413
BMD	—	—	–0.374	0.415	–0.639	0.619	–0.060†	0.604	0.248	0.503	–0.681
BMD%	—	—	—	–0.627	0.889	–0.798	0.093	–0.811	–0.387	–0.770	0.846
TBL	—	—	—	—	–0.392	0.661	0.079	0.708	0.504	0.765	–0.513
TBL%	—	—	—	—	—	–0.788	0.149	–0.776	–0.285	–0.668	0.925
SHR	—	—	—	—	—	—	0.363	0.987	0.751	0.823	–0.756
SHR%	—	—	—	—	—	—	—	0.316	0.815	0.140	0.252
SW	—	—	—	—	—	—	—	—	0.760	0.898	–0.726
SW%	—	—	—	—	—	—	—	—	—	0.686	–0.158
SHL	—	—	—	—	—	—	—	—	—	—	–0.578

[1]BMC = bone mineral content; BMD = bone mineral density; TBL = tibia length; SHL = shank length; SHW = shank weight; SHR = SHW:SHL.

†$P > 0.05$.

tive traits. Although some graphs suggest evidence for multiple QTL in adjacent intervals for the same trait, only results for the most significant position are presented in Tables 3 and 4, because only single QTL models were tested.

In total, 56 and 37 QTL were detected at the 5% chromosome-wise level for the 12 traits examined in the broiler-Leghorn cross and the broiler-Fayoumi cross, respectively, not counting potential multiple QTL in adjacent intervals. Thirteen QTL would be expected to be significant at the suggestive threshold by chance alone, given the 12 traits examined. Therefore, over 4 and 3 times as many QTL were detected at this level than were expected by chance in the broiler-Leghorn cross and the broiler-Fayoumi cross, respectively. Of the 56 suggestive QTL in the broiler-Leghorn cross, 14 QTL were significant at the 5% genome-wise level (Table 5). Of the 37 suggestive QTL in the broiler-Fayoumi cross, 10 QTL were significant at the 5% genome-wise level. Over the 12 traits examined, only 1 QTL would be expected to be significant at this level by chance alone. Thus, 10-fold more QTL were identified at this level than were expected. There were no QTL affecting skeletal integrity-related traits detected on Gga 3, 10, 11, 13, 18, 27 and E46 in the broiler-Leghorn cross, whereas there were no QTL detected on Gga 3, 5, 10, 12, 13, 17, 18, 27; E46; and E47 in the broiler-Fayoumi cross. The phenotypic trait variances explained by QTL ranged from 2.83 to 17.24 in the broiler-Leghorn cross and from 2.92 to 9.81 in the broiler-Fayoumi cross (Tables 3 and 4).

BMC and BMC%. For the broiler-Leghorn cross, 5 QTL for BMC were detected on Gga 1, 4, 6, 8, and 17, and 6 QTL for BMC% were detected on Gga 4, 5, 6, 8, 12, and 17 (Table 3). The additive effect suggested that broiler alleles were superior to the Leghorn alleles, except for the QTL for BMC on Gga 6 and for BMC% on Gga 12. Four of the 11 QTL showed overdominance, and heterozygotes concerning breed origin of the allele had higher BMC or BMC% than either of the homozygotes. For the broiler-Fayoumi cross, QTL for BMC were identified on Gga 1, 7, and 8, and QTL for BMC% were identified on Gga 1, 8, and 15 (Table 4). Broiler alleles tended to be associated with higher BMC or BMC% than the Fayoumi alleles, except for BMC% on Gga 1. One of the 6 QTL showed overdominance, and 1 showed complete dominance. Heterozygotes had higher BMC than either of the homozygotes. The total trait variances explained by QTL for BMC and BMC% were 20.94 and 24.13% in the broiler-Leghorn cross and 15.97 and 15.30% in the broiler-Fayoumi cross, respectively (Table 5).

BMD and BMD%. For the broiler-Leghorn cross, 3 QTL for BMD were identified on Gga 4, 12, and 17, and 6 QTL for BMD% were identified on Gga 1, 2, 4, 7, 9, and Z (Table 3). Leghorn alleles tended to be associated with higher BMC or BMC% than the broiler alleles, except for BMD on Gga 4 and 17 and BMD% on Gga 1. Two of the 6 QTL showed high degrees of overdominance (Gga 4 and Z). Heterozygotes had lower BMD% than either of the homozygotes in the 2 QTL with overdominance effect. For the broiler-Fayoumi cross, 2 QTL for BMD were identified on Gga 1 and 8, and 3 QTL were identified on Gga 1, 2, and 7. The additive effect suggested that broiler alleles were superior to the Fay-

Table 2. The 5 and 1% chromosome-wise significance level, as determined by permutation test, for skeletal integrity traits by chromosome in the broiler-Leghorn cross and the broiler-Fayoumi F_2 cross

	Broiler-Leghorn cross		Broiler-Fayoumi cross	
Gga	5%	1%	5%	1%
1	7.15	9.21	7.20	9.23
2	6.00	7.97	7.01	9.15
4	5.69	7.60	5.47	7.40
5	5.77	7.92	5.09	7.06
6	5.00	6.93	5.23	7.18
7	5.42	7.36	4.49	6.39
8	4.69	6.55	5.42	7.47
9	5.27	7.20	4.95	6.91
12	4.92	6.82	4.98	6.86
14	5.04	6.89	4.31	6.21
15	5.11	7.11	4.69	6.80
17	4.22	6.82	4.86	6.63
24	4.18	6.00		
Z	4.84	6.72		

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Table 3. The QTL significant at the 5% chromosome-wise level for skeletal integrity by chromosome in the broiler-Leghorn cross[1]

Gga	Trait[2]	*F*-value	Location	Additive effect[3]	SE	Dominance effect[4]	SE	Variance (%)
1	BMC	7.96	687	0.19	0.05	–0.12	0.12	4.38
1	BMD%	10.32**	688	0.02	0.01	0.01	0.01	5.59
1	TBL%	7.92	0	–0.35	0.08	0.13	0.12	4.35
1	SHR	9.08*	0	0.17	0.04	–0.04	0.06	4.96
1	SHW	8.45	687	1.68	0.43	–0.40	0.61	4.63
1	SHL	9.85**	217	0.14	0.03	0.02	0.05	5.36
2	BMD%	6.15	397	–0.003	0.0003	0.001	0.0004	3.62
2	SHR	6.0	398	–0.002	6.40	–0.35	0.12	3.56
2	SHR%	6.06	295	0.003	0.003	0.01	0.003	3.56
2	SHW%	6.56	395	0.08	0.03	0.13	0.04	3.88
2	SHL%	7.3	398	0.007	0.01	0.04	0.01	4.30
4	BMC	11.53**	436	0.18	0.04	0.03	0.07	6.70
4	BMC%	5.71	276	0.004	0.001	–0.004	0.002	3.47
4	BMD	5.96	274	0.004	0.001	–0.002	0.002	3.74
4	BMD%	17.83***	422	–0.001	0.0002	–0.009	0.0004	9.09
4	TBL	11.12**	440	2.85	0.69	1.78	1.23	6.48
4	TBL%	10.42**	428	–0.37	0.09	–0.21	0.14	6.10
4	SHR	31.84***	429	0.34	0.05	0.11	0.07	16.56
4	SHR%	7.48	236	0.007	0.002	–0.004	0.002	4.47
4	SHW	33.43***	429	3.91	0.52	1.37	0.77	17.24
4	SHW%	17.2***	428	0.09	0.02	0.027	0.026	9.68
4	SHL	19.76***	429	0.21	0.04	0.14	0.06	10.96
4	SHL%	10.52**	436	–0.03	0.007	–0.013	0.012	6.15
5	BMC%	7.19	209	0.014	0.0036	–0.001	0.003	4.30
5	TBL	6.86	78	2.34	0.63	–0.46	0.95	4.10
5	SHR%	6.48	173	–0.007	0.002	0.003	0.003	3.82
6	BMC	6.17	92	–0.16	0.05	–0.09	0.07	3.70
6	BMC%	6.6	96	0.007	0.002	–0.004	0.003	3.88
6	SHL	15.47***	82	–0.23	0.05	0.03	0.06	8.79
7	BMD%	9.06**	51	–0.0009	0.0002	0	0.0003	5.41
7	TBL%	9.38**	51	–0.46	0.11	0.18	0.16	5.53
7	SHR	5.52	35	0.16	0.06	–0.23	0.09	3.33
7	SHR%	6.87	74	–0.005	0.002	–0.007	0.003	4.03
7	SHW%	7.34	220	–0.07	0.02	0.05	0.03	4.37
7	SHL%	15.65***	53	–0.04	0.007	0.006	0.01	8.89
8	BMC	5.37	56	0.08	0.03	0.09	0.05	3.24
8	BMC%	8.35*	56	0.004	0.001	0.005	0.002	4.99
8	SHR	5.73	14	0.31	0.11	0.08	0.12	3.45
8	SHR%	9.39*	66	0.008	0.002	–0.002	0.003	5.45
8	SHW%	13.19***	45	0.12	0.03	0.05	0.03	7.59
9	BMD%	6.24	147	–0.001	0.0003	–0.0001	0.0003	3.35
9	TBL%	5.46	155	–0.38	0.23	0.16	0.35	3.29
9	SHR%	7.75*	104	0.02	0.01	0.02	0.01	4.58
9	SHW%	7.24*	105	0.21	0.09	0.27	0.09	4.32
12	BMC%	7.15*	11	–0.009	0.003	0.004	0.002	4.30
12	BMD	5.25	7	–0.005	0.002	0.0004	0.002	3.16
14	TBL	7.39*	63	0.85	0.25	0.37	0.27	3.87
14	SHL	5.36	59	8.85	2.75	3.19	2.97	2.85
15	SHR	6.18	43	0.61	0.17	0.35	0.18	3.71
17	BMC	4.83	65	0.16	0.05	0.25	0.18	2.92
17	BMC%	5.42	71	0.007	0.002	0.01	0.009	3.19
17	BMD	8.46*	21	0.004	0.001	0.003	0.001	5.13
17	TBL%	5.32	31	–0.27	0.09	0.11	0.15	3.20
24	TBL%	4.67	29	–0.33	0.12	–0.17	0.17	2.83
24	SHR%	5.72	11	–0.006	0.006	–0.002	0.003	3.38
Z	BMD%	4.98	111	–0.0002	0.002	–0.0006	0.0002	2.93

[1]Estimated significance levels (*F*-value), location, gene effects, and percentage of F_2 variance explained by each QTL.

[2]BMC = bone mineral content; BMD = bone mineral density; TBL = tibia length; SHL = shank length; SHW = shank weight; SHR = shank weight:shank length.

[3]Additive (a) and dominance (d) QTL effects correspond to genotype values of +a, d, and –a, respectively, for individuals having inherited 2 broiler alleles, heterozygotes, and individuals with 2 inbred alleles. Positive additive effects indicate that broiler alleles increased the trait; negative additive effects indicate that the 2 inbred lines alleles decreased it.

[4]Dominance effects are relative to the mean of the 2 homozygotes.

*Significant at 1% chromosome-wise level; **significant at 5% genome-wise level ($F > 9.51$); and ***significant at 1% genome-wise level ($F > 11.88$).

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Table 4. The QTL significant at the 5% chromosome-wise level for skeletal integrity by chromosome in the broiler-Fayoumi cross[1]

Gga	Trait[2]	*F*-value	Location	Additive effect[3]	SE	Dominance effect[4]	SE	Variance (%)
1	BMC	11.68**	439	0.12	0.03	0.05	0.04	7.07
1	BMC%	8.54	248	–0.004	0.001	–0.004	0.002	5.26
1	BMD	11.6**	479	0.008	0.002	0.002	0.003	7.02
1	BMD%	10.46**	630	–0.0007	0.0002	0.0007	0.0003	6.85
1	TBL%	12.74***	437	–0.31	0.06	–0.03	0.06	7.67
1	SHR	16.69***	437	0.17	0.03	–0.01	0.04	9.81
1	SHW	15.42***	634	2.22	0.41	–0.95	0.45	9.12
1	SHL	7.52	628	0.17	0.04	–0.06	0.07	4.67
1	SHL%	13.03***	639	–0.02	0.005	–0.002	0.0007	7.83
2	BMD%	7.61	463	–0.001	0.0003	0.0007	0.0004	5.26
2	TBL%	9.87**	82	–0.35	0.08	–0.01	0.14	6.04
2	SHL%	15.35***	62	–0.03	0.006	–0.009	0.008	9.09
4	TBL%	6.81	226	0.22	0.17	–1.67	0.46	4.24
4	SHL%	6.07	105	–0.03	0.007	–0.006	0.01	3.81
6	TBL%	5.78	45	–0.29	0.08	–0.06	0.13	3.63
6	SHR%	6.27	47	–0.006	0.002	–0.006	0.003	3.95
7	BMC	6.28	133	0.46	0.13	0.29	0.14	3.93
7	BMD%	5.3	189	–0.0007	0.0002	0.0005	0.0004	3.21
7	TBL%	6.39	184	–0.31	0.10	0.32	0.18	4.00
7	SHL%	5.92	189	–0.02	0.007	0.03	0.01	3.72
8	BMC	8.02*	52	0.08	0.03	0.11	0.04	4.97
8	BMC%	9.43*	75	0.006	0.002	0.006	0.003	5.87
8	BMD	6.22	51	0.003	0.001	0.003	0.002	3.90
8	TBL	4.61	33	0.94	0.51	2.17	0.98	2.92
8	SHR	5.81	38	0.06	0.04	0.19	0.07	3.65
8	SHW	6.45*	38	0.64	0.41	2.29	0.75	4.04
8	SHL	5.11	43	0.03	0.04	0.17	0.06	3.22
8	SHL%	4.82	43	–0.006	0.005	–0.02	0.008	3.05
9	TBL%	5.57	121	–0.45	0.21	–0.03	0.23	3.50
9	SHR%	11.12**	51	–0.004	0.002	–0.008	0.003	6.76
9	SHW%	9.46*	51	–0.05	0.02	–0.05	0.02	5.81
11	SHR	5.1	49	0.18	0.06	0.07	0.07	3.21
14	SHR	7.04	60	0.49	0.13	0.12	0.15	4.20
14	SHW	5.74	60	5.00	1.48	0.89	1.64	3.45
15	BMC%	6.16	6	0.007	0.002	0.003	0.002	4.17
24	SHL%	6.00	40	0.002	0.006	0.02	0.01	3.76
Z	SHW%	5.74	32	0.15	0.50	1.25	0.50	3.42

[1]Estimated significance levels (*F*-value), location, gene effects, and percentage of F_2 variance explained by each QTL.

[2]BMC = bone mineral content; BMD = bone mineral density; TBL = tibia length; SHL = shank length; SHW = shank weight; SHR = shank weight:shank length.

[3]Additive (a) and dominance (d) QTL effects correspond to genotype values of +a, d, and –a, respectively, for individuals having inherited 2 broiler alleles, heterozygotes, and individuals with 2 inbred alleles. Positive additive effects indicate that broiler alleles increased the trait, negative additive effects indicate that the 2 inbred lines alleles decreased it.

[4]Dominance effects are relative to the mean of the 2 homozygotes.

*Significant at 1% chromosome-wise level; **significant at 5% genome-wise level ($F > 9.72$); and ***significant at 1% genome-wise level ($F > 12.18$).

oumi alleles for BMD, whereas the opposite effect was observed for BMD%. One of 5 QTL had overdominance effect, and heterozygotes had higher BMD% than either of the homozygotes (Table 4). The total trait variances explained by QTL for BMD and BMD% were 12.03 and 24.40% in the broiler-Leghorn cross and 10.92 and 15.32% in the broiler-Fayoumi cross, respectively (Table 5).

TBL and TBL%. For the broiler-Leghorn cross, QTL effects on TBL were detected on Gga 4, 5, and 14, and 6 QTL for TBL% were detected on Gga 1, 4, 7, 9, 17, and 24 (Table 3). For the broiler-Fayoumi cross, 1 QTL for TBL was identified on Gga 8, and 6 QTL for TBL% were identified on Gga 1, 2, 4, 6, 7, and 9 (Table 4). Two of the 7 QTL showed strong overdominance, and heterozygotes showed the lower TBL% at QTL on Gga 4 and higher TBL on QTL on Gga 8 than either of the homozygotes (Table 4). The additive effect suggested that the broiler alleles were superior to the Leghorn alleles for TBL, whereas the opposite effect was observed for TBL% except for the QTL on Gga 4 in both F_2 crosses. The total trait variances explained by the QTL were 14.45 and 19.20% for TBL and TBL% in the broiler-Leghorn and 2.92 and 21.45% in the broiler-Fayoumi cross, respectively (Table 5).

SHR and SHR%. For the broiler-Leghorn cross, QTL affecting SHR were found on Gga 1, 2, 4, 7, 8, and 15, and QTL for SHR% were found on Gga 2, 4, 5, 7, 8, 9, and 24 (Table 3). The additive effect suggested that broiler alleles were superior to the Leghorn alleles for SHR and SHR%, except for the QTL for SHR on Gga 2

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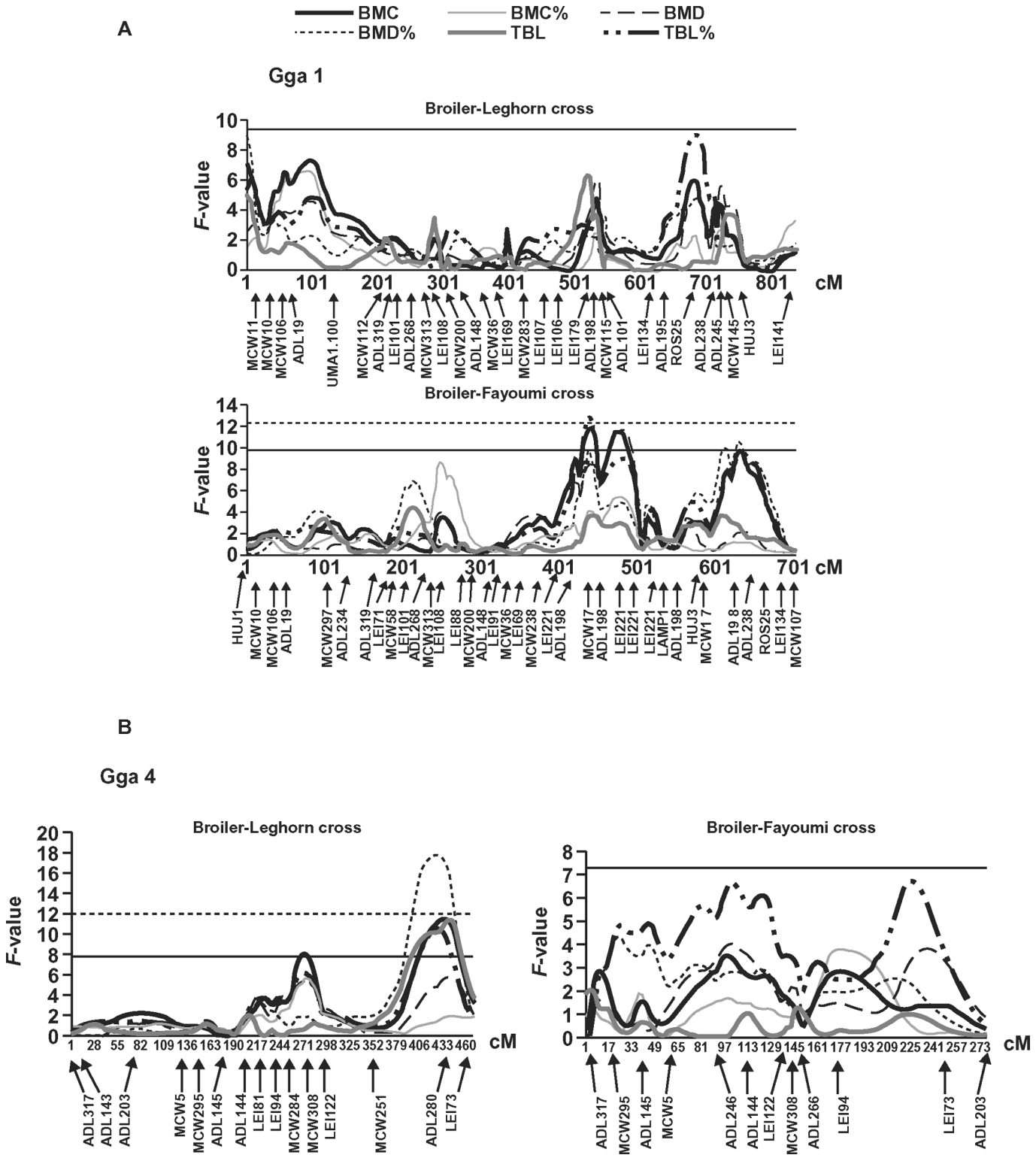

Figure 1. The *F*-value curves for evidence of QTL for bone mineral content (BMC), bone mineral density (BMD), tibia length (TBL), and their relative traits. The x-axis indicates the relative position on the linkage group. The y-axis represents the *F*-value. Arrows on the x-axis indicate the positions where a marker was present. Two lines are provided for 1% chromosome-wise (—) and 1% genome-wise (- - - -) significance.

and for SHR% on Gga 5, 7, and 24. One of the 13 QTL showed strong overdominance, and heterozygotes showed the highest SHR% at QTL on Gga 2. For the broiler-Fayoumi cross, QTL were identified for SHR on Gga 1, 8, 11, and 14 and for SHR% on Gga 6 and 9 in the broiler-Fayoumi cross (Table 4). Broiler alleles were superior to the Fayoumi alleles for SHR, whereas the opposite effect was observed for SHR%. One of the 6 QTL showed overdominance effect, and heterozygotes showed higher SHR than either of homozygotes. The

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Table 5. Number of QTL significant at the 5 and 1% chromosome-wise level (CHR) and genome-wise (GEN) level, respectively, by trait in the F_2 broiler-Leghorn and the broiler-Fayoumi crosses

	Broiler-Leghorn cross					Broiler-Fayoumi cross				
Trait[1]	5% CHR	1% CHR	5% GEN	1% GEN	Variance (%)	5% CHR	1% CHR	5% GEN	1% GEN	Variance (%)
BMC	4	—	1	—	20.94	1	1	1	—	15.97
BMC%	4	2	—	—	24.13	1	2	—	—	15.30
BMD	2	1	—	—	12.03	1	—	1	—	10.92
BMD%	3	1	1	1	24.40	2	—	1	—	15.32
TBL	1	1	1	—	14.45	1	—	—	—	2.92
TBL%	3	1	1	—	19.20	2	—	1	1	21.45
SHR	4	1	—	1	35.57	2	—	—	1	16.67
SHR%	6	1	—	—	29.29	1	—	1	—	10.71
SHW	1	—	—	1	21.87	—	3	—	1	20.03
SHW%	3	—	—	2	29.84	—	1	—	—	5.81
SHL	1	—	1	2	27.96	2	—	—	—	7.89
SHL%	1	—	1	1	19.34	4	—	—	2	31.26

[1]BMC = bone mineral content; BMD = bone mineral density; TBL = tibia length; SHL = shank length; SHW = shank weight; SHR = shank weight:shank length.

total trait variances explained by QTL for SHR and SHR% were 35.57 and 29.29% in the broiler-Leghorn cross and 16.67 and 10.71% in the broiler-Fayoumi cross, respectively (Table 5).

SHW and SHW%. The QTL effects for SHW were detected on Gga 1 and 4 and for SHW% on Gga 2, 4, 7, 8, and 9 in the broiler-Leghorn cross (Table 3). Broiler alleles showed associations with higher SHW and SHW% than the Leghorn alleles, except for the QTL on Gga 7. Four QTL were identified on Gga 1, 8, 14, and Z for SHW and 1 for SHW% on Gga 9 in the broiler-Fayoumi cross (Table 4). Broiler alleles showed higher SHW than the Fayoumi alleles, whereas broiler alleles had lower SHW% than the Fayoumi alleles. Two of the 4 QTL showed high degrees of overdominance, and heterozygotes had higher SHW than either of the homozygotes (Gga 8 and Z, Table 4). The total trait variances explained by the QTL for SHW and SHW% were 21.87 and 29.84% in the broiler-Leghorn cross and 20.03 and 5.81% in the broiler-Fayoumi cross, respectively (Table 5).

SHL and SHL%. Four QTL affecting SHL on Gga 1, 4, 6, and 14 and 3 QTL affecting SHL% on Gga 2, 4, and 7 were found in the broiler-Leghorn cross (Table 3). The additive effect indicated that broiler alleles were superior to the Leghorn alleles except for the QTL on Gga 4 and 7 for SHL%. One of the 7 QTL showed overdominance, and heterozygotes had higher SHL% than either of the homozygotes. Two QTL for SHL were identified on Gga 1 and 8, and 6 QTL for SHL% were identified on Gga 1, 2, 4, 7, 8, and 24 in the broiler-Fayoumi cross (Table 4). Four of the 8 QTL showed overdominance, and heterozygotes had larger SHL% than either of the homozygotes on Gga 7 and 24 and for SHL on Gga 8,

Table 6. Positional candidate genes for skeletal integrity QTL identified in both the Leghorn and Fayoumi crosses

Gga	Location	Line[1]	Trait[2]	Positional candidate gene(s)	Association previously reported
1	MCW11	LEG	SHR	Stem cell factor, bone marrow proteoglycan 2	—
1	ADL198	FAY	BMC, TBL%, SHR	—	—
1	ADL238	LEG, FAY	BMD%, SHW, SHL%	Progesterone receptor, fibroblast growth factor 8, Sox-10	Graham and Clark, 1997; Garofalo et al., 1999; Chimal-Monroy, 2003; Wardecka et al., 2002; Klein et al., 2002
2	LEI117-MCW247	FAY	TBL%, SHL%	Calcitonin receptor	
4	ADL260	LEG	BMC, BMD%, TBL, TBL%, SHR, SHW, SHW%, SHL, SHL%	Type IIb Na-Pi cotransporter, bone marrow stromal antigen, ADP ribosyl cyclase	Hilfiker et al., 1998; Sun et al., 1999
6	LEI92	LEG	SHL	Calmodulin-dependent protein kinase II, calcineurin-binding protein	—
7	ADL279-MCW183	LEG	TBL%, SHL%	Bone morphogenetic protein receptor type II	Nakamura et al., 2003
8	MCW147-MCW64	LEG, FAY	BMC, BMC%, SHW, SHR%, SHW%	Target of Egr1, transforming growth factor β receptor 3	Li et al., 2003; Cohen, 1997
9	ADL211	LEG, FAY	SHR%, SHW%	Angiotensin II, osteocrin	Leung, 2004; Thomas et al., 2003
12	ADL240	LEG	BMC%	—	—
14	ADL118	LEG	TBL	Tumor necrosis factor α	—
17	MCW330	LEG	BMD	—	—

[1]FAY = broiler-Fayoumi cross; LEG = broiler-Leghorn cross.

[2]BMC = bone mineral content; BMD = bone mineral density; TBL = tibia length; SHL = shank length; SHW = shank weight; SHR = shank weight:shank length.

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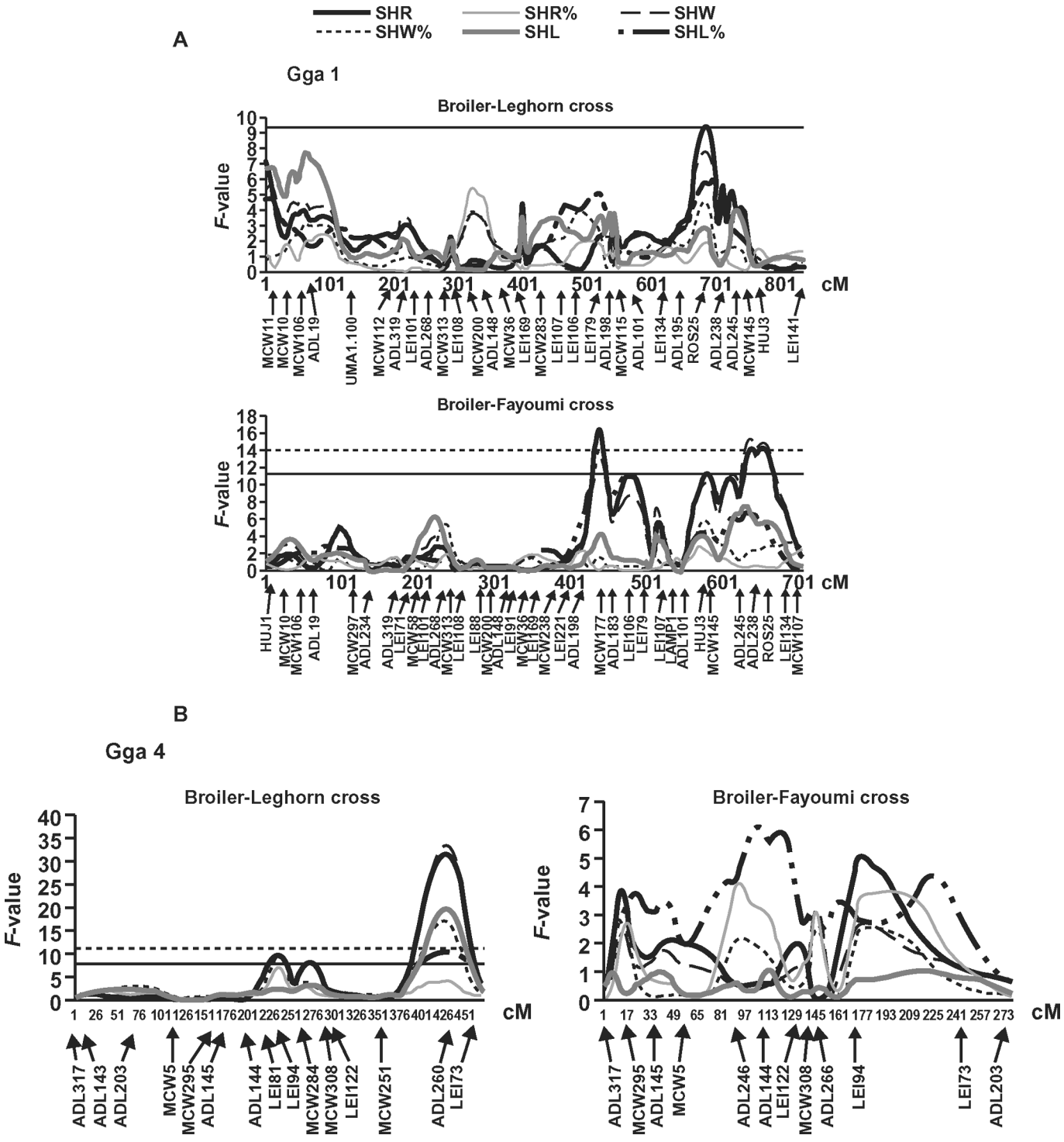

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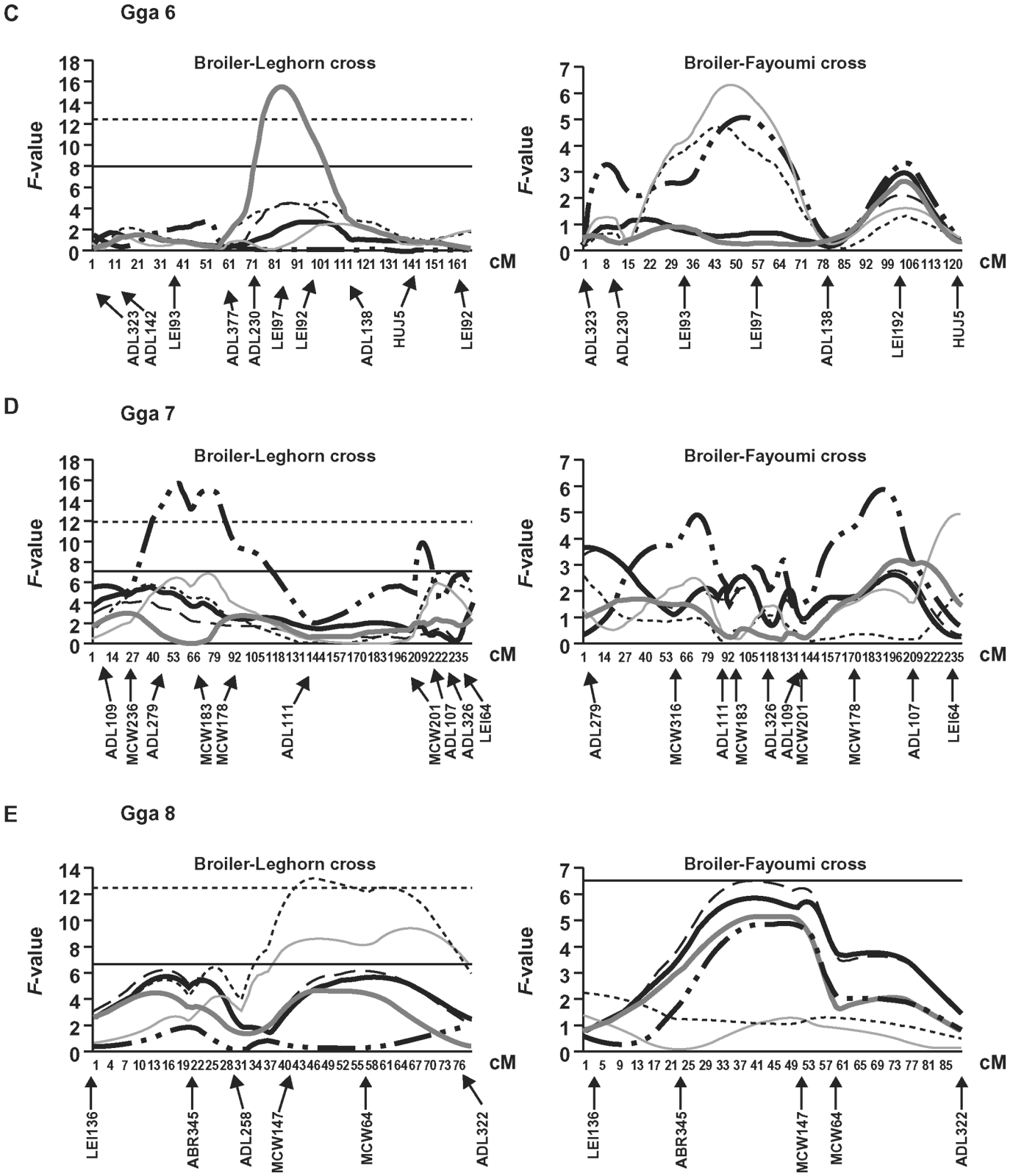

Figure 2. The *F*-value curves for evidence of QTL for shank length (SHL), shank weight (SHW), SHW:SHL (SHR), and their relative traits. The x-axis indicates the relative position on the linkage group. The y-axis represents the *F*-value. Arrows on the x-axis indicate the positions where a marker was present. Two lines are provided for 1% chromosome-wise (—) and 1% genome-wise (- - - -) significance.

but the opposite for SHL% on Gga 8. For SHL, broiler alleles were superior to the Fayoumi alleles, whereas the Fayoumi alleles were superior to the broiler alleles for SHL%, except for the QTL on Gga 24. The total trait variances explained by the QTL for SHL and SHL% were 27.96 and 19.34% in the broiler-Leghorn cross and 7.89

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and 31.26% in the broiler-Fayoumi cross, respectively (Table 5).

DISCUSSION

In the present study, 12 traits related to skeletal integrity were analyzed for QTL using a whole genome scan approach in 2 related F_2 resource populations. A total of 56 QTL were detected for the 2 crosses at the 5% chromosome-wise significance level. Of these QTL, 12 were significant at the 1% chromosome-wise level. Those QTL with the highest significance levels will be discussed further, including positional candidate genes (Table 6). In general, the traits measured in this study can be grouped into 2 categories, which are bone size (TBL, SHL, SHW, SHR) and bone mineralization (BMD and BMC), and are not always associated with the same locus. In light of these QTL regions and recent work on the genetics of skeletogenesis, multiple connections can be made relating the skeletal trait QTL and positional candidate genes with known functions in bone development, differentiation, and maturation (Karsenty, 1998). The skeletal system is comprised of 3 distinct cell types: chondrocytes (found in cartilage), osteoblasts, and osteoclasts (found in bone). These cell types have been shown to be regulated in developmental biology (skeletal patterning), differentiation, and function by transcription factors and specific sets of genes.

Developmental Patterning

In the embryo, limb formation is the most studied skeletal element and also the most understood. Limbs develop from cells derived from the somatic mesoderm and the somatic plate (Searls and Janners, 1971). Fibroblast growth factor (**FGF**) 8 is involved in initial limb bud formation (Crossley et al., 1996). Other FGF have been implicated in the elongation of the limb bud by regulating the diffusible factor sonic hedgehog (Laufer et al., 1997). In the present studies, a QTL region was identified on Gga 1 that contained the gene for FGF 9, which has been shown to be involved in skeletal development, specifically linear bone growth (Garofalo et al., 1999). The gene Sox-10 is also found in this region of Gga 1 and also functions to regulate chondrogenesis during limb development of the embryo (Chimal-Monroy et al., 2003). This region on Gga 1 has also been previously associated with a QTL for egg traits and skeletal traits in the chicken and the syntenic region in mouse (Klein et al., 2002; Wardecka et al., 2002).

Other than limb formation, not much else is known concerning the complexity of skeletal architecture and the influence of specific factors or genes, with the exception of mutations in several gene families including the bone morphogenetic proteins and the transforming growth factor-β proteins (**TGF-β**). Mutations or knockouts in these genes cause defects in skeletal patterning (Hogan, 1996; Thomas et al., 1996). The QTL was detected in regions where bone morphogenetic protein receptor type II and TGF-β receptor 3 are found on Gga 7 and Gga 8, respectively. The bone morphogenetic protein receptor type II functions as a TGF-β receptor and has been previously associated with osteogenesis (Nakamura et al., 2003). The QTL detected on Gga 8 is associated with both bone size and mineralization traits. The association of the TGF-β gene SNP has been previously described for the Iowa Growth and Composition Resource Population (Li et al., 2003), whereas TGF-β receptor 3 has been associated with bone formation and signaling through the TGF-β pathway (Cohen, 1997). Many genes related to skeletal patterning in embryo development were identified as potential positional candidates for QTL influencing skeletal traits in the population studies.

Chondrocytes

Arising from the preskeletal mesenchymal cells, chondrocytes can be identified by their expression of type IIb collagen; type IX, XI collagen; and matrix Gla protein. During differentiation, the chondrocytes begin to express type X collagen. This differentiation is stimulated by many factors including parathyroid hormone-related peptide and Sox-9. In the growing broiler, the priority is to elongate existing bones by endochondral growth at the epiphyseal growth plate and mineralize them by osteoblasts. Locally produced peptide growth factors play important autocrine and paracrine roles in normal growth plate physiology, including bone proteoglycan 2, which is involved in chondrocyte growth and differentiation (Leach and Twal, 1994). The gene for bone proteoglycan 2 is located on Gga 1 within the QTL region associated with bone size. Variation in this gene may affect the ability of the chondrocytes to elongate existing bone at the growth plate.

Osteoblasts

The deposition of bone matrix is performed by the osetoblast cell. Arising from the mesenchyme in embryo development, these cells are characterized by their expression of osteocalcin (Ducy and Karsenty, 1995) and differentiate under the regulation of the runt transcription factor gene family (Merriman et al., 1995). Other factors have also been described as regulating osteoblasts including osteocrin, a novel, unique vitamin D-regulated bone-specific protein (Thomas et al., 2003). The osteocrin gene is located on Gga 9 in the region where QTL were detected for bone size. Also in this region of Gga 9 is angiotensin II. Angiotensin peptides have multiple and novel actions including cell growth, antiproliferation, apoptosis, reactive oxygen species generation, hormonal secretion, proinflammatory and profibrogenic actions, as well as vasoconstriction and vasodilatation (Leung, 2004). Either of these genes are suitable positional candidates for genetic variation influencing skeletal traits at this locus.

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Osteoclasts

Bone resorption is performed by the osteoclast. These large multinucleated cells are derived from the monocyte lineage and rest in contact with the bone matrix. Osteoclast differentiation is controlled in part by osteoprotegrin (Simonet et al., 1997) involving calmodulin-dependent protein kinase II and calcineurin-binding protein (Zhang et al., 2005). The main effects on bone turnover (remodeling) are exerted on the osteoclasts. Progesterone is associated with bone remodeling and maintenance of mineralization (Graham and Clarke, 1997). The progesterone receptor gene is located on Gga 1 in the QTL region associated with bone mineralization and length. Tumor necrosis factor-α increases osteoclast formation and bone resorption both directly and by augmenting the sensitivity of maturing osteoclasts to the essential osteoclastogenic factor receptor activator of nuclear factor κ-B (Weitzmann and Pacifici, 2005). A QTL was detected on Gga 14 associated with bone size in the region of the positional candidate gene tumor necrosis factor-α.

Suppression of osteoclast function is also important in maintaining Ca stores. Calcitonin acts via its receptor to strongly inhibit osteoclast function to disrupt bone formation and resorption. The calcitonin receptor has been the target of therapy for osteoporosis (Munoz-Torres et al., 2004) and lies within the QTL region on Gga 2 associated with bone size.

Nutrition

Other factors influencing skeletal development in poultry have been studied including management techniques, bird mobility, and specialized feed additives, but the primary research area has been in vitamin and mineral nutrition. The importance of sufficient dietary Ca and P is undisputed as well as the effect of vitamin D for skeletal integrity. The mechanisms by which these nutrients are absorbed and utilized may provide suitable candidates for genetic influence on skeletal traits. The type IIb Na-P cotransporter is primarily expressed in the brush border membranes of the small intestinal epithelium, where it is considered to be responsible for P uptake in the intestine (Hilfiker et al., 1998). This gene is located within the QTL region on Gga 4 with a *F*-statistic value ranging from 10.5 to 33.4 explaining from 6.1 to 17.2% of the phenotypic variance by this locus. This region was also identified by Schreiweis et al. (2005a) as containing a putative QTL for bone size and mineralization. The specific role of this gene in the genetic basis of bone development and skeletal integrity deserves additional investigation.

In summary, QTL detected in the present study using a whole genome scan approach has provided an excellent framework for the fine-mapping of QTL affecting skeletal integrity straits. The positional genes described above will be strong candidate genes to be further explored to determine associations with bone traits in the chicken.

REFERENCES

Beamer, W. G., L. R. Donahue, and C. J. Rosen. 2002. Genetics and bone. Using the mouse to understand man. J. Musculoskelet. Neuronal. Interact. 2:225–231.

Bishop, S. C., R. H. Fleming, H. A. McCormack, D. K. Flock, and C. C. Whitehead. 2000. Inheritance of bone characteristics affecting osteoporosis in laying hens. Br. Poult. Sci. 41:33–40.

Chimal-Monroy, J., J. Rodriguez-Leon, J. A. Montero, Y. Ganan, D. Macias, R. Merino, and J. M. Hurle. 2003. Analysis of the molecular cascade responsible for mesodermal limb chondrogenesis: Sox genes and BMP signaling. Dev. Biol. 257:292–301.

Christians, J. K., V. Bingham, F. Oliver, T. T. Heath, and P. D. Keightley. 2003. Characterization of a QTL affecting skeletal size in mice. Mamm. Genome 14:175–183.

Cohen, M. M. 1997. Transforming growth factor β s and fibroblast growth factors and their receptors: Role in sutural biology and craniosynostosis. J. Bone Miner. Res. 12:322–331.

Crossley, P. H., G. Minorada, C. A. MacArthur, and G. R. Martin. 1996. Roles for FGF8 in the induction, initiation, and maintenance of chick limb development. Cell 84:127–136.

Deeb, N., and S. J. Lamont. 2002. Genetic architecture of growth and body composition in unique chicken populations. J. Hered. 93:107–118.

Ducy, P., and G. Karsenty. 1995. Two distinct osteoblast-specific *cis*-acting elements control expression of a mouse osteocalcin gene. Mol. Cell. Biol. 15:1858–1869.

Garofalo, S., M. Kliger-Spatz, J. L. Cooke, O. Wolstin, G. P. Lunstrum, S. M. Moshkovitz, W. A. Horton, and A. Yayon. 1999. Skeletal dysplasia and defective chondrocyte differentiation by targeted overexpression of fibroblast growth factor 9 in transgenic mice. J. Bone Miner. Res. 14:1909–1915.

Graham, J. D., and C. L. Clarke. 1997. Physiological action of progesterone in target tissues. Endocrinol. Rev. 18:502–519.

Haarbo, J., A. Gotfredsen, C. Hassager, and C. Christiansen. 1991. Validation of body composition by dual energy X-ray absorptiometry (DEXA). Clin. Physiol. 11:331–341.

Hilfiker, H., O. Hattenhauer, M. Traebert, I. Forster, H. Murer, and J. Biber. 1998. Characterization of a murine type II sodium-phosphate cotransporter expressed in mammalian small intestine. Proc. Natl. Acad. Sci. USA 95:14564–14569.

Hocking, P. M., M. Bain, C. E. Channing, R. Fleming, and S. Wilson. 2003. Genetic variation for egg production, egg quality and bone strength in selected and traditional breeds of laying fowl. Br. Poult. Sci. 44:365–373.

Hogan, B. L. 1996. Bone morphogenetic proteins in development. Curr. Opin. Genet. Dev. 6:432–438.

Ishimori, N., R. Li, K. A. Walsh, R. Korstanje, J. A. Rollins, P. Petkov, M. T. Pletcher, T. Wiltshire, L. R. Donahue, C. J. Rosen, W. G. Beamer, G. A. Churchill, and B. Paigen. 2006. Quantitative trait loci that determine BMD in C57BL/6J and 129S1/SvImJ inbred mice. J. Bone Miner. Res. 21:105–112.

Karsenty, G. 1998. Genetics of skeletogenesis. Dev. Genetics 22:301–313.

Klein, R. F., S. R. Mitchell, T. J. Phillips, J. K. Belknap, and J. Orwoll. 1998. Quantitative trait loci affecting peak bone mineral density in mice. J. Bone Miner. Res. 13:1657–1659.

Klein, R. F., R. J. Turner, L. D. Skinner, K. A. Vartanian, M. Serang, A. S. Carlos, M. Shea, J. K. Belknap, and E. S. Orwall. 2002. Mapping quantitative trait loci that influence femoral cross-sectional area in mice. J. Bone Miner. Res. 17:1752–1765.

Laufer, E., R. Dahn, O. E. Orozco, C. Y. Yeo, J. Pisenti, D. Henrique, U. K. Abbott, J. F. Fallon, and C. Tabin. 1997. Expression of Radical fringe in limb-bud ectoderm regulates apical ectodermal ridge formation. Nature 386:366–373.

Leach, R. M., and W. O. Twal. 1994. Autocrine, paracrine, and hormonal signals involved in growth plate chondrocyte differentiation. Poult. Sci. 73:883–888.

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Leung, P. S. 2004. The peptide hormone angiotensin II: Its new functions in tissues and organs. Curr. Protein Pept. Sci. 5:267–273.
Li, H., N. Deeb, H. Zhou, C. M. Ashwell, and S. J. Lamont. 2003. Chicken quantitative trait loci for growth and body composition associated with transforming growth factor-β genes. Poult. Sci. 82:347–356.
Li, H., N. Deeb, H. Zhou, C. M. Ashwell, and S. J. Lamont. 2005. Chicken quantitative trait loci for growth and body composition associated with the very low density apolipoprotein-II gene. Poult. Sci. 84:697–703.
Lilburn, M. S. 1994. Skeletal growth of commercial poultry species. Poult. Sci. 73:897–903.
Liu, Y. Z., Y. J. Liu, R. R. Recker, and H. W. Deng. 2002. Molecular studies of identification of genes for osteoporosis: The 2002 update. J. Endocrinol. 177:147–196.
Mandour, M. A., K. E. Nestor, R. E. Sacco, C. R. Polley, and G. B. Havenstein. 1989. Selection for increased humerus strength of cage reared broilers. Poult. Sci. 68:1169–1173.
Merriman, H. L., A. J. van Wijnen, S. Hiebert, J. P. Bidwell, E. Fey, J. Lian, J. Stein, and G. S. Stein. 1995. The tissue-specific nuclear matrix protein, NMP-2, is a member of the AML/CBF/PEBP2/runt domain transcription factor family: Interactions with the osteocalcin gene promoter. Biochemistry 34:13125–13132.
Mitchell, A. D., R. W. Rosebrough, and J. M. Conway. 1997. Body composition analysis of chickens by dual-energy x-ray absorptiometry. Poult. Sci. 76:1746–1752.
Munoz-Torres, M., G. Alonso, and M. P. Raya. 2004. Calcitonin therapy in osteoporosis. Treat. Endocrinol. 3:117–132.
Nakamura, Y., S. Wakitani, J. Nakayama, S. Wakabayashi, H. Horiuchi, and K. Takaoka. 2003. Temporal and spatial expression profiles of BMP receptors and noggin during BMP-2-induced ectopic bone formation. J. Bone Miner. Res. 18:1854–1862.
Sall, J., and A. Lehman. 1996. JMP Start Statistics: A Guide to Statistical and Data Analysis Using JMP and JMP IN Software. Duxbury Press, Eadsworth Publishing Co., Belmont, CA.
Schreiweis, M. A., P. Y. Hester, and D. E. Moody. 2005a. Identification of quantitative trait loci associated with bone traits and body weight in an F_2 resource population of chickens. Genet. Sel. Evol. 37:677–698.
Schreiweis, M. A., J. I. Orban, M. C. Ledur, D. E. Moody, and P. Y. Hester. 2005b. Validation of dual-energy X-ray absorptiometry in live White Leghorns. Poult. Sci. 84:91–99.
Searls, R. L., and M. Y. Janners. 1971. The initiation of limb bud outgrowth in the embryonic chick. Dev. Biol. 24:198–213.
Simonet, W. S., D. L. Lacey, C. R. Dunstan, M. Kelley, M. S. Chang, R. Luthy, H. Q. Nguyen, S. Wooden, L. Bennett, T. Boone, G. Shimamoto, M. DeRose, R. Elliott, A. Colombero, H. L. Tan, G. Trail, J. Sullivan, E. Davy, N. Bucay, L. Renshaw-Gegg, T. M. Hughes, D. Hill, W. Pattison, P. Campbell, and W. J. Boyle. 1997. Osteoprotegerin: A novel secreted protein involved in the regulation of bone density. Cell 89:309–319.
Slemenda, C. W., J. C. Christian, C. J. Willams, J. A. Norton, and C. C. Johnston. 1991. Genetic determinants of bone mass in adult women: A reevaluation of the twin model and the importance of gene interaction on heritability estimates. J. Bone Miner. Res. 6:561–567.
Slosman, D. O., J. P. Casez, C. Pichard, T. Rochat, F. Fery, R. Rizzoli, J. P. Bonjour, A. Morabia, and A. Donath. 1992. Assessment of whole-body composition with dual-energy x-ray absorptiometry. Radiology 185:593–598.
Sun, L., O. A. Adebanjo, B. S. Moonga, S. Corisdeo, H. K. Anandatheerthavarada, G. Biwas, T. Arakawa, Y. Hakeda, A. Koval, B. Sodam, P. J. Bevis, A. J. Moser, F. A. Lai, S. Epstein, B. R. Troen, M. Kumegawa, and M. Zaidi. 1999. CD38/ADP-ribosyl cyclase: A new role in the regulation of osteoclast bone resorption. J. Cell. Biol. 146:1161–1172.
Thomas, J. T., K. Lin, M. Nandedkar, M. Camargo, J. Cervenka, and F. P. Luyten. 1996. A human chondrodysplasia due to a mutation in a TGF-β superfamily member. Nat. Genet. 12:315–317.
Thomas, G., P. Moffatt, P. Salois, M. H. Gaumond, R. Gingras, E. Godin, D. Miao, D. Goltzman, and C. Lanctot. 2003. Osteocrin, a novel bone-specific secreted protein that modulates the osteoblast phenotype. J. Biol. Chem. 278:50563–50571.
Wardecka, B., R. Olszewski, K. Jaszczak, G. Zieba, M. Pierzchala, and K. Wicinska. 2002. Relationship between microsatellite marker alleles on chromosomes 1-5 originating from the Rhode Island Red and Green-legged Partrigenous breeds and egg production and quality traits in F_2 mapping population. J. Appl. Genet. 43:319–329.
Weitzmann, M. N., and R. Pacifici. 2005. The role of T lymphocytes in bone metabolism. Immunol. Rev. 208:154–168.
Whitehead, C. C. 2004. Overview of bone biology in the egg-laying hen. Poult. Sci. 83:193–199.
Whitehead, C. C., H. A. McCormack, L. McTeir, and R. H. Fleming. 2004. High vitamin D_3 requirements in broilers for bone quality and prevention of tibial dyschondroplasia and interactions with dietary calcium, available phosphorus and vitamin A. Br. Poult. Sci. 45:425–436.
Zhang, L., M. A. McKenna, N. Said-Al-Naief, X. Wu, X. Feng, and J. M. McDonald. 2005. Osteoclastogenesis: The role of calcium and calmodulin. Crit. Rev. Eukaryot. Gene Expr. 15:1–13.
Zhou, H. J., N. Deeb, C. M. Ashwell, and S. J. Lamont. 2006. Genome-wide linkage analysis to identify chromosomal regions affecting phenotypic traits in the chicken. I. Growth and average daily gain. Poult. Sci. 85:1700–1711.
Zhou, H. J., and S. J. Lamont. 1999. Genetic characterization of biodiversity in highly inbred chicken lines by microsatellite markers. Anim. Genet. 30:256–264.
Zhou, H., A. D. Mitchell, J. P. McMurtry, C. M. Ashwell, and S. J. Lamont. 2005. Insulin-like growth factor-I gene polymorphism associations with growth, body composition, skeleton integrity, and metabolic traits in chickens. Poult. Sci. 84:212–219.

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