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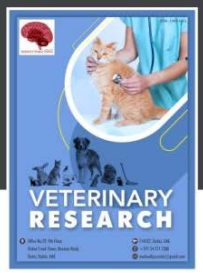

Genetic Mapping of Quantitative Trait Loci Affecting Skeletal Architecture in Japanese Quail

H. Charati, A.K. Esmailizadeh, R. Jabbari Ori, H. Moradiyan and S. Sohrabi
Department of Animal Science, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, 76169-133 PB, Iran

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Corresponding Author:
H. Charati
Department of Animal Science, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, 76169-133 PB, Iran

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Abstract: Quantitative Trait Loci (QTL) are DNA segments linked to traits. In this study, a three-generation resource population was developed using two distinct Japanese quail strains, wild and white to map QTL underlying skeletal architecture. Eight pairs of white and wild birds were crossed reciprocally and 34 F1 birds were produced. The F1 birds were intercrossed to generate 422 F2 off spring. All of the animals from three generations (472 birds) were genotyped for eight microsatellite markers on chromosome 1. The phenotypic data were collected on the F2 birds. QTL analysis was conducted applying the line-cross model and the least-squares interval mapping approach. The results indicated QTL affecting skeletal architecture traits on chromosome 1. The F2 phenotypic variance explained by the detected additive QTL effects ranged from 0.0-2.23 for different traits. The identified QTL interacted significantly with sex (QTL for tibia bone weight, humerus bone length, femur bone diameter, right leg weight) and hatch (QTL for left leg length, breast bone weight, femur weight, femur bone weight, femur meet weight).

INTRODUCTION

There is the need to select chickens with better skeletal structure to support the high growth rate and the musculature (Zhou *et al*., 2007; Zhang *et al*., 2010). Bone defects and deformity in poultry production lead to economic losses. Leg abnormalities, reduced feed utilization and growth rate (Cook, 2000) as well as problems for animal welfare (Zhang *et al*., 2010). Genetic composition plays an important role in the development of skeletal architecture (Cook, 2000). Therefore, incorporation of the major gene effects using the Marker-Assisted Selection (MAS) protocols into a breeding program could be one of the solutions to reduce these problems (Zhang *et al*., 2010).

Recent successes in mapping Quantitative Trait Loci (QTL) that contribute to phenotypic variation in humans and model organisms make it possible to address important questions concerning the evolution of the system as a whole in vertebrates as well as in biomedical research on the genetics of bone growth. QTL analysis allows researchers to link two types of information phenotypic data (trait measurements) and genotypic data (usually molecular markers) in an attempt to explain the genetic basis of variation in complex traits. The goal of this process is to identify the action, interaction, number and precise location of these regions (Cook, 2000; Zhou *et al*., 2007).

Studies on the skeletal morphology using QTL analysis and genetic architecture of skeletal traits are

becoming more and more common (Zhou *et al*., 2007; Farber and Medrano, 2007; Yu *et al*., 2007). Many studies have also successfully detected numerous QTL for economically important traits such as growth and body composition in chickens by using crossbred experimental populations (Wang *et al*., 2012). The objective of the current study was to identity QTL for skeletal traits in a white and wild intercross Japanese quail population.

In chickens, Rosario *et al*. (2006) identified markers associated with performance and carcass traits on chromosomes 1, 3 and 4. Applying a single marker approach to a multi generational chicken population, Atzmon *et al*. (2008) identified 729 associations with egg production, body weight and carcass traits, 150 of which were significant. In chickens, previous studies identified QTL affecting body weight, feed intake, carcass traits and organs weights on four regions of chromosome 1 (Nones *et al*., 2006) and also on chromosomes 2-5 (Ruy *et al*., 2005; Baron *et al*., 2011).

Despite many efforts to construct linkage maps and identification of QTL in chicken genome, very little information is available in mapping genomic regions underlying quantitative traits in Japanese quail. Minvielle *et al*. (2005) found QTL for body weight at 5 and 70 weeks of age and for feed intake on chromosome 1 in an F2 population of Japanese quail. Esmailizadeh e*t al*. (2012) have recently identified highly significant QTL for liveweights (weight at 3-6 weeks of age) in a half-sib population of a commercial strain of Japanese quail. However, to the knowledge there was no published QTL result on carcass traits in Japanese quail. In this study, we used an F2 population derived from the cross of a white line and a wild line from which phenotypes were available for a series of traits that are known to vary in birds that suffer from Skeletal Architecture.

MATERIALS AND METHODS

Resource populations and data recording: An F2 population specially designed for QTL mapping studies was originated from wild (meat type) and white (layer type) strains of Japanese quail. The white (S) and Wild (W) founder strains were intercrossed to produce 34 F1 parents (9 males and 25 females). The F1 birds including 17 SW and 17 WS reciprocal half of cross progeny were generated by S males×W females and W males×S females reciprocal crosses, respectively. The SW males were intercrossed to WS females while the WS males were intercrossed to both SW and WS females generating 422 F2 off spring (246 males and 176 females) including 153 SWWS, 230 WSSW and 39 WSWS birds, respectively. The F2 population was created in five consecutive hatches. The total resource mapping population consisted of 472 birds.

The parents were kept in group cages and fed a layer diet *ad libitum*. The F2 progeny were raised for 5 weeks on a floor covered with wood shavings in an environmentally controlled room with continuous artificial lighting and at a temperature which was decreased gradually from 37-25°C. The progeny received water and a mash starter diet (0-21 days) and a mash growing diet (22-35 days) *ad libitum*.

The phenotypic measurements included Cold Carcass Weight (CCW), Breast Weight (BW), Breast Bone Weight (BBW), Breast Meat Weight (BMW), Femur Weight (FW), Femur Meat Weight (FMW), Femur Bone Weight (FBW), Femur Bone Length (FBL), Femur Bone Diameter (FBD), Tibia Bone Weight (TBW), Humerus Bone Weight (HBW), Humerus Bone Length (HBL), Humerus Bone Diameter (HBD), Right Leg Weight (RLW), Right Leg Length (RLL), Right Leg Diameter (RLD), Left Leg Weight (LLW), Left Leg Length (LLL), Left Leg Diameter (LLD).

The weight of bones and meat were measured using a digital scale (0.01 g precision) and the bones length and diameter were measured using a digital caliper (0.01 mm precision).

DNA markers and genotyping: Eight microsatellite markers with an average distance of 29 cM between markers located on chromosome 1 were chosen based on the polymorphism information content values of the loci (Kayang *et al*., 2002) and their positions (Kayang *et al*., 2004).

To determine the genotypes of the individuals for the microsatellite markers, genomic DNA was isolated from whole blood samples of all the mapping of the birds (i.e., 16 parents, 34 F1 and 422 F2 birds) by salting-out DNA extraction procedure. Marker sequence amplifications were carried out by PCR in total 25 mL reaction mixtures per each individual sample. This mixture included 2 mL of template DNA, 2.5 mL PCR buffer, 1 mL MgCl2, 0.5 mL dNTP mix, 0.3 mL Taq DNA polymerase and 16.5 mL sterile water. The reaction conditions were 95°C for 4 min, 30 cycles of 94°C for 30 sec, annealing at the 25 temperature set for each primer (43-55°C) for 1 min, 63°C for 2 min and an extension at 72°C for 4 min (Table 1). PCR products were run on 8% denaturing polyacrylamide gels using electrophoresis. Individual PCR product fragment sizes for the microsatellite markers were determined by visualising the band pattern via silver nitrate staining method.

The descriptive statistical analyses were conducted using ASReml (Gilmour *et al*., 2006) and residuals were checked for normality. The QTL analysis was carried out by the linear regression method (Haley *et al*., 1994) for F2 outcross pedigrees. The genetic model at the QTL assumed that the original strains were fixed for different alleles, although, genes could be segregating elsewhere.

Table 1: Summary of general characteristics of the microsatellite markers on Japanese quail chromosome 1 used in this study

Marker	Position (cm)[A]	Oligo sequence		TA[B]
		Reverse	Forward	
GUJ0055	0	5´-GCATACTGCAATATACCTGA-3´	5´-TTGACATACTTGGATTAGAGA-3´	55
GUJ0052	19	5´-AAACTACCGATGTAAGTAAG-3´	5´-ATGAGATATATAAGGAACCC-3´	43
GUJ0048	57	5´-AACGCATACAACTGACTGGG-3´	5´-GGATAGCATTTCAGTCACGG-3´	55
GUJ0013	91	5´-ACCAAACCCGAGATCCGACA-3´	5´-AGCGTTCGCGTTCCTCTTTC-3´	55
GUJ0056	122	5´-GTTACATCCATCCTGCCTCA-3´	5´-CTCTTGAGCCTACCAGTCTG-3´	55
GUJ0098	172	5´-GCATAACTGAACTACCACGC-3´	5´-GCATCAGTTCCATCAGCTAG-3´	55
GUJ0068	197	5´-TAGGAGAGGTCACGATTTGC-3´	5´-ATCTTAACTCGCCCAGCCTT-3´	54
GUJ0090	206	5´-GCCTTCAGAGTGGGAAAT-3´	5´-TCTCACAGAAACAGCTCC-3´	55

[A]Marker position on chromosome based on Japanese quail sex averaged linkage map (Kayang *et al*., 2002); [B]TA, annealing temperature (8°C)

At the first stage of the analysis, the probability of an F2 offspring being each of the four QTL genotypes (QQ, Qq, qQ and qq) at each position in the genome was calculated conditionally upon the marker genotype. Subsequently, the following three linear models for the additive (a), dominance (d) and imprinting (i) effects of the QTL at a given position were analyzed by least squares for each trait:

$$y_{ijkl} = \mu + H_i + S_j + aP_{ak} + e_{ijkl} \quad (1)$$

$$y_{ijkl} = \mu + H_i + S_j + aP_{ak} + dP_{dk} + e_{ijkl} \quad (2)$$

$$y_{ijkl} = \mu + H_i + S_j + aP_{ak} + dP_{dk} + iP_{ik} + e_{ijkl} \quad (3)$$

Where:

y_{ijkl} = The observed phenotype of individual l
μ = The mean of the population
H_i and S_j = The fixed effects of hatch and sex, respectively
a, d and I = The estimated additive, dominance and imprinting effects of QTL
P_{ak} = The conditional probability of animal k to carry the allele of wild strain
P_{dk} = The conditional probability of animal k to be heterozygous
P_{ik} = The conditional probability of animal k is heterozygous and inherited the wild strain allele from its sire
e_{ijkl} = The random residual error

To investigate whether the putative QTL was different in males vs. females F2 offspring, QTL by sex interaction effect was also included in model 3. Additive QTL effect by hatch interaction was also analyzed. The GridQTL portal under an F2 module at http://www.gridqtl. org.uk/ was utilized for QTL analysis (Seaton *et al*., 2006). Applying the above mentioned models, the F-statistic profiles were generated at 1-cM intervals along the chromosome to identify the most likely QTL position. Significance thresholds for analyses were calculated using a permutation test (Churchill and Doerge., 1994). Data permutation with 10000 replicates was used to determine the empirical distribution of the test statistic under the null hypothesis of no QTL. QTL effects that exceeded the chromosome-wide F-critical threshold at a $p<0.05$ and F-critical threshold of $p<0.01$ were considered evidence for significant QTL.

Percentage of the trait variance among the F2 birds explained by the detected QTL (V_{QTL}) was calculated as:

$$V_{QTL} = 100 \times (RMS\text{-}FMS)/RMS$$

where, RMS is the residual mean square from the reduced model, omitting desired effect of QTL and FMS is the residual mean square from the full model including desired effect of QTL.

RESULTS AND DISCUSSION

Descriptive statistics of the traits: The number of observations, mean, minimum and maximum, standard deviation and coefficient of variation for the traits recorded on the 419 F2 females are given in Table 2. The overall means of the traits were 29 g for BMW, 0.29 for RLW, 2.79 g for FBD and 32.71 g for BW. The maximum of BBW was 6.87 g.

Polymorphic Information Content (PIC): In this study, all of the marker loci were polymorphic and the average number of alleles per locus was 8. The Polymorphic Information Content (PIC) shows the useful information provided by a marker on the genome. The PIC values vary among the markers where some markers are fully informative and others have a PIC<0.5. Based on the classification of Botstein *et al*. (1980) (highly informative PIC>0.50; reasonably informative 0.50>PIC>0.25 and slightly informative PIC<0.25) these contents of the polymorphic markers were highly informative. The useful information contents of the markers used in this study in different parts of the chromosome 1 of Japanese quail are presented in Table 3 and Fig 1.

Additive effects of QTL: In total, 21 chromosome-wide significant QTL were found through the scanning of

Table 2: Phenotypic observation and analysis of the F2 population

Trait	N	Mean[A] (mm, g)	Min (mm, g)	Max (mm, g)	r.s.d.[B]	CV (%)
CCW	421	104.500	141.00	460.30	13.220	12.650
BW	399	32.710	12.52	47.81	5.608	0.171
BBW	399	3.518	1.75	6.87	0.724	0.205
BMW	397	29.000	12.33	43.20	5.212	0.179
FW	399	9.339	4.94	13.25	1.580	0.169
FMW	394	8.338	0.85	91.41	4.437	0.532
FBW	394	0.555	0.32	0.90	0.073	0.131
FBL	394	39.030	29.60	42.50	1.708	0.043
FBD	398	2.795	1.97	3.61	0.228	0.081
TBW	400	0.599	0.33	0.99	0.077	0.128
HBW	399	0.620	0.36	0.99	0.098	0.158
HBL	398	37.870	23.06	45.20	1.911	0.050
HBD	396	2.920	1.50	4.70	0.322	0.110
RLW	400	0.298	0.13	0.61	0.044	0.147
RLL	400	31.390	25.13	39.21	1.280	0.040
RLD	400	2.478	1.64	3.06	0.187	0.075
LLW	398	0.291	0.19	0.50	0.037	0.127
LLL	398	31.170	26.39	34.00	1.091	0.035
LLD	400	2.471	1.52	2.94	0.184	0.074

A Trait mean adjusted for fixed effects included in the model; BResidual standard deviation after fitting the basic fixed effects (see the text)

Table 3: The useful polymorphic information content of each marker

		Information			Genotyped individuals (%)			
Marker	Position (cm)	Additive	Dominance	Imprinting	P (%)	F1 (%)	F2 (%)	Alleles
GUJ0055	0	0.42	0.26	0.67	16 (100%)	34 (100%)	402 (95%)	3
GUJ0052	19	0.26	0.02	0.26	16 (100%)	34 (100%)	418 (98%)	3
GUJ0048	57	0.20	0.04	0.24	16 (100%)	34 (100%)	407 (96%)	2
GUJ0013	91	0.31	0.20	0.22	16 (100%)	34 (100%)	414 (98%)	2
GUJ0056	122	0.20	0.00	0.20	15 (93%)	34 (100%)	401 (95%)	2
GUJ0098	172	0.29	0.04	0.26	16 (100%)	34 (100%)	416 (98%)	2
GUJ0068	197	0.15	0.08	0.20	16 (100%)	34 (100%)	407 (96%)	2
GUJ0090	206	0.55	0.17	0.25	16 (100%)	34 (100%)	409 (96%)	3

Table 4: Summary of Quantitative Trail Loci (QTL) associated with internal organs in F2 population of Japanese quail

			QTL effect[B]			V_{QTL}[C]			
Trait	Position (CM)[A]	F-value	Additive (s.e)	Dominance (s.e)	Imprinting (s.e)	Additive	Dominance	Imprinting	Closest marker
BMW	103	9.02*	2.1902 (0.729)	-	-	1.98	-	-	GUJ0013
FBD	206	10.24*	0.0630 (0.019)	-	-	2.23	-	-	GUJ0090
BW	95	6.19*	1.5750 (0.697)	3.104 (1.294)	-	1.48	-	-	GUJ0013
HBL	76	6.97*	-1.2660 (0.482)	3.215 (1.047)	-	0.86	0.02	-	GUJ0013
FW	100	5.56*	0.4840 (0.206)	0.844 (0.418)	-	1.52	0.00	-	GUJ0013
FBD	206	5.20*	0.0610 (0.020)	0.018 (0.041)	-	2.23	0.00	-	GUJ0090
CCW	91	5.68*	3.4850 (1.459)	5.192 (2.476)	-0.140 (0.070)	1.44	0.00	0.0	GUJ0013
RLW	178	6.47**	0.0120 (0.005)	0.003 (0.011)	-0.022 (0.005)	0.00	0.00	0.0	GUJ0098
BMW	99	4.91*	1.8560 (0.697)	3.362 (1.406)	0.115 (0.687)	1.98	0.01	0.0	GUJ0013
HBL	76	4.64*	-1.2740 (0.491)	3.221 (1.051)	0.038 (0.460)	0.86	0.02	0.0	GUJ0013

[A]QTL location based on the Japanese quail sex averaged linkage map (Kayang *et al*., 2002); [B]The additive and dominance effects were defined as the deviation of animals homozygous for the wild allele or heterozygous, respectively from the mean of two homozygotes; [C]QTL variance (the reduction in residual variance of the F2 population obtained by inclusion of a QTL at the given position); *p<0.05; **p<0.01

chromosome 1. These locations were related to BMW, FBD, BW, HBL, FW, FBD, CCW, RLW, LLL, BBW, FBW, FMW, BW and TBW.

In model 1 which accounts for only additive effects of QTL, two chromosome-wide significant QTL underlying BMW and FBD were found at 103 and 206 cm of the linkage map, respectively. The additive effects of both QTL were positive and the closest marker loci to two of the detected QTL (QTL for BMW and FBD) were GUJ0013 and GUJ0090, respectively (Table 4 and Fig. 2). The percentage of the F2 phenotypic variance explained by the detected additive QTL effects for BMW and FBD were 1.98 and 2.23, respectively (Table 4).

Additive and dominance effects of QTL: In model 2 that includes the additive and dominance effects of QTL,

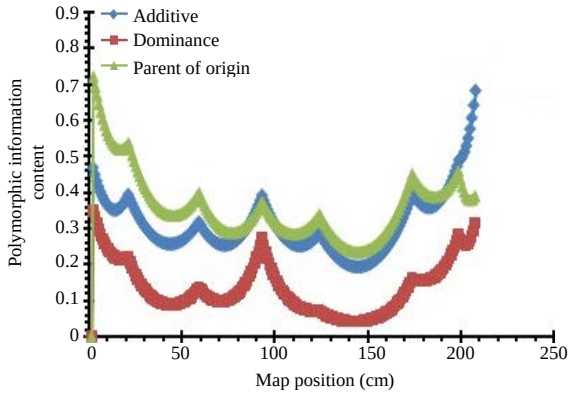

Fig. 1: The useful polymorphic information contents of the markers used in this study in different parts of the chromosome 1 of Japanese quail for the additive, dominance and imprinting effects

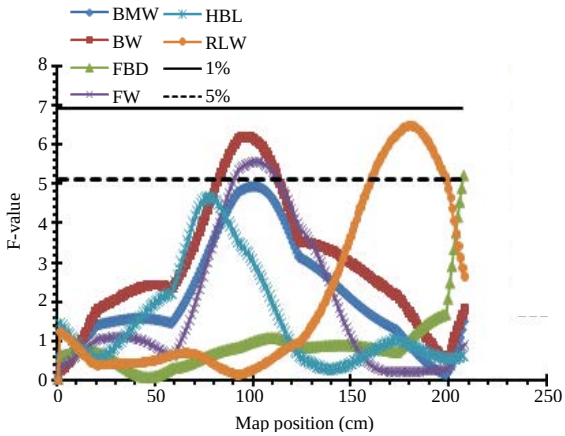

Fig. 3: Test statistic curves resulted from the additive quantitative trait loci by hatch interaction model on chromosomes 1 using an intercross between two Japanese quail strains

Fig. 2: Test statistic curve resulted from the additive quantitative trail loci model on chromosomes 1 using an intercross between two Japanese quail strains

five chromosome-wide significant QTL underlying BW, HBL, FW and FBD were found at 95, 76, 100 and 206 cm of the linkage map, respectively. The closest marker locus to QTL for BW, HBL and FW was GUJ0013 while the nearest marker to QTL for FBD was GUJ0090 (Table 4).

Additive, dominance and imprinting (parent-of-origin) effects of QTL: In the third analysis where the additive, dominance and imprinting (parent-of-origin) effects of QTL were jointly modeled, four chromosome-wide significant QTL underlying CCW, RLW, BMW and HBL were found at 91, 178, 99 and 76 cM of the linkage map, respectively. QTL that surpassed the suggestive or significant linkage threshold are summarized in Table 4-6.

Table 4 shows the location of the significant QTL their positions on the chromosome, the maximum F values obtained at this position their genetic effects and the reduction of the residual variance obtained by fitting a QTL at this location. The F2 phenotypic variance percentage explained by the detected QTL for additive effects was 1.98 and 2.23 for BMW and FBD, respectively. The peak value of the test statistic (F = 5.6) of the detected QTL for CCW on chromosome 1 was very close to the GUJ0013 marker.

Interaction between QTL and hatch: Interaction of the additive QTL effect and hatch was significant for LLL, BBW, FW, FBW and FMW (Table 5 and Fig. 3). Interaction of the additive QTL effect and hatch for BMW, FBD and BW were positive while the additive QTL effect for TBW was negative.

Interaction between QTL and sex: Additive QTL effects for RLW, BW, BMW, TBW, FBD ($p<0.05$) and HBL ($p<0.01$) had significant interaction with gender (Table 6 and Fig. 4). The peak values of the F-statistics for the RLW and BW in this analysis were detected at 4.6 and 4.7 cm, respectively from the beginning of the linkage group. The F2 phenotypic variance percentage explained by the detected QTL was 0.02 for BMW (Table 6).

The number of individuals in the experimental designs for QTL mapping should be several hundreds to maximize the power of the statistical tests (Tatsuda and Fujinaka, 2001). Therefore when F2 birds are produced they often have to be reared over a long-term period as it is difficult to produce adequate number of offspring per family all at the same time. In this respect, Japanese quail (*Coturnix coturnix japonica*) is an ideal for QTL analysis

Table 5: Summary of Quantitative Trait Loci (QTL) results obtained from modeling QTL by hatch interaction

Trait	Position (cm)[A]	F-value	QTL additive effect H1 (s.e)	H2 (s.e)	H3 (s.e)	H4 (s.e)	H5 (s.e)	V_{QTL}[B]	Closest marker
LLL	126	4.12**	4.616 (1.041)	-0.587 (1.011)	0.096 (0.946)	-0.559 (0.785)	0.257 (0.832)	0.03	GUJ0056
BBW	206	2.90*	-0.282 (0.144)	0.037 (0.204)	0.381 (0.143)	0.136 (0.116)	0.181 (0.122)	0.03	GUJ0090
FW	91	3.08*	0.414 (0.427)	-0.146 (0.448)	0.933 (0.386)	-0.009 (0.329)	1.211 (0.414)	0.02	GUJ0013
FBW	100	3.33*	0.049 (0.023)	-0.020 (0.024)	0.042 (0.021)	-0.010 (0.017)	0.059 (0.022)	0.00	GUJ0013
FMW	109	3.56**	-6.228 (1.528)	0.088 (1.518)	1.018 (1.414)	-0.041 (1.165)	1.145 (1.395)	0.03	GUJ0056

[A]QTL location based on the Japanese quail sex averaged linkage map (Kayang *et al*., 2002); [B]QTL variance (proportion of phenotypic variance of the F2 population explained by QTL); *p<0.05; **p<0.01

Table 6: Summary of Quantitative Trait Loci (QTL) results obtained from modeling QTL by sex interaction

Trait	Position (cm)[A]	F-value	QTL additive effect Male[A] (s.e)	Female[A] (s.e)	V_{QTL}[B]	Closest marker
RLW	206	4.66*	0.016 (0.005)	-0.002 (0.005)	0.00	GUJ0090
BW	105	4.79*	3.239 (1.065)	0.667 (1.178)	1.01	GUJ0013
BMW	102	5.60*	3.136 (0.978)	1.006 (1.070)	0.02	GUJ0013
HBL	85	6.59**	0.341 (0.588)	-2.254 (0.628)	1.02	GUJ0013
FBD	206	5.31*	0.051 (0.026)	0.076 (0.029)	0.00	GUJ0090
TBW	198	5.16*	-4.000 (0.011)	-0.039 (0.012)	0.00	GUJ0068

[A]QTL location based on the Japanese quail sex averaged linkage map (Kayang *et al*., 2002); [B]QTL variance (proportion of phenotypic variance of the F2 population explained by QTL); *p<0.05; **p<0.01

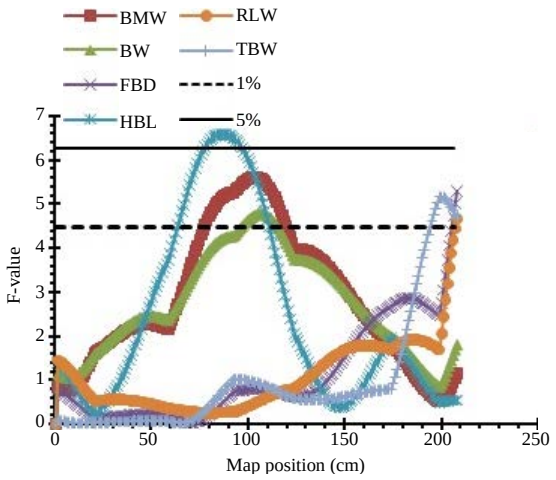

Fig. 4: Test statistic curves resulted from the additive quantitative trait loci by sex interaction model on chromosomes 1 using an intercross between two Japanese quail strains

due to its short generation interval to control the individuals under the same conditions, resistance to diseases and high egg production.

In the present study, 19 traits related to skeletal architecture were analyzed for QTL using the scanning of chromosome 1 an F2 resource population. A total of 21 QTL were detected for at the 5% chromosome-wise significance level; of these QTL, 5 were significant at the 1% chromosome-wise level. A number of QTL mapping studies have been performed on crosses between genetically and phenotypically divergent lines of quail. These studies have focused on identifying QTL responsible for body weight (Esmailizadeh *et al*., 2012; Sohrabi *et al*., 2012) feed-efficiency, growth and egg traits (Minvielle *et al*., 2005; Minvielle *et al*., 2006; Minvielle *et al*., 2007).

The QTL variance suggests the contribution of specific trait loci to the total phenotypic variance of the trait. At each position, mapping QTL using desirable model determines whether a significant amount of the variance in a quantitative trait can be attributed to a QTL at that position. QTL variance defined as the reduction of the residual variance obtained by fitting a QTL at the corresponding location was relatively small for the detected loci (0.0-2.23%). Similarly, Sohrabi *et al*. (2012) reported relatively small QTL variance for hatching weight and growth traits (0.6-3.7%) in this F2 population of Japanese quail. These results are in contrast to the relatively high QTL variance (3.3-12.5%) for QTL segregating in the wild type Japanese quail was reported by Esmailizadeh *et al*. (2012). Given the relatively small sample size and the nature of the half-sib design used in the study of Esmailizadeh *et al*. (2012) it is possible that the effects were overestimated. Relatively large progeny group sizes are needed to detect a medium-sized QTL in each half-sib group otherwise the experiment will have low power to detect the QTL or the detected effect will be overestimated (Esmailizadeh *et al*., 2008). The low variation explained by the QTL detected in the present study implies that other factors or other QTL in other chromosomes may underlie the variation in this trait.

Other researchers have investigated the development of quail skeleton (Dadasheva and Guryeva, 1993; Nakane and Tsudzuki, 1999). This study is an important

first step in the effort to locate QTL responsible for variation of skeleton in the Japanese quail. In summary, several QTL influencing skeletal traits (right leg length, left leg length and humerus bone length) were identified in this study, contributing to an overall understanding of the genetic architecture regulating skeleton. Dunn *et al*. (2007) reported significant QTL on chromosome 1 for bone index and the component traits of tibiotarsal and humeral breaking strength in an F2 population derived from White Leghorn chicken. Additive effects for tibiotarsal breaking strength represented 34% of the trait standard deviation and 7.6% of the phenotypic variance of the trait.

A QTL by sex interaction was assessed to investigate whether the effect differed between the two sexes. We identified significant QTL by sex interaction for TBW so that the absolute QTL additive effect was higher in F2 males (4.0) than in the females (0.03). Schreiweis *et al*. (2005) identified significant QTL for tibia bone mineral density, tibia area and tibia length at 35 weeks of age at positions 102, 171 and 169 cm on chromosome 3 in chicken, respectively. Generally, a QTL by sex interaction can be considered as a genotype by environment interaction, considering sex as an organismal environment for gene expression (Alexei *et al*., 2010). Conducting a full genome scan with a QTL by sex interaction model or conducting the analysis separately for each sex could help to detect these kinds of interactions. However, the larger number of tests conducted could also lead to an increase in false positive results. Further experiments are needed to confirm QTL by sex interactions detected in the experiment before application in selection. In a number of studies, QTL by sex interaction was tested only for locations that were significant in the initial analysis using models without sex interaction which does not detect QTL with sex-antagonistic effects and has less power to detect QTL with sex-specific and sex-biased effects (Ikeobi *et al*., 2002; Ikeobi *et al*., 2004; Nones *et al*., 2006).

We identified significant QTL for humerus bone length with additive and dominance effects. Schreiweis *et al*. (2005) detected significant QTL for humerus length at 0 cm on chromosome 6 in chicken.

CONCLUSION

Genes controlling body weight and size often have pleiotropic effects on skeletal phenotypes. Large individuals typically have more bone tissue and bones that with stand greater biomechanical stress than small individuals do. Thus, genes affecting body weight or body size can be important indirect regulators of skeletal phenotypes. Previously, we identified QTL affecting hatching weight, body weights at 5 weeks of age that overlap with QTL for BMW, BMW, HBL, FW, trait in present study. Thus, these loci may contain pleiotropic genes.

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