Animal Science Papers and Reports vol. 32 (2014) no. 4,369-383
Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

Detection of quantitative trait *loci* affecting carcass traits and internal organs on chromosome 3 in an F2 intercross of Japanese quail

Hadi Charati*, Ali Esmailizadeh Koshkoiyeh, Roghayeh Jabbari Ori, Hassan Moradian , Ahmad Ayatollahi Mehrgardi

Department of Animal Science, Faculty of Agriculture,
Shahid Bahonar University of Kerman, Kerman, PB 76169-133, Iran

(Accepted August 25, 2014)

Molecular technologies based on markers indicating differences among individuals at the DNA level can play an important role in genetic improvement of carcass traits through marker or gene assisted selection. The purpose of this study was to map quantitative trait *loci* (QTL) of chromosome 3 affecting carcass traits on Japanese quail using microsatellite markers. Two white and wild strains of Japanese quail were crossed reciprocally and the F_1 generation was created. The F_2 generation was generated by intercrossing F_1 birds. Phenotypic data including weights of hot and cold carcasses, carcass parts and internal organs were collected from 422 F_2 birds. The total mapping population (472 birds) was genotyped for microsatellite markers. QTL analysis was performed using the least squares regression interval mapping method. Significant QTL were identified for hot and chilled carcass weights, liver weight, head percentage, uropygial gland percentage, intestine percentage, ovary weight, uropygial gland weight, pancreas percentage (0-36 cM with an additive effect), proventriculus percentage, head weight (6-20 cM with a dominance effect), and gizzard percentage (0 cM with an imprinting effect).

KEY WORDS: Japanese quail / carcass traits / chromosome 3 / internal organs / microsatellite markers / QTL mapping

Positioning *loci* associated with quantitative trait *loci* (QTL) is the first step toward identification of genes responsible for variation in quantitative traits. Detecting genomic regions regulating economically important traits can increase the response

*Corresponding author: hadicharati@yahoo.com

of breeding programs, especially for those that are difficult to improve by phenotypic selection. A number of tools for genome analyses developed during the last decade have facilitated identification of genes controlling complex traits. This has opened promising prospects for predictive medicine in humans and marker-assisted selection (MAS) in plants and animals of economic interest [Knott *et al*. 1998, Ober *et al*. 2001].

The Japanese quail (*Coturnix japonica*) belong to the class of Aves, order: *Galliformes*, family: *Phasianidae*, genus: *Coturnix*. The number of chromosomes for this species is 78, including 27 mini pairs, 6 medium pairs, and 6 big pairs. Domestic quails, derived from the Japanese quail (*Coturnix japonica*) as laying, meat, and laboratory animals have produced a flourishing industry. At present there are about 1,050 million quails worldwide, including 200 million quails in China alone. Quail ranks second to chicken in the Chinese poultry industry [Chang *et al*. 2007].

QTL have been mapped in pigs [Knott *et al*. 1998, Walling *et al*. 1998], mice [Flint *et al*. 1995, Brockman *et al.* 1998], and chickens [Vallejo *et al*. 1998, van Kaam *et al*. 1998, van Kaam *et al.* 1999a, van Kaam *et al*. 1999b]. Many studies have also successfully detected numerous QTL for economically important traits such as growth and body composition in chickens using crossbred experimental populations [Wang *et al*. 2012]. The chicken QTLdb (http://www.animalgenome. org) contains 2451 QTL involving 248 different traits from 125 publications. Numerous QTL affecting growth and fat traits were identified on chicken chromosomes 3, 5 and 7 [Wang *et al*. 2012].

Rosário *et al*. [2006] identified markers associated with chicken performance and carcass traits on chromosomes 1 (GGA1), 3 (GGA3) and 4 (GGA4). Atzmon *et al*. [2006] used single-marker analyses and found 44 significant associations out of the 456 marker-trait combinations of associations with traits related to growth and fatness in a commercial chicken line. Applying a single marker approach to a multi-generational 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 feed intake, body weight, organ weights and carcass traits on four regions of chromosome 1 [Nones et al. 2006], and on chromosomes 2, 3, 4, and 5 [Baron *et al.* 2011, Ruy *et al.* 2005]. Quantitative trait loci affecting lung, spleen, and bursa weights were identified on chicken chromosomes 3, 10 and 17 by Park *et al.* [2006]. In chickens previous studies identified QTL affecting body weight, feed intake, carcass traits and organ weights on four regions of chromosome 1 [Nones *et al.* 2006], and on chromosomes 2, 3, 4, and 5 [Baron *et al*. 2011, Ruy *et al*. 2005].

Despite many efforts to construct linkage maps and identification of QTL in the chicken genome, very little information is available on mapping of genomic regions underlying quantitative traits in the 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 F_2 population of the Japanese quail. Esmailizadeh *et al*. [2012] have recently identified highly significant QTL for live weights (weight at 3, 4, 5, and 6 weeks of age) in a half-sib population of a commercial strain of the Japanese quail. However, to

our knowledge, there have been no published reports on QTL associated with carcass traits in the Japanese quail. Thus, the objective of this study was to map QTL for carcass traits and internal organs on Japanese quail chromosomes 3 in an F_2 resource population.

Material and methods

Experimental population and data recording

Two strains of the Japanese quail, Pharaoh (meat type) and white (layer type), were used to create an F_2 resource population designated for QTL mapping studies. Both strains originated in Japan and were imported from Canada to Iran in 1990. The white (W) and Pharach (P) founder strains were intercrossed to produce 34 F_1 parents (9 males and 25 females). The F_1 birds included 17 WP and 17 PW reciprocal progeny. The WP males were intercrossed to PW females, while the PW males were intercrossed to both WP and PW females generating 422 F_2 offspring (245 males and 177 females) including 153 WPPW, 230 PWWP and 39 PWPW birds, respectively. The F_2 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 F_2 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 to 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 F_2 birds were not fed in the 12-hour period *prior to slaughter,* while they had free access to water. Before slaughter of the F_2 progeny, all the birds were weighed. After slaughter, defeathering and evisceration, the hot carcass weight and the head weight with skin were obtained using a digital balance accurate to 0.01 g. The internal organs including heart, gizzard, liver, proventriculus, intestine, pancreas, spleen, ovary and testes were separated and weighed during evisceration. Carcasses were refrigerated at a temperature of +4°C for 24 hours and chilled carcass weight was recorded. The uropygial gland and bursa of fabricius were separated from the chilled carcass and weighed. The chilled carcass was dissected into five parts including the breast muscle, wings with skin, leg muscles (thigh and lower thigh), the neck and back. The weights of these five carcass parts were recorded and the percentages of the carcass parts relative to the carcass weight were calculated. The weight of the total separatable fat from whole carcass was also recorded.

DNA Markers and Genotyping

Blood samples of all the animals (i.e. 16 parents, 34 F_1, and 422 F_2 birds) were collected at slaughter using 2.5 mL blood collection tubes containing EDTA as an anticoagulant and stored at -20°C. DNA was extracted from 500 µL of the blood using a modified salting-out technique, where proteins and other contaminants were

precipitated from the cell lysate using high concentrations of ammonium acetate. The precipitates were removed by centrifugation and the DNA was recovered by alcohol precipitation. Three microsatellite markers covering 100% of the map of chromosome 3 were used to genotype 16 parental, 34 F_1, and 422 F_2 birds. Three primer pairs of microsatellite markers were designed according to the literature [Kayang *et al*. 2002], as shown in Table 1.

Table1. Summary of general characteristics of microsatellite markers on Japanese quail chromosome 3

Marker	Position (cM)[A]	Oligo sequence Reverse	Forward	TA[B]
GUJ0099	0	5′-TTTTAAGTTTCCCCAGGCAG-3′	5′-CTCTTATCCATCCTTCCTTC-3′	55
GUJ0035	27	5′-GGGCAATAAAAGAAAGACTG-3′	5′-AATACTGGTTTTGTGATGGC -3′	55
GUJ0041	38	5′-TGAAACATACCTGAGTGCTA-3′	5′-AAAATGTCTGCAAAATGGGC-3′	57

[A]Marker position on chromosome based on the Japanese quail sex averaged linkage map [Kayang *et al.* 2004].
[B]T_A, annealing temperature.

Polymerase chain reaction (PCR) was performed for genotyping all birds for each marker. The reactions contained 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 amplification conditions for PCR were 94°C for 4 minutes, 30 cycles of 94°C for 30 s, annealing at the temperature set for each primer (55-57°C) (Tab. 1) for 45 s and 2 minutes at 63°C, followed by a final extension step of 4 minutes at 72°C. The PCR products were then separated on 8% denaturing polyacrylamide gels with a molecular weight marker on an electrophoresis system at 200 V for 3 to 4 hours. Individual PCR product fragment sizes for the microsatellite markers were determined by visualizing the band pattern via the silver nitrate staining method.

Statistical analysis

Prior to statistical analyses of QTL the residuals of single traits were checked for normality. The QTL analysis was carried out by the linear regression method [Haley *et al*. 1994] for F_2 outcross pedigrees. The genetic model at the QTL assumed that the original strains were fixed for different alleles, although genes could be segregating elsewhere. Hence, it was possible to combine information about the QTL across the families as pointed by Knott *et al.* [1998]. At the first stage of the analysis the probability of an F_2 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:

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

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

$$[3] \quad y_{ijkl} = \mu + H_i + S_j + aP_{ak} + dP_{dk} + gP_{gk} + e_{ijkl}$$

where:

y_{ijkl} – observed phenotype of individual l;

μ – overall mean of the population;

H_i, S_j – fixed effects of hatch and sex;

a, d, g – additive, dominance and parental imprinting effects of QTL, respectively;

P_{ak} – conditional probability of animal k to carry the allele of wild strain;

P_{dk} – conditional probability of animal k to be heterozygous;

P_{ik} – conditional probability of animal k being heterozygous and inheriting the wild strain allele from its sire;

e_{ijkl} – random residual error.

To investigate whether the putative QTL was different in male vs. female F_2 offspring, the QTL by sex interaction effect was also included in model 3. The additive QTL effect by hatch interaction was also analyzed. The GridQTL portal under an F_2 module at http://www.gridqtl.org.uk/ [Seaton *et al*. 2002] was used for QTL analyses. 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 et al. 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 the F-critical threshold of $P<0.01$ were considered evidence for a significant QTL effect.

The percentage of the variance explained by the detected QTL (V_{QTL}) was calculated as:

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

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

Results and discussion

The number of records (N), means, minimum and maximum, standard deviation, the range and coefficient of variation for the traits studied are given in Table 2. In this study all the marker loci were polymorphic and the average number of alleles per locus was 3. The allele sizes ranged from 114 to 284 bp. The information content (IC) shows useful information provided by a marker on the genome**.** The IC values vary among the markers, with some markers being fully informative and others with $IC<0.5$. Values for the polymorphism information content (PIC) were 0.539, 0.697 and 0.590 for markers GUJ0035, GUJ0041 and GUJ0099, respectively. Based on

Table 2. Summary statistics for phenotypic traits of the F2 population

Trait	N	Mean[A]	Minimum	Maximum	RSD[B]	CV (%)
Live body weight prior to slaughter	422	152.40	83.70	199.20	17.58	11.6
Hot carcass weight	422	112.00	48.30	166.00	14.33	12.8
Chilled carcass weight	421	104.50	141.00	460.30	13.22	12.6
Head weight	422	5.90	4.06	7.53	0.29	5.0
Neck weight	422	4.20	2.12	6.91	0.46	11.1
Breast weight	422	36.90	14.39	54.08	3.10	8.4
Back weight	422	17.30	8.54	30.95	1.83	10.6
Wings weight	422	9.80	4.69	13.52	1.09	11.1
Legs weight	422	21.40	9.91	30.08	1.64	7.7
Carcass fat weight	422	0.70	0.05	5.54	0.70	100.0
Heart weight	422	1.30	0.59	2.01	0.16	13.2
Gizzard weight	422	4.00	1.86	6.05	0.45	11.3
Liver weight	422	3.40	1.85	9.64	0.55	15.8
Proventriculus weight	421	0.60	0.05	0.92	0.10	15.5
Intestine weight	422	7.00	3.46	15.14	1.14	16.2
Pancreas weight	422	0.50	0.05	0.94	0.10	21.3
Spleen weight	416	0.10	0.02	0.23	0.03	32.4
Uropygial gland weight	400	0.30	0.03	0.65	0.07	26.0
Bursa of fabricius weight	410	0.10	0.03	0.33	0.04	32.7
Ovary weight	177	0.16	0.10	6.40	0.45	286.4
Testes weight	245	0.26	0.10	3.20	0.36	138.5
Head percentage	421	5.22	3.64	8.67	0.93	18.0
Neck percentage	421	4.02	2.53	6.93	0.43	10.8
Breast percentage	421	35.29	3.90	47.63	2.91	8.2
Back percentage	421	16.72	10.01	35.53	1.63	9.8
Wings percentage	421	9.43	5.64	13.06	1.04	11.1
Legs percentage	421	20.55	13.09	24.10	1.24	6.0
Carcass fat percentage	421	0.64	0.60	4.10	0.64	100.0
Heart percentage	421	1.09	0.61	1.99	0.44	41.0
Gizzard percentage	420	3.50	2.24	7.56	0.46	13.4
Liver percentage	421	3.08	1.75	8.93	0.55	18.1
Proventriculus percentage	419	0.56	0.60	1.12	0.96	173.1
Intestine percentage	421	6.21	3.36	13.83	1.15	18.5
Pancreas percentage	421	0.43	0.50	0.88	0.97	225.5
Spleen percentage	415	0.78	0.20	0.20	0.86	110.3
Uropygial gland percentage	399	0.26	0.30	0.60	0.74	285.2
Bursa of fabricius percentage	409	0.11	0.30	0.37	0.38	352.1
Ovary percentage	177	0.13	0.10	3.90	0.90	692.3
Testes percentage	245	0.22	0.10	2.38	0.91	416.6
Carcass efficiency	417	73.50	27.46	97.73	3.13	4.2

[A]Trait mean adjusted for fixed effects included in the model.
[B]Residual standard deviation after fitting basic fixed effects (see the text).

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 across chromosome 3 of the Japanese quail are presented in Figure 1.

In model 1, which only accounts for additive effects of QTL, eight chromosome-wide significant QTL underlying cold carcass weight, hot carcass weight, ovary weight, intestine %, gizzard %, pancreas %, liver % and uropygial gland % were found at 9, 7, 32, 24, 19, 36, 0 and 23 cM of the linkage map, respectively. The additive effects

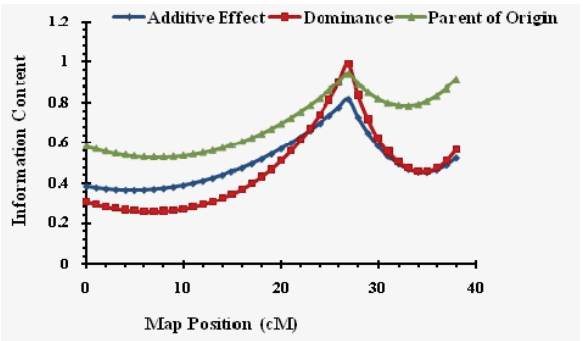

Fig. 1. Useful information contents of markers used in this study in different parts of chromosome 3 of Japanese quail for additive, dominance and imprinting effects.

of both QTL were position and the closest marker locus to f our of the detected QTL (QTL for ovary weight, intestine %, gizzard % and uropygial gland %) was GUJ0035 and the nearest markers to the other QTL (QTL for pancreas % and chilled carcass weight, hot carcass weight, liver %) were GUJ0041 and GUJ0099, respectively.

In model 2, which includes additive and dominance effects of QTL, ten chromosome-wide significant QTL underlying chilled carcass weight, hot carcass weight, head weight, head %, back %, liver weight, ovary weight, liver %, proventriculus % and uropygial gland % were found at 0, 0, 20, 1, 1, 0, 31, 0, 6, and 15 cM of the linkage map, respectively. The additive and dominance effects of all the detected QTL were negative except for chilled and hot carcass weights. The closest marker locus to the QTL for ovary weight and uropygial gland % was GUJ0035, while the nearest marker to the QTL for liver weight, liver %, proventriculus %, chilled and hot carcass weight was GUJ0099.

In the third analysis, where the additive, dominance and imprinting (parent-of-origin) effects of QTL were jointly modeled, fourteen chromosome-wide significant QTL underlying hot carcass weight, chilled carcass weight, head weight, head %, back %, ovary weight, uropygial gland weight, intestine %, gizzard %, pancreas %, liver %,

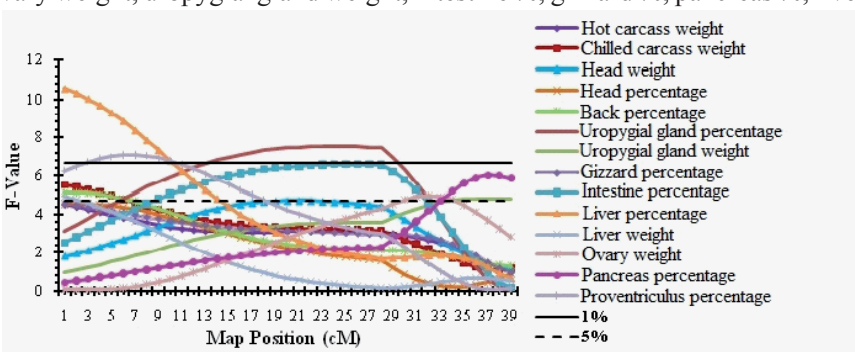

Fig. 2. Test statistic curve resulted from the additive quantitative trait loci model on chromosomes 3 using an intercross between two Japanese quail strains.

uropygial gland %, proventriculus % and liver weight were found at 0, 0, 20, 1, 1, 31, 35, 24, 0, 36, 0, 23, 6 and 0 cM of the linkage map, respectively. QTL that surpassed the suggestive or significant linkage thresholds are summarized in Tables 3, 4 and 5.

Table 3 shows the location of 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 (Fig. 2).

The additive QTL effects for chilled and hot carcass weights were significant (Tab. 3). The maximum F-statistics for hot carcass and chilled carcass weights were detected at 4.5 and 5.5 cM, respectively, from the beginning of the linkage group. The F_2 phenotypic variance percentage explained by the detected QTL for additive effects was 1.4 and 1.6 for both carcass weights, respectively. QTL for head weight were mapped at 20 cM, while the other two QTL affecting head % and back % were detected at 1 cM on chromosome 3 (Tab. 3). The type of action for the detected QTL for head weight was dominance, while the effect of the QTL for head % was additive. The dominance QTL effect for head weight was positive, while the additive QTL effect for head % was negative. The peak value of the test statistic (F = 5.1) of the detected QTL for back % on chromosome 3 was very close to the GUJ0035 marker. Additionally, this QTL explained 1.2% of the F_2 phenotypic variance for back % (Tab. 3).

Table 3. Quantitative trait *loci* associated with carcass traits in F2 population of Japanese quail

Trait	Position (cM)[A]	F-value	QTL effect[B]			V_{QTL}[C]			Closest marker
			additive (SE)	dominance (SE)	imprinting (SE)	additive	dominance	imprinting	
Intestine percentage	24	6.6*	0.20(0.07)	-	-	1.4	-	-	GUI0035
Pancreas percentage	36	6.0*	0.20(0.009)	-	-	1.1	-	-	GUI0041
Uropygial gland percentage	23	7.5*	0.01(0.008)	-	-	1.6	-	-	GUI0035
Liver weight	0	4.8*	0.52(0.10)	-0.38(0.16)	-	1.0	1.0	-	GUI0099
Ovary weight	31	4.9*	0.42(0.15)	-0.35(0.20)	-	3.3	1.3	-	GUI0035
Liver percentage	0	10.5**	0.41(0.10)	-0.52(0.16)	-	2.5	2.0	-	GUI0099
Proventriculus percentage	6	7.0**	0.02(0.01)	-0.06(0.01)	-	1.1	2.2	-	GUI0099
Hot carcass weight	0	4.5*	-0.30(.11)	0.30(0.18)	-	1.4	0.4	-	GUI0099
Chilled carcass weight	0	5.5*	-0.31(0.11)	0.38(0.17)	-	1.6	0.7	-	GUI0099
Head weight	20	4.7*	-0.03(0.06)	-0.37(0.10)	-	0.3	1.6	-	GUI0035
Head percentage	1	4.5*	0.10(0.04)	-0.20(0.70)	-	0.7	1.1	-	GUI0099
Back percentage	1	5.1*	0.20(0.11)	-0.51(0.17)	-	0.8	1.2	-	GUI0099
Uropygial gland weight	35	4.7**	0.57(0.10)	-0.28(0.14)	-0.14(0.07)	0.0	0.0	2.0	GUI0041
Gizzard percentage	0	4.5*	0.28(0.11)	-0.19(0.17)	-0.26(0.08)	1.4	0.0	1.4	GUI0099

[A]QTL location based on the Japanese quail sex averaged linkage map [43].
[B]Additive and dominance effects were defined as deviation of animals homozygous for the wild allele or heterozygous, respectively, from the mean of two homozygotes.
[C]QTL variance (reduction in residual variance of the F_2 population obtained by inclusion of a QTL at the given position).
*P<0.05; **P<0.01.

The interaction of the additive QTL effect and hatch was significant for intestine %, pancreas %, liver %, gizzard %, ovary %, back weight and back % (Tab. 4 and Fig. 3). Additive QTL effects for pancreas %, liver %, uropygial gland %, pancreas weight, back weight and back % showed a significant interaction with sex (Tab. 5 and

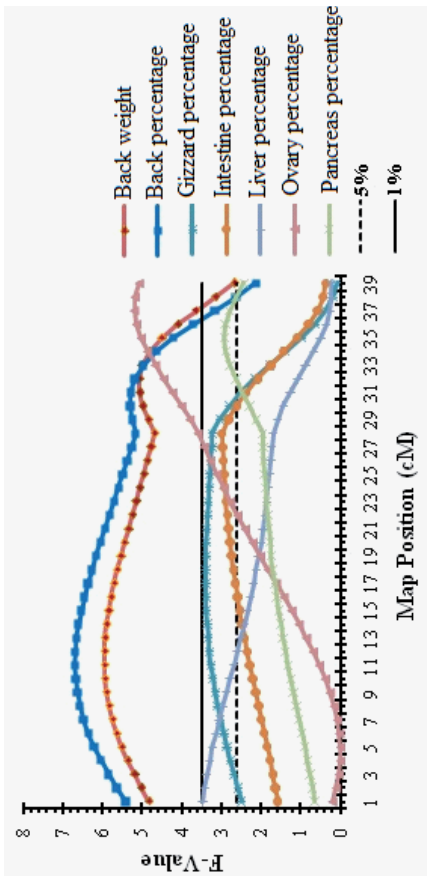

Fig. 3. Test statistic curves resulted from the additive quantitative trait loci by hatch interaction model on chromosome 3 using an intercross between two Japanese quail strains.

Fig. 4).

The peak values of the F-statistics for back weight and back % in this analysis were detected at 4.09 and 4.29 cM, respectively, from the beginning of the linkage group. The F_2 phenotypic variance percentage explained by the detected QTL was 0.8 (Tab. 5).

QTL were detected for carcass traits, which are important traits in poultry breeding. This study adds new important information from a chromosome wide search for QTL in the Japanese quail and it is the first report on the detection and positioning of loci affecting carcass traits on chromosome 3 in the Japanese quail. The contribution of the detected QTL to the F_2 phenotypic variance ranged from 0.0 to 3.3%. In a comprehensive review of studies

Table 4. Summary of quantitative trait *loci* (QTL) results obtained from modeling QTL by hatch interaction

Trait	Position (cM)[A]	F value	QTL additive effect					V_{QTL}[B]	Closest marker
			H1 (SE)	H2 (SE)	H3 (SE)	H4 (SE)	H5 (SE)		
Pancreas percentage	34	2.9*	-0.01(0.02)	0.05(0.02)	0.06(0.02)	-0.01(0.02)	0.008(0.02)	1.1	GUJ0041
Intestine percentage	27	2.9*	0.02(0.16)	0.31(0.16)	0.51(0.17)	-0.09(0.15)	0.26(0.17)	1.4	GUJ0035
Liver percentage	0	3.4*	0.52(0.21)	0.61(0.27)	0.34(0.27)	-0.07(0.21)	0.52(0.23)	2.5	GUJ0099
Ovary percentage	36	5.1**	-0.007(0.13)	-0.03(0.32)	-0.05(0.47)	-0.01(0.21)	1.13(0.22)	3.1	GUJ0041
Gizzard percentage	16	3.3**	0.02(0.19)	0.50(0.21)	0.78(0.23)	0.17(0.19)	-0.13(0.23)	1.4	GUJ0035
Back weight	10	5.9**	-5.46(0.19)	-0.10(0.21)	1.18(0.33)	-0.27(0.20)	0.34(0.22)	0.8	GUJ0099
Back percentage	10	6.6**	-0.03(0.22)	-0.09(0.24)	1.41(0.25)	-0.31(0.22)	0.38(0.24)	0.8	GUJ0099

[A]QTL location based on the Japanese quail sex averaged linkage map [43].
[B]QTL variance (proportion of phenotypic variance of the F2 population explained by QTL).
*P<0.05; **P<0.01.

Table 5. Summary of quantitative trait *loci* (QTL) results obtained from modeling QTL by sex interaction

Trait	Position (cM)[A]	F-value	QTL additive effect		V_{QTL}[B]	Closest marker
			male A (SE)	female A(SE)		
Pancreas weight	37	5.09*	-0.60(0.10)	0.40(0.10)	1.00	GUJ0041
Pancreas percentage	36	5.69**	-0.003(0.01)	-0.04(0.01)	1.10	GUJ0041
Liver percentage	0	8.01**	0.09(0.16)	0.56(0.12)	2.50	GUJ0099
Uropygial gland percentage	26	4.23*	0.01(0.01)	0.02(0.00)	1.60	GUJ0035
Back weight	27	4.09*	0.32(0.10)	0.32(0.08)	0.80	GUJ0035
Back percentage	27	4.29*	0.36(0.12)	0.03(0.09)	0.80	GUJ0035

[A]QTL location based on the Japanese quail sex averaged linkage map [43].
[B]QTL variance (proportion of phenotypic variance of the F2 population explained by QTL).
*P<0.05; **P<0.01.

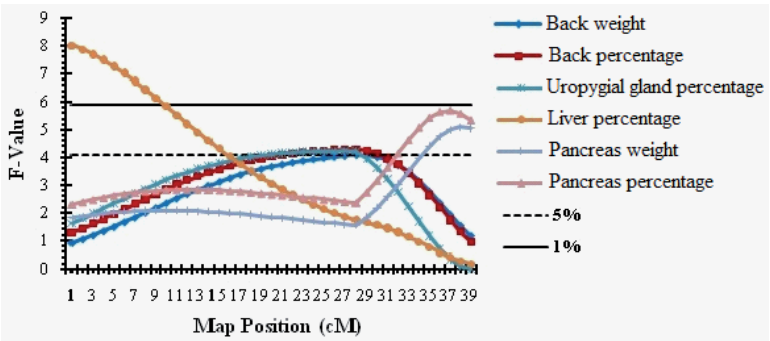

Fig. 4. Test statistic curves resulted from the additive quantitative trait loci by the sex interaction model on chromosomes 3 using an intercross between two Japanese quail strains.

conducted to identify QTL in chickens [Sazanov *et al*. 2010], no QTL for growth or carcass traits was mapped between LEI0143 and ROS0308 (0-69 cM according to the Chicken Consensus Map, [Schmid *et al*. 2005] on GGA11, but a suggestive linkage for length of intestine at 9 weeks of age between ROS0111 and ADL0308 (37-69 cM, Consensus Map) was found in this region by Navarro et al. [2005]. The additive effects of these QTL were positive for chilled and hot carcass weight, while the dominance effects for head weight and back % were negative. Van Kaam *et al*. [1999a, 1999b] performed a genome scan for growth and carcass composition using a crossing between two broiler lines and only one QTL reached the genome-wide significance level. This carcass QTL was located on the chicken chromosome 3 at 0 cM.

A QTL by sex interaction was assessed to investigate whether the effect differed between the two sexes. Significant QTLs by sex interactions were found for both back weight and back %. This QTL explained 0.8% of the phenotypic variance (Tab. 5 and Fig. 4). Generally, a QTL by sex interaction can be considered as a genotype by environment interaction, considering sex as an organismal environment for gene expression [Sazanov *et al.* 2010]. Conducting a full genome scan with a QTL using the sex interaction model or conducting the analysis separately for each sex could

facilitate detection of such interactions. However, a 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 our experiment before their application in selection. In a number of studies the QTL by sex interaction was tested only for locations that were significant in the initial analysis using models without sex interaction [Ikeobi *et al*. 2002, Ikeobi *et al*. 2004, Nones *et al.* 2006], which does not detect QTL with sex-antagonistic effects and has less power to detect QTL with sex-specific and sex-biased effects.

The lack of a significant sex by QTL interaction for carcass weight is interesting, as it suggests that detected QTL were segregating in both sexes, although the females were heavier than the males. Moreover, no QTL was detected for carcass fat weight as an important economic trait. This may be due to the fact that the variation in total carcass fat weight among the F_2 birds in this experiment was too large (Tab. 2). Therefore, an analysis of marker-QTL data for carcass fat weight at maturity may help to further elucidate the nature of gene action for fatness in the Japanese quail.

There are a number of QTL studies that identified QTL for liver weight and liver % on chicken chromosomes. For example, Han et al. [2012] reported a QTL affecting liver weight positioned at 12 cM on chicken chromosome 21. In addition, d'André Hirwa *et al*. [2010] identified a significant QTL for liver weight at 562 cM on chicken chromosome 1. Moreover, Zhou et al. [2006] reported QTL affecting liver weight located at 61.9, 89.4, 40.5 and 26.4 cM on chicken chromosomes 6, 7, 10, and 18, respectively. Several QTL affecting liver % were detected by Zhou *et al.* [2006] at positions 289.2, 229.5, 118.3, 99.4, 89.1, 61.1, 112.6, and 61.7 cM on chicken chromosomes 1, 4, 5, 6, 7, 9, 10, and 26, respectively. The identification of QTL affecting different liver weight and liver % measurements on chromosome 3 in the Japanese quail in the present study is in agreement with the QTL reported for these traits on the systemic regions of chicken chromosomes, suggesting that in addition to the structural genomic conservation, functional genomics conservation also exists between the two species.

We detected significant QTL for gizzard % located at 0 cM on chromosome 3 of the Japanese quail. Gizzard is responsible for grinding the feed, thus facilitating digestion and consequently the absorption of nutrients. Quantitative trait loci for gizzard weight were detected in several chromosomes in the chicken. Gao [2009] and Tercic [2009] detected a QTL for the same trait on chromosomes 1 and 5. Moreover, Navarro *et al.* [2005] reported QTL affecting gizzard weight located at 187, 116 and 143 cM on chicken chromosome 1, 2, and 5, respectively. Few reports are available for QTL associated with intestine length or weight in chicken, while no QTL study on intestine % in the Japanese quail and chicken has been reported. However, significant QTL affecting intestine length were found in other chromosomal regions of the chicken in other studies [Gao *et al.* 2009, Ambo *et al*. 2009, Zeng *et al.* 2011]. In this study we identified one QTL affecting intestine % in the Japanese quail (Tab. 3).

Genomic imprinting is a process, through which the expression of a gene is dependent on the sex of the parent from which it was inherited, with the repressed allele generally considered to be the imprinted one [John *et al.* 1996]**.** The design of our study allowed us to search for potential imprinting effects. In this study the imprinting effect (parent-of-origin effect) of QTL located at 35 and 0 cM on chromosome 3 were significant for uropygial gland weight and gizzard %.

We identified five QTL located at 0 - 20 cM and flanked by GUJ0099 and GUJ0035 for carcass and carcass parts. The high association between the weights of body parts and whole body weight could confound the identity of genes controlling variability in body weight with those involved in carcass composition variability [Lagarrigue *et al.* 2006]. Since there are high genetic correlations between chicken body weights and carcass traits, direct selection for live body weight could produce indirect genetic gains for carcass, weight and carcass parts [Gaya et al. 2006, Sandercock *et al.* 2009]. The detected QTL for carcass weight had a positive dominance effect, while this QTL had a negative dominance effect on back weight. The so-called cryptic QTL is believed to be caused by no or limited selection for the trait, drift, and pleiotropic effects of the QTL allele on other traits that are under selection, or a close linkage and linkage disequilibrium with QTL that are under selection [Sazanov *et al*. 2010].

In conclusion, a number of QTL were detected across the Japanese quail chromosome 3, which affected carcass weight and carcass parts. Our results point out to candidate regions affecting traits of great economic relevance to the Japanese quail breeding. Although this paper adds value to our current understanding of the inheritance of carcass traits in the Japanese quail, it should be noted that the results from the initial QTL studies cannot be implemented directly to the breeding programs, especially when experimental designs such as backcross or F_2 populations are employed. The comparative analysis regarding the location of QTL on different Japanese quail and the syntenic chicken chromosomes, in combination with their association with phenotype may improve our ability to understand the genetic complexity of economically important traits in the Japanese quail.

REFERENCES

1. AMBO M., MOURA ASAMT., LEDUR MC., PINTO LFB., BARON EE., RUY DC., NONES K., CAMPOS RLR., BOSCHIERO C., BURT DW., 2009 – Quantitative trait loci for performance traits in a broiler x layer cross. ***Animal Genetics*** 40, 2, 200-208.
2. ATZMON G., BLUM S., FELDMAN M., CAHANER A., LAVI U., HILLEL J., 2008 – QTLs detected in a multigenerational resource chicken population. ***Journal of Heredity*** 99, 528-538.
3. ATZMON G., RONIN YI., KOROL A., YONASH N., CHENG H, HILLEL J., 2006 – QTLs associated with growth traits and abdominal fat weight and their interactions with gender and hatch in commercial meat-type chickens. ***Animal Genetics*** 37, 352-358.
4. BARON EE., MOURA AS., LEDUR MC., PINTO LF., BOSCHIERO C., RUY DC., NONES K., ZANELLA EL., ROSARIO MF., BURT DW., COUTINHO LL., 2011 – QTL for percentage of carcass and carcass parts in a broiler x layer cross. ***Animal Genetics*** 42, 117-124.

5. BROCKMAN GA., HALEY CS., RENNIE U., KNOTT SA., SCHWERIN M., 1998 – Quantitative trait loci affecting body weight and fatness from a mouse line selected for extreme high growth. ***Genetics*** 150, 369-381.
6. BOTSTEIN D., WHITE RL., SKOLNICK M., DAVIS RW., 1980 – Construction of a genetic linkage map in man using restriction fragment length polymorphisms. ***American Journal of Human Genetics*** 32, 314-331.
7. CHANG GB., CHANG H., LIU XP., ZHAO WM., JI DJ., MAO YJ., SONG GM., SHI XK., 2007 – Genetic Diversity of Wild Quail in China Ascertained with Microsatellite DNA Markers. ***Journal of Animal Science*** 12, 1783- 1790.
8. CHURCHILL GA., DOERGE RW., 1994 – Empirical threshold values for quantitative trait mapping. *Genetics* 138, 963-971.
9. DANDRE-HIRWA C., YAN W., WALLACE P., NIE Q., LUO C., LI H., SHEN X., SUN L., TANG J., LI W., ZHU X., YANG G., ZHANG X., 2010 – Effects of the thyroid hormone responsive spot 14alpha gene on chicken growth and fat traits. ***Poultry Science*** 89, 9, 1981-1991
10. ESMAILIZADEH AK., BAGHIZADEH A., AHMADIZADEH M., 2012 – Genetic mapping of quantitative trait loci affecting bodyweight on chromosome 1 in a commercial strain of Japanese quail. ***Animal Production Science*** 52, 64-68.
11. FLINT J., CORLEY T., DEFRIES JC., FULKER DW., GRAY JA., MILLER S., 1995 – A simple genetic basis for a complex psychological trait in laboratory mice. ***Science*** 269, 1432-1435.
12. GAO Y., DU ZQ., WEI WH., YU XJ., DENG XM., FENG CG., FEI J., FENG JD., LI N., HU XX., 2009 – Mapping quantitative trait loci regulating chicken body composition traits. ***Animal Genetics*** 40, 6, 952-954.
13. GAYA LG., FERRAZ JB., REZENDE FM., MOURAO GB., MATTOS EC., ELER JP., MICHELAN-Filho T., 2006 – Heritability and genetic correlation estimates for performance and carcass and body composition traits in a male broiler line. ***Poultry Science*** 85, 837-843.
14. GILMOUR AR., GOGEL BJ., CULLIS BR., THOMPSON R., 2006 – ASREML user guide release 2.0. (VSN International Ltd: Hemel Hempstead, UK).
15. HALEY C., KNOTT S., ELSEN J., 1994 – Mapping quantitative trait loci in crosses between outbred lines using least squares. ***Genetics*** 136, 1195-1207.
16. HAN R., WEI Y., KANG X., CHEN H., SUN G., LI G., BAI Y., TIAN Y., HUANG Y., 2012 – Novel SNPs in the PRDM16 gene and their associations with performance traits in chickens. ***Molecular Biology Reports*** 39, 3, 3153-3160.
17. IKEOBI CON., WOOLLIAMS BJA., MORRICE DR., LAW A., WINDSOR D., BURT DW., HOCKING PM., 2004 – Quantitative trait loci for meat yield and muscle distribution in a broiler layer cross. ***Livestock Production Science*** 87, 143-151.
18. IKEOBI CON., WOOLLIAMS BJA., MORRICE DR., LAW A., WINDSOR D., BURT DW., HOCKING PM., 2002 – Quantitative trait loci affecting fatness in the chicken. ***Animal Genetics*** 33, 428-435.
19. JOHN RM., SURANI MA., 1996 – Imprinted genes and regulation of gene expression by epigenetic inheritance. ***Current Opinion in Cell Biology*** 8, 348-353.
20. KAYANG BB., VIGNAL A., INOUE-MURAYAMA M., MIWA M., MONVOISIN JL., ITO S., MINVIELLE FA., 2004 – A first generation microsatellite linkage map of the Japanese quail. ***Animal Genetics*** 35, 195-200.
21. KAYANG BB., INOUE-MURAYAMA M., HOSHI T., MATSUO K., TAKAHASHI H., MINEZAWA M., MIZUTANI M., ITO S., 2002 – Microsatellite loci in Japanese quail and cross-species amplification in chicken and guinea fowl. ***Genetics Selection Evolution*** 34, 233-253.

22. KNOTT SA., MARKLUND L., HALEY CS., ANDERSSON K., DAVIES W., ELLEGREN H., FREDHOLM M., HANSSON I., HOYHEIM B., LUNDSTROM K., MOLLER M., ANDERSSON L., 1998 – Multiple marker mapping of quantitative trait loci in a cross between outbred wild boar and large white pigs. ***Genetics*** 149, 1069-1080.
23. LAGARRIGUE S., PITEL F., CARRE W., ABASHT B., LE-Roy P., NEAU A., AMIGUES Y., SOURDIOUX M., SIMON J., COGBURN-Aggrey S., LECLERCQ B., VIGNAL A., DOUAIRE M., 2006 – Mapping quantitative trait loci affecting fatness and breast muscle weight in meat-type chicken lines divergently selected on abdominal fatness. ***Genetics Selection Evolution*** 38, 85-97.
24. MINVIELLE F., KAYANG B., INOUE-MURAYAMA M., MIWA M., VIGNAL A., GOURICHON D., NEAU A., MONVOISIN J., ITO S., 2005 – Microsatellite mapping of QTL affecting growth, feed consumption, egg production, tonic immobility and body temperature of Japanese quail. ***BMC Genomics*** 6, 87.
25. NAVARRO P., VISSCHER PM., KNOTT SA., BURT DW., HOCKING PM., HALEY CS., 2005 – Mapping of quantitative trait loci affecting organ weights and blood variables in a broiler layer cross. ***British Poultry Science*** 46, 430-442.
26. NONES K., LEDUR MC., RUR DC., BARON EE., MELO CMR., MOURA ASAMT., ZANELLA EL., BURT DW., COUTINHO LL., 2006 – Mapping QTLs on chicken chromosome 1 for performance and carcass traits in a broiler x layer cross. ***Animal Genetics*** 37, 95-100.
27. OBER C., ABNEY M., MCPEEK MS., 2001 – The genetic dissection of complex traits in a founder population. ***American Journal of Human Genetics*** 69, 1068-1079.
28. PARK HB., JACOBSSON L., WAHLBERG P., SIEGEL PB., ANDERSSON L., 2006 – QTL analysis of body composition and metabolic traits in an intercross between chicken lines divergently selected for growth. ***Physiological Genomics*** 25, 2, 216-23.
29. ROSARIO MF., LEDUR MC., MOURA ASAMT., COUTINHO LL., GARCIA AAF., 2006 – Identification of markers potentially associated to QTLs for performance traits in an experimental chicken population. 30th International Conference on Animal Genetics. Porto Seguro, 98.
30. RUY DC., NONES K., BARON EE., LEDUR MC., MELO CMR., AMBO M., CAMPOS RLR., COUTINHO LL., 2005 – Strategic marker selection to detect quantitative trait loci in chicken. ***Journal of Agricultural Science*** 62, 111-116.
31. SANDERCOCK DA., NUTE GR., HOCKING PM., 2009 – Quantifying the effects of genetic selection and genetic variation for body size, carcass composition, and meat quality in the domestic fowl (Gallus domesticus). ***Poultry Science*** 88, 923-931.
32. SAZANOV A., SAZANOVA A., BARKOVA O., JASZCZAK K., 2010 – QTL in chicken: a look back and forward - a review. ***Animal Science Papers and Reports*** 28, 307-314.
33. SCHMID M., NANDA I., HOEHN H., SCHARTL M., HAAF MT., BUERSTEDDE JM., ARAKAWA H., CALDWELL RB., WEIGEND S., BURT DW., SMITH J., GRIFFIN DK., MASABANDA JS., GROENEN MA., CROOIJMANS RP., VIGNAL A., FILLON V., MORISSON M., PITEL F., VIGNOLES M, GARRIGUES A, GELLIN J., RODIONOV AV., GALKINA SA., LUKINA NA., BEN-ARI G., BLUM S., HILLEL J., TWITO T., LAVI U., DAVID L., FELDMAN MW., DELANY ME., CONLEY CA., FOWLER VM., HEDGES SB., GODBOUT R., KATYAL S., SMITH C., HUDSON Q., SINCLAIR A., MIZUNO S., 2005 – Second report on chicken genes and chromosomes 2005. ***Cytogenetic and Genome Research*** 109, 415-479.
34. SEATON G., HALEY CS., KNOTT SA., KEARSEY M., VISSCHER PM., 2002 – QTL Express: mapping quantitative trait loci in simple and complex pedigrees. ***Bioinformatics*** 18, 339-340.
35. TERCIC D., HOLCMAN A., DOVC P., MORRICE DR., BURT DW., HOCKING PM., HORVAT S., 2009 – Identification of chromosomal regions associated with growth and carcass traits in an F (3) full sib intercross line originating from a cross of chicken lines divergently selected on body weight. ***Animal Genetics*** 40, 5, 743-748.

36. VALLEJO RL., BACON LD., LIU HC., WITTER RL., GROENEN MA., HILLEL J., CHENG HH., 1998 – Genetic mapping of quantitative trait loci affecting susceptibility to Marek's disease virus induced tumors in F-2 intercross chickens. ***Genetics*** 148, 349-360.
37. VAN KAAM JBCHM., GROENEN MAM., BOVENHUIS H., VEENENDAAL A., VEREIJKEN ALJ., VAN-ARENDONK JAM., 1999a – Whole genome scan in chickens for quantitative trait loci affecting growth and feed efficiency**.** ***Poultry Science*** 78, 15-23.
38. VAN KAAM JBCMH., GROENEN MAM., BOVENHUIS H., VEENENDAAL A., VEREIJKEN ALJ., VAN-ARENDONK JAM., 1999b – Whole genome scan in chickens for quantitative trait loci affecting carcass traits. ***Poultry Science*** 78, 1091-1099.
39. VAN KAAM JBCMH., VAN- ARENDONK JAM., GROENEN MAM., BOVENHUIS H., VEREIJKEN ALJ., CROOIJMANS R., VAN- DER- POEL JJ., VEENENDAAL A., 1998 – Whole genome scan for quantitative trait loci affecting body weight in chickens using a three generation design. ***Livestock Production Science*** 54, 133-150.
40. WALLING GA., ARCHIBALD AL., CATTERMOLE JA., AL E., 1998 – Mapping of quantitative trait loci on porcine chromosome 4. ***Animal Genetics*** 29, 415-424.
41. WANG SZ., HU XX., WANG ZP., LI XC., WANG QG., WANG YX., TANG ZQ., LI H., 2012 – Quantitative trait loci associated with body weight and abdominal fat traits on chicken chromosomes 3, 5 and 7. ***Genetics and Molecular Research*** 11, 2, 956-965.
42. ZENG F., XIE L., PANG X., LIU W., NIE Q., ZHANG X., 2011 – Complementary deoxyribonucleic acid cloning of avian G0/G1 switch gene 2, and its expression and association with production traits in chicken. ***Poultry Science*** 90, 7, 1548-1554.
43. ZHOU H., DEEB N., EVOCK-CLOVER CM., ASHWELL CM., LAMONT SJ., 2006 – Genome-wide linkage analysis to identify chromosomal regions affecting phenotypic traits in the chicken II. Body composition. ***Poultry Science*** 85, 10, 1712-1721.