# Microsatellite mapping of quantitative trait loci affecting carcass traits on chromosome 1 in half-sib families of Japanese quail (*Coturnix japonica*)

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**Abstract** The objective of this study was to identify the quantitative trait loci (QTL) affecting carcass traits on chromosome 1 in Japanese quail. The populations comprised of 422 progeny in 9 half-sib families. Phenotypic data on carcass weight, carcass parts, and the internal organs were collected on 422 progeny. Nine half-sib families were genotyped for 8 microsatellite markers covering chromosomes 1. Quantitative trait loci effects were estimated using interval mapping regression method Chromosome wide significant QTL were identified for the weight of bursa of fabricius at 24 cM (P < 0.01), uropygial gland at 102 cM (P < 0.05) and breast muscle at 199 cM (P < 0.05) on chromosome 1. The detected QTL segregated in 7 out of the 9 half-sib families, and the size of QTL effect ranged from 0.68 to 25.1 in unit of the trait residual standard deviation. The current study adds significant new information for better understanding of the inheritance of carcass traits in Japanese quail.

**Keywords**: carcass traits, Japanese quail, half-sib family, QTL mapping, microsatellite markers

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#### Introduction

Quantitative traits are influenced not only by several genes and their interactions but also by the surrounding environment. Identification of the genes which control economic traits is of great importance in agriculture. Since polymorphic DNA markers are available in many species, construction of well saturated genetic maps and genetic dissection of complex quantities traits are expected (Vallejo et al., 1998).

The simple way of scoring and high polymorphism are among the unique features which makes microsatellites one of the most popular molecular markers used in various fields (Zane et al., 2002) including molecular genetic evaluation and mapping of poultry chromosomes (Gholizadeh et al., 2007). Also, microsatellites are used for mapping locations within the genome, specifically in genetic linkage analysis and marker assisted selection to locate a gene or a mutation responsible for a given trait.

Knowledge of the Japanese quail (*Coturnix japonica*) genome paves the way in finding better solutions

for increasing the efficiency of meat and egg production as sources of high quality protein for human nutrition. The quail's 16-d embryonic developmental period, combined with its sexual maturation period of 8 weeks, results in generational times that are substantially shorter than those of the chicken (Huss et al., 2008). This useful feature makes the quail a suitable case to be genetically analyzed (Groenen et al., 1998).

Many studies have identified QTL for economically important traits such as growth and carcass traits in chickens (Van Kaam et al., 1999; McElroy et al., 2006; Uemoto et al., 2009). Some studies on the construction of linkage map using microsatellite markers has been conducted on quail (Kayang et al., 2000; Kayang et al., 2002). In 2004, a linkage map of quail using microsatellite markers was developed (Kayang et al., 2004). Minvielle et al. (2005) reported genome-wide significant or suggestive QTL for clutch length, body weight (5 and 70 weeks of age) and feed intake on chromosome 1, age at first egg and egg number on chromosome 6,

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and eggshell weight and residual feed intake on chromosome 20 in an F2 population of Japanese quail. Esmailizadeh et al. (2012) located significant QTL affecting body weight at 3, 4, 5 and 6 weeks of age, average daily gain, and Kleiber ratio, an indirect criterion for feed efficiency at 0-23 cM on chromosome 1 in a halfsib population of Japanese quail comprising of 300 birds from three half sib families. Sohrabi et al. (2012, 2014) also found locations of QTL underlying hatching weight and growth traits and estimated their additive and dominance effects on chromosome 1 in an F<sub>2</sub> population. By using an F2 experimental population, Jabbari et al. (2014) also found similar results (by traits) on chromosome 3. Ahmadi et al. (2014), by construction of an F2 experimental population of Japanese quail, reported significant loci affecting breast weight and some other carcass parts weight on chromosome 5.

Since the poultry meat may be transported over long distances, the carcass has to be processed further to keep its quality. Slaughtering and dissection in sib tests make the detection of phenotypes on the relative yield of carcass parts difficult and costly. Detecting genomic regions, however, can facilitate genetic progress (Baron et al., 2011). Since none of the previous studies on detection of QTL affecting carcass traits in Japanese quail did not use half sib families, the objective of this study was to identify quantitative trait loci (QTL) affecting carcass traits on chromosome 1 in a half sib population of Japanese quail.

#### Materials and methods

Experimental population and phenotypic measurements

Parents were randomly selected from a commercial strain of Japanese quail. Nine unrelated males were randomly mated to 27 female birds to create a resource paternal half-sib mapping population consisting of 422 progeny (246 males and 176 females). The half-sib population was created in 5 consecutive hatches. The parents were kept in group cages and fed a layer diet ad libitum. The progeny from 9 sire families were raised for 5 weeks on a floor covered with wood shavings in an environmentally controlled room with continuous artificial lighting and at the temperature which was decreased gradually from 37 to 25°C. The progeny received water, a mash starter diet (0–21 days), and a mash growing diet (22–35 days) ad libitum.

The F2 birds were slaughtered at 35 days of age and hot carcass weight, intestine weight, pancreas weight, liver weight, spleen weight, heart weight, pre-stomach weight, gizzard weight, testis weight, ovary weight, and head weight were recorded. The carcasses were kept at 4°C for 24 h and, then, cold carcass weight, total carcass fat weight, uropygial gland weight, bursa of fabricius weight, femur weight, breast weight, neck weight, back weight, and wing weight were recorded. It should be noted that the trait measurement was performed by one person.

## DNA samples, microsatellite markers, and genotyping

Blood samples of the 9 sires and their progeny were collected at slaughter using 2.5 mL blood collection tubes containing EDTA as the anticoagulant. Afterwards, they were kept at -20°C. The genomic DNA was extracted from the whole blood using an optimized and modified salting-out method (Miller et al., 1988).

Eight microsatellite (GUJ0055, GUJ0052, GUJ0048, GUJ0013, GUJ0056, GUJ0098, GUJ0068, and GUJ0090) located on Japanese quail chromosome 1 were selected based on the polymorphism information content values of the loci (Kayang et al., 2002) and their positions (Kayang et al., 2004) and the 9 male birds and their offspring were genotyped for these markers.

Polymerase chain reaction (PCR) was performed for genotyping the 9 male birds and their progeny for each marker. The reactions consisted of 2 µL of template DNA (100 ng of genomic DNA), 2.5 µL PCR buffer (1 × PCR buffer), 1 μL MgCl2 (3.0 μM MgCl2), 0.5 μL dNTP mix (200 µM dNTP), 0.3 µL Tag DNA polymerase (1U), 1 µL (0.25 µM) of each forward and reverse primer, and 16.5 µL sterile water in the total volume of 25 µL. PCR amplifications were performed in 0.2 ml tubes using a 30 cycle program. The PCR procedure was performed at 4 min at 94°C, followed by 30 cycles of 30 s at 94°C, 1 min at annealing temperature (varying from 55-57°C, depending on the marker being amplified) and 1 min at 72°C, followed by a final extension step of 4 minutes at 72°C. The PCR products were run on 8% denaturing polyacrylamide gels using electrophoresis. Individual PCR product fragment sizes for the microsatellite markers were determined by visualizing the band pattern via silver nitrate staining method.

#### Descriptive statistics and QTL mapping analysis

Basic descriptive statistics, including the number of observations (N), minimum values, maximum values, means, and residual standard deviation (RSD) were calculated using ASReml software (Gilmour et al., 2006)

fitting a model including the mean of the half sib population for each trait, fixed effects of hatch (5 levels), and sex (2 levels) carcass weight as covariate and residual random term.

QTL analysis was conducted using the least squared regression interval mapping approach (Knott et al., 1996). QTL effects were estimated using 2 analyses for individual families and across families. For interval mapping, the following model was used:

$$y_{ijkl} = \mu_i + a_i x_{ij} + H_k + S_l + e_{ijkl}$$
 (1)

where  $y_{ijkl}$  is the observed phenotype of individual n, offspring of sire i;  $\mu_i$  is the mean of sire family i;  $\alpha_i$  is the allele substitution effect of the QTL within family i;  $x_{ij}$  is the probability that animal n inherits the (arbitrarily assigned) first allele of sire i, given the observed marker genotypes and marker/QTL positions;  $H_k$  and  $S_l$  are the fixed effects of hatch (5 levels) and sex (2 levels), respectively, and  $e_{ijkl}$  is the residual random effect.

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 were determined empirically by permutation testing (Churchill and Doerge, 1994). Data permutation with 10,000 replicates was used to determine the empirical distribution of the test statistic under the null hypothesis of no QTL. QTL analysis was performed using GridQTL portal (Seaton et al., 2006).

#### **Results and discussion**

An example of visualized band patterns of the PCR products is represented in Figure 1. Descriptive analysis of

all the studied traits was listed already by Charati et al. (2014). The effect of hatch was significant (P < 0.001) for all the traits, except for the uropygial gland weight. Effect of sex was significant for carcass weight (P < 0.001), back weight (P < 0.05), bursa of fabricius weight (P < 0.01), gizzard weight (P < 0.001), head weight (P < 0.001), pre-stomach weight (P < 0.05), spleen weight (P < 0.01), and wing weight (P < 0.01).

The half-sib progeny was distributed across 5 hatches with on average 41 offspring ranging from 32 (sire families 7 and 8) to 78 (sire family 1) (Table 1).

Information content was calculated at 1 cM intervals across all the investigated regions in the population for each analysis. The information content of an individual marker is the proportion of animals in which the allele inherited from the sire can be unambiguously identified (Table 2). Average information content across the 9 families and all of the investigated intervals along chromosome 1 (between and at the loci of the markers) was 0.39, ranging from 0.31 to 0.56 (Figure 2). The measure of information content can range from 0 (absence of information) to 1 (maximal information content). It should be noted that information content is less between markers than at the marker loci, as shown in Figure 2. The decrease in the information content can be due to the loss of informative markers in 1 or more families. In such a case, the relative genotype has been detected using the markers placed at farther distance (Knott et al., 1998). Factors such as the number of cases with no genotypes and the imbalance of allele segregation in markers can affect the amount of useful information gained by the markers (Esmailizadeh et al., 2008b).

The most likely position, F-statistic values, and alle-

**Table1.** Distribution of the informative and uninformative progeny across the 9 half-sib

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Family	1	2	3	4	5	6	7	8	9	Sum
Informative	77	39	60	35	36	27	29	25	55	383
Uninformative	1	13	1	1	0	12	3	7	1	39
Total	78	52	61	36	36	39	32	32	56	422

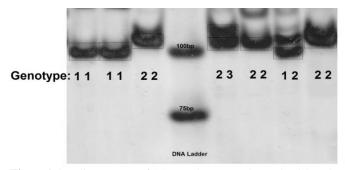
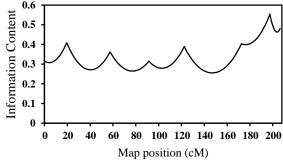


Figure 1. banding pattern of SSR markers on polyacrylamide gel



**Figure 2.** The useful information content (the PIC values) across chromosome 1 in 9 half-sib families of Japanese quail

Table 2. The useful information content of each marker

Marker	Position (cM)	Distance (cM)	Genotype	d individuals (%)	Information	Alleles
	Position (CM)	Distance (CM)	Sires	Half-sib progeny	IIIIOIIIIatioii	
GUJ0055	0	0	9 (100%)	402 (95%)	0.31	3
GUJ0052	19	19	9 (100%)	417 (98%)	0.31	3
GUJ0048	57	38	9 (100%)	407 (96%)	0.36	2
GUJ0013	91	34	9 (100%)	414 (98%)	0.31	2
GUJ0056	122	31	9 (100%)	401 (95%)	0.39	2
GUJ0098	172	50	9 (100%)	416 (98%)	0.40	2
GUJ0068	197	25	9 (100%)	407 (96%)	0.56	2
GUJ0090	206	9	9 (100%)	409 (96%)	0.48	3

lic effects of detected QTL using individual family analysis for carcass traits are given in Table 3. In this analysis, the detected QTL was segregated in 7 out of 9 half-sib families and 17 chromosome-wide significant QTL was mapped for 11 traits through the scanning of chromosome 1. In total, 5 QTL were significant at 1% chromosome-wide level and 12 QTL were significant at 5% chromosome-wide level for 11 out of 17 traits evaluated in the half-sib population.

Individual family analysis in family 1 showed 4 chromosome-wide significant QTL underlying femur weight (P < 0.05), heart weight (P < 0.05), liver weight (P < 0.05), and uropygial gland weight (P < 0.05) at 61, 172, 122, and 123 cM of the linkage map, respectively. In family 2, two chromosome-wide significant QTL underlying bursa of fabricius weight (P < 0.05) and uropygial gland weight (P < 0.01) were found at 104 and

110 cM of the linkage map, respectively. In family 3, three chromosome-wide significant QTL underlying breast weight (P < 0.01), head weight (P < 0.01), and uropygial gland weight (P < 0.05) were found at 199, 171, and 206 cM of the linkage map, respectively. In family 4, two chromosome-wide significant QTL underlying breast weight (P < 0.01) and uropygial gland weight (P < 0.05) were found at 187 and 152 cM of the linkage map, respectively. In family 5, two chromosome-wide significant QTL underlying intestine weight (P < 0.05) and pancreas weight (P < 0.05) were found at 21 and 91 cM of the linkage map, respectively. In family 6, two chromosome-wide significant OTL underlying back weight (P < 0.05) and bursa of fabricius weight (P < 0.01) were found at 0 and 153 cM of the linkage map, respectively. There was no evidence for significant QTL for families 7 and 8. Finally, in family

Table 3. Summary of significant QTL from individual family analyses

Trait 1 Location	Logation (aM)	Family	Effect   CE	E statistic	F-statistic 7	Threshold <sup>2</sup>	Elantrina mantran
	Location (CIVI)	raillily	Effect ± SE	F- statistic -	P<0.05	P<0.01	<ul> <li>Flanking marker</li> </ul>
FeW	61	1	$0.74 \pm 0.23$	9.9 *	7.2	10.9	GUJ0048
HtW	172	1	$1.26 \pm 0.45$	7.9 *	7.4	11.2	GUJ0098
LiW	122	1	$-0.68 \pm 0.24$	8.2 *	7.4	10.9	GUJ0056
UGW	123	1	$0.70 \pm 0.25$	8.1 *	7.3	10.8	GUJ0056
BFW	104	2	$-5.69 \pm 1.85$	9.5 *	6.4	10.1	GUJ0013
UGW	110	2	$8.37 \pm 2.15$	15.2 **	6.7	10.9	GUJ0056
BrW	199	3	$-0.98 \pm 0.26$	13.7 **	6.9	10.8	GUJ0068
HdW	171	3	$1.26 \pm 0.35$	13.2 **	6.5	9.9	GUJ0098
UGW	206	3	$0.83 \pm 0.31$	7.3 *	6.6	10.3	GUJ0090
BrW	187	4	$-2.39 \pm 0.67$	12.7 **	7.2	11.4	GUJ0068
UGW	152	4	$2.67 \pm 0.80$	11.1 *	7.5	12.2	GUJ0098
InW	21	5	$25.10 \pm 8.71$	8.3 *	8.1	13.0	GUJ0052
PaW	91	5	$-1.14 \pm 0.39$	8.7 *	8.6	13.3	GUJ0013
BaW	0	6	$-15.37 \pm 5.24$	8.6 *	6.6	10.4	GUJ0055
BFW	153	6	$-2.06 \pm 0.54$	14.8 **	6.5	10.3	GUJ0098
BrW	0	9	$-1.68 \pm 0.55$	9.3 *	7.8	12.0	GUJ0055
PSW	206	9	$1.35 \pm 0.42$	10.6 *	7.3	11.5	GUJ0090

<sup>1</sup>FeW: Femur weight, FeW: HtW: Heart weight, LiW: Liver weight, UGW: Uropygial gland weight, BFW: Bursa of fabricius weight, BrW: Breast weight, HdW: Head Weight, InW: Intestine weight, PaW: Pancreas weight, BaW: Back weight, PSW: Pre-stomach weight <sup>2</sup> Chromosome wide significance thresholds from permutation test

<sup>\*, \*\*;</sup> P < 0.05 and P < 0.01 chromosome-wide significant of QTL respectively

9, two chromosome-wide significant QTL underlying breast weight (P < 0.05) and pre-stomach weight (P < 0.05) were found at 0 and 206 cM of the linkage map, respectively.

Size of the QTL effect ranged from 0.68 to 25.10 in the unit of the trait residual standard deviation for different traits. Maximum QTL effect was related to liver weight in family 9 and minimum QTL effect was associated with intestine weight in family 5. Given that a paternal half-sib design was used in this study, the estimated QTL effect in this design was an allele substitution effect, which marked the difference between parental Q and q alleles of the offspring and the effects of other genes on average between the two groups were assumed to be the same. It should be noted here that the allele substitution effect could also include dominance effect, because additive QTL effect was estimated as the mean difference between the 2 homozygous genotypes

of QTL (QQ and qq). Simulation results showed that, when the number of progenies was low, size of the QTL effect was overestimated (Esmailizadeh et al., 2008a). Therefore, it is likely that, in the families with fewer offspring, the size of QTL effect would be overestimated.

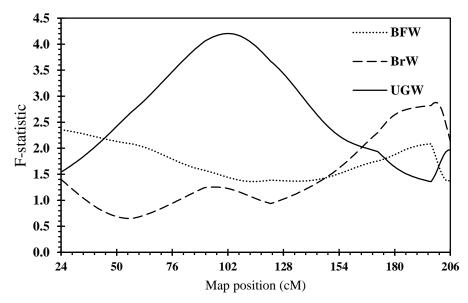
Across family analysis showed 3 chromosome-wide significant QTL underlying bursa of fabricius weight (P < 0.05), breast weight (P < 0.05), and uropygial gland weight (P < 0.01), which were found at 24, 199, and 102 cM of the linkage map, respectively. The flanking marker locus to QTL for the bursa of fabricius weight was GUJ0052, for that of breast weight was GUJ0090, and the nearest marker to QTL for uropygial gland weight was GUJ0013. Table 4 shows the position of the significant QTL on the chromosome, the maximum F value obtained at each position, and the reduction of the residual variance obtained by fitting the QTL at the detected location (Figure 3).

Table 4. Summary of significant QTL from across families' analyses

Trait <sup>1</sup>	Location (cM)	$V_{OTL}^2$	F-Statistics	F-statistic Threshold <sup>3</sup>		- Flanking marker
Han	Location (Civi)	V QTL	r-Staustics	P<0.05	P<0.01	- Flanking marker
BFW	24	6.2	2.4 *	2.2	3.1	GUJ0052
BrW	199	4.0	2.9 *	2.5	3.5	GUJ0090
UGW	102	6.3	4.2 **	2.8	3.5	GUJ0013

<sup>&</sup>lt;sup>1</sup>BFW: Bursa of fabricius weight, BrW: Breast weight, UGW: Uropygial gland weight

<sup>\*,\*\*;</sup> P < 0.05 and P < 0.01 chromosome-wide significant of QTL respectively



**Figure 3.** F-statistic curves resulted from the joint analysis of 9 half-sib families on chromosomes 1 of Japanese quail. The lower and upper horizontal lines represent 5 and 1% chromosome-wide significant levels of linkage, respectively. BFW: Bursa of fabricius weight, BrW: Breast weight, UGW: Uropygial gland weight

<sup>&</sup>lt;sup>2</sup>QTL variance (the reduction in residual variance of the half sib population obtained by inclusion of a QTL at the given position)

<sup>&</sup>lt;sup>3</sup>Chromosome wide significance thresholds from permutation test

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The proportion of the progeny phenotypic variation explained by the significant QTL ranged from 4.0 to 6.3%. The QTL variance shows the contribution of specific trait loci to the total phenotypic variance of the trait. In each position, mapping QTL using the desirable model determines whether a significant amount of the variance in a quantitative trait can be associated with the QTL in that position. QTL variance determined as the reduction of the residual variance acquired by fitting the QTL in the corresponding location was relatively small for the detected loci (Sohrabi et al., 2012). Considering the nature of the half-sib design used in the study, the effects were likely to be overestimated. Fairly large progeny group sizes were required to identify a medium-sized QTL in each half-sib group; otherwise, the experiment would either have low power to detect the

QTL or the detected effect would be overestimated (Van der werf et al., 2007).

There are a large number of QTL studies that have identified QTL for carcass traits on chicken chromosome 1. As the chicken and quail are two species of animals belonging to the same family with identical karyotype (2n = 78), QTL for the traits identified in this study on chromosome 1 might be comparable with those reported in chicken (Kayang et al., 2006). Moradian et al. (2014) used an F2 population originated from two strains of Japanese quail to identify significant QTL for breast weight, carcass weight, carcass fatness, pre-stomach weight, and uropygial gland. In contrast, in the current study, due to the structure of available data, a half sib design was used. In this study, 3 significant QTL were detected from across family analyses on chromosome 1 in Japanese quail. Significant QTL affecting breast weight was found at 199 cM. Five QTL affecting breast weight at 92, 94, 128, 441, and 459 cM have been found in previous studies in chicken on chromosome 1 (Ikeobi et al., 2004; Atzmon et al., 2006; Lagarrigue et al., 2006; Atzmon et al., 2008). Another significant QTL identified in this study was the bursa of fabricius weight QTL at 24 cM. In chicken, however, suggestive QTL affecting this trait was found on chromosomes 17 and 26 at 31 and 32 cM (Park et al., 2006). In this study, significant QTL underlying uropygial gland weight was detected at 102 cM, but there is no published QTL for this trait in chicken. It should be noted that, due to the marker-poor linkage map in Japanese quail compared with the marker-rich linkage map in the chicken, the positions of the identified QTL for these traits on Japanese quail chromosome 1 might not be comparable to those of the detected OTL on chromosome 1 in chicken.

The current study found significant QTL for a number of internal organs which have not been subjected in

previous studies. The development of internal organs, supposed to be coordinated with the development of the whole body (Zhang et al., 2007; Esmailizadeh and Mohammadabadi, 2010), reflects body structure which may influence the productive potential. As a result, investigation on QTL mapping of the weights of the internal organs would be helpful in understanding the genetic basis of internal organ development and this will benefit understanding of the genetic background of internal organ growth in Japanese quail in particular and the birds in general (Zhang et al., 2007).

Results of this study point out to candidate regions affecting traits of economic relevance to the Japanese quail breeding. The comparative analysis concerning the location of QTL on different Japanese quail and the syntenic chicken chromosomes, in combination with their association with phenotype can expand our ability to understand the genetic complexity of economically important traits in Japanese quail. Isolation of additional microsatellites, single nucleotide polymorphisms (SNPs), and microsatellites in ESTs and genes are required to allow for the construction of a high resolution linkage map for fine mapping of the detected QTL and discovering new potential genes of interest.

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### نقشه یابی جایگاه های ژنی موثر بر خصوصیات مرتبط با لاشه روی کروموزوم شماره یک بلدرچین ژاپنی در خانواده های ناتنی

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چکیده هدف از مطالعه حاضر شناسایی جایگاه های ژنی موثر بر خصوصیات لاشه روی کروموزوم شماره ۱ بلدرچین ژاپنی بود. جمعیت نقشه یابی مورد استفاده در این پژوهش از ۴۲۲ نتاج حاصل از ۹ خانواده ناتنی تشکیل شده بود. داده های فنوتیپی شامل وزن لاشه، وزن اجزای لاشه و وزن اندامهای داخلی مربوط به نتاج جمع آوری شد. ۸ نشانگر ریزماهواریه مربوط به کروموزوم شماره ۱ در ۹ خانواده ناتنی تعیین ژنوتیپ شده و نقشه یابی با استفاده از روش درون فاصله ای مبتنی بر رگر سیون با استفاده از این ژنوتیپ ها انجام گرفت. جایگاه های ژنی موثر بر وزن بورس فابریسیوس در موقعیت ۲۴ سانتی مورگان (۲۰/۰۵) و وزن سینه در موقعیت ۲۴ سانتی مورگان (۲۰/۰۵) وی کروموزوم شماره ۱ شناسایی شد. جایگاه های شناسایی شده در این پژوهش در ۷ خانواده از ۹ خانواده ناتنی تفرق یافته اند و اندازه اثر این جایگاه ها ز ۲۹/۰ تا ۲۵/۱ واحد برحسب انحراف استاندارد باقیمانده بود. این پژوهش اطلاعات جدیدی درخصوص توارث صفات مرتبط با لاشه در بلدرچین ژاپنی ارائه می کند.