Genetic Mapping of a Body Weight Trait in Chicken

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Abstract The indigenous chicken line, Satsumadori (SD), is characterized by slow growth and widely varied body weight. We carried out linkage analysis on chicken growth traits using a resource population between a SD male and a White Leghorn female, comprising 232 F₂. We chose 72 microsatellite loci among 244 loci publicly available of 19 autosomal linkage groups, in terms of their utilities and locations. A quantitative trait loci (QTL) affecting body weight at 13 weeks was mapped at 122.8 cM on chromosome 1 (LOD score=5.16). The closest loci were ADL19 and ADL192. The difference in body weight of F₂ were associated with allele types of ADL19 and ADL192 ($P < 0.01$). In 52 SD males, we found three alleles of ADL19, each of which was assumed to be associated with the body weight at 13 weeks ($P = 0.062$). Therefore, ADL19 will be applicable to marker-assisted selection for body weight in SD-crossed commercial populations.

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Recently, many studies have been carried out on quantitative trait loci (QTL) analysis of livestock related to economic traits or diseases. A lot of DNA markers like microsatellites have been developed and mapping on the linkage map is being extensively performed^{2-14, 17, 19-22, 25-28}. In addition, experimental populations are being developed and linkage analysis is being carried out on these markers for various traits or diseases^{1, 16, 18, 29, 30}. Georges *et al.*¹⁶ have identified five chromosomes giving very strong evidence for the presence of a QTL controlling milk production : chromosomes 1, 6, 9, 10 and 20. Andersson *et al.*¹¹ found evidence for QTLs on chromosome 4 with large effects on growth, length of the small intestine and fat deposition in pigs. In chickens, Groenen *et al.*¹⁸ found that the QTL for body weight at 48 days of age in broilers is on chromosome 1, van Kaam *et al.*³⁰ found that the QTL for feed intake in broilers is on chromosome 1, and Vallejo *et al.*²⁹ reported two significant QTLs and two suggestive QTLs for Marek's disease on four chromosomal subregions : chromosomes 2, 4, 7 and 8.

On the other hand, the special chicken Hyogo-Ajidori of Hyogo Prefecture, is a three-way cross, produced by crossing an inferior White Plymouth Rock with a Hyogo, which is a two-way cross between a Satsumadori (SD) male and a Nagoya female. SD is a native breeds in Japan and no improvement has been done on its growth. Among these three breeds, the growth of SD is the worst, and body weights vary widely among individuals. As a result, a similar tendency is also seen in the growth of Hyogo and Hyogo-Ajidori. To improve the productivity of Hyogo-Ajidori, we carried out QTL analysis on the growth of SD males.

Materials and Methods

Experimental population

Two male and ten female F₁s were produced from a SD male and a White Leghorn (WL) female, and 129 male and 103 female of F₂s were obtained by crossing F₁ individuals. These 246 animals were used as the experimental population. The body weight just before the experiment (at about 1 year of age) of the

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SD male was 4.3 kg. The F_2 s were hatched in three periods, reared in young-chick cages until 4 weeks of age, and in chick-rearing cages until 13 weeks of age. Thirteen weeks is the target shipment age for Hyogo-Ajidori. Feeds on the market for stud young chicks (CP 19.0% and ME 2,900 kcal/kg) and for stud middle-large chicks (CP 14.5% and ME 2,750 kcal/kg) were fed respectively until 4 weeks of age and thereafter. During the experimental period, feed and water were supplied at *ad libitum* consumption and no illumination. The body weights of the 232 F_2 individuals at 13 weeks of age were measured.

52 SD males

Apart from the above experimental population, 52 SD males were reared in the same way and their body weights were measured at 13 weeks of age. There was no parent-offspring relationship between the experimental SD male and these 52 SD males. They were from several parent groups.

DNA samples

Blood samples were collected from the 246 individuals of the experimental population. 40 μ l of blood was diluted to 200 μ l with 160 μ l of PBS (–), and DNA was extracted with a QIAamp Blood Kit (QIAGEN). The average DNA concentration was 167 μ g/ml, and all were adjusted to 20 μ g/ml.

Microsatellite markers (markers)

We used the 244 primers: Comprehensive Mapping Kit#1, 120 primer pairs^{4, 6, 7, 10, 11, 20, 22, 25} and Comprehensive Mapping Kit #2, 124 primer pairs^{4, 6, 7, 10–12, 20–22, 25} which are open on the homepage of the Chicken Genome Project (<http://poultry.mph.msu.edu/>).

PCR

PCR amplifications were carried out in 16.5 μ l reaction mixtures containing 20 ng DNA, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl₂, 200 μ M each of the 4 dNTPs, 0.5 units AmpliTaq Gold DNA polymerase (Perkin-Elmer) and 5 pmol of each of the primer pairs. The reaction was performed by pre-denaturing at 95°C for 9 min, then 43 cycles of denaturation at 94°C for 30 sec, annealing at the temperature set for each primer (43–60°C) for 1 min, and finally an extension at 60°C for 10 min.

Electrophoresis and DNA Genotyping

Primers were divided into several groups by the length and fluorescence of the PCR products. Electrophoresis was carried out with 5.7% polyacrylamide gel (36 cm) on a sequencer (373S; ABI, Perkin-Elmer) for 11 h and the results were analyzed with software from Genescan and Genotyper (ABI, Perkin-Elmer).

QTL analysis

QTL analysis was performed using MAPMAKER/QTL²³. The positions of the markers on each linkage group and the distances between markers were referred to the map of East Lansing¹⁵ on the homepage of the Roslin Institute (<http://www.ri.bbsrc.ac.uk/cgi-bin/mapviewer?species=chicken>).

Results

Body weights of F_2 individuals

Body weights and standard deviations are shown in Table 1 for F_2 individuals at 13 weeks of age, hatched in three periods. No significant difference was seen in each group for both males and females. Correction of the body weight differences between males and females was done by subtracting the average body weight of the females from that of the males, and adding the value obtained to the body weight of the females.

Information on markers

There were 38 markers with unclear fluorescence and PCR temperature among the 244 markers used. There were 14, 9 and 44 markers which failed to identify the genotypes in SD, in WL and in both SD and WL respectively, due to the failure of DNA amplification in PCR. There were also 33 markers with which the genotypes of SD and WL were identical. These 138 markers in total, therefore, could not be used in the genotype identification. The heterogeneity of SD was 37.2%, and 39.9% in WL. Among the 206 markers examined, there were 149 ADL (Avian Disease and Oncology Laboratory, Michigan State University, East Lansing, MI) markers and 57 MCW (Microsatellite Chicken Wageningen, The Netherlands) markers. In comparison, 35.6% of the ADL markers, and 8.8% of the MCW markers could not identify the genotypes due to the failure of DNA amplification in PCR for SD. Similarly, 32.2% of

Table 1. Body weight of F₂ individuals at 13 weeks of age

Sex		Hatch 1	Hatch 2	Hatch 3	Total
Male	Number of birds	53	48	28	129
	Mean $\pm$ S.D.	1,682 $\pm$ 232	1,742 $\pm$ 165	1,634 $\pm$ 155	1,694 $\pm$ 197
	Maximum	2,100	2,080	1,930	2,100
	Minimum	820	1,350	1,310	820
Female	Number of birds	43	23	37	103
	Mean $\pm$ S.D.	1,368 $\pm$ 212	1,308 $\pm$ 151	1,375 $\pm$ 135	1,357 $\pm$ 175
	Maximum	1,880	1,600	1,620	1,880
	Minimum	700	990	1,070	700
Male and female (corrected)	Number of birds	96	71	65	232
	Mean $\pm$ S.D.	1,692 $\pm$ 222	1,710 $\pm$ 166	1,678 $\pm$ 148	1,694 $\pm$ 186
	Maximum	2,217	2,080	1,957	2,217
	Minimum	820	1,327	1,310	820

the ADL markers and 8.8% of the MCW markers could not identify the genotype in WL. The heterogeneity in ADL markers with which genotypes were identified, was 32.3% in SD and 38.6% in WL. Similarly in MCW markers, it was 46.2% in SD and 42.3% in WL. Among the 106 markers which had been judged to be usable, 21 were found to be inapplicable due to the failure of PCR in F₂. In addition, where there was only one marker in a linkage group, the marker was not used, and where there were several markers on the same position, only one was used in the analysis. Therefore, 72 markers of 19 autosomal linkage groups were finally chosen for the QTL analysis (Table 2).

QTL analysis

One QTL was found in the linkage analysis, between genotype and body weight at 13 weeks of age in the F₂ (Fig. 1). This QTL was on a position between ADL19 and ADL192, and 6.0 cM from ADL19 on chromosome 1 (122.8 cM of chromosome 1), and the distance from ADL19 to ADL192 is 23.5 cM. The LOD score was 5.16, showing the highest value. Among the markers used, ADL19 and ADL192 were the nearest loci to this part. The variance explained of this QTL was 18.3%.

Effects on F₂ body weight by the ADL19 and ADL192 alleles, and in 52 SD males by the ADL 19 allele

Table 3 shows the relationship between the ADL19 and ADL192 alleles nearest to the QTL, and body weight at 13 weeks of age in the F₂, and the ADL19 allele and body weight at 13 weeks of age in 52 SD

males. The ADL19 allele type of SD was shown by AA (93 bp), and in WL by BB (95 bp). The ADL 192 allele type of SD was shown by AA (139 bp) and in WL by AB (137 bp, 139 bp). In either case, there were significant differences in body weight depending on the allele ($P < 0.01$), and the F₂ body weight of B type was heavier than that of A type. In 52 SD males, there were three alleles in ADL19 and all were homozygous (AA : 93 bp, CC : 103 bp, DD : 109 bp). The majority was occupied by one allele and a difference in body weight was seen in different alleles ($P = 0.062$). The alleles of ADL192 were all identical.

Discussion

In QTL analysis, it is important to repress as much as possible the impact of environment on the target trait, and the number of individuals in a family line used in analysis can be several hundred. Because of this, when F₂ individuals are produced, the individuals have to be reared dividing into several periods, as it is difficult to control all the individuals at the same time. In this case, environmental factors like rearing location and season will have some impact on the target trait, and the larger the rearing area and the longer the generation alteration time, as in large livestock, the larger the impact will be. From this respect, chickens are favorable for use in linkage analysis since it is possible to make a number of family lines in a short time, and to control the individuals in the same conditions. Crooijmans *et al.*¹²⁾ described that chickens are an excellent model system for the application of

QTL Affecting Growth in Chicken

Table 2. Linkage groups and markers used in QTL analysis

Linkage group	Number of markers	Genome coverage (cM)	Average interval (cM)
Chromosome 1	13	394.80	30.37
Chromosome 2	9	460.04	51.12
Chromosome 3	5	287.13	57.43
Chromosome 4	5	111.62	22.32
Chromosome 5	6	167.24	27.87
Chromosome 6	4	44.99	11.25
Chromosome 7	2	112.89	56.45
Chromosome 8	4	50.13	12.53
E 27	2	5.80	2.90
E 29	3	29.65	9.88
E 30	2	32.60	16.30
E 31	2	79.96	39.98
E 35	2	23.58	11.79
E 36	2	118.37	59.19
E 41	2	13.18	6.59
E 46	2	22.77	11.39
E 47	2	13.59	6.80
E 48	2	14.52	7.26
E 53	3	71.38	23.79
Total	72	2,054.24	28.53

Fig. 1. Lod scores on chromosome 1.

Table 3. Relationship between the allele of ADL19 and ADL192 and body weight at 13 weeks of age in F₂, and the allele of ADL19 and body weight at 13 weeks of age in 52 SD males

Bird	Marker	Allele type	Number of birds	Body weight (g)
F ₂	ADL19	AA	24	1,578 ^c
		AB	70	1,673 ^b
		BB	52	1,759 ^a
	ADL192	AA	109	1,652 ^b
		AB	54	1,736 ^a
		BB	10	1,792 ^a
52 SD males	ADL19	AA	39	1,802 ^e
		CC	9	1,658 ^f
		DD	4	1,915 ^d

^{a, b, c} Values in the same bird and same marker with different superscripts are significantly different, $P < 0.01$.

^{d, e, f} Values with different superscripts are suggestive different, $P < 0.10$.

genetic mapping technology because of its short generation interval and the possibility to generate large full-sib families. Vallejo *et al.*²⁹⁾ stated a similar position. Also in this study, no significant difference in average body weight was found in three hatch groups, and the data was effective for QTL analysis.

On markers, ADL markers are those developed from the experimental population produced by the back-crossing of Red Jungle Fowl and WL, the so-called East Lansing reference population¹⁵⁾. Besides, MCW markers have been developed by back-crossing of WLs of different lineage which are called the Compton reference population³⁾. The difference in utility between SD and WL was small, but difference between the kinds of markers was as large as 4-fold. This indicates that in an experimental population, different kinds of markers may have different problems in PCR, even under the same conditions. The heterogeneity of MCW markers was higher than that of ADL markers. A high heterogeneity indicates easy identification of a heredity pattern of markers from parents to the next generation. Based on these results, MCW markers are considered to be more

useful than ADL markers in this experimental population. Besides, new markers have been reported by Crooijmans *et al.*¹³⁾, so MCW markers are thought to be effective in linkage analysis hereafter.

From the results of the QTL analysis, the LOD score showed a high value of 5.16. The LOD score threshold was derived by Lander and Botstein²⁴⁾, and a LOD score above 3 indicates a significant linkage. According to this, the LOD score of 5.16 in this study is thought to be a relatively strong linkage. Groenen *et al.*¹⁸⁾ suggested that the QTL for body weight at 48 days of age in broilers was on 220 cM of chromosome 1, and van Kaam *et al.*³¹⁾, though using suggestive linkage, suggested that the QTL for body weight at 48 days of age in broilers was on 240 cM and the QTL for growth was on 235 cM. Although we cannot compare this data directly with the results of our study due to the differences in chicken breeds, days of age, and the experimental population which was referred, it is certain that the QTL affecting growth of chickens is on chromosome 1. In this study, we used the existing marker-kit, but contrary to our expectation, it turned out to be that a number of markers could not be used in the QTL analysis. As a result, we failed to cover the whole genome and there are still some long distances between the markers. Though it is possible that the QTL is in a position outside the parts covered in this study, from the results of other studies to date, any QTL concerning growth is found in only one place on the genome^{18, 30, 31)}. van Kaam *et al.*³⁰⁾ described the possibility that a single QTL affects growth. In addition, the distance of markers within which a QTL was found in this study is 23.5 cM, which is relatively short. Though we did not use very many markers in this study, we were fortunate to have found the position of a QTL affecting growth. In fact, when we compared the allele of the marker nearest to the QTL (ADL19 and ADL192), and the body weights of the F₂ at 13 weeks of age, we found a significant difference in body weight by allele. Furthermore, we compared the allele of ADL19 and the body weights of 52 SD males at 13 weeks of age, then in spite of some lean to an allele, the difference in body weight by allele was seen ($P = 0.062$), and specifically the body weight of allele DD was 15% more than that

of CC. These results indicate the existence of a QTL for growth on this part, and the marker-assisted selection for body weight in SD-crossed commercial populations is possible using marker ADL19.

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