Genetic Linkage Mapping of RAPD Markers Segregating in Korean Ogol Chicken - White Leghorn Backcross Population

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ABSTRACT : This study was carried out to construct mapping population and to evaluate the methods involved, including polymorphic DNA marker system and appropriate statistical analysis. As an initial step to establish chicken genome mapping project, White Leghorn (WL) and Korean Ogol chicken (KOC) were used for generating backcross population. From 8 initial parents, total 280 backcross progenies were obtained and 40 were used for genotyping and linkage analysis. For development of novel polymorphic markers for KOC, Random Amplified Polymorphic DNA (RAPD) markers specific for this chicken line were generated. Also included in this study were six microsatellite markers from East Lansing map as reference loci. For segregation analysis, 15 RAPD markers and 6 microsatellites were used to genotype the backcross population. Among the RAPD markers that we developed, 2 pairs of markers were identified to be linked and another 4 RAPD markers showed linkage with microsatellites of known map. In summary, this study showed that our backcross population generated from the mating of KOC to WL serves as a valuable genetic resource for genotyping. Furthermore, RAPD markers are proved to be valuable in linkage mapping analysis. *(Asian-Aust. J. Anim. Sci. 2001. Vol. 14, No. 3 : 302-306)*

Key Words : Genome Mapping, Backcross Population, KOC, RAPD, Microsatellites, Linkage Analysis

INTRODUCTION

Because chickens represent a valuable agricultural commodity as a source of eggs and high-protein meat, commercial poultry breeding flocks need to be well pedigreed and closely monitored for both physiological and disease-related characteristics. Additionally, the chickens offer a number of advantages for the construction of linkage maps since its genome size is small relative to other domesticated animals (approximately 1.2×10^9 bases, Olofsson and Bernardi, 1983), and high reproductive potential. However, compared to other livestock species, the progress in chicken genome mapping has been slow mainly due to its large number of microchromosomes and lack of integrated research efforts in poultry genetics.

The first chicken genetic map based on DNA markers was published by Bumstead and Palyga in 1992. Following this, second genetic map (Levin et al., 1993, 1994) consisting primarily of RFLPs became avaliable, but was not suited for total genome scan in large populations. Recently, however, large numbers of microsatellite markers have become available allowing higher density mapping possible (Khatib et al., 1993; Crooijmans et al., 1993, 1995, 1996; Cheng and Crittenden, 1994; Cheng et al., 1995; Gibbs et al., 1995, 1997; Ruyter-Spira et al., 1996; Groenen et al., 1998). Nevertheless, the number of microsatellite markers is not sufficient enough for the whole-genome scan and sequencing the flanking part of microsatellite is still labor-intensive and requires a major collaboration.

Another polymorphic DNA marker system, Random Amplified Polymorphic DNA (RAPD), has been widely applied in various areas of genetic studies for the detection of polymorphism due to its simplicity and ease of use with less sophisticated equipments (Williams et al., 1990; Cushwa and Medrano, 1996).

In this study, RAPD and microsatellite markers were used in our initial attempts to construct mapping populations and to evaluate different DNA mapping strategies as well as appropriate statistical analysis.

MATERIALS AND METHODS

Genetic stocks

Korean Ogol chicken (KOC) obtained from a breeder at Yunsan district, Chungnam Providence and White Leghorn (WL) chicken which had been reared since early 1980s at the National Livestock Research Institute, Suwon were used in this study. All experimental chickens have been maintained in the experimental farm at the Seoul National University since 1992.

Backcross population for segregation analysis was obtained by reciprocal mating of KOC males with two WL females. Hybrid F1 males or females were

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backcrossed to their recurrent parents, respectively. F1 male progenies of WL females were mated to 5 full-sib WL female individuals which were full-sib with the parental individuals. F1 females were also simply mated with their male parent. A total of 280 backcross progenies were obtained. Of four possible segregating populations, 3 families consisted of 3 F1 female offsprings from the mating of a WL male and their backcross progenies were used for linkage test. Prescreened polymorphic random primers and microsatellite markers were used for segregation analysis.

DNA extraction

Venous blood was collected from the wing vein into vacuum blood collection tubes containing heparin. Five milliliters of blood samples were diluted in 1 X SSC (0.15 M NaCl, 15 mM trisodium citrate, pH 7.0) and washed twice by centrifugation. One hundred microliters of the pellet was resuspended with 300 μl of high TE (100 mM Tris, 40 mM EDTA pH 8.0) and lysed with 300 μl of lysis buffer (100 mM Tris, 40 mM EDTA pH 8.0, 0.2% SDS). Lysates were extracted twice with 24:1 (v/v) mixture of chloroform : isoamyl alcohol and once with phenol:chloroform: isoamyl alcohol (25:24:1) as described (Sambrook et al., 1989). Purified DNA was precipitated by addition of 2.5 volumes of ethanol, rinsed with 70% ethanol and dissolved in TE, pH 8.0 or dialyzed extensively with TE buffer (pH 8.0). The concentration and amount of purified DNA were determined by agarose gel electrophoresis and a spectrophotometer, and the final concentration of DNA was adjusted to 5 ng/μl with double-distilled H_2O.

RAPD analysis

RAPD analysis was performed as described (Williams et al., 1990). Polymerase chain reaction (PCR) was carried out in a 25 μl reaction containing 50 to 100 ng of genomic DNA, 1X reaction buffer (50 mM KCl, 10 mM Tris-Cl, 1.5 mM $MgCl_2$, 0.01% Triton X-100), dNTPs (each 200 μM), 0.5 units of Taq DNA polymerase (Bioneer, Daejon, Korea) and 100 nM of primer (University of British Columbia, Canada), was overlaid with same volume of mineral oil.

The G+C contents of these 10 oligonucleotide primers were 50-80%. Amplification was conducted in a Perkin Elmer Cetus DNA Thermal Cycler. Initial denaturation was performed for 5 min at 95℃ and followed by 50 cycles of denaturation at 95℃ for 1min, annealing at 39℃ for 1min, extension at 72℃ for 2 min. Final extension was carried out at 72℃ for 7 min after cycles were completed. The amplified products were analyzed by electrophoresis on 1.5% agarose gel.

Microsatellite analysis

The microsatellite loci were selected from the panel of chicken mapping set I, which was obtained from the US chicken mapping board.

PCR analysis of microsatellites was carried out using fluorescently labelled PCR primers (Fam, Hex and Tet, Perkin-Elmer). PCR reactions were performed using the 384-well based PCR workstation (ABI 877 workstation, Applied Biosystems, Perkin-Elmer) in a 2.5 μl volume containing 200 μM of each dNTP, 0.1 unit/reaction of Taq polymerase and 0.5 pmol of each primer. Thermal cycling was; 3 min at 93℃ followed by 10 cycles of 30s at 93℃, 30s at annealing temperatures, and 30s at 72℃, then 25 cycles in the same condition except 30s at 88℃ for denaturation. The final elongation was prolonged to 7 min. Each PCR product was mixed by PCR workstation for appropriate volume to obtain a peak signal around 1000, then heated to 92℃ for 2 min after the addition of 0.5 μl of the internal standard (Genescan 350, Applied Biosystems) and 2.5 μl of formamide. Standard 5% polyacrylamide denaturing sequencing gel was used for separation using the ABI 377 sequencer (Applied Biosystems). Size determination was performed using the GeneScan 672 (version 3.1) software (Applied Biosystems) according to the manufacturer's manual.

Linkage analysis

The linkage analysis was performed using the Mapmaker (version 2, Lander et al., 1987). Segregating polymorphic markers were scored as 'H' (heterozygote for recurrent parents), 'A' (homozygote for recurrent parent) and '-' for missing data, respectively. The entire set of markers was then processed through the Mapmaker computer program

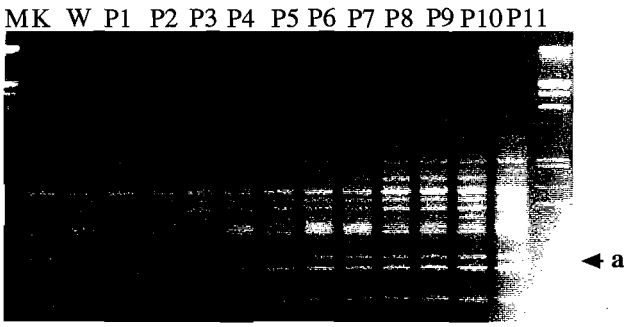

Figure 1. Segregation pattern of the KOC-specific RAPD marker from #392 in a family consisted of male KOC (K), female WL (W) and their 11 progenies (P1-P11). Arrow indicates the segregating band.

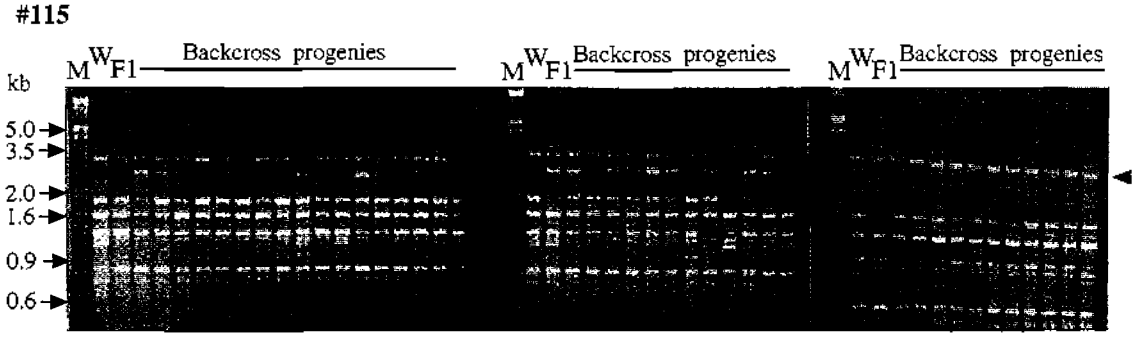

Figure 2. Segregation of the polymorphic RAPD markers in the KOC backcross population with the primer #115. Arrowhead indicates the segregating bands. M: lambda DNA Hind III / EcoR I, W: WL male, F1: female offspring of the mating between WL and KOC.

(Lander et al., 1987) using two-point (LOD 3.0) analysis to confirm the order and recombination fraction for the markers.

RESULTS AND DISCUSSION

Backcross mating plan was accomplished to design hybrid F1 individuals being mated to their recurrent parents. All possible combinations of matings were available in reciprocal fashion. The reference population generated by backcross mating has been most successful in mice and chickens (Copeland and Jenkins, 1991; Bumstead and Palyga, 1992; Crittenden et al., 1993). The rationale of backcross reference

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*R013    AAHHAAAHHHAHAAAAAAAAHAAHAHAHHHHAHAHAHHHHH
*R114a   HHAHHAHAHAHHAHAHHAAHHAHAAAAHHHHHHHHHAAHHH
*R114c   AAAHHHAHAAHAAHHAHAAHHAAHAHAHAHHHAAHAAAAH
*R115    HHAHHHAAAHHAAHAHAHAAAAAAAHHHHHHHHAAHAAAH
*R124a   HHAAHAAHAAAAHHAA-AHHAAAHHAAAA----------
*R147    AHHHAAAAHAHHAAHHAAAAAHHHHAHHH----------
*R159    HHAHHAAHAAAAHHHAAAHHAAAHAAAAAHHAHHHAHAAA
*R167    AAHHAHHAHAAAAHAA-HAHHAHAHAHAAAHHHAAAHAHH
*R301a   HHAAAAHAHHHHHHHHHAAHHHHHAHHHHHHAHHAHHHH
*R327a   HHHHAAHHAAAAAHAHAAHAAHAHAHAHH----------
*R327b   HHHHAHHHHAAHAHAHHHHAHAAAHAHAA----------
*R351    HAHAHAAAAAHHAHHHHAHHAAAHHHHHAAAAHAHHHHH
*R392    AAHHAAAHAAAAAAAAAAHAHHAHAHAHHHAHAHAHHAH
*R490    HHHAHHAAHAHAHHHAHAAHHAHHAHHAH----------
*R497    AAAAAHHAHAHAAAHHHHAHHHHAHHHAHHHAAAAHHHAH
*MCW04   AAHAAAHHHAAHHAAAAHAHHAHHHHAAAHAHAAHHHHAA
*ADL154  HHAAHAAHAHAAHHAHHAAAHHHAHHAHAHAAAHHAHAAH
*ADL171  HHAAHAA-AAHAHHAHHAAAHA-HAHAHAHAAAHHAAAAH
*ADL190  AHHHAHAAHAAHHHHAA-HAAAAHHAHHAAHH-HAHAAHA
*ADL032  AAAAHAHAHAHH-AHAHAHAHAAAAAAAA--HHHHAAHHH
*ADL136  AAHAAAHHAAAHAAAHHHAHHHHAHHAAAHHAAAAHAHAA
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Figure 3. F2 backcross data of 21 RAPD and microsatellite markers for inputing to the Mapmaker computer program. The genotype symbols followed are "H" (presence of KOC specific bands), "A" (absence of KOC specific bands) and "-" (missing data for the individual at this locus).

population was that the parental lines should be highly inbred so that each polymorphism would be represented by only two alleles in the backcross, thereby simplifying the scoring the polymorphic progeny even when the locus has multiple alleles. In our previous results of genetic analysis of KOC with other chicken breeds, largest genetic distance was found between KOC and WL than Rhode Island Red or Cornish (Lee et al., 1995; Hwang et al., 1997). From 8 parental individuals, each duplicated males and females per breed, all possible combinations of matings were carried out using 82 different F1 hybrids. From these, only hybrid females were selected for mating with their male parents, resulting in a total of 280 backcross progenies.

For typing KOC specific markers, only offsprings of a KOC male parent were analysed. In this study, as an initial mapping effort, only 43 individuals were genotyped and tested for combinational linkage. For the analysis of RAPD markers specific for KOC, we initially tested 300 random oligonucleotide primers. The number of bands per primer was ranged from 2 to 11 with the average 6.7 bands. However, on the basis of consistent amplification and polymorphism, we selected 13 primers for following segregation analysis.

As seen in figure 1, clear segregation could be observed with the primer #392 (arrow). For the segregation analysis, 40 backcross progenies were used with their parents. Figure 2 shows the typical genetic segregation pattern of an RAPD marker in a backcross pedigree.

As the standards for polymorphic RAPD loci, we included the microsatellites. From initial 20 loci, we selected 6 loci which were polymorphic and gave consistent amplification.

With the resultant genotypes from 15 RAPD bands and 6 microsatellites, the two-point analysis was performed using the MapMaker (Lander et al., 1987) using the Cosambi mapping function. In figure 3, all genotypes at 21 loci were presented. For simple

Table 1. Lod-table of pair-wise analysis between a pair of markers. Upper row represents the recombination fraction and lower row the LOD scores

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	R013	R114a	R114c	R115	R124a	R147	R159	R167	R301a	R327a	R327b	R351	R392	R490	R497	MCW04	ADL154	ADL171	ADL190	ADL032
2	0.47																			
	0.00																			
3	0.50	0.45																		
	0.00	0.09																		
4	0.50	0.40	0.40																	
	0.00	0.35	0.35																	
5	0.50	0.50	0.43	0.50																
	0.00	0.00	0.12	0.00																
6	0.31	0.41	0.50	0.50	0.50															
	0.93	0.19	0.00	0.00	0.00															
7	0.50	0.50	0.38	0.50	0.11	0.50														
	0.00	0.00	0.55	0.00	4.29	0.00														
8	0.38	0.44	0.49	0.50	0.50	0.50	0.50													
	0.46	0.14	0.01	0.00	0.00	0.00	0.00													
9	0.50	0.30	0.50	0.50	0.50	0.41	0.50	0.50												
	0.00	1.43	0.00	0.00	0.00	0.19	0.00	0.00												
10	0.50	0.50	0.50	0.41	0.43	0.45	0.38	0.50	0.50											
	0.00	0.00	0.00	0.19	0.12	0.07	0.37	0.00	0.00											
11	0.50	0.41	0.50	0.50	0.50	0.50	0.50	0.29	0.50	0.45										
	0.00	0.19	0.00	0.00	0.00	0.00	0.00	1.15	0.00	0.07										
12	0.50	0.47	0.47	0.50	0.39	0.45	0.50	0.50	0.43	0.48	0.50									
	0.00	0.02	0.02	0.00	0.28	0.07	0.00	0.00	0.20	0.01	0.00									
13	0.30	0.50	0.43	0.50	0.50	0.45	0.50	0.50	0.50	0.21	0.50	0.50								
	1.43	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	2.31	0.00	0.00								
14	0.50	0.38	0.38	0.48	0.46	0.50	0.48	0.46	0.31	0.50	0.50	0.41	0.50							
	0.00	0.37	0.37	0.01	0.03	0.00	0.07	0.03	0.93	0.00	0.00	0.19	0.00							
15	0.50	0.50	0.50	0.50	0.50	0.45	0.50	0.38	0.35	0.50	0.50	0.50	0.50	0.41						
	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.46	0.79	0.00	0.00	0.00	0.00	0.19						
16	0.40	0.50	0.50	0.50	0.46	0.50	0.50	0.44	0.50	0.50	0.48	0.50	0.45	0.50	0.43					
	0.35	0.00	0.00	0.00	0.03	0.00	0.00	0.14	0.00	0.00	0.01	0.00	0.09	0.00	0.20					
17	0.50	0.50	0.45	0.50	0.36	0.50	0.43	0.50	0.45	0.45	0.50	0.50	0.47	0.50	0.50	0.50				
	0.00	0.00	0.09	0.00	0.50	0.00	0.20	0.00	0.09	0.07	0.00	0.00	0.02	0.00	0.00	0.00				
18	0.50	0.39	0.29	0.42	0.31	0.50	0.37	0.50	0.45	0.41	0.50	0.45	0.47	0.33	0.50	0.50	0.16			
	0.00	0.37	1.51	0.21	0.86	0.00	0.58	0.00	0.09	0.20	0.00	0.09	0.02	0.66	0.00	0.00	4.24			
19	0.42	0.50	0.50	0.50	0.44	0.32	0.45	0.41	0.50	0.50	0.36	0.50	0.47	0.50	0.50	0.50	0.50	0.50		
	0.21	0.00	0.00	0.00	0.07	0.79	0.09	0.29	0.00	0.00	0.50	0.00	0.02	0.00	0.00	0.00	0.00	0.00		
20	0.50	0.32	0.43	0.50	0.48	0.50	0.49	0.50	0.50	0.50	0.50	0.46	0.50	0.50	0.50	0.50	0.50	0.46	0.50	
	0.00	1.00	0.15	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.06	0.00	
21	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.46	0.50	0.50	0.41	0.50	0.50	0.50	0.28	0.25	0.47	0.50	0.50	0.50
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.19	0.00	0.00	0.00	0.82	2.27	0.02	0.00	0.00	0.00

backcross segregation, only the presence or absence of KOC specific bands was indicated.

Also, the LOD scores were presented in table 1 for all pair-wise markers. As indicated with shadow, several pairs of markers showed significant linkage. Among RAPD markers, R327a and R392 as well as R124a and R159 showed genetic linkage.

Relative positions of 4 RAPD markers on known chicken map were determined with the help of microsatellites; R114a-R301a-ADL032, R497-ADL136-MCW04, ADL171-ADL154-R114c (figure 4).

Additionally, as shown in figure 4(E), ADL032, R114a and R301a were positioned at East Lansing linkage group E30 (Cheng et al., 1997). R114c was positioned at chromosome 8. Comparing the relative distance between ADL171-ADL154 which was

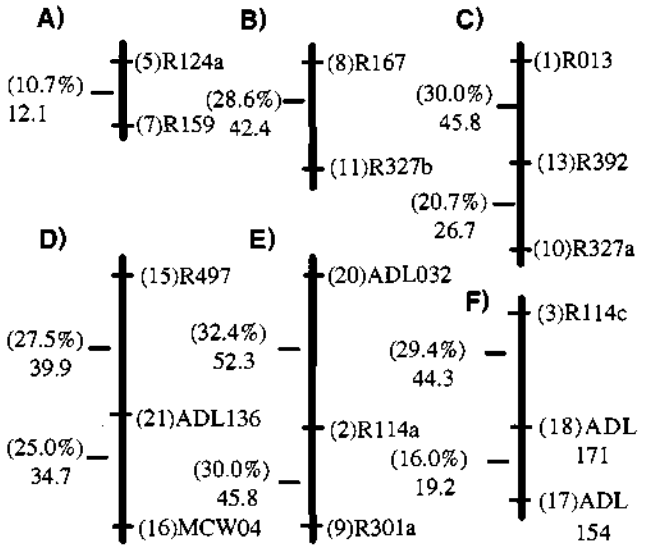

Figure 4. Diagram showing six groups of linked markers. Each pair shows significant level of LOD score for linkage determination.

determined in this study with that of the East Lansing map, only the small difference was identified (19.2 vs 16.6) (figure 4 F). This result indicates that the construction of mapping population using polymorphic markers for genetic mapping has been successful.

Our results add data to the existing chicken genome resources. It has also been shown that the backcross population used in this study may serve as a valuable genetic resource for genotyping.

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