IDENTIFICATION OF RAPD MARKERS IN CROSSES BETWEEN INBRED LINES OF RHODE ISLAND RED AND WHITE LEGHORN

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SUMMARY

Reciprocal crosses were made between an inbred brown eggshell line and an inbred white shell line. The F1s were backcrossed to parental lines and DNA from parents and backcross progeny were tested for polymorphisms using RAPD primers. Sixteen polymorphic bands out of 100 primers were found, including fourteen autosomal and two sex-linked. These markers will be used in future studies of these lines for detection of QTLs for egg production traits.

INTRODUCTION

Systematically searching for QTL requires construction of a saturated genetic map (Lander and Botstein, 1989). Random amplified polymorphic DNA (RAPD) molecular markers, first suggested by Williams *et al.* (1990), are good candidates for this purpose. Arbitrary primers are used and no prior DNA sequence information is required. Most applications have been in plants (Martin *et al.*, 1991; Quiros *et al.*, 1991; and Heun and Helentjaris, 1993). Levin *et al.* (1993) constructed a genetic map of the chicken Z chromosome by using a backcross between inbred lines of Red Jungle Fowl and White Leghorn.

In chickens, most egg production traits are quantitative, and polygenic inheritance is assumed. However, some loci with large effects may exist. Wei *et al.* (1992), based on results from crosses of two white shell inbred lines, suggested that at least two major genes affected eggshell pigments. To verify major genes, crosses between diverse inbreds and use of RAPD-PCR would be a way to search for markers for traits, especially in the case where little information on DNA sequence is available. The objective of this study was to identify a group of RAPD markers to be used for detecting QTLs in several important egg traits, including eggshell color.

MATERIALS AND METHODS

Reciprocal crosses were conducted between a brown eggshell Rhode Island Red inbred line (DO) and a white eggshell White Leghorn inbred line (ES). Two types of F1s (DE from the cross between a DO male and ES females, and ED from the reciprocal cross) were backcrossed to both parental lines. Reciprocal backcrosses were also made between DE and both parents. Therefore, six types of F2 backcross progeny were obtained: DDDE (DO as sires and DE as dams), DEDD (DE sires and DO dams), DDED (DO sires and ED dams), EEDE (ES sires and DE dams), DEEE (DE sires and ES dams), and EEED (ES sires and ED dams). This mating design allowed major autosomal genes as well as sex-linked genes to be distinguished. Eggshell color and five other egg production traits were recorded for individual hens for marker - QTL analysis.

Blood samples for DNA isolation were obtained from parents in backcross and from all backcross progeny. DNA isolation followed a phenol/chloroform extraction using a procedure provided by L.B. Crittenden at the Avian Disease and Oncology Lab at East Lansing, MI.

One hundred single 10-mer random primers from Operon Technologies, Inc., and Biotechnology Laboratory, University of British Columbia were used to search for polymorphic bands between lines. Two individual DNA samples (one male and one female) from each of DO and ES lines and two from F1 groups were randomly chosen for the preliminary search. If suitable polymorphic bands were obtained, either 64 female birds from the backcross to DO lines or 53 females from the backcross to ES lines were genotyped, depending on which line showed recessive alleles (no bands) in the parent.

RAPD-PCR was conducted under the following conditions. A total reaction volume of 25 µl contained 19 ng of DNA templates, 18 ng single 10-mer oligonucleotide primer, 0.1 mM of each of the four deoxyribonucleotide triphosphates, 0.65 unit of thermostable *Taq* DNA polymerase (Promega Corporation, Madison, WI), 1.5 mM $MgCl_2$ and 2.5 µl 10x reaction buffer (both $MgCl_2$ and buffer were supplied with the *Taq* enzyme), and 10^{-6} M tetramethylammonium chloride. All reaction mixtures were overlaid with 30 µl mineral oil before being placed in an Eppendorf Thermal Cycler (Eppendorf, Fremont, CA). Thermal cycler was programmed for an initial incubation at 94°C for 2 min; 45 cycles each with denaturing at 94°C for 0.5 min, annealing at 35°C for 1 min, and extension at 72°C for 1 min; and a final cycle at 72°C for 5 min. Amplification products were detected by electrophoresis in 1.5% agarose gels, followed by staining with ethidium bromide and photographing under UV light.

For each polymorphic RAPD band, band presence or absence was expected in a ratio of 1:1 in the backcross if the band was inherited autosomally. Chi-square statistics were used for testing segregation ratio for distortion (Green, 1963). Recombination fractions between markers were calculated and tested using Green's methods. Haldane cM (Haldane, 1919) as pair-wise map units were evaluated using Two-Point analysis in *Mapmaker* 3.0 (Lincoln *et al.*, 1993). Sex-linked RAPD markers were detected by checking segregation patterns in reciprocal subgroups of each backcross population. Chi-square statistics and *Mapmaker* were also used for linkage analysis of sex-linked markers.

RESULTS AND DISCUSSION

Sixteen polymorphic bands were obtained from 12 primers out of 100 screened (Table 1). Range of band size was 1240 bp to 290 bp. Seven polymorphic bands were found in progeny obtained from backcrossing to DO parents and nine were found in progeny from backcrossing to ES parents. Two of these bands were sex-linked on Z chromosome of DO origin.

Table 1. RAPD markers segregating between DO and ES lines.

RAPD[1] marker	Band size[2]	Primer sequence 5' to 3'	Backcross toDO/ES	Sex-linked?	+/-[3]	χ^2 for segregation distortion
Du01	485	AGTCCTCGCC	DO	No	32/32	0
Du02	1240	as for Du01	DO	No	21/43	7.56**[4]
Du03	1180	TGGTGGACCA	DO	No	35/27	1.03
Du04	290	GAATGCGACG	DO	No	31/33	0.06
Du05	940	ATGACGTTGA	DO	No	39/25	3.06
Du06	290	CTGAGGAGTG	DO	No	29/35	0.56
Du07	920	GGGCTAGGGT	DO	No	33/31	0.06
Eu01	940	AGCAGGTGGA	ES	No	32/21	2.28
Eu02	600	GTCCTCGTAG	ES	No	28/25	0.17
Eu03	1210	CGGTGGCGAA	ES	No	22/31	1.53
Eu04	300	as for Du05	ES	No	30/23	0.92
Eu05	870	CCGGACACGA	ES	No	32/21	2.28
Eu06	1200	CAGGACATCG	ES	No	23/30	0.92
Eu07	920	GACGGAAGAG	ES	No	22/31	1.53
Es01	1140	as for Eu05	ES	Yes	15/9[5]	1.50
Es02	940	as for Eu05	ES	Yes	12/12	0

[1]. Internal lab nomenclature [2]. Approximate estimates based on comparison to φX174 RF DNA HaeIII digest standard [3]. "+/-" is the ratio for individuals with bands present or absent in backcross populations. [4]. * P<0.05, ** P<0.01, and *** P<0.001 [5]. For sex-linked bands, band segregation only occurred in DEEE subgroup in the backcross to ES. There were 24 individuals in this subgroup.

Segregation of each autosomal polymorphic band was expected to be in a 1:1 ratio in both backcross populations. Chi-square tests showed that all segregations were not significantly different from expected except for marker Du02 (P<.05). This one unexpected segregation ratio may be by chance due to the number of comparisons made.

Among 42 pair-wise autosomal linkage calculations, three linkages with high LOD scores or χ^2 values were obtained (Table 2 and 3): Du01 with Du06 (12.3 cM), Du03 with Du05 (14.9 cM), and Eu01 with Eu05 (8.2 cM). Five loose linkages were also obtained but were not significantly different from independent loci. A linkage group including Eu01, Eu03, Eu05, and Eu07 (Table 3) may exist, and a Multipoint analysis of *Mapmaker* gave a likely order of Eu05-Eu01-Eu03-Eu07.

Table 2. Pair-wise RAPD linkage analysis in backcrosses with DO and F1 as parents.

	Du01	Du02	Du03	Du04	Du05	Du06	Du07
Du01		–	–	–	–	12.3	–
Du02	48.4		82.1	–	–	9.67	–
Du03	51.6	40.3	0.51		14.9	–	–
Du04	45.3	56.3	62.9*		8.31	–	–
Du05	48.4	43.8	12.9***	56.3		–	–
Du06	10.9***	43.8	46.8	50.0	50.0		–
Du07	45.3	40.6	41.9	46.9	40.6	46.9	

The upper right triangle gives the results from two points analysis in the *Mapmaker*. The top numbers are Haldane cM and the lower numbers are corresponding LOD scores. A dash indicates no linkage between two loci. The lower left part is from two point analysis using Green's method (1963). Recombination fractions were tested by χ^2 using * for P<0.05, ** for P<0.01, and *** for P<0.001.

The chicken genome is expected to be between 2800-3300 cM total length (Bitgood and Shoffner, 1992). Only a few linkages among fourteen autosomal RAPDs would be expected. Independence of most RAPD bands in this study reflected coverage of markers on the genome, which could be used for global searching for QTLs. However, linkages among markers are necessary for precisely locating QTLs (Lander and Botstein, 1989). Linkages are not estimable between two groups of RAPDs in backcross populations since RAPDs are dominant markers and each backcross only gave one phase (coupling) of linkage. To connect RAPDs from both groups, an intercross between F1s should be conducted and would allow additional linkage to be detected.

Table 3. Pair-wise RAPD linkage analysis in backcrosses with ES and F1 as parents.

	Eu01	Eu02	Eu03	Eu04	Eu05	Eu06	Eu07
Eu01		–	56.9	–	8.2	–	–
Eu02	49.1		1.21	–	9.80	–	–
Eu03	34.0*	52.8	–		–	78.6	–
Eu04	49.1	45.3	45.3	–	70.3	0.50	70.3
Eu05	7.5***	49.1	37.7	45.3	0.70	–	0.70
Eu06	58.5	39.6	50.9	43.4	–	–	–
Eu07	49.1	56.5	37.7	52.0	58.5	–	–
					49.1	47.2	

Table structure is the same as for Table 2.

Two RAPD bands were Z-linked: Es01 and Es02 (Table 4). No unusual segregation ratios were found (P>0.05). Calculated recombination fraction was 20.1% (P<.01), or 26.9 Haldane cM with LOD score of 1.89.

Many important traits have been reported to be sex-linked in poultry. The chicken Z chromosome has been proposed to be 210 cM, and RAPDs were found widely distributed on it (Levin *et al.*, 1993). Identification of sex-linked markers will allow mapping of genes contributing to those traits. Levin *et al.* (1993) report 13 Z-linked RAPD bands using much divergent populations. The two found in this study are in addition to their findings.

Table 4. Segregation of Z-linked markers in backcrosses with ES and F1 as parents.

Subgroup	Number of birds	sex chromosome origin	expected +/-	observed +/-	
				Es01	Es02
EEDE	24	$Z^{E}W^{E}$	all -	24-	24-
DEEE	24	$Z^{E}W^{E}$ & $Z^{D}W^{E}$	12+/12-	15+/9-	12+/12-
EEED	5	$Z^{E}W^{D}$	all -	5-	5-

Reproducible results from RAPD use is a major consideration and has been discussed by several authors (Yu and Pauls, 1992; Ellsworth *et al.*, 1993). In this paper, we only reported markers with easily reproducible bands even though there were about 20 other polymorphic bands seen but not used. Condition-sensitive bands usually had large or small sizes or appear relatively faint. These might be used but additional work is needed to optimize the reaction conditions, including adjustments of annealing temperature, magnesium ion concentration, primer and DNA template concentration, and amount of *Taq* polymerase. We also found differences between primers coming from two sources that might be caused by different variations of the G-C content in oligonucleotide primers. Most reproducible bands were obtained with primers having a G-C content higher than 60%.

Despite some problems due to repeatability and dominance, this study showed that RAPD method is a fast and relatively simple analytical process for identification of markers in animals, especially for species lacking previous DNA sequence information.

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