DNA Fingerprint Bands Correlated with the Egg Weight Performance of Hens

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ABSTRACT : Beijing White Chickens laying larger eggs and smaller eggs were respectively used as parental individuals for mating to produce the F1 progeny and then the F1 progeny individuals mated to produce 125 individuals of the F2 progeny. Three bands associated with the egg weight performance were identified from DNA fingerprints of the 125 individuals generated with a bovine minisatellite probe BM6.5B. The simple linear correlation analysis showed that the coefficients of correlation between frequencies of the three bands (DB1, DB2 and DB3) and egg weights were -0.6, -0.6 and 0.9, respectively.

(**Key Words**: DNA Fingerprints, Bovine Minisatellte, Egg Weight Trait, Genetic Correlation)

INTRODUCTION

Minisatellite DNAs are a kind of hypervariable repetitive sequences in eukaryotic genomes, and the repetitive sequences consist of many repeat units in which each unit embodies a core sequence. The variations of minisatellites originate from the variable number of repeat units at the same minisatellite locus in a population. Not only are there high homologies between the core sequences of each units in the same minisatellite locus, but also between the core sequences of each unit in different minisatellite loci from the same species or different species (Jeffreys et al., 1985; Jeffreys, 1987). Therefore, under certain conditions a minisatellite probe from a species genome can hybridize to minisatellite DNAs of other species genomes to produce hypervariable DNA fingerprints (Wetton et al., 1987). Many studies reportedly reveal that minisatellite probes 33.15 and 33.6 isolated from human genome can generate highly variable DNA fingerprints of domestic animals (Hillel et al., 1989; Jeffreys et al., 1987). The minisatellite probe BM6.5B used in this study was screened out and cloned from Chinese bovine genome with human minisatellite probe 33.6 and could produce hypervariable DNA fingerprints of chickens also (Huang et al., 1995).

A DNA fingerprint covers variations of many loci, usually more than ten, in a genome. So, some genetic markers of QTL (quantitative trait locus) can be more easily found out with DNA fingerprinting (Georges et al., 1990; Lamont et al., 1996). It has been frequently reported that DNA fingerprinting has been applied to searching for fingerprint bands (candidate markers) correlated with some quantitative traits of domestic animals (Meng et al., 1996; Lamont et al., 1996). For example, Plotsky et al. (1993) found out one specific band (S6.6), which was negatively correlated with chicken abdominal fat content (percentage) significantly, from DNA fingerprint patterns of half-sib families of meat chickens with minisatellite probes 33.6 and R18.1. The egg weight trait of chickens shows middle-strength heritability. This study will be expected to reveal DNA fingerprint bands (DNA fragments) correlated with the egg weight trait of half-sib families of chickens analyzed by DNA fingerprinting with bovine minisatellite BM6.5B. More-over, these DNA fragments will be used for linkage analyses and as molecular genetic markers in assistant selections of chicken breedings.

MATERIALS AND METHODS

Experiment materials

The chicken individuals tested in this study originated from the pure lines of Beijing White Chicken. Four male birds with higher average weights of eggs (values calculated through their sibs) mating with nine female birds with lower average weights of eggs, and four male birds with lower average weights of eggs (values calculated through their sibs) mating with nine female

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birds with higher average weights of eggs produce F1 progeny individuals. F1 individuals are randomly selected for mating to produce 125 individuals of F2 progeny tested in this study.

Methods of experiments and statistic analyses

Genomic DNA was extracted from chicken blood cells and used for endonuclease digesting and Southern blotting (Meng, 1994). Bovine minisatellite probe BM 6.5B was labelled using ^{32}P-dCTP (3000Ci/mmol) by random priming (Feiberg & Vogelsterl, 1983) and a high specific activity of 7.6 × 10^9 cpm/μg of probe DNA was obtained. Blot membranes were prehybridized for 1.5 hours and hybridied for 13.5 hours with the minisatellite probe at 60℃, respectively, in the same solution of 1mM EDTA (pH 8.0), 7% SDS and 0.5M phosphate buffer (pH 7.2). The probe concentration was 2.86 × 10^6 cpm/ml of hybridization solution. The hybridized membranes were washed twice for 20 minutes each time at 60℃ in the solution of 2 × SSC 0.1% SDS.

One hundred and twenty five female birds (F2) were divided into 12 groups according to their egg weights (egg weight from 36-week-old femals), each group containing ten or eleven individuals. DNA mixes (8 μg) were equally prepared from every individual of each group, and digested to completion with the restriction endonuclease HaeⅢ, electrophrosized and transferred onto membranes. The membranes were probed with BM 6.5B to produce DNA fingerprints of 12 groups of birds. Then, a gradient of band intensity among the different groups (high intensity in one extreme group decreasing towards the other extreme group) would be identified. For the next step, 125 chicken individuals of 12 groups were arranged from smaller to larger according to its egg weight, and all the individuals were fingerprinted with the same probe under the same conditions (prehybridization and hybridization) to find out how many individuals in one group holding one specific band identified by DNA mixes procedure.

The correlation analyses were made on the average egg weights of each group and the frequences of one specific band presenting in each group. The correlation coefficients were calculated based on the formula $r = \frac{SPxy}{\sqrt{SSxSSy}}$ and made on significant test (T test). In the formula, $SPxy = \sum(x-\bar{x})(y-\bar{y})$, $SSx = \sum(x-\bar{x})^2$, $SSy = \sum(y-\bar{y})^2$, r represents the correlation coefficient, x the frequencies of one specific band presenting in each group, y the average egg weights of each group.

RESULTS AND DISCUSSIONS

Characteristic bands from DNA fingerprints of mixes and individuals

Multiple-locus DNA fingerprints can reflect the variations of the genomes and may be used to study genetic structures and improvements of domestic populations (Tixier-Boichard et al., 1996; Zhu et al., 1996). It was easy to identify a gradient of band intensity in DNA fingerprints produced with DNA mixes and such a band can be believed as a characteristic band associated with a certain quantitative trait (such as egg weight) (Plotsky et al., 1990). One hundred and twenty five chicken individuals were divided into 12 groups and used for DNA fingerprinting with the probe BM6.5B. The intensities of three bands were found to show regular distributions in each group, and among the three bands, two bands (DB1 and DB2) were getting more and more intensity from smaller egg group to larger egg group, one band (DB3) was getting more and more intensity from smaller egg group to larger egg group. It was deduced depending on the above decribed that the three bands appearance in 12 groups could be correlated with the egg weight trait.

It became simple and highly accurate to locate some bands associated with quantitative traits from DNA fingerprints of mix samples. However, how many birds in a certain group holding some associated band were not known until DNA fingerprinting of all individuals was produced. DNA fingerprints of 27 chicken individuals (their egg weights was 65.2 g to 76.1 g from left to right in figure 1) were showed in figure 1. DB1 and DB2

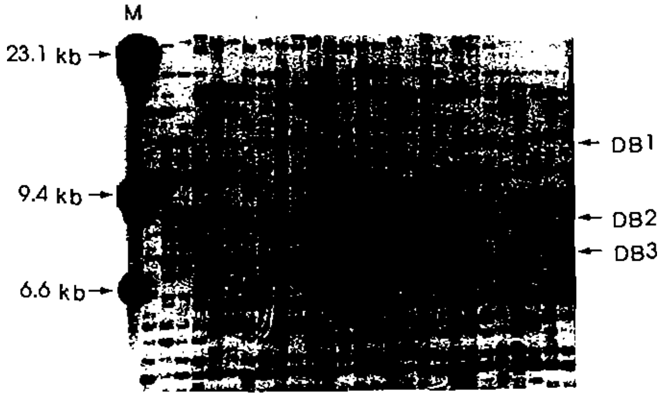

Figure 1. DNA fingerprints of 27 hen individuals produced with probe BM6.5B (M: Molecular markers of lambda DNA/HindⅢ. In the figure, the individual number is ordered as No. 1 to No. 27 from left to right. The average egg weight of the individuals is getting higher and higher with 65.2g of No. 1 and 76.1 g of No. 27.).

showed a tendency to appear in the individuals with smaller egg weight, and the band DB3 showed a tendency to appear in the individuals with larger egg weight. Plotsky et al. (1990) prepared firstly DNA fingerprints of DNA mix samples and then individual DFPs of the 53 ckickens. Afterwards, they analysed the correlations between DNA fingerprint bands and abdominal fat weights or abdominal fat percentage. The result revealed a highly negative correlation (-0.93) between one band P6.1 and abdominal fat weights or abdominal fat percentage.

Correlation analyses between the frequencues of certain bands and average egg weights

Table 1 lists the number of 12 groups of chickens possesing bands DB1, DB2 and DB3, respectively and the values of average egg weights. Analyses of simple linear correlations between average egg weights and band frequencies (indicated as percentage) revealed that the correlation coefficients between the frequencies of bands DB1, DB2 and DB3 and the average egg weights were -0.6, -0.6 and 0.9, respectively. They correlations were significant or extremely significant by t-test test ($p < 0.05$, $p < 0.01$). The results implied that the bands DB1, DB2 and DB3 showed significantly negative or positive correlations with the locus controlling the egg weight trait. These associated bands (DNA fragments) will be isolated and cloned to be used as locus-specific probes or molecular genetic markers.

For the next step, these probes (or markers) will be used for linkage analyses. If the markers are closely linked to the egg weight locus, they will be applied to laying-chicken breedings and identifying the major gene controlling egg weight trait by chromosome walking.

Table 1. Average egg weights and numbers of individuals showing bands DB1, DB2 and DB3 per group of chickens respectively

Group Nos.	1	2	3	4	5	6	7	8	9	10	11	12
Individual number	11	11	11	10	10	10	10	10	10	10	11	11
Average egg weight(g)	51.3	54.7	56.4	58.0	60.1	60.8	62.2	63.2	64.4	65.5	67.4	72.1
S. E.	3.05	0.72	0.48	0.54	0.29	0.25	0.35	0.32	0.30	0.46	1.03	2.2
No. of DB1*	6	3	4	1	3	2	0	0	1	0	0	2
Frequency1)	54.5	27.3	36.4	10	30	20	0	0	10	0	0	18.2
No. of DB2*	7	7	5	4	6	7	3	3	2	0	4	3
Frequency2)	63.6	63.6	45.5	40	60	70	30	30	20	10	36.4	27.3
No. of DB3*	1	4	3	4	3	4	7	5	5	6	7	9
Frequency3)	9.1	36.4	27.3	40	30	40	70	50	50	60	63.6	81.8

* Means the numbers of the individuals possessing bands DB1, DB2 and DB3 in each group, respectively. 1), 2) and 3) Mean the frequencies (in percentage) of the individuals possessing bands DB1, DB2 and DB3 in each group, respectively.

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