Research Notes

Microsatellite Polymorphism Between and Within Broiler Populations[1]

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ABSTRACT Two independent broiler chicken populations were genotyped with microsatellite markers to determine genetic polymorphisms within and among broiler populations. Birds were genotyped with primers from the US Poultry Genome Mapping Kits 1 and 2. The 59 primer sets selected for this study provided wide genomic coverage. All 59 primer sets amplified a polymerase chain reaction product in Population L, whereas 57 primer sets produced a product in Population C. The average allele number per line per microsatellite was 2.8 and 2.9 for Populations L and C, respectively. Considering the 57 primer pairs generating product in both lines, 72.3% of the total alleles were unique to one or the other population. This study illustrates the high polymorphism level in broiler populations of microsatellites amplified from primers developed from Red Jungle Fowl or White Leghorn sequences.

(*Key words*: microsatellite, polymorphism, molecular marker, broiler chicken)

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INTRODUCTION

The use of microsatellites has become a standard technique for molecular genetic evaluation and mapping of chickens (e.g., Khatib et al., 1993; Cheng, 1997). Microsatellites consist of tandem repeats of core sequences of di-, tri-, or tetranucleotide units. The polymorphic variants are likely generated by unequal crossover between the repeat units during meiosis. To date, more than 600 dinucleotide microsatellite markers have been mapped to the East Lansing map (Dodgson et al., 1998). Microsatellites are used in a wide range of applications in the genetic evaluation of chicken lines, including bulk-segregate analysis (eg., Khatib et al., 1994) and estimation of genetic relationships of native chicken populations (Ruyter-Spira et al., 1997; Takahashi et al., 1998). However, only a limited number of investigations have used microsatellites across broiler chicken populations (Crooijmans et al., 1996; Vanhala et al., 1998; Karaca et al., 1999). By using microsatellite primers designed from either White Leghorn or database sequences, Crooijmans et al. (1996) and Vanhala et al. (1998) estimated allele frequencies in broiler lines (5.2 and 5.7, respectively).

To be of maximum usefulness as a tool for genetic analyses, microsatellite primers should be able to be applied in populations other than the source of the primer sequence. The microsatellite loci evaluated in this study were dinucleotide repeats, isolated from either Red Jungle Fowl or White Leghorn chicken DNA libraries, and have been mapped in the East Lansing Red Jungle Fowl by White Leghorn reference population cross (Crittenden et al., 1993; Cheng and Crittenden, 1994; Cheng et al., 1995). The microsatellite primers were designed based on unique flanking regions of these isolated repeats (Cheng et al., 1995). The objective of this study was to evaluate the efficacy and informativeness within and between broiler lines of microsatellite primers developed from Red Jungle Fowl or White Leghorn sequences.

MATERIALS AND METHODS

Experimental Animals

Two broiler chicken populations from two different primary breeding companies were utilized. Population L was from a male line of commercial broiler breeders. Population C was from the F1 cross of two lines that had been divergently selected from a dam line of commercial broiler breeders for antibody response to *Escherichia coli* vaccine (Yonash et al., 1996). Six individuals were genotyped from each population.

DNA Isolation, Microsatellite Amplification, and Genotyping

Individual DNA was isolated from 50 μL of blood in EDTA by a phenol/chloroform protocol (Dunnington et al., 1990), quantified by spectrophotometry, and diluted

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TABLE 1. Comparison of polymorphisms of 59 microsatellites in two independent broiler populations

Polymorphism statistics	Population L	Population C	Populations L and C
Polymorphic primers (%)	91.5	89.5	98.3
Mean number of alleles/primer	2.8	2.9	4.4
Total number of alleles	165	163	253

to 50 *n*g/μL. All amplifications were performed under the same conditions.

Genomic DNA (50 *n*g) was amplified with 1 unit *Taq* polymerase,[4] 1.5 m*M* $MgCl_2$, 200 μM dNTP, and 50 pmoles of each primer in a total volume of 25 μL (Cheng et al., 1995). The amplification went through 30 cycles of 94 C denaturation, 45 C annealing, and 72 C extension, each for 1 min, and a final extension for 5 min at 72 C. Amplified products (1.5 μL) were analyzed by an ABI Prism 377 sequencer[5] at the Nucleic Acid Facility, Iowa State University. Genotype data were analyzed by Genescan software, version 2.1.[5]

RESULTS AND DISCUSSION

The 59 fluorescently tagged microsatellite primers were selected from the US Poultry Genome Mapping Kits 1 and 2, which give wide genomic coverage of the chicken genome (Dodgson et al., 1998). All 59 primer sets amplified product in Population L, whereas 57 of 59 primers amplified product in Population C. The microsatellites were highly polymorphic within the broiler populations, 91.5 and 89.5% polymorphic for Populations L and C, respectively (Table 1). Sequences of several of the amplified products confirmed that they contained the expected microsatellite core repeats (data not shown). The ability of these primers to efficiently amplify product illustrates that the unique microsatellite-flanking primer sequences are conserved across widely divergent chicken populations. The highly polymorphic nature of these microsatellites demonstrates their utility as informative molecular markers for broiler lines, even though the primer sequences were derived from other types of populations.

The mean number of amplified alleles per primer was 2.8, 2.9, and 4.4, respectively, for Population L, Population C, and both lines combined (Table 1). When comparing the products of the 57 primers that amplified DNA in both populations, 77.2% of the primers shared at least one allele between populations; however, 72.3% of the individual alleles were unique to one of the populations. Previous studies have utilized microsatellite primers derived from White Leghorn DNA or database sequences to analyze broiler DNA (Crooijmans et al., 1996; Vanhala et al., 1998). From pooled DNA samples, the estimated mean number of alleles per primer was 3.6 within individual broiler lines and 5.2 for combined broiler lines (Crooijmans et al., 1996). Vanhala et al. (1998) estimated the number of alleles per primer to be 5.7 for a commercial broiler line. The slightly lower allele frequencies of the present study, both for individual and combined populations, may be the result of the smaller number of individuals genotyped per line. Six individuals per population were genotyped in the present study; whereas, Crooijmans et al. (1996) genotyped pools of 60 individuals per line from nine broiler lines, and Vanhala et al. (1998) genotyped 12 to 31 broilers.

The present study utilized substantially more primers (n = 59) and, thus, had more comprehensive coverage of the genome than the previous studies of broiler populations (17 and 9 primers, respectively; Crooijmans et al., 1996; Vanhala et al., 1998). Because the studies by Crooijmans et al. (1996) and Vanhala et al. (1998) shared five primers, they analyzed a total of 21 primers in broiler lines. By assuming a maximal coverage of 20 cM on each side of a marker and accounting for primers located less than 20 cM apart, the primers selected for this study covered over 57% of the genome. The previous studies had maximal coverages of 21 and 12% of the genome (Crooijmans et al., 1996; Vanhala et al., 1998, respectively) or a combined coverage (accounting for shared primers) of approximately 28% of the genome. The current study almost doubled the genomic coverage relative to previous studies, and it used 59 primers not used in the previous studies, thus giving substantial additional characterization of microsatellites in broiler populations.

In summary, this study demonstrates on a genome-wide level that the Red Jungle Fowl and White Leghorn derived microsatellite primers from the US Poultry Genome Mapping kits 1 and 2 are very efficient and highly informative as genetic markers in evaluation of broiler populations. These microsatellites were extremely proficient at amplifying highly polymorphic PCR product, both within and between broiler populations. The high level of microsatellite polymorphism demonstrated between broiler populations suggests that crosses of broiler lines will generate populations in which microsatellites will be very efficacious for mapping.

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