MICROSATELLITE POLYMORPHISM IN COMMERCIAL BROILER AND LAYER LINES

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SUMMARY

Polymorphism for 8 microsatellite markers was determined in highly selected commercial broiler (9) and layer (6) lines using mixed (60 animals) blood samples. The average number of alleles per marker was 6.3 over all lines, 5.9 over broiler lines and 3.1 over layer lines. The average number of marker alleles within a line was 3.3, 4.0, and 2.1 for all, broiler and layer lines, respectively. The average percentage of heterozygous animals was 44, 55 and 28 for all, broiler and layer lines. In broilers and layers, 50% and 5% of the marker-line combinations showed a heterozygosity of more than 60%.

INTRODUCTION

A marker is an identified genomic site. Marker alleles represent polymorphism at this site. Markers can be used in animal breeding programmes: marker-assisted selection (Soller, 1978) and marker-assisted introgression (Hillel *et al.*, 1990; Groen and Timmermans, 1992; Hospital *et al.*, 1992). Marker-assisted selection exploits marker-quantitative trait loci associations; due to linkage disequilibrium the marker explains part of the within-family variance, thus providing additional information for selection purposes (Lande and Thompson, 1989). Marker-qtl associations can be determined by association studies in resource populations. For this purpose, the establishment of a marker map from linkage studies in reference populations is useful. The degree of marker polymorphism, i.e. the number of alleles and the percentage of heterozygous animals, determines the number of informative gametes used to estimate associations and linkages (Van der Beek and Van Arendonk, 1993). High marker polymorphism within a line or population under selection will enhance marker-assisted selection (Dekkers and Dentine, 1990).

Marker polymorphism is used to determine genetic variation within and between breeds: genetic distance, heterozygosity and inbreeding (Nei, 1987, Kuhnlein *et al.*, 1989, 1990; Lynch, 1990; Groen, 1993). Genetic distance might be used to predict heterotic performance of crossbred lines (Glodek, 1974; Goddard and Ahmed, 1982).

Different types of locus specific genetic markers are available, among others minisatellites, microsatellites, RAPD's, and RFLP's. The application of microsatellites is currently thought to be very important as they are numerous and randomly distributed in the genome, they seem highly polymorphic and they show co-dominant inheritance (Burke *et al.*, 1991). No literature on polymorphism for locus specific microsatellites in highly selected commercial layer and broiler lines is available.

The aim of this paper is to describe microsatellite polymorphism in highly selected commercial layer and broiler lines.

MATERIALS AND METHODS

Microsatellite isolation. A genomic library of the DNA of a white leghorn chicken was constructed (partially filled in *Sau*3A fragments of 300-3000 bp), in the partially filled *Xho*I site of lambda *Zap*II vector (strategene, La Jolla, CA, USA). Approximately 85,000 clones were screened using the synthetic polynucleotides $(TG)_{13}$, $(CAC)_5$ and $(GGAT)_4$ which were labelled at the 5' end with [γ-^{32}P]ATP. This resulted in the isolation of 178 $(TG)_n$ clones, 5 $(CAC)_n$ and only 1 $(GGAT)_n$ clone. After isolation of the positive clones, the

pBluescript plasmids were isolated and linear PCR sequencing of the repeat and its flanking regions was performed in a total volume of 10 μl using a DNA thermal cycler (Perkin-Elmer-Cetus, Norwalk, CT, USA). A set of six oligonucleotides ($(TG)_5X$, where X = A, C or G and $(GT)_5$ Y, where Y = T, A of G) was used to obtain the sequence of the immediate flank of the cloned microsatellite. From this sequence information, a 24-bp PCR primer was synthesized and used to obtain the sequence of the repeat and the other flanking region. Of the 178 positive $(TG)_n$ clones 153 gave a very strong signal. Thus far, for 80 $(TG)_n$ containing clones the repeat and flanking regions were sequenced, and for 50 clones primer pairs were synthesized. These locus specific microsatellites were tested for polymorphism using a panel of five unrelated animals derived from three different breeds of layers and two different breeds of broilers. About 75-80% of the microsatellites were polymorphic in the test panel, with the number of alleles varying from 2 to 6. More details on the microsatellite isolation are in Crooijmans *et al.* (1993, 1994).

Population screening. Blood samples of 60 animals (20 and 40 randomly chosen males and females) of a single line were mixed, and genomic DNA was extracted from these mixed samples. Nine broiler (Cornisch and Rock) and 6 layer (White Leghorn) lines were sampled. PCR reactions were carried out in a total volume of 20μl containing 10-50 ng genomic DNA, 1.5 m*M* $MgCl_2$, 50 m*M* KCl, 10 m*M* Tris.HCl pH = 8.3, 1 m*M* tetra-methylammoniumchloride (TMAC), 200 μ*M* dNTP, 0.5 Unit Tth DNA polymerase (HT Biotechnology) and 30 ng of each primer, one of which was labelled at the 5' end. 35 cycles of PCR were performed, each cycle consisting of 1 min at 94° C, 2 min at 62° C and 3 min at 72°C. PCR amplication products were separated on a 6% denaturating polyacrylamide gel (sequagel-6: National Diagnostics). Analyses were performed using an Automated Laser Fluorescent (ALF) DNA Sequencer (Pharmacia). The size of the detected alleles was calculated using the computer program Fragment Manager (Pharmacia). Frequencies of an allele within a line were determined from relative (to total peak area within the sample of the line) fluorescent area of the corresponding peak. Analyses was in duplo, starting with two different mixed blood samples. Thus far, complete information on all lines is available for 8 microsatellites.

Parameters. The number of alleles observed per marker was determined; over all line, over broiler lines, and over layer lines. Moreover, the number of alleles per marker per line, averaged over all lines, over broiler lines, and over layer lines were determined. Heterozygosity was calculated per line per marker, according to Nei (1987)

$$H_l = [2n/(2n-1)][1 - {}_i\Sigma^{ml}(p_{li}^2)],$$

assuming Hardy-Weinberg equilibrium, where n is the number of animals in the sample (60 in this study), ml is the number of alleles at locus l, and p_{li} is the frequency of the ith allele at locus l. Heterozygosity per marker was averaged over all lines, over broiler lines and over layer lines.

RESULTS

The number of alleles per marker over all lines was on average 6.3 (Table 1) with a maximum of 12 and a minimum of 2. Within a line, the average number of alleles per marker was 3.3. Some microsatellites were monomorphic in layer lines. MCW16 and MCW18 each had 8 alleles in one broiler line. The percentage of heterozygous animals for a single marker was 44 averaged over all lines, and 55 and 28 averaged over broiler and layer lines.

Table 1. Microsatellite polymorphism in broiler and layer lines

Marker	Total #alleles observed			Average # alleles/line			Perc. heterozygosity[1]		
	All	Broiler	Layer	All	Broiler	Layer	All	Broiler	Layer
MCW-004	7	7	3	3.1	4.0	1.7	46.3	63.2	20.8
MCW-005	12	10	8	4.1	4.7	3.3	62.0	66.9	54.8
MCW-009	2	2	2	2.0	2.0	2.0	31.4	30.4	33.0
MCW-014	5	4	2	2.7	3.3	1.7	44.5	56.8	25.9
MCW-016	9	9	2	4.5	6.3	1.7	47.3	68.1	16.0
MCW-018	8	8	2	4.4	6.3	1.5	47.9	71.4	12.6
MCW-036	4	4	3	3.0	3.0	3.0	43.5	51.7	31.1
MCW-041	3	3	3	2.2	2.3	2.0	30.9	34.1	26.1
Averaged	6.3	5.9	3.1	3.3	4.0	2.1	44.2	55.4	27.5

[1] calculated as $H_l = [2n/(2n-1)][1 - {}_i\Sigma^{ml}(p_{li}^2)]$, assuming Hardy-Weinberg equilibrium, where n is the number of animals in the sample (60 in this study), ml is the number of alleles at locus l, and p_{li} is the frequency of the ith allele at locus l (Nei, 1987).

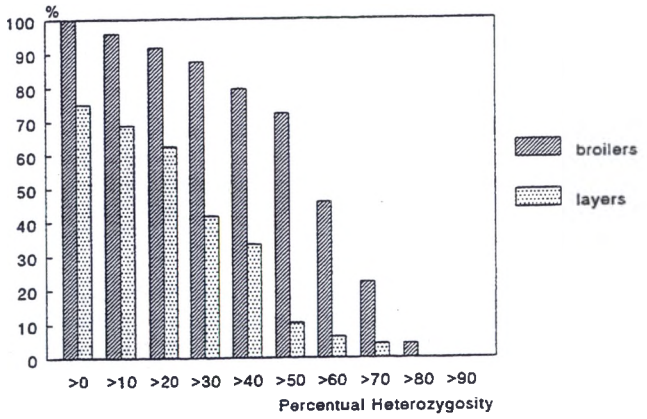
In total, 72 and 48 marker-line combinations were tested in broilers and layers, respectively. Figure 1 shows the proportion of marker-line combinations exceeding a given degree of heterozygosity. For example, in broilers 50% of the marker-line combinations had a heterozygosity higher than 60%; 25% had a heterozygosity higher than 70%. In layers, only 5% of the marker-line combinations had a heterozygosity higher than 60%.

DISCUSSION

The observed degree of polymorphism of the analyzed microsatellites is moderate. An important reason could be that the lines analyzed are highly selected, especially the layer lines. A moderate degree of polymorphism will limit the efficiency of marker-assisted selection when using microsatellites in these commercial lines.

Prediction of heterotic performance from genetic distance between commercial lines is of practicle importance. For an accurate estimation of genetic distance, a high number of loci is required rather than a high expected polymorphism per locus (Nei, 1987).

Figure 1. Percentual number of marker-line combinations (72 in broilers, 48 in layers) exceeding a given degree of heterozygosity.



In this study, only 8 microsatellites were analyzed and hardly any selection was practiced during isolation. Selection in markers for a given line can be performed to obtain higher degrees of polymorhism. However, selection on polymorphism increases the number of microsatelltes to be isolated. Markers can be highly heterozygous in one line, while monomorphic in an other line. This means, that selection of markers should be primarily based on polymorphism within the line used for linkage or association studies or within the line marker-assisted selection will be practiced.

In conclusion, the observed polymorphism of microsatellites is moderate: 6.3 alleles per marker over all lines, with on average 3.3 alleles and 44% heterozygous animals within a line. In broilers and layers, 50% and 5% of the marker-line combinations showed a heterozygosity of more than 60%. These results should be considered when studying optimum designs for association and linkage studies and when estimating potential benefits from marker-assisted selection.

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