HYPERVARIABLE MARKERS IN THE CHICKEN GENOME

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SUMMARY

The distribution and abundance of hypervariable DNA markers in the chicken genome was examined using multilocus DNA fingerprinting. Minisatellite alleles at loci detected using the Jeffreys probes 33.6 and 33.15 in a family containing two parents and thirteen offspring were found to segregate independently, and band transmission followed mendelian expectation. A low level of cosegregation (6.2%) and apparent allelism (13.8%) was found, and a higher than previously described level of homozygosity (27%) was attributed to the fact that the parents originated from two inbred lines. Locus-specific probes were isolated from two chicken genomic DNA libraries and tested on a panel of four unrelated individuals. Early results suggest that these probes detect loci with observed heterozygosity values of 80% to 100%. It is proposed to use the locus-specific probes in strain identification, genome mapping (including resolution of quantitative trait loci) and marker assisted selection.

INTRODUCTION

In the poultry industry, chickens which have been bred specifically for egg or meat production have become valuable commercial assets. The breeding history and varietal composition of lines of chickens entering final commercial crosses is therefore not disclosed by breeding companies. The concept of the chicken breed has now become commercially redundant and has been replaced by the production optimized inbred line (Abplanalp 1986). Reliable genetic characterization of inbred lines is therefore desirable for both commercial protection and as a means of verifying the origins of birds in cases of unsatisfactory productivity. This characterization has recently been shown to be possible using DNA fingerprinting (Jeffreys *et al.* 1985, Burke and Bruford 1987) by Kuhnlein *et al.* (1989). Identification of the origins of chickens where the product is often sold as a one day old fertilized egg can only be reliably tested at the molecular genetic level. We are currently applying both multilocus and locus-specific DNA fingerprinting to this problem .

The chicken genome, although the most widely studied of avian genomes, is still very poorly covered in terms of linkage groups and chromosome markers (Somes 1987). Widely dispersed polymorphic markers will obviously be of importance in mapping genes for such traits as disease resistance and economically important quantitative variation (Soller and Beckmann 1986). This could lead to the use of markers in selection programmes for such traits (Stam 1986). Locus-specific hypervariable probes should form an important component of a set of such markers, and are being isolated with these applications in mind.

MATERIALS AND METHODS

Multilocus DNA fingerprints were obtained from chicken DNA digested with *Hae* III as previously described (Burke and Bruford 1987, Burke *et al.* 1989). Gels

consisted of 1% agarose in 1X TBE (0.089M Tris, 0.089M Borate, 2mM EDTA, 0.5µg/ml ethidium bromide, pH 8.3), were 30cm long and hybridizations carried out in 1X SSC at 65°C did not include competitor DNA.

Locus-specific probes were isolated from clones obtained from two genomic DNA libraries of different size fractions (11-21kb and 2-7kb). DNA fractions were ligated into charomid (cosmid) vectors (Saito and Stark 1986) as described elsewhere, (Armour *et al.* 1990, Hanotte *et al.* 1990). Positive clones were selected following transfer onto Amersham Hybond-N nylon membranes (Buluwela *et al.* 1989) and hybridization with 33.6 and 33.15 in phosphate buffer (0.263M Na_2HPO_4, 7% SDS, 1mM EDTA, 1% BSA, Westneat *et al.* 1988) at 65°C. Washing was carried out at 65°C in 40mM Na_2HPO_4, 1% SDS for 15 min., and 0.1X SSC, 0.01% SDS for 15 min. Probes were isolated and prepared as in Armour *et al.* (1990).

Locus-specific DNA fingerprints were obtained using DNA prepared from four unrelated chickens and digested with *Mbo* I. Gels consisted of 0.8% agarose in 1X TBE, and were blotted onto nylon membranes as in Reed and Mann (1985). Locus-specific probes were oligolabelled with ^{32}PdCTP (Feinberg and Vogelstein 1983), and hybridized to membranes overnight at 65°C with 10µg/ml chicken competitor DNA (prepared as in Maniatis *et al.* 1982). The membranes were washed as above, and autoradiography was carried out using Fuji RX or Amersham MP X-ray film for 1-3 days with two intensifying screens.

RESULTS

1. DNA fingerprinting

A pedigree consisting of two parents and thirteen offspring was fingerprinted using the enzyme/probe combinations of *Hae* III with 33.6 (see Figure 1) and 33.15. The family comprised a cross between birds from two different commercial inbred lines of egg producing chickens, with 8 male and 5 female offspring. Table 1 shows the segregation analysis for all detected minisatellite loci in this family (Jeffreys *et al.* 1985, Burke and Bruford 1987). The mean transmission frequency for 51 loci was 0.493, and the mode of inheritance therefore conforms with mendelian expectation. The mean number of loci scored in the parents using both probes was 36 (including homozygous loci weighted for observed linkage rate).

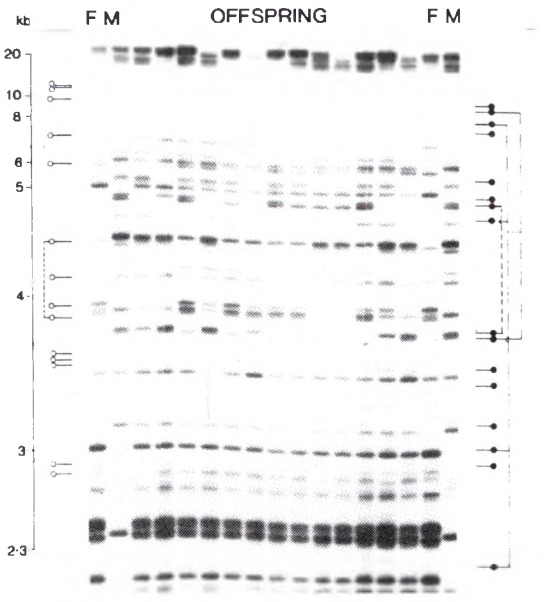

Figure 1

Figure 1 Pedigree of 2 parents and 13 offspring probed with Jeffreys probes 33.6. Cosegregating heterozygous maternal (o) and paternal (•) bands are joined by continuous lines; allelic pairs are joined by broken lines.

Table 1 Segregation analysis in a chicken pedigree.

Transmission to no. of offspring (r)	Female: Transmission of Single fragment Obs.	Exp.	Female: Transmission of Pair (++ or --) Obs.	Exp.	Male: Transmission of Single fragment Obs.	Exp.	Male: Transmission of Pair (++ or --) Obs.	Exp.
0	0	0.0	0	0.0	0	0.0	0	0.0
1	0	0.0	2	0.6	0	0.0	5	0.4
2	2	0.3	4	3.3	0	0.0	3	2.6
3	0	1.1	9	12.3	0	0.2	6	9.6
4	3	2.7	30	30.6	3	0.8	29	24.1
5	3	4.6	65	55.1	3	2.1	37	43.4
6	5	5.8	67	73.5	4	3.8	58	57.8
7	5	5.5	78	73.5	9	5.0	65	57.8
8	7	3.9	50	55.1	3	5.0	39	43.4
9	1	2.1	30	30.6	2	3.8	21	24.1
10	1	0.8	8	12.3	0	2.1	10	9.6
11	0	0.2	5	3.3	0	0.8	2	2.6
12	0	0.0	3	0.6	0	0.2	1	0.4
13	0	0.0	0	0.0	0	0.0	0	0.0
Total	27		351		24		276	

Mean transmission frequency = 0.493
All but one of each set of cosegregating fragments or pair of apparently allelic fragments in a parent (see Figure 1) were excluded so that each scored locus is represented once only.

The cosegregation of bands was investigated as in Burke and Bruford (1987). As expected in crosses between inbred lines, a large proportion of apparently homozygous loci was detected (27%). Resolvable linked and allelic bands comprised 6.2% and 13.8% of the total respectively, and once these had been removed from the analysis the remaining loci were found to assort independently.

2. Locus specific probes

Four unrelated chicken DNA samples were probed with each of the isolated chicken minisatellites. Figure 2 shows examples of the results obtained using probes cGgMS1, cGgMS18, cGgMS20 and cGgMS121. To date, 25 probes have been tested, 11 of which produce polymorphic single locus minisatellite patterns. Nine probes produced satellite patterns; this was not surprising as we have shown elsewhere

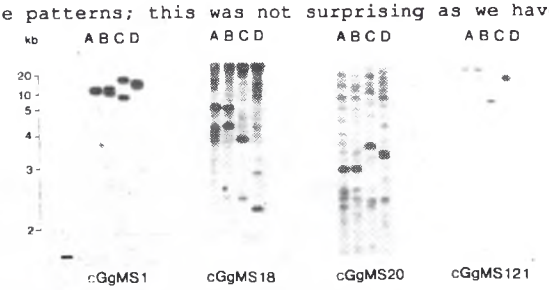

Figure 2

Figure 2. DNA samples from 4 unrelated chickens digested with *Mbo*I and probed using locus-specific minisatellite probes cGgMS1, cGgMS18, cGgMS20 amd cGgMS121. A = ISA line 1, B = ISA line 2, C = North Holland Blue, D = Red Junglefowl.

that certain chicken satellite sequences are recognised by minisatellite polycore probes (Bruford *et al.* 1990). The mean heterozygosity of the 11 polymorphic probes (using pairwise comparisons of alleles due to the small sample size) was 0.91 (s.d. 0.118) (Wong *et al.* 1987), and the mean number of alleles in the four individuals tested was 4.91 (s.d. 1.45).

DISCUSSION

The minisatellite sequences clearly represent a dispersed, independently segregating polymorphic set of markers in the chicken genome. Linkage, although at a slightly higher level than previously detected in avian genomes (Burke and Bruford 1987, Burke *et al.* 1989) only occurs in 6.2% of the loci detected in multi-locus analysis. We have shown elsewhere (Bruford *et al.* 1990) that other multi-locus probes also detect independently segregating markers in the chicken genome. Thus the potential for whole genome mapping using minisatellite loci as a base component seems high. Highly heterozygous single locus markers have successfully been isolated and further characterization of these is currently in progress. Early results show that some of these probes may be suitable for the identification of commercial lines and this is being investigated further. Localization of quantitative trait loci and the use of marker assisted selection are at the moment not possible using this small set of probes, but as their number increases it seems likely that these goals will become achievable.

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