Assessment of Inbreeding by DNA Fingerprinting: Development of a Calibration Curve Using Defined Strains of Chickens

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ABSTRACT

By analyzing DNA fingerprints of chickens from seven well-defined genetic groups, a calibration curve was established relating the degree of inbreeding with the average band frequency, allelic frequency and band sharing. The probe used was bacteriophage M13 DNA and digestion of the genomic DNA was carried out with the *Msp*I restriction enzyme. The analysis also provided an estimate of the average allelic frequency at a hypervariable locus and the average mutation frequency per locus and generation. The values of 0.24 and 1.7×10^{-3}, respectively, are similar to the estimates for humans using other probes and hybridization protocols. It is suggested that the calibration curve established can be used for determining inbreeding not only in chickens, but also in other species.

HYPERVARIABLE minisatellite regions or variable number of tandem repeat (VNTR) loci give rise to multiple alleles at a high frequency (JEFFREYS 1987; NAKAMURA *et al.* 1987). Such loci consist of tandem repeats of short segments of DNA and alleles arise from variations in the number of repeats (JEFFREYS, WILSON and THEIN 1985a). Different alleles can be distinguished by cutting DNA with a restriction enzyme which recognizes sites flanking the region of repetitive DNA and thus yields DNA fragments whose lengths vary with the number of repeats.

Minisatellites from different loci fall into families whose sequences are related (NAKAMURA *et al.* 1987). Southern blots hybridized with a minisatellite probe therefore yield a complex banding pattern called DNA fingerprint. A single hybridization usually resolves about 30 bands. Since in heterozygotes both alleles of a locus are generally visible, this represents at least 15 loci out of the 1000–1500 hypervariable loci estimated to be present per genome (WYMAN and WHITE 1980; WONG *et al.* 1986; NAKAMURA *et al.* 1987; KNOWLTON *et al.* 1986).

DNA fingerprints are highly individual specific (JEFFREYS, WILSON and THEIN 1985b) and have therefore found wide applications in forensic medicine (JEFFREYS, BROOKFIELD and SEMEONOFF 1985; GILL, JEFFREYS and WERRETT 1985), as well as ecological studies aimed at establishing mating behavior (WETTON *et al.* 1987; BURKE *et al.* 1989). However, DNA fingerprinting can be used not only to establish relationships among individuals, but is also a powerful tool for research in population genetics. Using genetic groups of chickens as a model, we have previously shown that an index of genetic distance, based on DNA fingerprinting pattern correctly reflected the history and relationships of the different populations (KUHNLEIN *et al.* 1989).

A further application of DNA fingerprinting in population genetics is the assessment of inbreeding. In this communication we report on the analysis of strains and lines of chickens, with known degrees of inbreeding, by DNA fingerprinting and the establishment of a calibration curve relating the inbreeding coefficient to band variability and band sharing. It is suggested that this calibration curve can be used to assess inbreeding in experimental and commercial populations of chickens, as well as in rare or localized breeding populations of any vertebrates.

MATERIALS AND METHODS

Genetic groups: All genetic groups studied were White Leghorns. Their origin and characteristics are described in Table 1, and their inbreeding coefficients are listed in Table 3. All these strains and lines are currently maintained at the Animal Research Centre of Agriculture Canada in Ottawa, Canada, except lines 6_3 and 7_2 which are kept at the Regional Poultry Research Laboratory of the U.S. Department of Agriculture, East Lansing, Michigan. The inbreeding coefficients of strains 7, 8, 9, S and WG were computed on individual pedigree basis up to 1980 and subsequently estimated from the size of mating populations and type of mating (FALCONER 1960). The contribution of the initial inbreeding of the founder population to the current inbreeding coefficient has been estimated to be <0.001. The highly inbred lines 6_3 and 7_2 were derived by brother-sister matings (STONE 1975). The current inbreeding coefficient is >0.98 (L. B. CRITTENDEN, personal communication).

DNA fingerprinting: DNA was extracted from 100 μl of

TABLE 1

Description of genetic groups

S	White Leghorn strain selected for susceptibility to Marek's disease at Cornell University until 1971. Maintained since at Ottawa without selection (HUTT and COLE 1957; GAVORA, EMSLEY and COLE 1979)
7	Formed from four commercial White Leghorn strains in 1958 and maintained since without selection (GOWE and FAIRFULL 1980)
8	Derived from strain 7 in 1969 and selected since for high egg number and related traits (GOWE and FAIRFULL 1980)
9	Derived from strain 7 in 1969 and selected for high egg production rate and related traits (GOWE and FAIRFULL 1980)
WG	Inbred line derived from strain 9 (GAVORA, KUHNLEIN and SPENCER 1989)
6_3	Highly inbred White Leghorn line derived at the Regional Poultry Research Station of the USDA, East Lansing, Michigan, USA (STONE 1975). Resistant to Marek's disease
7_2	Same as line 6_3, except Marek's disease susceptible (STONE 1975)

heparinized blood according to JEFFREYS and MORTON (1987) and dissolved in 5 mM Tris-HCl, 0.1 mM EDTA (pH 7.5). A 20-μl mixture containing 5 μg of DNA and 18 units of *Msp*I restriction enzyme and *Msp*I buffer was incubated at 37° for 2–4 hr, electrophoresed in a 1% agarose gel (1 V/cm for 16.5 hr) and transferred to nitrocellulose membranes by capillary blotting. Prehybridization, hybridization and washing were carried out according to the method of VASSART *et al.* (1987) using ^{32}P-labeled M13 mp9 single strand DNA as a probe (Pharmacia). Labeling was carried out with an oligo-prime labeling kit (Pharmacia) and yielded specific activities between 5×10^8 and 10^9 dpm per μg of DNA. Fifty nanograms of probe were used per hybridization reaction. The filters were exposed to Kodak XAR-5 film at −70° for 24–62 hr with one or two Cronex intensifying screens.

Evaluation of DNA fingerprints: DNA fingerprints of six randomly selected chickens per genetic group were scanned with a computer-linked densitometer and the eight most intensive bands were marked on each DNA fingerprint. The scans were then compared for the mutual presence of these bands. Bands were scored as identical (*i.e.*, representing the same allele) if they had the same apparent molecular weight and relative intensities differing by less than a factor of two (homozygote versus heterozygote). An example of this evaluation is given in Table 2. The molecular weights of the bands scored were between 25 and 2.5 kb.

Calculation of the mutation rate to new alleles: Extrapolation of the dependence of the average band frequency on inbreeding to 100% inbreeding provides an estimate of the mutation rate (v) to a new allele per generation and locus. For $v \ll 1$ in a 100% inbred line, the expected number of new bands (u) per generation is

$$u = 2vN_0A$$

N_0 is the number of bands which would have been scored in the absence of mutation (each representing two copies of a minisatellite locus due to homozygosity) and A is the number of animals tested. The average band frequency (b) will then be

$$b = (N_0 + u/A)/(N_0 + u)$$

The two equations can be solved for v, yielding the expression

$$v = (1 - b)/(2Ab - 2)$$

In our determination six animals were scored and the average band frequency extrapolated to 100% inbreeding was 0.983. This yields a mutation rate of 1.7×10^{-3} per locus and generation.

Regression analyses and the determination of confidence intervals were carried out according to standard procedures (ZAR 1974).

TABLE 2

Evaluation of band frequencies and allelic frequencies in strain S

Allele	Chicken #1	#2	#3	#4	#5	#6	Band frequency[a]	Allelic frequency[b]
1	+[c]	+[c]	−	+[c]	−	−	0.500	0.360
2	+[c]	+[c]	+[c]	+[c]	+[c]	+[c]	1.000	1.000
3	+[c]	+[c]	+[c]	+[c]	+[c]	+[c]	1.000	1.000
4	+[c]	−	−	−	−	+[c]	0.333	0.226
5	+[c]	+[c]	−	−	−	−	0.333	0.226
6	+[c]	+[c]	+[c]	+[c]	+[c]	+[c]	1.000	1.000
7	+[c]	+[c]	+[c]	+[c]	+[c]	+[c]	1.000	1.000
8	+[c]	−	+[c]	+[c]	−	+[c]	0.667	0.514
9	−	+[c]	−	+[c]	−	+[c]	0.500	0.360
10	−	+[c]	−	+[c]	+[c]	−	0.500	0.360
11	−	−	+[c]	−	−	−	0.167	0.108
12	−	+	+[c]	+	+[c]	+	0.667	0.514
13	+	−	+[c]	+	+[c]	+	0.833	0.707
14	−	−	−	−	+[c]	+[c]	0.333	0.226
15	−	+	−	−	+[c]	+	0.500	0.360
	Average band frequency						0.62 ± 0.07	
	Average allelic frequency						0.53 ± 0.08	

[a] Average and standard error. Analyzing five chickens instead of six gave average band frequencies between 0.61 and 0.69, with the average being 0.63 ± 0.01. Scoring the seven most intense bands only gave an average band frequency of 0.62 ± 0.07.

[b] Allelic frequency calculated from the band frequency (see text).

[c] Eight bands which had the highest intensity in the particular lane.

RESULTS AND DISCUSSION

To study the dependence in the variability of the DNA fingerprinting pattern on the degree of inbreeding, we examined 7 different breeding populations of chickens whose degree of inbreeding ranged from 2.5% to >98% (Tables 1 and 3). The DNA fingerprinting patterns of chickens with inbreeding coefficients of >98% and 39%, respectively, are shown in Figure 1. The banding patterns of chickens from the highly inbred line were identical, whereas considerable variation was observed among chickens of the less inbred strain.

As an index of the uniformity (U) of the DNA fingerprinting pattern within a strain, the average

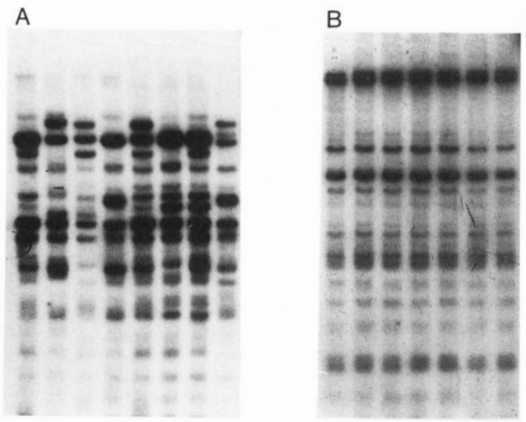

FIGURE 1.—DNA fingerprints of chickens from two strains with different inbreeding coefficients. Panel A, strain S with an inbreeding coefficient of 39%. Panel B, highly inbred line 7_2 with an inbreeding coefficient >98%.

band frequency was computed according to the equation

$$U = (1/N) \cdot \sum_{i=1}^{N} v_i$$

where N is the number of different bands scored and v_i is the frequency of band i in the breeding population. Assuming that each band represents an allele of a hypervariable minisatellite region, this index is equal to the average frequency of genotypes which have a particular allele in common. With an increasing degree of inbreeding one would expect that more and more alleles become fixed, resulting in the extreme case in an average genotypic frequency approaching one. In an outbred population on the other hand, the average genotypic frequency would be determined by the genetic variability of the founder population and the average number of alleles per locus which are distinguishable in the DNA fingerprinting pattern.

It is obviously desirable to score as many bands and individuals as possible. However, the number of lanes on a gel are limited, distant lanes are hard to compare and scoring of faint bands is unreliable. We therefore restricted ourselves to analyzing six chickens per genetic group and limited the number of bands scored in each chicken (see MATERIALS AND METHODS and Table 2 for details). Evaluation of a duplicate DNA fingerprint in one of the genetic groups (Table 3) indicated that this scoring method was reproducible and nearly identical values where obtained when five chickens or fewer bands were analyzed (footnote to Table 2).

Table 3 lists the average band frequencies of seven strains or lines of chickens. As expected, it increases with the degree of inbreeding. Linear regression analysis yields a correlation coefficient of 0.996 ($P < 0.01$), indicating that the dependence of the band frequency on inbreeding is well represented by a linear approximation (Figure 2).

An alternative measure for band variability within a strain is band sharing (WETTON *et al.* 1987). Band sharing (S) for two individuals is computed as

$$S = 2N_{AB}/(N_A + N_B)$$

where N_{AB} is the number of bands shared and N_A and N_B are the total number of bands scored in individual A and B, respectively. This measure can be extended to several individuals by averaging over all possible pairwise combinations. As expected, average band sharing increases with the inbreeding (Table 3). The relationship between band sharing and the inbreeding coefficient is nonlinear and has to be fitted by a higher order approximation.

If the inbreeding coefficient (f) of a strain is known, the allelic frequencies (p_i) can be estimated from the band frequencies (v_i) based on the equation (FALCONER 1960):

$$v_i = \underbrace{(p_i)^2}_{\text{homozygotes}} + \underbrace{f p_i (1 - p_i)}_{\text{heterozygotes}} + \underbrace{2 p_i (1 - p_i) - 2 f p_i (1 - p_i)}_{\text{heterozygotes}}$$

This equation can be solved for p_i. The average value of p_i for the different strains are listed in Table 3. Its dependence on inbreeding is close to linear (Figure 2; correlation coefficient of 0.999).

The intercept at $f = 0$ (no inbreeding) provides an estimate for the average frequency of alleles which can be distinguished at hypervariable loci. A value of 0.243 was obtained (95% confidence interval ±0.026). A second estimate of the same parameter can be obtained from the number of different bands observed and the number of bands scored per chicken (Table 3). Assuming that different alleles at a locus will always result in hybridization signals of comparable intensity—an assumption which appears to be substantiated by WYMAN and WHITE (1980), WONG *et al.* (1986), NAKAMURA *et al.* (1987) and JEFFREYS *et al.* (1988)—we expect that in the less inbred strains which contain few homozygotes each fingerprint will reveal two alleles per locus. Hence, the average of 10.6 bands scored per chicken (strains 7, 8 and 9, Table 3) represent 5.3 loci. Since an average of 22.5 different alleles were scored per strain the average allelic frequency was 0.236.

In comparison, NAKAMURA *et al.* (1987) determined the number of different alleles at 76 different hypervariable minisatellite loci in 60 to a 120 unrelated human subjects. Approximating the allelic frequency at each loci by the reciprocal value of the number of different alleles observed and averaging over all loci, an overall average allelic frequency of 0.255 is obtained (95% confidence interval ±0.028). A second estimate by JEFFREYS, WILSON and THEIN (1985a) using fewer probes and subjects yielded an average of 0.322 (95% confidence interval ±0.144).

Extrapolation of the band frequency to $f = 1$ (100%

TABLE 3

Average band frequencies, allelic frequencies and band sharing in seven genetic groups of chickens with different degrees of inbreeding

Genetic[a] group	Inbreeding coefficient	No. of different bands scored	Average No. bands scored per chicken	Band frequency[b]	Allelic frequency[c]	Band sharing
7	0.026	25	10.7 ± 0.7	0.43 ± 0.05	0.27 ± 0.03	0.44 ± 0.05
8	0.103	21	10.2 ± 0.6[d]	0.48 ± 0.05	0.33 ± 0.05	0.51 ± 0.03
9	0.126	21	10.5 ± 0.5	0.50 ± 0.05	0.33 ± 0.04	0.52 ± 0.04
9[e]	0.126	22	11.3 ± 0.5	0.52 ± 0.05	0.34 ± 0.04	0.53 ± 0.04
S	0.39	15	9.3 ± 0.3	0.62 ± 0.07	0.53 ± 0.08	0.69 ± 0.02
WG	0.762	13	10.5 ± 0.4	0.81 ± 0.05	0.78 ± 0.05	0.81 ± 0.02
7_2	>0.98	8	8.0 ± 0.0	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
6_3	>0.98	8	8.0 ± 0.0	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00

[a] Genetic groups are described in Table 1.
[b] The average band frequency is equal to the average frequency of genotypes which carry an allele corresponding to a band.
[c] Calculated from the band frequencies as indicated in the text. The reciprocal value of the average allelic frequency is equal to the average number of alleles per locus.
[d] Average and standard error.
[e] Duplicate DNA fingerprint of strain nine chickens.

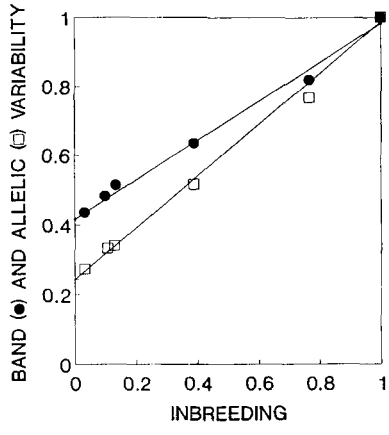

FIGURE 2.—Dependence of band variability (●) and apparent allelic variability (□) on inbreeding. Linear regression analysis through the data points from seven strains (Table 3) yielded correlation coefficients of 0.996 and 0.999, respectively. The intercepts at 0 inbreeding were 0.417 (±0.035) and 0.243 (±0.026), and the slopes were 0.566 (±0.059) and 0.744 (±0.044). The values in parenthesis define the 95% confidence interval.

inbreeding) provides an estimate of the frequency of new alleles arising per generation and locus (see MATERIALS AND METHODS). A value of 1.7×10^{-3} was obtained. This mutation rate is close to the theoretical value of JEFFREYS, WILSON and THEIN (1985b) who estimated that, based on population simulations, new alleles should arise at a frequency of $0.5–1.5 \times 10^{-3}$ per gamete and generation for a minisatellite 10 kb long. It is also close to the experimentally determined rate reported by JEFFREYS *et al.* (1988). In the latter study an extensive analysis of five different-tandem repetitive hypervariable loci in 40 families comprising a total number of 475 subjects yielded mutation rates between 0 and 0.052 with an average of 1.2×10^{-3} per locus and generation.

Considering the differences in the sizes of the populations and the classes of loci analyzed, it is quite surprising that our values obtained by extrapolation are in such good agreement with those obtained through direct experimental analysis. It indicates that the average band frequency adequately reflects the degree of inbreeding and that the curve of the relationship between band variability and coefficients of inbreeding can be used to determine the degree of inbreeding in unknown populations by DNA fingerprinting. This may be of practical use in assessing inbreeding in commercial flocks of chickens or populations of livestock species, thus providing information about genetic variability and potential response to selection. Further, it can be used to determine the degree of inbreeding in geographically isolated breeding populations or among endangered species.

Whether the same calibration curve can be used for other species or families of minisatellites remains to be determined. However, the good agreement between mutation rate and allelic frequencies observed in this study with the M13 probe and in humans with other probes indicates that such extrapolation may be meaningful.

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