Relationship Between Band Sharing Levels of DNA Fingerprints and Inbreeding Coefficients and Estimation of True Inbreeding in Turkey Lines[1]

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ABSTRACT A regression analysis between band sharing of DNA fingerprints and calculated inbreeding coefficients was conducted using six experimental turkey lines. The DNA fingerprints were produced from 18 individual DNA samples per line representing different families. The DNA was digested with a *Hae*III restriction enzyme and hybridized with Jeffreys' 33.6 probe. The band sharing within lines ranged from 0.42 to 0.62. The inbreeding coefficients of the lines were calculated based on population sizes and variation in family sizes. The inbreeding coefficients varied from 2.5 to 45%. Regression analysis between the two variables yielded a highly significant ($P \leq 0.0001$) linear model with a correlation coefficient of 0.992. The linear model was used to estimate the actual inbreeding in these lines.

(*Key words*: DNA fingerprinting, band sharing, inbreeding coefficient, correlation, turkey)

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INTRODUCTION

In a mammalian genome, about 95% of DNA sequences are not encoding or regulatory sequences. Most of the sequences are repeat sequences with a primordial nature (Ohno and Yomo, 1991). These sequences usually consist of tandem repeats with a "core" sequence called a minisatellite and are highly variable in the number of repeats (Jeffreys, 1987). Differences in these loci between individuals can be detected using a technique called DNA fingerprinting. The technique produces a banding pattern usually containing over 30 bands from genomic DNA digested with a restriction enzyme and hybridized with a repeat sequence probe in a Southern blot. The band patterns are highly specific for each individual (Jeffreys *et al.*, 1985a), and these bands are inherited in a co-dominant Mendelian fashion (Jeffreys *et al.*, 1985a; Bruford *et al.*, 1992). It is reasoned that the similarity between banding patterns increases as the relationship between two individuals become closer (Lynch, 1991). Because the technique targets a large number of loci that are believed to be distributed over the entire genome, DNA fingerprints may be very useful indicators of overall differences in the entire genome between individuals.

Inbreeding is a genetic parameter of concern in animal lines used for the genetic im provement of economic traits. Falconer (1982) defined inbreeding as the mating between individuals that are related to each other by ancestry. The inbreeding coefficient, a measure of inbreeding, is the probability of two genes at a locus being identical by descent in an individual. The inbreeding coefficient can be estimated based on actual pedigrees or population sizes and variation of family sizes (Falconer, 1982). However, calculated inbreeding may not reflect true inbreeding of populations, because many other factors also change inbreeding. Moreover, the calculation of inbreeding requires complete records of populations, which may not be available.

The similarity of DNA fingerprints among individuals within a line probably is a good indicator of inbreeding. The DNA fingerprints from an inbred human population were more similar to each other than those from outbred human populations (Bellamy *et al.*, 1991). A similar result was found in naked mole-rat colonies (Reeve *et al.*, 1990). Moreover, similarity of DNA fingerprints was correlated to relationship coefficients (Mannen *et al.*, 1993; Butler *et al.*, 1994). The degree of band sharing (BS) between individuals can be used for precise assessment of lion kinships by calibrating with coefficients of relatedness (Gilbert *et al.*, 1991). A study conducted by Kuhnlein *et al.* (1990) showed that band frequencies and allelic frequencies estimated from DNA fingerprints were highly related to inbreeding coefficients of chicken lines in a linear manner and BS

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was also correlated to inbreeding in a nonlinear fashion. The purpose of the present study was to investigate the relationship between BS of DNA fingerprints and inbreeding in some experimental turkey lines and to estimate true inbreeding in those populations.

MATERIALS AND METHODS

Turkey Lines

Six closed experimental turkey lines were used in the DNA fingerprinting analysis. The RBC1 line was a randombred control population derived from a wide genetic background (McCartney, 1964). The RBC2 line was a randombred control line established from the crossing of two commercial lines of large-bodied turkeys (Nestor *et al.*, 1969). The E line was a subline of the RBC1 line developed by family selection for increased egg production (Nestor, 1980). The F line was derived from the RBC2 line by mass selecting for increased 16-wk BW (Nestor, 1977a). The RBC3 line was another randombred control line developed from reciprocal crosses of a commercial sire line and the F line (Noble *et al.*, 1995). The FL line was a line derived from the F line by mass selecting for increased shank width (Nestor *et al.*, 1985).

All randombred control populations were maintained with 36 or more parental pairs (Nestor, 1977b). The number of parents in the selected lines varied. The E line was maintained with 36 or 48 parental pairs until the sixth generation. Seventy-two parental pairs were used to reproduce the E line thereafter. The F line was maintained with 36 parental pairs for the first 21 generations. Thirty-six males and 72 females (1 sire mated to 2 dams) were used to reproduce the F line thereafter. Thirty-six pairs were used to maintain the FL line for the first 12 generations. Thereafter, 36 sires were mated to 54 females. All parents were mated at random except that full-sib matings were avoided to reduce the increase in inbreeding. The RBC1, RBC2, and RBC3 lines had been maintained for 32, 26, and 6 generations, whereas the E, F, and FL lines had been selected for 32, 26, and 14 generations, respectively.

Calculation of Inbreeding Coefficients

With the exception of the FL line, the inbreeding coefficient of each line used was assumed to be zero at the time when the lines were established. For the FL line accumulated inbreeding in the F line was taken into consideration. The total accumulated inbreeding coefficients were the sum of the increments in each generation that were calculated using the equations described by Falconer (1982) based on variation in family sizes.

DNA Fingerprinting

The methods used to produce DNA fingerprints and to determine BS levels have been reported by Zhu *et al.* (1995). In brief, individual DNA samples were obtained from 18 males per line representing different families. A 1.5-kb inserted fragment of a human genomic clone of Lambda 33.6 (Jeffreys *et al.*, 1985a) was used as a probe. Approximately 10 μg of each DNA sample was digested with 15 U of a *Hae*III restriction enzyme for over 3 h in 30 μL total volume and separated by electrophoresis on 0.8% agarose gels for 36 h at 1.5 V/cm. The average number of scored bands ranged from 37 to 40 per sample. Band comparisons of DNA fingerprints were performed using two computer programs. The BS between individuals from the same lines was calculated according to the formula: $BS = 2N_{ab}/(N_a + N_b)$ (Wetton *et al.*, 1987), where N_{ab} was the number of scored bands shared by samples a and b; N_a and N_b were the total numbers of scored bands in samples a and b, respectively.

Statistical Analysis

Regression analysis of BS and inbreeding coefficients was carried out using a regression procedure provided in the SAS® software package (SAS Institute, 1988). The slopes and intercepts of two linear regression lines were compared according to the equations described by Zar (1984).

RESULTS AND DISCUSSION

The total inbreeding coefficients of the six turkey lines ranged from 2.5 to 44.9%, and the BS increased as the inbreeding became larger (Table 1). Regression analysis using a simple linear model based on the data of the BS and calculated inbreeding coefficients in the lines yielded a highly significant linear model ($P \leq 0.0001$). The linear model was $Y = 0.48X + 0.40$, where Y was the

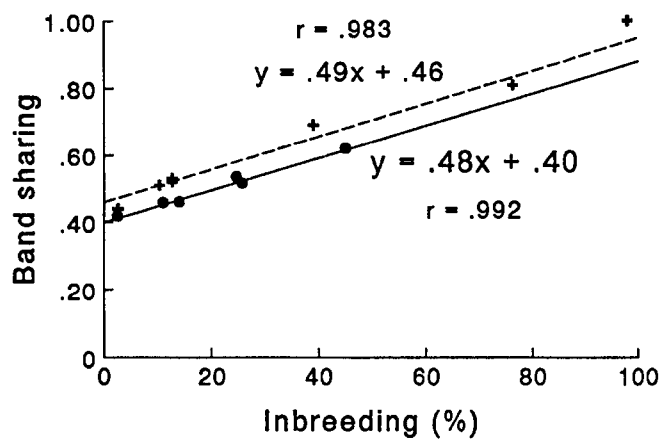

FIGURE 1. Plots of average band sharing and inbreeding coefficients of turkey lines and chicken strains. Dots and solid line are based on data from the turkey lines. Crosses and dash line indicate data from chicken strains (adapted from Kuhnlein *et al.*, 1990). Two highly inbred chicken lines (6_3 and 7_2) were excluded from regression analysis. Two slopes were not significantly different, but two intercepts were different.

TABLE 1. Average band sharing ± SEM (BS) of DNA fingerprints and inbreeding coefficients (IC) in six turkey lines and eight chicken[1] strains or lines

Turkeys			Chickens		
Lines[2]	IC	BS[3]	Strains[4]	IC	BS
RBC3	2.5	0.42 ± 0.09	7	2.6	0.44 ± 0.05
RBC2	11.0	0.46 ± 0.12	8	10.3	0.51 ± 0.03
RBC1	14.0	0.46 ± 0.09	9	12.6	0.52 ± 0.04
F	24.7	0.54 ± 0.11	9′	12.6	0.53 ± 0.04
FL	25.7	0.52 ± 0.11	S	39.0	0.69 ± 0.02
E	44.9	0.62 ± 0.09	WG	76.2	0.81 ± 0.02
			6_3	98.0	1.00 ± 0.00
			7_2	98.0	1.00 ± 0.00

[1]The data have been published by Kuhnlein *et al.* (1990).

[2]RBC1, RBC2, and RBC3 = randombred control populations; E = a subline of RBC1 selected for increased egg production; F = a subline of RBC2 selected for increased 16-wk BW; FL = a subline of the F line selected for increased shank width.

[3]The band sharing of the turkey lines was reported by Zhu *et al.* (1995).

[4]7 = an unselected strain established from four commercial White Leghorn strains; 8 = a subline of Strain 7 selected for reproduction traits; 9 and 9′ = replicates selected for reproduction traits; S = a strain selected for susceptibility to Marek's disease; WG = an inbred line derived from Strain 9; and 6_3 and 7_2 = two highly inbred White Leghorn lines selected for resistant and susceptible to Marek's disease, respectively.

average BS in DNA fingerprints and X was the calculated inbreeding coefficient (Figure 1). A higher order curve did not increase the goodness of fit of the model. Because the inbreeding coefficients were estimated from the population sizes, not from the actual pedigrees, the mean of the BS in each line, instead of individual BS, was used to calculate the correlation coefficient between the two parameters. The correlation coefficient was 0.992.

With the linear model above, the BS can be estimated to be 0.40 when the inbreeding coefficient was zero. It is probably not true that BS was 0.40 at zero inbreeding. These experimental lines were derived from a limited number of commercial lines that had been selected for many generations. On the other hand, average BS among these lines and some commercial lines was about 0.25 (Zhu *et al.*, 1995). Almost the same average BS levels were measured between some chicken lines (Dunnington *et al.*, 1994) and between unrelated individuals of human populations (Jeffreys *et al.*, 1985b). Therefore, there was likely some inbreeding in the founder populations that was not included in the calculation of inbreeding coefficients.

At the other end of the model, the BS levels of lines would be only 0.88 instead of 1 when their accumulated inbreeding coefficients reach 100%. Although the extension of inbreeding to 100% in this model may not be appropriate and needs further investigation, the result may be explained by mutations or other factors reducing the increment of BS as inbreeding rises. The mutation rate for bands in DNA fingerprints has been observed to be 0.004 in humans, which was based on the observation that one new band was found in 270 examined bands (Jeffreys *et al.*, 1985a). The incremental rate of inbreeding per generation ranged from approximately 0.4% in the randombred control lines to 1.2% in the E line. It would take about 85 to 250 generations for these lines to reach an inbreeding coefficient of 100%. In such a long period of time, mutations can become a significant factor in influencing BS.

It appears that the calculated inbreeding coefficients may not reflect the true inbreeding of these lines because of mutations and inbreeding in the founder populations. If BS between two truly unrelated individuals is assumed to be 0.25, which is reasonable based on BS obtained from chicken, turkey, and human populations (Jeffreys *et al.*, 1985b; Dunnington *et al.*, 1994; Zhu *et al.*, 1995), the inbreeding would be zero at this BS level. The populations are also assumed to have 100% of true inbreeding when BS is 1. The actual inbreeding coefficients can be calculated to be 22.0, 27.4, 27.4, 35.5, 38.2, and 48.9% in the RBC3, RBC2, RBC1, FL, F, and E lines, respectively, using the linear model.

Data from the chicken strains used by Kuhnlein *et al.* (1990) also showed that average BS increased with the inbreeding coefficients (Table 1), but the relationship between BS and inbreeding was reported to be nonlinear. However, if the regression analysis was based on the means of BS levels in each chicken strain, the regression analysis yielded another highly significant ($P \leq 0.0001$) linear model, $Y = 0.55X + 0.45$, with a correlation coefficient of 0.993. Interestingly, the slopes of the two linear models were similar ($P > 0.20$), though their intercepts were different ($P \leq 0.001$). The difference in intercepts probably was due to the differences in the laboratory methods used, including the probes and the restriction enzymes, the number of bands scored, and the measurement of inbreeding in founder populations. Kuhnlein *et al.* (1990) produced six DNA fingerprints per strain from individual DNA digested with *Msp*I restriction enzyme, separated on 1% agarose gels at 1 V/cm for 16.5 h, and hybridized with labeled M13. The number of scored bands averaged from 8 to 25 per sample. Inbreeding in the Kuhnlein *et al.* (1990) study was based on pedigrees and population sizes.

Because mutations play a role in reducing BS, the populations used in a linear regression study should

have similar increment rates of inbreeding coefficients to minimize the influence of mutations on regression analysis. An inbred line produced by mating between brothers and sisters will reach over 98% inbreeding in 20 generations (Falconer, 1982), whereas an outbred population with 1% increment of inbreeding per generation needs over 98 generations to reach that inbreeding level. The number of mutations will be different when both lines reach 100% inbreeding. Therefore, highly inbred lines should be excluded from those outbred populations. If the two highly inbred chicken lines (reproduced by full-sib mating) used by Kuhnlein *et al.* (1990) were excluded, the slope of the regression would be 0.49 (Figure 1), which was much closer to the one obtained from the turkey lines in the present study. The similarity of the two slopes indicates that the models probably reflect the true relationship between BS and inbreeding.

According to the results, the linear model may be used to estimate the inbreeding of populations based on the BS of DNA fingerprints. However, the populations should be outbred and the method used to produce DNA fingerprints and to measure BS should be the same procedure including probes and restriction enzymes.

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