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DNA fingerprinting: a tool for determining genetic distances between strains of poultry

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Summary. DNA fingerprinting, a technique based on the detection of hypervariable minisatellite regions in DNA restriction fragments, was tested for its applicability to conduct population genetics in poultry. Using MspI digestion and phage M13 DNA as a probe, between 25 and 35 minisatellite-containing DNA fragments were observed per bird. Comparison of the banding pattern of offspring with their parents revealed that the bands were inherited as stable genetic traits. The variability of the DNA fingerprinting pattern was reduced in inbred strains. DNA fingerprints of chickens from five well-defined populations of known genetic relationships were analyzed and indices of genetic distances were computed. They correctly reflected the history of these strains, indicating that DNA fingerprinting may be a powerful tool to characterize genetic relationships between different breeding populations of the same species.

Key words: DNA fingerprinting – Poultry – Inheritance – Inbreeding – Genetic distance

Introduction

DNA fingerprinting is a technique for detecting genetic differences at a large number of hypervariable DNA loci in a single test (Jeffreys 1987). Such hypervariable loci are regions which contain tandem repeats of short DNA segments. Variability arises from differences in the number of repeats. DNA fingerprinting has already been widely used for identifying individuals and for parentage testing (Jeffreys et al. 1985; Burke and Bruford 1987; Wetton et al. 1987), but its suitability to conduct population genetic studies has not yet been demonstrated. For this purpose we analysed chickens from five well-defined strains of poultry whose genetic relationships were known, and tested whether the relative genetic distances computed from the DNA fingerprinting patterns correctly reflect the history of these strains.

Materials and methods

All strains except the French Broiler Breeder lines were maintained at the Animal Research Center in Ottawa. Strains S and K are White Leghorn strains developed at Cornell University in 1936. They were derived from a common genetic base, except that the founders of strain K included a few commercial birds (Kimber) introduced in 1936 and 1940. Thereafter, the strains have been reproduced as closed populations. Strain S was selected for susceptibility to Marek's disease whereas strain K was selected for resistance to Marek's disease, high egg production and egg weight (Hutt and Cole 1947; Cole 1968; Cole and Hutt 1973; Gavora et al. 1979). Since 1966, the two strains have been maintained at the Animal Research Centre in Ottawa by random mating without selection. Strain 7 is a random-bred White Leghorn strain of broad genetic base which was synthesized in 1960 from four North American commerical stocks and has since been maintained without selection (Gowe and Fairfull 1984). Strain WG was derived from a substrain of strain 7 in 1969 and was developed by inbreeding with selection for resistance to Marek's disease and high egg production. Strain NH is a meat-type breed of New Hampshire and has been maintained as a closed population for more than 20 generations (Grunder et al. 1972). The two lines of French Broiler Breeder were maintained at the Macdonald College Poultry Unit. The lines were selected for six generations using a high (fatty line) or low (lean line) ratio of abdominal fat to live weight in males at 9 weeks of age (Leclercq et al. 1980).

DNA was extracted from heparinized blood according to Maniatis et al. (1982) and dissolved in 5 m*M* TRIS pH 7.5, 0.1 m*M* EDTA pH 8.0. A 20 µl mixture containing 5 µg DNA and 18 units of MspI and MspI buffer was incubated at 37 °C for 2–4 h, electrophoresed in a 1% agarose gel (1 V/cm for 16.5 h) and transferred to nitrocellulose membranes. Prehybridization, hybridization and washing was carried out according to the method of Vassart et al. (1987), using ^{32}P-labelled M13 mp9 single strand DNA as a probe. ^{32}P-labelling was carried out with

an oligo-prime labelling kit (Pharmacia) and yielded specific activities between 5×10^8 and 10^9 dpm per μg of DNA. Fifty nanograms of probe was used per hybridization reaction. The filters were exposed to Kodak XAR-5 film at −70 °C for 24–62 h with one or two Cronex intensifying screens. The resultant autoradiographs were scanned with a computer-linked densitometer.

The index of genetic distance (D) between two populations was calculated as $D = -\ln(I)$, where I is the genetic identity index determined from the DNA fingerprinting patterns of six birds from each population according to the equation:

$$I = \frac{1}{N} \cdot \sum_{i=1}^{N} \frac{2\, v_i^{(1)} \cdot v_i^{(2)}}{[v_i^{(1)}]^2 + [v_i^{(2)}]^2}.$$

N is the number of different bands scored in the two strains and $v_i^{(1)}$ and $v_i^{(2)}$ are the frequencies of band i in populations 1 and 2, respectively. The index was adapted from the genotypic identity measure of Hedrick (1971) and is the probability of drawing the same allele from both strains in successive trials standardized by the average probability of drawing the allele from the same strain. Genetic variability within poultry strains was determined according to the equation:

$$V = 1 - \frac{1}{N} \cdot \sum_{i=1}^{N} v_i$$

where v_i is the frequency of band i and N is the number of bands scored.

The average number of bands which could be scored per bird varied between 25 and 35, depending on the quality of the blot and the exposure time during autoradiography but not on the strain being analyzed. For determining genetic distances between two strains, DNA samples of representative birds were, therefore, run on a single blot.

Results and discussion

The inheritance of DNA fingerprinting patterns was tested in two families of chickens, each consisting of the two parents and three offspring (Fig. 1). All of the 28 bands which could be scored reliably in each offspring could be traced back to one of the respective parents. The result indicates that, as has been shown for other birds and species (Wetton et al. 1987; Burke and Bruford 1987; Jeffreys et al. 1985; Jeffreys and Morton 1987; Jeffreys et al. 1987; Vassart et al. 1987), the DNA fingerprinting patterns are inherited as stable genetic traits.

The variability of DNA fingerprinting patterns within strains of poultry decreases with the degree of inbreeding (Fig. 2). Analysis of six chickens of strain S (39% inbreeding, Gavora et al. 1979) revealed polymorphisms in 95% of the bands scored, whereas in the inbred line WG (76% inbreeding, Gavora et al., unpublished results) only 75% of the bands were polymorphic. The indices of band variability were 0.51 and 0.24, respectively.

In order to establish whether DNA fingerprinting can be used to determine genetic relationships, four strains with similar indices of band variability (and, hence, inbreeding) were analysed. Indices of genetic distance ('Materials and methods') computed between pairs of strains reflected their relationships and history (Table 1, Fig. 3). Strain NH, a closed population maintained without selection for more than 20 generations, was most distant from strains S, K and 7. This data is compatible with NH being a meat-type breed of New Hampshires (Grunder et al. 1972), whereas the other three strains are egg-type White Leghorns. Of the White Leghorn strains, S and K are closer to each other than either one is to strain 7. This closeness reflects that strains S and K were derived from a common genetic base in 1936, whereas strain 7 was formed in 1959 from four commercial stocks of White Leghorns virtually unrelated to strains S and K.

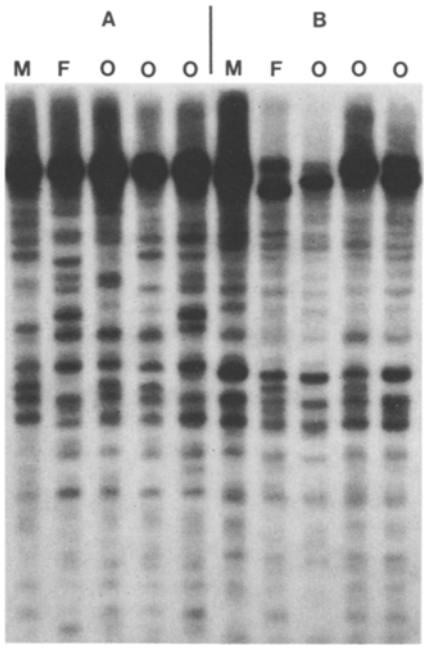

Fig. 1. DNA fingerprints of two families of French Broiler Breeders (A, B). Families A and B are from the lean and fatty line, respectively. Male and female parents are indicated by M and F, respectively, and offspring by O. The top bands were evaluated on a less heavily exposed autoradiograph

Table 1. Genetic distances between five strains of chickens[a]

	S	7	NH	WG[b]
K	1.25	1.66	2.22	2.15
S	–	1.75	2.38	2.62
7		–	2.23	1.87
NH			–	3.12

[a] The genetic distances were computed as indicated in the text by analyzing the DNA fingerprints of 6 chickens per strain using a computer-linked autoradiography scanner. Nearly identical values were obtained by evaluating five chickens per strain

[b] WG is an inbred line (Fig. 2). The genetic variability computed from the DNA fingerprinting pattern was 0.24. All other strains had a variability between 0.51 and 0.52

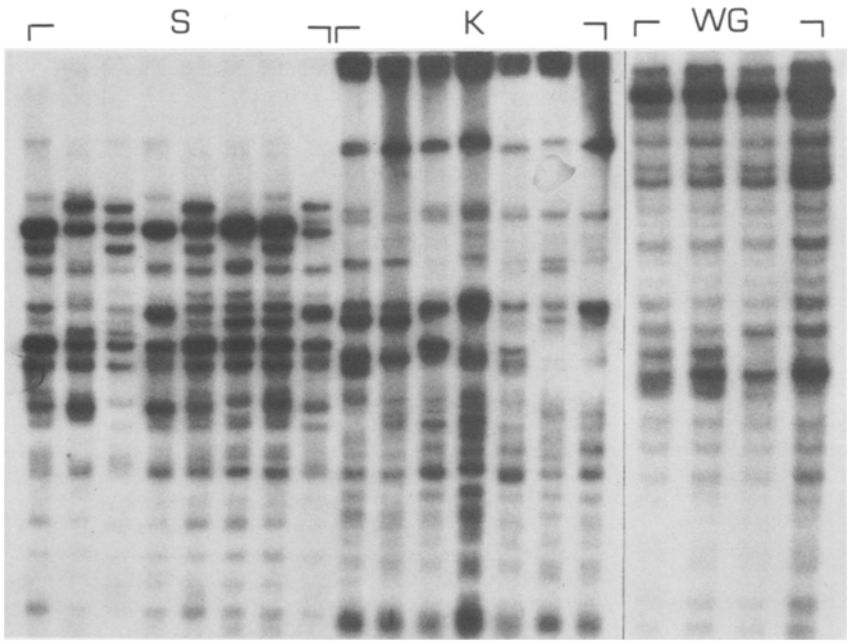

Fig. 2. DNA fingerprints of chickens from strains S and K and inbred line WG

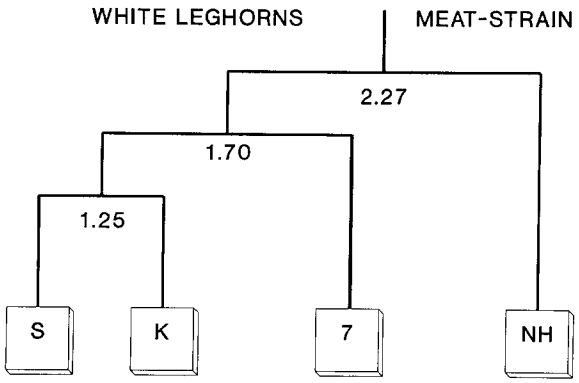

Fig. 3. Genetic relationship among the four strains of chickens based on the genetic distances shown in Table 1. The branch point of line WG is not shown. Its genetic distances from the other strains are large as expected from the high degree of inbreeding which is reflected by a low genetic variability (Table 1). Nevertheless, the relative genetic distances place it closest to its parent strain, 7

The relative distances between populations are expected to increase due to differential selection, genetic bottlenecks and inbreeding. Thus, the inbred line WG was the most distant from all other strains. This line was derived from a substrain of strain 7 in 1969 and was developed by inbreeding with selection for resistance to Marek's disease and high egg production. The relative genetic distances (Table 1) place WG next to strain 7 followed by strains K, S and NH, an order which is consistent with WG being derived from strain 7 and being a White Leghorn strain. Notably, WG is closer to strain K than S, which may reflect that WG and K had both been selected for Marek's disease resistance and high egg production, whereas S had been selected only for Marek's disease susceptibility.

In this analysis, the proposed index of genetic distance correctly delineated genetic relationships between populations within the same species. Whether this method is also applicable to establishing evolutionary relationships between species remains to be determined. In our analysis, the largest genetic distance observed was that between the inbred White Leghorn line WG and the New Hampshire meat strain NH. When 6 birds from each strain were compared, only 3 bands were common to both strains out of a total of 86 bands scored, indicating that the distance between these 2 strains is at the limit of resolution of this method. However, multiple hybridizations with probes recognizing different families of minisatellites or hybridization with probes which recognize more stable minisatellites than those detected by M13 DNA should make it feasible to establish genetic relationships between more remote breeding populations or even between different species.

DNA fingerprinting might have practical use in poultry breeding. It provides a means to identify strains of poultry and to determine the genetic variability and, hence, the selection potential within a breeding population. Measurements of genetic distances using DNA fingerprinting may prove useful in predicting and optimizing heterosis which is known to be associated with egg production traits.

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