J. Appl. Genet. 38(2), 1997, pp. 173-178

DNA fingerprints generated by R.18.1 DNA probe in two stocks of chickens: Green Legged Patridgenous (GLP) and Rhode Island Red (RIR)

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Abstract. Hybridization of a multi-locus DNA probe, R 18.1, to genomic DNA from poultry showed a highly polymorphic fingerprint pattern. The detected DNA fingerprints are individually specific and differ between Green Legged Patridgenous (GLP) and Rhode Island Red (RIR) stock of chickens. The average numbers of detected bands in RIR were 18.68 and in GLP – 15.33, but the average band sharing levels were 0.619 and 0.431, respectively. The level of polymorphism may be connected possibly with a higher level of inbreeding in the examined stock of chicken.

Key words: DNA fingerprints, molecular markers, R 18.1 multi-locus DNA probe, chickens.

Introduction

Recent strategies in molecular biology have made it possible to use different techniques for the development of genomic maps. Molecular depicted linkage maps help to closely monitor genomic organization and may be used as a source of information to improve animals breeding. During the last two years the creation of a genetic linkage map of the chicken has been in progress. These maps show distances and positional relationships of genes and/or other genetic elements. The most commonly used techniques are the analysis of restriction

Received: July 1996.
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fragment length polymorphism and microsatellites or the variable number of tandem repeat sequences. The randomly amplified polymorphic DNA (RAPD) technique can also be used to analyse the genome of any species in a short time and without prior sequence knowledge (WELSH, MCCLELLAND 1990).

The aim of the genome mapping is to obtain a detailed knowledge of the number and arrangement of genes on chromosomes either by mapping cloned genes into a gene map or by detecting marker-trait linkages in specific crosses. This can improve agriculturally useful animals by enhancing economically important traits, which can not be done by breeding techniques.

Highly polymorphic DNA markers, such as simple sequence repeats or microsatellites have made it possible to detect many loci and to construct linkage maps.

Minisatellite probes such as Jeffreys 33.6 and 33.15 (JEFFREYS et al. 1985a), bacteriophage M13 (VASSART et al. 1987), R 18.1 (HABERFELD, HILLEL 1991) have already been used to detect a high level of polymorphism. The obtained DNA fingerprints serve as a powerful tool for the identification of used individuals, parentage testing and linkage analysis (GILL et al. 1985, JEFFREYS et al. 1985b, 1986).

In the mammals haploid genome is 3.3×10^9 bp and it is 2.75 times larger than the avian haploid genome size of 1.2×10^9 bp (LEVIN 1980, OLOFSSON, BERNARDI 1983). This might influence the frequency and types of microsatellite-like repeats (MORAN 1993). In the genome of vertebrates probably as many as 10^5 microsatellites are present (LITT, LUTY 1989, LOVE et al. 1990).

DNA fingerprinting is a procedure using multilocus probes that detect mini- and microsatellite regions of tandem repeats hypervariably regions.

DNA fingerprints patterns are readily used for the identification of individuals, families, breeds and lines of animals.

This technique can also identify DNA markers linked to quantitative trait loci (QTL) (DUNNINGTON et al. 1992, PLOTSKY et al. 1993). Most DNA fingerprinting bands represent independent loci, and therefore this pattern reflects a broad screening of the genome (HILLEL et al. 1989).

For the present study, we want to establish lines of chickens based on crosses between individuals from the two breeds of chickens: Green Legged Patridgenous preservable flocks and highly productive Rhode Island Red. The fingerprinting pattern for these two highly inbred flocks is going to be obtained and analysed, using R 18.1 multilocus DNA probe. The objective of this study was to evaluate the genetic variation within and among highly inbred chicken lines, thus providing a basis for the future genetic map studies.

Material and methods

Chickens

For the present map study project we have chosen two lines of chickens: Green Legged Patridgenous preservable flocks – 12 male and Rhode Island Rock – 41 female, as the parental lines. These lines of chickens differ in egg productivity, polymorphism of proteins in eggs and blood and in egg defects (weak shell, shell-less egg). Green Legged Partigenous breed is a Polish native breed of chickens maintained as a preservable flock, characterized by a small body and egg size, and by a very low egg production (about 40 eggs in the first 100 days of laying). A highly productive Rhode Island Red strain will serve as a highly productive commercial layer (about 81 eggs in the first 100 days of laying) to produce crossings (Table 1). These lines of chickens were maintained in separate cages and kept in genetic purity in the Institute of Genetics and Animal Breeding in Jastrzębiec (Poland). They were free of major poultry pathogens.

Table 1. Mean values (± SD) for studied traits of Rhode Island Red (RIR) and Green Legged Patridgenous (GLP) chicken stocks

Traits	GLP	RIR
Egg weight (g)	48.9 ± 4.1	59.4 ± 4.0
Egg shell strength (kg)	2.86 ± 0.92	3.22 ± 0.58
Egg laying during first 100 days	39.0 ± 11.5	80.8 ± 10.8

Preparation of genomic DNA

DNA was isolated from avian red blood cells as described by HILLEL et al. (1989).

Southern blotting and hybridization

10 µg of DNA samples were digested with 2-3 units per 1 µg of DNA of restriction endonuclease: Hinf I or Hae III (N.E. Biolabs). After digestion and checking the quantity and purity of DNA (OD 260/280 should be 1.8-2.0), 8 µg of digested DNA were placed in a well of 0.8% agarose (Sigma) gel (20 cm long) in 1 × TBE buffor. Electrophoresis was run at 30 volts for about 48-60 hours, until the molecular weight marker is at the desired position. After electrophoresis blotting on Hybond N^+ membrane (Amersham) was done dur-

ing at least 16 hours. After DNA fixation on the membrane, genomic probe R.18.1 (HABERFELD, HILLEL 1991) was labelled with ^{32}P-dCTP according to FEINBERG and VOGELSTEIN (1984). Hybridization to the genomic probe R 18.1 was carried out overnight at 65°C in 25 ml of 0.263 M Na-phosphate buffer and 7% SDS.

Blots were washed twice in 0.263 M Na-phosphate, 1% SDS and followed by two washes in 1 × SSC, 0.1% SDS for 15 min. at 65°C each.

Autoradiography was performed during 1-3 days at –80°C, using Kodak films (Amersham) in the presence of an intensifying screen.

Determination of band sharing

Band sharing (BS) was calculated according to NEI and LI (1979) and it included only clearly distinguishable bands larger than 2 kb. $BS = 2(N_{ab})/(N_a+N_b)$, where BS = level of band sharing between lines of chickens a na b, N_{ab} = numbers of bands shared between lines a and b, N_a = the total number of bands for line a, and N_b = total number of bands for line b.

Results and discussion

We used the R 18.1 probe developed in the Department of Genetics, Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot, Israel, which gives highly polymorphic DNA patterns after the digestion of chicken DNA with Hinf I (HABERFELD et al. 1991). R 18.1 is constructed of 1027 bp, contains six poly (GT) stretches and hybridizes to the genomic DNA of animals. The same probe hybridized to cattle, sheep and human DNA revealed fewer polymorphic loci.

In our experiment the hybridization of the probe R 18.1 to Hinf I digested chicken DNA showed a highly polymorphic DNA pattern (Figs. 1-4). In the analysis of 41 unrelated individuals of Rhode Island Red female chickens and 12 unrelated Green Legged Patridgenous males, the average number of 18.68 bands per individual was detected in female and 15.33 in male. The band sharing between unrelated individuals was calculated to be 0.619 for female and 0.431 for male (calculated according to NEI, LI, 1979, HABERFELD et al. 1991).

The hybridization pattern of the used microsatellite probe in our case is characterized by a lower number of polymorphic DNA fingerprint bands. It is comparable to loci detected by the 33.6 Jeffreys probe in broiler chickens – 20.0 bands, and layers – 17.25 bands (HILLEL et al. 1989). In the same paper,

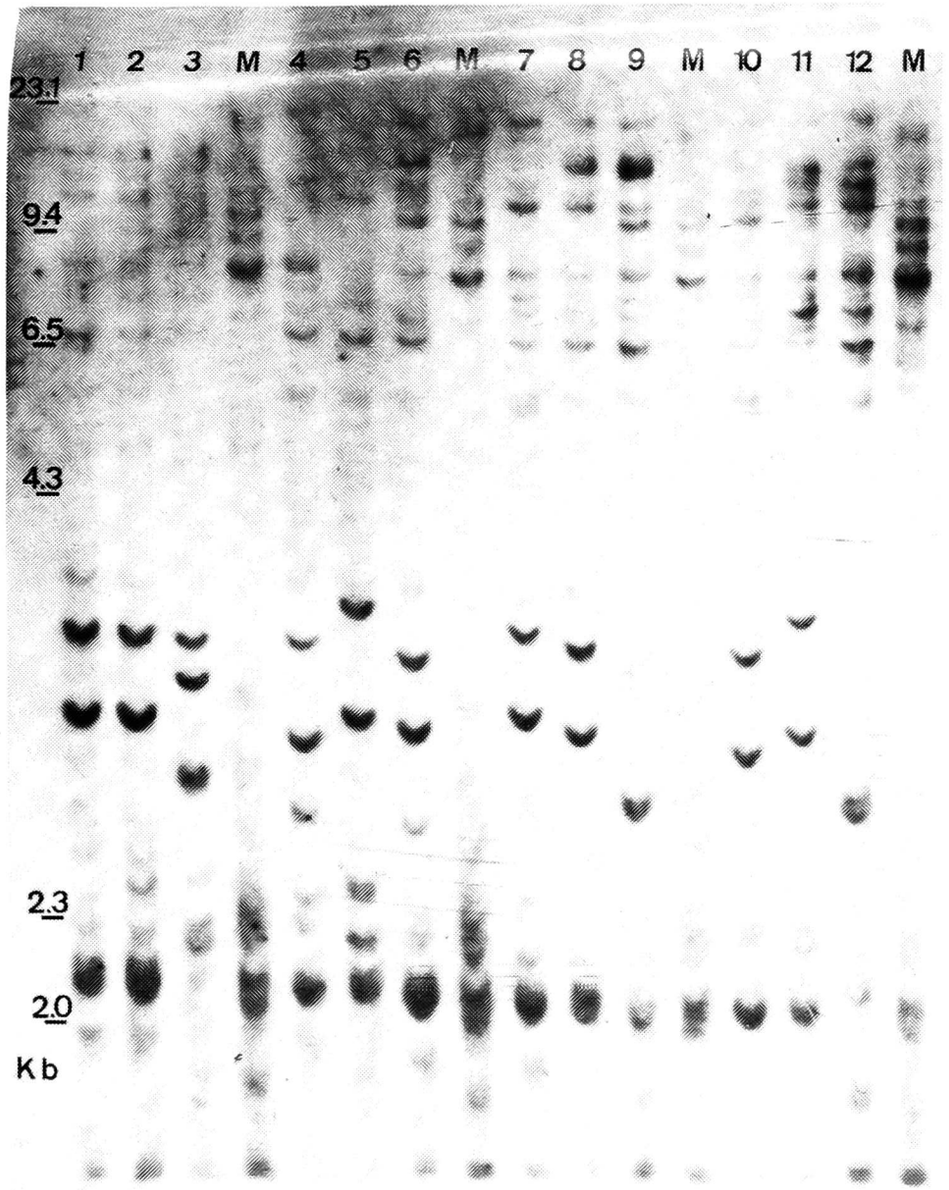

Fig. 1. DNA fingerprints pattern of 12 unrelated male Green Legged Patridgenous preservable flock obtained in hybridization of DNA with R 18.1 probe. 1-12 – numbers of cocks, M – mix from 41 female Rhode Island Red chickens

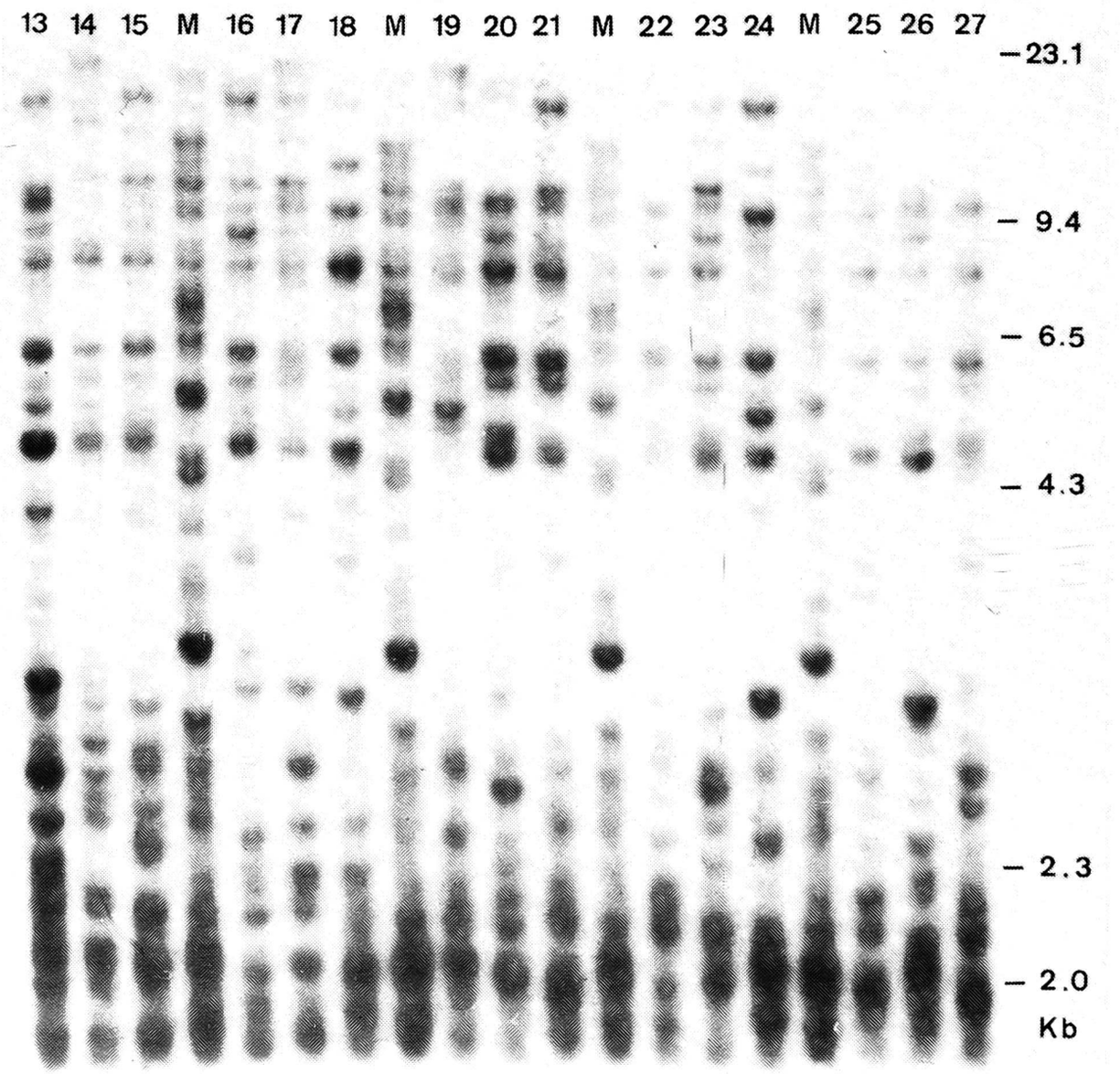

Fig. 2. DNA fingerprints pattern obtained in hybridization of DNA from unrelated individuals (no. 13-27) of Rhode Island Red female chickens and mixes (M) from 12 male Green Legged Patridgenous chickens to R 18.1 probe

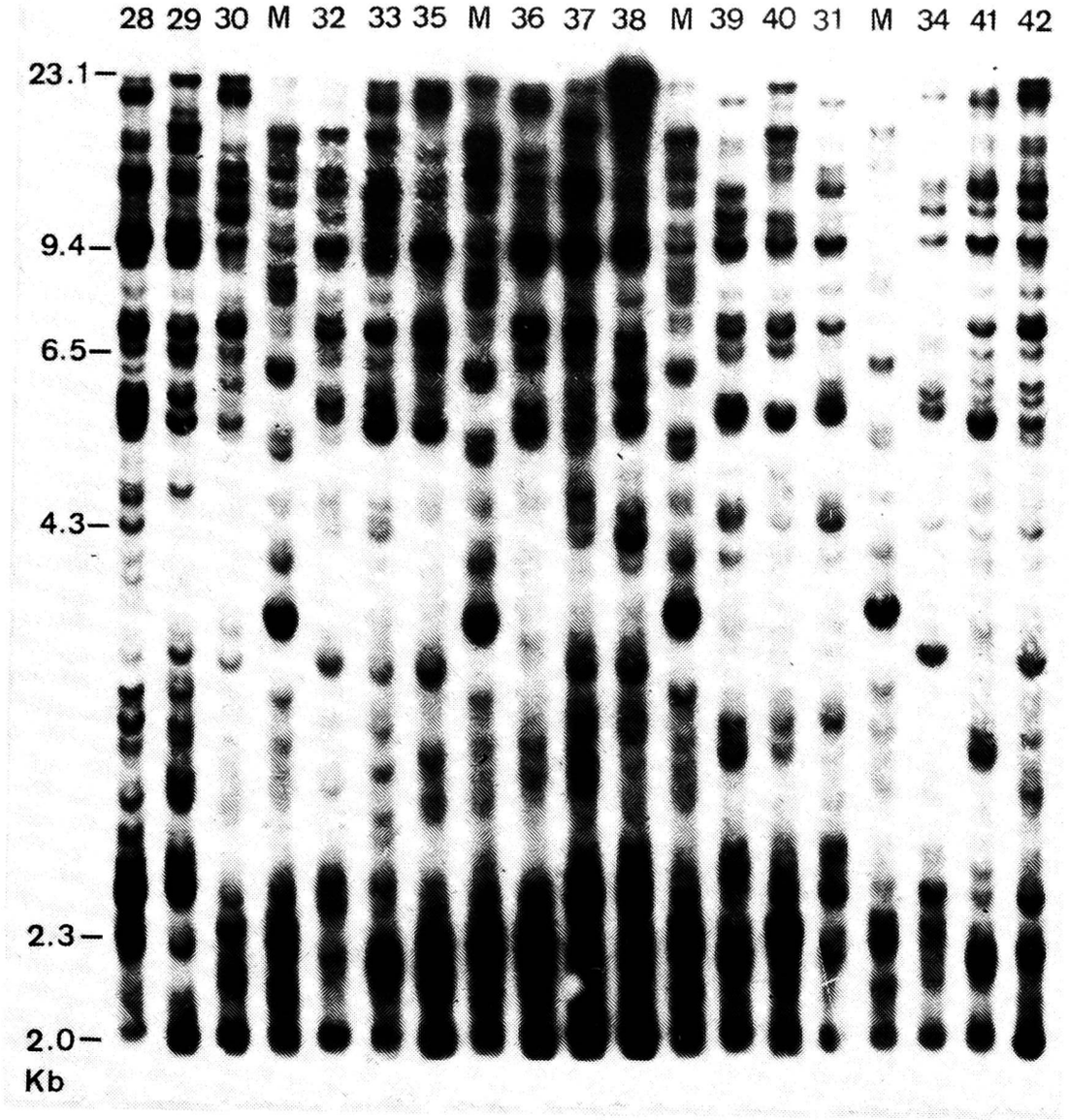

Fig. 3. DNA fingerprints pattern of unrelated female Rhode Island Red chickens (no. 28-42) and mixes (M) of 12 Green Legged Patridgenous chickens hybridized to R 18.1 probe

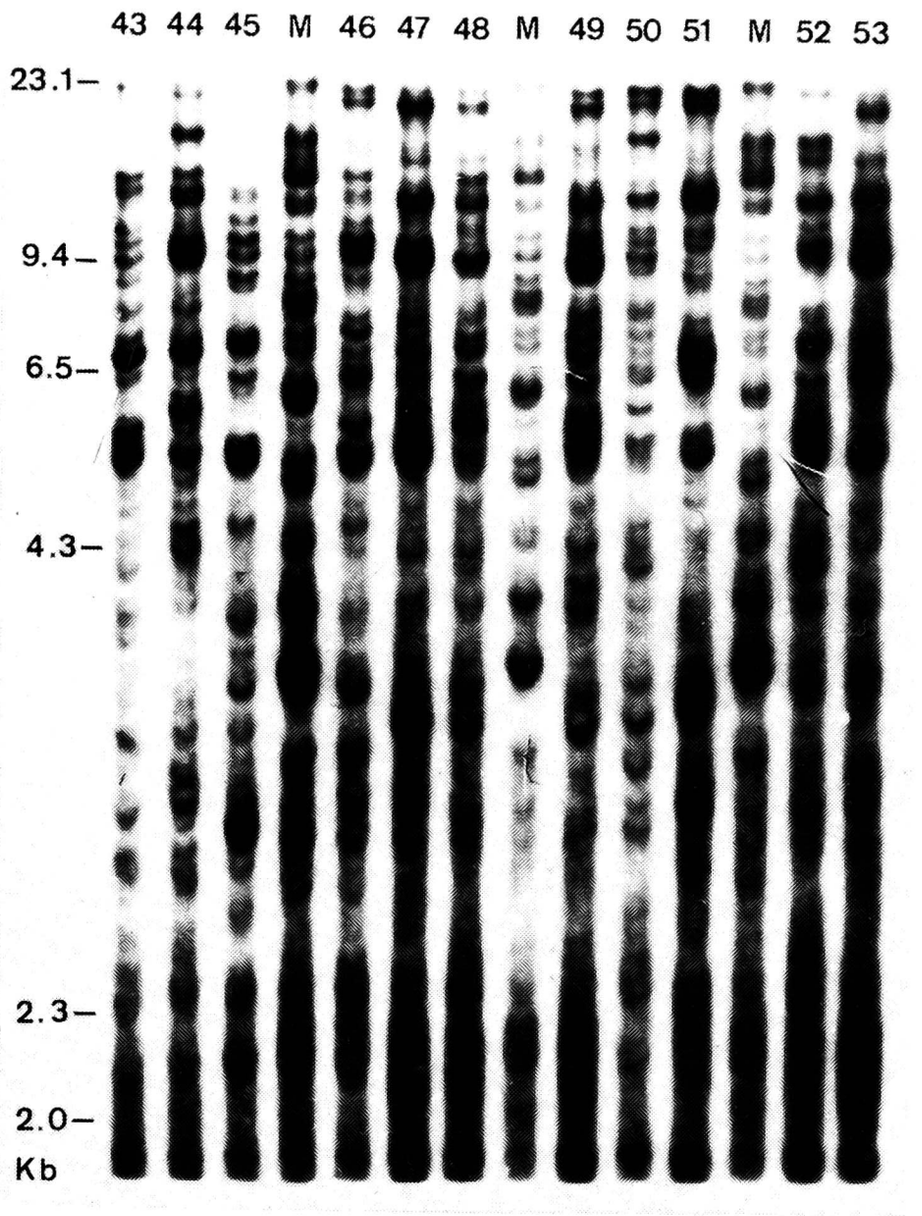

Fig. 4. DNA fingerprints pattern of Rhode Island Red unrelated female chickens (no. 43-53) and mixes of 12 Green Legged Patridgenous cocks (M) hybridized to R 18.1 probe

when 11 unrelated broilers were examined with the same probe, the mean of 28.5 bands was observed. Fingerprint loci detected by the probe 18.1, the same as in our work, in broiler chickens was 27.8, which was shown by HABERFELD et al. (1991).

The level of band-sharing in our chickens is 0.431 for sires and 0.619 for dams. It was higher than in broilers and layers examined by HILLEL et al. (1989), where it was 0.25 and 0.21, respectively. In our case the low level of polymorphism may be possibly connected with a small population of tested chickens. This pattern should be tested more carefully by examining bigger populations of chickens.

On the basis of our data, we have chosen 10 sires and 10 dams to obtain crosses (F_1 generation) between these two lines of chickens. They were characterized by the lowest bands sharing to know the organization and localization of genes controlling quantitative trails loci (QTL).

10 family groups (F_1 generation) are actually crossed to each other (brother × sister) to get a progeny. The F_2 generation will be used to map QTL with the aid of microsatellites markers. In conclusion, we would like to point out the validity of this approach to poultry breeding, especially after a higher development of microsatellite probes containing poly (GT), poly (GTG) and poly (GAC/TA) sequences reported by some authors (ALI et al. 1986, EPPLEN 1988, SCHAFER et al. 1988, KASHI et al. 1990, HABERFELD, HILLEL 1991). These probes detect microsatellite loci and in the case of chickens, they may be considered as an important genetic markers to construct linear models. These models can be used for the selection of important quantitative traits within families. It is also probable that the use of DNA fingerprints may help to eliminate an unwanted genome from a given population of animals or for the selection of a desired one.

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