GENETIC VARIATION IN JUVENILE GROWTH TRAITS OF CHICKENS AS INFLUENCED BY DNA FINGERPRINT MARKERS

S.K. Mishra[1], V. Thiagarajan[2], A.M. Nainar[2] and P. Ramadass[2]

[1] Project Directorate on Poultry, Hyderabad, India- 500030
[2] Department of Animal biotechnology, Madras Veterinary College, Chennai, 600007, India

INTRODUCTION

Amongst the various available DNA markers, DNA fingerprints are the most documented in genetic studies of chickens (Dunnington *et al*., 1994 and Lamont *et al*., 1996). The utility of the DNA fingerprints (DFP) as genetic markers linked to quantitative trait loci (QTL) coding for economically important traits of chickens has been examined over various laboratories (Dunnington *et al*., 1992 ; Dolf *et al*., 1993 and Lamont *et al*., 1996). Most studies documented so far, are based on DFP probes: 33.6 (Jeffreys *et al*., 1985) and R18.1 (Haberfeld *et al*., 1991). Since majority of the loci detected in chicken genome by 33.6 are not linked to the loci detectable by R18.1 (Haberfeld *et al*., 1991), additional minisatellite loci could be surfaced by bringing in newer multi-locus probe (MLP)- restriction enzyme (RE) combinations, to explore greater number of QTLs segregating in different resource populations. Relying on these assumptions, a study was conducted to generate a DFP based marker system using above two probes in a variant genetic background and evaluate them as genetic markers linked to QTLs controlling few juvenile traits in meat type chickens.

MATERIALS AND METHODS

Genetic resources and sample generation. The study utilized two genetically distinct closed chicken populations, differing significantly from each other for body weight at 6 weeks of age (BW6). A single F_1 male was backcrossed to low weight line females to produce a half sib group of 260 chickens. The whole sib-ship, maintained under standard management and *ad libitum* feeding conditions, was individually evaluated for live-weight at 4, 6 and 8 weeks of age (BW4, BW6 and BW8) and over-all body fatness (indirectly through blood parameters) viz. plasma triglyceride (TGL) concentration, very low-density lipoprotein (VLDL), total cholesterol (TCHOL), during market ages

Genetic analysis of the population was carried out using the DNA profiling data. Selective genotyping was attempted on the whole of back-cross (BC_1) progeny by identifying the highest and the lowest 10% individuals by ranking the whole sib-ship in descending order, trait wise. DNA pooling was carried out for the above selectively genotyped progeny, to yield 2 distinct pools: high pool (HP) and low pool (LP). The respective pools and the individual chickens were fingerprinted in various combinations.

DNA isolation, Processing and Fingerprinting. DNA was isolated for the entire sibship individually, following a standard phenol- chloroform based procedure. DNA fingerprinting was carried out on the selected BC_1 progeny, as per Plotsky et al. (1993) employing RE: AluI,

using probes 33.6 and R18.1 sequentially. The DNA profiles thus generated as fingerprints (DFP) were analyzed by standard gel analysis software.

Analysis of DFP patterns and the statistics applied. The DNA fingerprints (DFP) were analyzed for the number of the distinct bands per each lane, number of polymorphic bands across lanes and average band sharing rates (BS) between pairs of DFP lanes. Statistical procedures included : tail analysis (TA) and linear regression of the individual phenotypic values on each important polymorphic DFP band. The TA was carried out as per Plotsky *et al*. (1993) by comparing the DFPs of contrasting DNA pools (HP/LP) for each trait, in a single blot.

Linear model analysis. The effect of DFP bands on phenotypic values of the BC_1 progeny was estimated using a multiple regression analysis through the SAS statistical package (SAS Institute Inc., 1993). All the six traits were analyzed individually. Here in, the quantitative (trait) values were the dependent variable whereas the covariates : dam and sex of the bird and all the important polymorphic bands, were the independent variables. The DNA profiles of the 30 BC_1 individuals were used for this analysis. The partial regression coefficients obtained for each co-variate was examined for its statistical significance.

RESULTS AND DISCUSSION

DFP analyses and BS rates between parental lines. The DNA fingerprint profiles were primarily used to generate polymorphic DNA profiles to support QTL analyses. RE: *Alu* I was selected as the enzyme of choice based on the findings of Mishra *et al*. (2000). On average, the *AluI*-profiles yielded 30 to 31 bands per individuals with probe 33.6, and 25 bands with probe R 18.1. Up to 16 polymorphic bands were recorded with R18.1 while the 33.6 produced up to 14 polymorphic bands. The average BS between the parental lines was 0.51 using combined profiles of R18.1 and 33.6. Moderate level of BS so observed in this study finds support from the report of Tixier-Boichard *et al*., (1996) who had recorded BS values in the range of 0.2 to 0.25 from unrelated line comparisons.

TA and DFP marker analysis. The TA revealed a number of DFP bands with disparate intensity across the HP and LP tails for each of the trait studied. On an average, 4 bands showed differential intensity and up to 3 bands were distinctly variant across pools for either of the probes. Based on the TA, 13 polymorphic bands per probe per HP-LP pair, were identified and subjected to the linear model analysis. The various DFP profiles unraveled through R18.1 and 33.6 along with TA comparing the HP-LP for BW6 in a randomly sampled 10 individuals are depicted in the figures 1 and 2 respectively. Differences as recorded between the two contrasting pools with respect to presence or absence of polymorphic DFP bands and their intensity for almost all the traits studied are supported by the findings of Lamont et al. (1996) and Lakshmanan *et al.* (1994) who had also identified multiple trait-associated intensity shifts for most of the egg production traits evaluated.

Table 1. Multiple regression analysis

Regression coefficients and S.Es for important DFP bands (Trait wise)				
DFP Bands	BW6	BW8	TGL	TCHOL
R18.1-origin bands				
19.55Kb	-142.18± 64.66*	-172.33 ±96.31	6.4 ± 13.81	-5.71 ± 12.03
15.65Kb	40.7± 54.81	38.59 ±79.40	-18.81 ± 10.02	-14.43 ± 8.31*
15.2Kb	70.6 ± 50.43	220.06 ± 64.66**	-26.97 ± 8.97**	-17.92 ± 8.34*
13.9Kb	-65.8 ± 51.65	-192.17± 66.05 **	15.07 ± 10.22	21.80 ± 7.37**
12.8Kb	-132.27± 64.18*	6.85 ± 99.6	10.89 ± 14.47	1.77 ±11.37
11.2Kb	-87.08 ±55.19	-146.22 ±79.64	15.77 ± 10.16	17.32 ± 8.21*
10.25Kb	76.94 ±56.03	150.12 ± 78.17	-27.44 ± 9.97*	-17.13 ± 8.64
33.6-origin bands				
24.7Kb	-45.56 ±78.65	-121.71 ± 108.59	31.2 ± 13.72*	22.97 ± 11.65
8.8Kb	-147.33±47.98**	-71.48 ± 81.88	7.78 ± 10.94	-1.12 ± 9.38
4.83Kb	141.0 ± 53.33*	89.30 ± 82.10	-8.72 ± 11.55	-11.70 ± 9.36
4.75Kb	115.77 ± 51.91*	-28.88 ± 81.23	11.63 ± 11.42*	17.39 ± 8.86

* indicates significance at p< 0.05.
** indicates significance at p< 0.01.

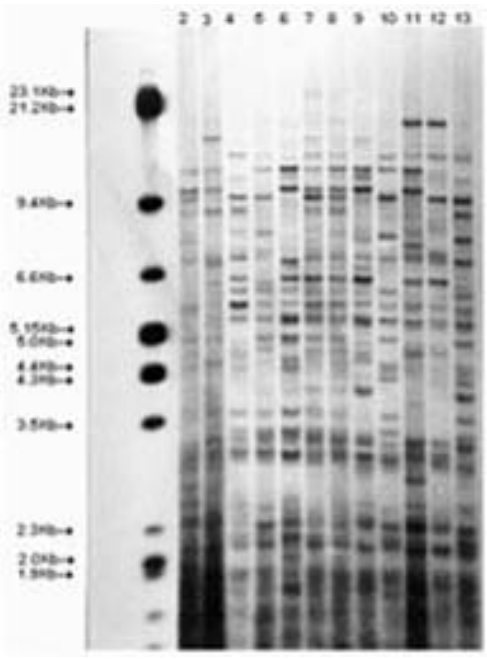

Figure 1. DNA finger prints showing *Alu*I-R18.1 profiles of randomly sampled BC_1 Individuals. Lanes 7 and 8 are the HP-LP pairs for BW6

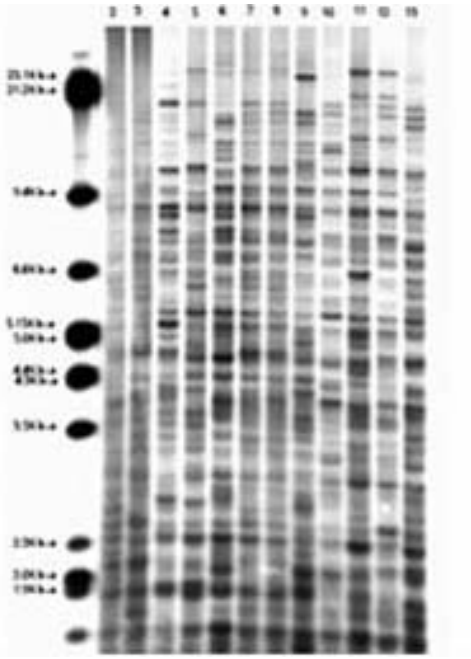

Figure 2. DNA finger prints showing *Alu*I-33.6 profiles of randomly sampled BC_1 Individuals. Lanes 7 and 8 are the HP-LP pairs for BW6

Linear model analysis. The results of multiple regression analysis for 4 traits are presented in the table 1. The analysis revealed significant ($p<0.05$) DFP band effects for two R18.1 origin bands and two 33.6 origin bands for BW6. Two R18.1 origin DFP bands also significantly ($p<0.01$) affected BW8. Similarly, two bands each, originating from either of the probes were significantly affecting the TGL values of the BC_1 progeny. The TCHOL was significantly affected by four R18.1 origin bands. A 15.2Kb band that was positive in its effect on BW8 exhibited negative influence on both TCHOL and TGL. None of the analyzed- DFP bands bore any significance on BW4 and VLDL. Analysis of the other two co-variates showed that sex effects were largely non-significant but the dam effects were significant ($p < 0.01$) in affecting all the traits. The significant band effects for the four 33.6 origin bands and the five R18.1-origin bands as realized, would otherwise mean that the phenotypic expression of the BC_1 broilers was influenced by the occurrence of these bands, in their profiles apart from other genetic factors.

Partial regression of the phenotypic performance of chickens upon DFP band's occurrence, along with other co-variates (sex, dam etc.) has also been attempted by earlier workers (Plotsky *et al*., 1993 ; Dolf *et al*.,1993 and Lamont *et al*., 1996). The significant band effects realized from the above bands (2 to 4 bands each) influencing the BW6, BW8, TGL and TCHOL as recorded in this study were therefore, in the expected direction and indicate the potentiality of these bands as genetic markers for the said traits.

CONCLUSION

As evident from the results, the significant association of the DFP bands with BW6, BW8, TGL and TCHOL and the distinct TA associated shifts for all these traits are highly suggestive of primary linkages of the said DFP bands with the studied traits. However, the exact nature of association of these markers with the said traits would need to be studied in details for quantifying their effect on the respective traits.

ACKNOWLEDGEMENTS

The authors thank Sir. A. J. Jeffreys and Dr. J. Hillel for providing their probes for this study. The support of the CSIR, New Delhi for the CSIR-SRF grant is also acknowledged.

REFERENCES

Dolf, G *et al*. (1993) In DNA Fingerprinting: State Of The Science, P.371-377, Editors S.D.J. Pena *et al*. Birkhauser Verlag, Basel, Switzerland.

Dunnington, E.A *et al*.(1992) *Poul.Sci*. **71** : 1251-1258.

Dunnington, E.A *et al*. (1994) *Poul. Sci.***73** : 1218-1225.

Habarfeld, A *et al*.(1991) *Anim. Genet.* **22** : 299 -305.

Jeffreys, A.J *et al*.(1985) *Nature* **314** : 67-73.

Lakshmanan *et al.* (1994) *Proc 5th WCGALP* **21** : 205-208

Lamont, S.J *et al*.(1996) *Anim. Genet.***27** : 1-8.

Mishra, S.K *et al*. (2000) *Proc 21st world's Poultry Congress*, Montreal, Canada.

Plotsky, Y *et al*.(1993) *Anim. Genet.* **24** : 105-110.

Tixier-Boichard, M. *et al*.(1996) *Anim. Genet.* **27** : 163-169.

SAS Institute. (1993) SAS User's Guide: Version 6, SAS Institute Inc. Cary, North California.