# PRELIMINARY ANALYSIS OF ENDOGENOUS VIRAL GENES IN FAYOUMI AND RHODE ISLAND RED CHICKENS

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## SUMMARY

DNA sequences related to avian leukosis virus have been identified in the genome of chickens from a Fayoumi strain, kept with a random mating system, and 2 Rhode Island Red lines, divergently selected on residual feed intake. Digestion with SacI enzyme and hybridization with RAV-2 sequences showed 14 different bands in the Fayoumi and 8 in the RIR. Inheritance pattern of the bands was consistent with the existence of one endogenous viral locus per band. The Fayoumi showed a higher variability for the number of bands per animal (from 0 to 7) than the RIR lines (from 3 to 7). Level of heterozygosity appeared also higher in the Fayoumi. Such observations might be related to different genetic origins and selection objectives between the 3 populations. Comparison with the classification known in White Leghorns was not straightforward, further analysis of DNA polymorphism and viral expression are needed.

## INTRODUCTION

Endogenous viral genes (*ev*-genes) consist in DNA sequences related to an avian leukosis virus (ALV), which have been integrated into the chicken genome. Transcription of *ev*-genes can be either complete and lead to the production of an infectious virus belonging to the sub-group E of avian leukosis viruses, or it can be incomplete and lead to the production of some viral proteins (group-specific antigen = gs ; envelope protein = chick-helper-factor or chf), or the gene can be completely silent and be used as a simple marker of the chromosomal region where it stays.

Integration seems to be stable enough so that *ev*-genes behave in a mendelian way, their polymorphism has been particularly well described in White Leghorns where 21 different *ev*-genes have been identified ( Rovigatti and Astrin, 1983; Smith, 1986). However the list is not closed and recent studies have revealed the presence of yet unidentified *ev*-genes in White Leghorns ( Kuhnlein *et al.*, 1989). Some observations made on chickens other than White Leghorns suggested that the pattern of *ev*-genes might be quite different in non Leghorn strains (Smith, 1986).

The purpose of this study was thus to describe the polymorphism of ALV-related DNA sequences in a Fayoumi strain and in two Rhode Island Red (RIR) strains.

## MATERIAL AND METHODS

The Fayoumi strain represents an old egyptian breed, well adapted to hot climate and poor feed. It has been kept as a closed experimental line at Jouy-en-Josas since 1978 (Mérat and Bordas, 1982). Except for body weight, which has been increased with a mild selection pressure, no particular selection objective has been applied. The sample of birds studied consisted in

5 sires and 20 dams, together with 49 male and female chicks sampled among the progeny hatched in 1989.

The two RIR strains originated from a common basis in 1975 and have been, since then, divergently selected for residual feed intake (Bordas and Mérat, 1984). In 1988 lines differed for feed conversion, with 2.74 in the R- and 3.22 in the R+, with a similar laying rate. The sample of birds studied for the R- line consisted in 9 sires and 18 dams together with 24 male and female chicks sampled among their progeny hatched in 1988, the design was 9 sires, 17 dams and 26 chicks for the R+ line.

The breeding flock is not kept in specific pathogen free conditions and birds are vaccinated against major contagious diseases, including Marek's disease. A sample of egg albumen was taken for all the dams and an ELISA assay for the viral p27 protein (modified after Clark and Dougherty, 1980) was used to check that exogenous leukosis was not present.

Blood samples were collected on EDTA and 250 µl of whole blood was kept to extract genomic DNA. Hemolysis was performed with 50 ml buffer containing 0.14 M $NH_4CL$ and 0.017 M TRIS-HCL (pH 7.5), after mild centrifugation the pellet was resuspended in 3 ml buffer containing 50 mM NaCl, 10 mM TRIS-HCL(pH 7.5), 10 mM EDTA, 0.4% SDS with the addition of 0.1 mg/ml proteinase K. The samples were incubated overnight at 42 °C. They were then extracted with phenol and chloroform-isoamyl alcohol (24:1) and precipitated with isopropanol. The precipitate was recovered with a glass rod, washed with ethanol 70%, and dissolved in 2 ml 10 mM TRIS-HCL (pH 7.5) and 1 mM EDTA. DNA yield lied between 500 µg and 800 µg, final concentration was adjusted around 250 µg/ml.

Ten µg DNA were incubated for 3 hours at 37 °C with 80 units SacI restriction enzyme using the proper reaction buffer as specified by the supplier ( Boehringer Manheim ). Owing to a large volume of incubation, digested DNA was precipitated with ethanol 100%, supernatant was removed and DNA was washed with ethanol 70% which was then evaporated, the pellet being resuspended in 20 µl sterile water. Samples were then subjected to gel electrophoresis on a 0.7% agarose gel in TEA buffer (Maniatis *et al.*, 1982) at 2 V/cm for 17 hours. The Raoul I scale supplied by Appligene was used as a size marker. After the run, the gels were stained with ethidium bromide to visualize the DNA. The gels were then treated for 15 mn with 0.15 N HCL . Blotting was carried out onto a charged nylon membrane (Biotrace, Gelman Labs.) in 0.4 M NaOH. After transfer, the membranes were washed with 2xSSPE (Maniatis *et al.*, 1982).

Prehybridization was carried out in plastic bags at 42 °C for at least 4 h in 5xSSPE, 1% SDS, 50% formamide, 0.1% Denhart, 5% Dextran and 200 µg/ml salmon sperm DNA. Hybridization was carried out for 40 h at 42° C, with the same mixture as before except that it contained no salmon DNA but 25 ng of denatured $^{32}P$-labelled RAV-2 for a blot of 400 $cm^2$. After hybridization, the membranes were washed twice for 5 mn at room temperature with 2xSSPE, then 15 mn at 65 °C with 2xSSPE and 0.5% SDS, a final wash was performed at 65 °C for 15 mn with 0.5xSSPE. The membranes were blotted dry and autoradiographed at -80°C using Kodak Safety Film AR and intensifying screens, time of exposure varied from 24 h to 96 h.

The plasmid pRAV-2 is a pBR322 plasmid as described by Kuhnlein *et al.*,(1989) and was a generous gift from Dr. L.B. Crittenden, Regional Poultry Research Laboratories, East Lansing, Michigan. The inserted RAV-2 sequences were separated from the pBR322 with a SalI digestion followed by electrophoresis in low-melting agarose and phenol extraction. A mixture of 100 ng RAV-2 sequences and 250 pg linearized pBR322 was labelled with the random-primed DNA labelling kit of Amersham using 120 µCi of $dC^{\alpha}TP$. The

addition of a minimal amount of pBR322 was useful to visualize the Raoul I scale on the autoradiography.
Reference genomic DNAs containing either ev1, ev3, ev6, ev12 or ev21 were a generous gift of Drs. E.J. Smith and L.B. Crittenden (RPRL, East Lansing, Michigan).

## RESULTS

All the dams were free of exogenous leukosis, according to the ELISA test, so that the detected DNA sequences should represent *ev*-genes. The total number of the different bands observed was 14 in the Fayoumi strain and 8 in the RIR lines. Some characteristics of the individual banding pattern are shown in table 1.

Table 1- Variability in the number of bands per animal according to the strain

| | Fayoumi strain | R- line | R+ line |
|---|---|---|---|
| Average number | 3.5 | 4.3 | 4.7 |
| Standard deviation | 1.6 | 0.8 | 0.9 |
| Minimum | 0 | 3 | 3 |
| Maximum | 7 | 6 | 7 |

Frequencies of the different bands have been calculated for each strain (table 2). They varied from 1% (one animal only had the given band) to 54% in the Fayoumi strain and from 2% to 100% in the RIR lines.

Table 2- Frequencies of the different *ev*-related bands according to the strain, expressed as %

| Fayoumi strain 74 animals | | R- line 51 animals | | R+ line 52 animals | |
|---|---|---|---|---|---|
| kb | % | kb | % | kb | % |
| 25 | 54 | 25 | 67 | 25 | 92 |
| 18 | 19 | | | | |
| 16 | 14 | | | | |
| 15 | 39 | | | | |
| 12.5 | 28 | 12.5 | 33 | 12.5 | 27 |
| 11.5 | 5 | | | | |
| 10.6 | 36 | 10.6 | 100 | 10.6 | 100 |
| 10 | 7 | | | | |
| | | 9 | 100 | 9 | 98 |
| 8.4 | 9 | | | | |
| 7.8 | 22 | 7.8 | 14 | 7.8 | 12 |
| 7.4 | 20 | 7.4 | 14 | 7.4 | 67 |
| | | 7 | 2 | 7 | 19 |
| 6.2 | 53 | | | | |
| 4.5 | 1 | | | | |
| 4.4 | 50 | 4.4 | 98 | 4.4 | 60 |

Generally the frequencies were lower in the Fayoumi and the polymorphism was higher than in the RIR. The 2 RIR lines shared the same

bands with some differences in the frequencies, particularly for the 25kb and 7.4kb, more frequent in the R+, and the 4.4kb, more frequent in the R-. These trends should be followed in subsequent generations of selection.

The segregation of the bands was studied in some families; particularly the progeny of the "null" dam in the Fayoumi strain offered a good opportunity to follow the pattern of inheritance of the sire's bands : no band was uniformly transmitted and each band could be transmitted with or without any of the others, which showed that they could not be allelic and corresponded each to different and independent loci. The sire was thus carrying 6 different ev-loci in an heterozygous state. Families were less informative in RIR lines since parents were most often sharing several bands, uniform transmission of a band to all the progeny was frequent and suggested homozygosity in the parents.

## DISCUSSION

The present results show interesting differences between lines, with a higher level of polymorphism and probably also of heterozygosity of *ev*-genes in the Fayoumi than in the RIR. The 3 populations have about the same genetic size but the Fayoumi has a very special origin and the RIR are being selected, which might explain the different patterns found. Also, levels of inbreeding will have to be calculated in a next step. The finding of a "null" animal is a rare event, yet already found in White Leghorns (Gavora *et al.*, 1989).

Comparison with the classification established in White Leghorns is still difficult. Some bands appear to be similar but genotypes should be further confirmed with the use of a second enzyme, as BamHI, and viral phenotype should be investigated, at least for the gs antigen using a feather pulp test (Kuhnlein et al, 1989). Use of reference *ev* gave expected bands, however a band of similar size with SacI digestion may still correspond to a different DNA sequence according to the strain. For instance, a simultanous study run with a SacI digestion on birds carrying the K mutation, either in a White Leghorn or in a dual-purpose line, showed the expected linkage in the White Leghorn (Bacon *et al.*, 1988), while no linkage appeared in the other line, where a 9.2kb band was present in both k and K hens; this suggests that if a linkage does exist in this line, the linked *ev*-gene can not be identified in the same way as was the *ev*21 in the White Leghorn.

## REFERENCES


BACON, L.D., SMITH, E.J., CRITTENDEN, L.B. and HAVENSTEIN, G.B. 1988. Poult.Sci. 67: 191-197.

BORDAS, A. and MERAT, P. 1984. Ann.Agric.Fenn. 23: 233-237.

CLARK, M. and DOUGHERTY, R.M. 1980. J. Gen. Virol. 47: 283-291.

GAVORA, J.S., KUHNLEIN, U. and SPENCER, J.L. 1989. J.Anim.Breed.Genet. 106: 217-224.

KUHNLEIN, U., GAVORA, J.S., SPENCER, J.L., BERNON, D.E. and SABOUR, M. 1989. Theor.Appl.Genet. 77: 26-32.

MANIATIS, T., FRITSCH, E.F. and SAMBROOK, J 1982. Molecular cloning : a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor,NY.

MERAT, P. and BORDAS, A. 1982. Ann.Génét.Sél.anim. 14: 241-244.

ROVIGATTI, U.G. and ASTRIN, S.M. 1983. Curr.Top.Microbio. Immunol. 103: 1-21.

SMITH, E.J. 1986. In: Avian leukosis, de Boer G.F. (ed), Nijhof, Boston, pp 101-120.