VARIATIONS IN EV-GENE PATTERNS OF LAYER AND BROILER TYPE CHICKENS

H.J.M. Aarts, M.C. van der Hulst, G. Beuving and F.R. Leenstra
Spelderholt, Centre for Poultry Research and Information Services,
Spelderholt 9, 7361 DA Beekbergen, The Netherlands.

SUMMARY

The ev-gene patterns of White Leghorn (WL; 6 lines), Medium Heavy brown egg laying (MH; 4 lines), White Plymouth Rock or broiler breeder dam (WPR; 8 lines) and Cornish or broiler breeder sire (2 lines) type chickens were compared. Southern blot analysis of SstI-digested genomic DNA of 152 chickens revealed that the number of ev-genes in a WL type chicken is about half the number of that in a chicken from the MH, WPR or Cornish type. Also, the number of different genes found within a line was much lower in WL lines (x=10.0 bands/line) as that in the three other types (x= 16.0 in the broiler type lines and 20.5 in the MH lines). When the mean number of different genes per line is divided by the mean number of genes per chicken the same ratio, approximately 4, is found within each of the four types of chickens.

All ev-genes detected contained the "env" (3') part of the viral genome. Only 8 ev-fragments, found in 7 animals of 2 lines, lacked the "gag" (5') part of the viral genome.

INTRODUCTION.

The genomes of almost all vertebrate animals contain endogenous viral sequences. Sequences related to the genomic RNA of Avian Leukosis Virus (ALV) are widespread among the genomes of chickens. More than 20 different ev-genes in chickens have been described (Smith, 1977). Some of these ev-genes are active and can interfere with ALV-infection (Crittenden et al., 1984). Ev-genes are of interest not only because of their possible role in ALV-infection but also because of their random distribution within the host genome. This random distribution makes them suitable for use as molecular markers for quantative traits or other loci (Smith and Crittenden, 1986; Bacon et al., 1987; Kuhnlein et al.,1989).

Except for a few studies (Gudkov et al., 1981; Chernov et al., 1984), the knowledge concerning ALV-related ev-genes is almost exclusively derived from studies of White Leghorns and does not give a complete picture of the patterns of variation of ev-genes within the entire chicken population. In this report the results from Southern blot analysis of the genomic DNA of 152 chickens of various lines by using probes which detect ev-gene sequences were described.

MATERIALS AND METHODS

Chickens. Blood samples of 152 chickens, from 20 commercial lines (4 - 10 chickens/line) were collected. Six of the 20 lines were of the WL, four of the MH, eight of the WPR and two of the Cornish type.

DNA analysis. Seven μg of chicken genomic DNA was digested to completion with the enzyme SstI. The DNA fragments were separated on a 0.7% TAE-agarose gel (TAE buffer: 40 mM TRIS-acetate, 2 mM EDTA) and blotted to a nylon filter (Hybond-N, Amersham). EcoRI-digested RCAS-plasmid ("RCAS"

probe, Hughes et al., 1987), the 1.4 Kb BamH1 fragment of the RCAS-plasmid ("gag" probe; coding region for p27 and part of p19) and the 1.2 kB EcoRI/KpnI fragment of the RCAS-plasmid ("env" probe; coding region for gp85) were labeled with ^{32}P-αATP (Multi-prime, Promega) and were used as probe to detect ev-gene specific sequences. After incubation the filters were washed twice with 2xSSC/0.1% SDS at 65 °C for 30 min and twice with 0.1xSSC/0.1% SDS at 65 °C for 20 min. Lambda digested with HindIII was used as a marker to determine the lengths of the hybridizing fragments.

Statistics. Student's t test was used to compare the number of (different-) bands found within and among each line and within each type of chicken.

RESULTS

Identification. The ev-gene pattern from 152 chickens was studied by Southern blotting analysis (Southern, 1975). The chromosomal DNA was cut by using the restriction enzyme SstI and "RCAS" (Hughes et al., 1987) was used as probe. RCAS contains, besides plasmid DNA, the whole genome of Rous sarcoma virus (RSV) without the src region. Sub-probes from the RCAS plasmid ("gag" and "pol"; see Materials and methods) were used to describe the ev-genes more accurately. Only 8 "RCAS"-hybridizing fragments (4 in 3 individuals of one WL line and 4 in 4 individuals of one WPR line) did not hybridize with the "gag"-subprobe (see table 1). In both lines, the bands lacking gag showed restriction length polymorphisms. All fragments which were found to contain ev-gene sequences also hybridized with the "env" sub-probe.

No deletions or insertions detectable by southern blots were present within the gag regions of the ev-genes within the DNAs investigated (data not shown).

The ev-genes found in this set of animals were identified by the length of the hybridizing SstI fragment. They were not identified according to the nomenclature used in literature for the ev-genes present in the genomes of WL-type chickens, because the total number of different SstI fragments found in the MH, WPR and Cornish types of chickens exceeds far the number of ev-genes described (Smith, 1987). Furthermore almost all ev-genes investigated here seem to be complete in contrast to the relatively high percentage of incomplete ev-genes found by others (Tereba, 1981; Smith, 1987). Also polymorphisms and the complexity of the ev-gene patterns makes it impossible to identify all hybridizing fragments only by restriction enzyme mapping according to the ev-genes described. The use of ev-gene specific flanking probes will facilitate identification of, and development of nomenclature for, ev-genes in chicken lines other than leghorns.

Variation of ev-patterns among different types of chickens. The average number of ev-genes per chicken and the number of different ev-genes per chicken line was determined to estimate the complexity of the ev-gene patterns within WL, MH, WPR and Cornish type chickens. Table 1 clearly shows that chickens from the WL type exhibit less complex hybridizing patterns, compared to the other lines. The number of hybridizing fragments found within the DNA of WL type chickens varies between 1 and 4 with a mean of 2.4. More hybridizing fragments were found whithin the genomes of chickens of the Cornish, WPR and MH types; means of 4.2, 4.3 and 4.8, respectively.

The mean numbers of different hybridizing fragments found within

each line for the WL, WPR, Cornish and MH types is 10.0, 16.0, 16.0 and 20.5, respectively. When the mean number of different fragments per line is divided by the mean number of fragments per chicken the same ratio (L/x), approximately 4.0 (var.: 3.7-4.3; see Table 1), is found within each of the four types of chickens.

Table 1 The average number of different ev-gene fragments per chicken and per line in WL, MH, WPR and Cornish type chickens.

type	line	x	n	L	L/x	x	L	L/x
WL	1	2.1 (0.6)	8	9	4.3			
	2	2.2 (0.6)	9	8	2.2			
	3	2.6 (0.8)	9	16	6.2			
	4	2.5 (0.9)	8	15	6.0			
	5	3.0 (0.0)	4	5	1.7			
	6	1.8 (0.4)	6	7	3.9			
						2.4 (0.4)	10.0 (4.1)	4.2
MH	1	5.6 (0.5)	5	21	3.8			
	2	6.3 (1.2)	9	30	4.8			
	3	3.3 (0.7)	6	14	4.2			
	4	3.9 (0.7)	10	16	4.1			
						4.8 (1.2)	20.5 (6.0)	4.3
WPR	1	5.6 (2.1)	6	26	4.6			
	2	3.7 (0.9)	7	21	5.7			
	3	3.7 (1.5)	6	16	4.3			
	4	4.9 (0.7)	9	10	2.1			
	5	3.7 (0.7)	9	8	2.2			
	6	4.3 (0.4)	8	17	4.0			
	7	4.5 (0.7)	10	18	4.0			
	8	4.1 (0.8)	7	12	2.9			
						4.3 (0.6)	16.0 (5.5)	3.7
Cornish	1	3.1 (1.0)	9	15	4.8			
	2	5.2 (0.7)	6	17	3.3			
						4.2 (1.1)	16.0 (1.0)	3.8

x) average number of ev-gene fragment per chicken.
n) number of chickens.
L) number of different ev-gene fragments within a line.
x) average number of ev-gene fragments per line.
L) average number of different ev-gene fragments per line.
standard deviation is given between parentheses.

Variation of ev-gene patterns among lines of the same type. Not only is variation found in the ev-gene patterns among different types (WL, Cornish, WPR, MH) of animals but also among lines from the same type and also among animals of the same line. There were no significant differences between the

mean number of ev-genes per chicken among the WL lines or among the WPR lines. The MH lines can be separated into two groups, two lines of animals which contain approximately 6 ev-genes and two lines of animals containing 3-4 ev-genes. The values, 3.1 and 5.2, found for the two Cornish lines are significantly different from each other ($p<0.05$). The number of different fragments within each line (L) and the ratio L/x are depicted in Table 1. Only within the MH-lines this ratio is uniform. Within the other type of lines the ratio fluctuates and varies between 1.7 (WL-5) and 5.7 (WPR-2).

DISCUSSION

On average, the mean number of different ev-genes found within a line is a magnitude of four times greater than the mean number of "RCAS" hybridizing Sst1 fragments found within a chicken. The exact meaning of this (factor L/x) is as yet not clear. Either it could mean that each ev-gene locus contains at least four alleles or that this factor represents a common feature of the ev-gene loci within the chicken genome. Ev-gene specific or ev-gene specific flanking probes could clarify this matter.

From the mean number of endogenous viral bands per chicken, it is obvious that the number of endogenous viral gene loci is higher in MH and broiler type chickens than in WL type chickens. Both new integrations of viral genomes and polymorphism within the ev-gene loci could be responsible for the variation in the hybridizing patterns. The mean cause for the higher number of hybridizing bands in the MH, WPR and Cornish compared to the WL type chickens might be an increase in the number of ev-gene loci in the MH and broiler type chickens or a decrease of ev-gene loci in WL type chickens by intense selection. When the last hypothesis is true the MH, WPR and Cornish types would be closer to the "natural" state. The difference in ev-gene number might be coincidence, but it is worthwhile to examine if these differences in ev-gene loci were caused by the selection history of the different types of lines.

REFERENCES

Bacon, L.D., Smith, E., Crittenden, L.B., and Havenstein, G.B. 1987 Poultry Sci. 67:191-197.

Chernov, M.V., Gudkov, A.V., Obukh, I.B. 1984. Fol. Biol. (Praha) 30:342-348.

Crittenden, L.B., Smith, E.J., and Fadly, A.M. 1984. Avian Dis. 28: 1037-1056.

Gudkov, A.V., Obukh, I.B., Serov, S.M., and Naroditsky, B.S. 1981. J. gen. Virol. 57: 85-94.

Hishinuma, F., Debona, P.J., Astrin S., and Skalka, A.M. 1981. Cell 23: 155-164.

Hughes, S.H., Greenhouse, J.J., Petropoulos, C.J., and Sutrave, P. 1987. J. Virol. 61: 3004-3012.

Kuhnlein, U., Sabour, M., Gavora, J.S., Fairwfull, R.W., Bernon, D.E., 1989. Poultry Sci. 68: 1161-1167.

Smith, E.J., and Crittenden, L.B. 1986. Avian Path. 15: 395-406.

Smith, E.J. (1987). In: Avian leukosis, ed. de Boer, G.F., Martinus Nijhoff Publishing, Boston.

Southern, E.M. 1975. J. Mol. Biol. 98: 503-517.

Tereba, A. 1981. J. Virol. 40: 920-926.