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Different endogenous viral loci in Cornish and White Plymouth rock chickens

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Summary. Endogenous viral (*ev*) loci were studied in three broiler lines. In 5 birds of each of line cw1 and line cw2 (White Plymouth Rock lines) 19 and 14, respectively, different *Sst*I *ev*-junction fragments were found, while in 8 R line birds (Cornish type) 15 different *Sst*I junction fragments were found. Further characterization of the line R loci with a second restriction enzyme, *Bam*HI, revealed that these junction fragments represent 25 different loci, of which at least 21 have not been reported previously. *Sst*I RFLP analysis of progeny from crosses between chickens of the three broiler lines and White Leghorns demonstrated that within line R and cw1 approximately 90% of the *ev* loci were hemizygous. In line cw2 at least 50% of the *ev* loci were hemizygous. There was no evidence for polymorphic loci, and only two *ev* loci were found to be linked genetically. Intertype crosses revealed that overall differences in the RFLP patterns observed between Cornish, White Plymouth Rock and White Leghorn chicken lines were due to the presence of different *ev* loci in each of the lines rather than to polymorphism. The few shared *ev* loci always contained similar allelic fragments.

Key words: *ev*-loci – Avian Leukosis Virus – Commercial broiler chicken lines – Hemizygosity – RFLP

Introduction

The chicken genome contains DNA sequences, known as endogenous viral *ev* loci, that are related to the Avian Leukosis Virus (ALV) (Astrin 1978). Studies on these loci have been directed mainly at the clarification of their nomenclature, the elucidation of their structure (Hughes et al. 1981 a, b; Smith 1987), their possible role in ALV susceptibility (Crittenden et al. 1982, 1984) and their cosegregation with important economic traits (Kuhnlein et al. 1989 a, b; Iraqi et al. 1991). Recently, some studies have been made on their distribution and diversity within poultry. Considerable variation was seen in the RFLP patterns of chickens from either the same or different lines (Aarts et al. 1991; Boichard et al. 1990; Boulliou et al. 1991; Iraqi et al. 1991; Smith 1987). Most individual *ev* loci have been found to be low or intermediate frequencies, but some seem to be fixed (Boulliou et al. 1991; Iraqi et al. 1991; Tereba and Astrin 1980). As most of the *ev* loci within chicken populations have been reported to be present at low or intermediate frequencies the number of hemizygous loci should theoretically be relatively high. In the study reported here, progeny of crosses between chickens of different lines were analysed by RFLP mapping to test this assumption. The hypothesis – many loci with few alleles per locus rather than few loci with many alleles – was also tested.

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Materials and methods

Broiler chicken lines

Line R is a White Cornish line individually selected for high 6-week body weight and is derived from a commercial broiler sire flock (Leenstra et al. 1986). Lines cw1 and cw2 are commercial White Plymouth Rock broiler lines.

White Leghorn lines

Line WLB originates from a basic commercial line and has been kept as a closed population for at least 20 generations. Line *ev*0 originates from line 0, a chicken line lacking ALV-related endogenous viral sequences (Astrin et al. 1979).

Crosses

In the first experiment 8 hens of line R, 5 cocks of line cw1 and 5 cocks of line cw2 were mated with *ev*0 birds. Progeny embryos were collected from each cross. The DNA of these embryos was used for Southern blot analysis. In a second experiment, the cw1 and cw2 cocks were crossed with 8 line R hens as well as to halfsibs of these hens. The same cw1 and cw2 cocks were also crossed with White Leghorn layer hens. From the cw1 × R, cw2 × R, cw1 × WL and cw2 × WL crosses 130 male progeny were raised, and their blood DNA was analysed by Southern blotting (Southern 1975). Semen from 15 selected males was collected to inseminate *ev*0 hens. The DNA from approximately 20 progeny of each cross was subjected to Southern blot analysis.

Genomic DNA isolation

From embryos. One-week-old chicken embryos were homogenized in 4 ml GIT buffer (4 *M* guanidine isothiocyanate, 0.025 *M* sodium acetate, pH 6, 0.84% B-mercaptoethanol), and 2 ml of TLE (0.2 *M* TRIS-HCl, 0.1 *M* LiCl, 5 m*M* EDTA, pH 8.2)-saturated phenol was added to the homogenate. After mixing for 10 min 2 ml of a chloroform-isoamylalcohol mixture (v/v: 24/1) was added, and the homogenate was mixed for another 10 min prior to incubation for 20 min at 50 °C. The homogenate was subsequently centrifugated for 10 min at 3,900 rpm and the DNA in the aqueous phase precipitated with ethanol (96%). The DNA clump was rinsed with ice-cold ethanol (80%) and then dissolved in 2 ml TE buffer (10 m*M* TRIS-HCl, 1 m*M* EDTA, pH 8.0). The DNA solution was incubated overnight at 37 °C with proteinase K (100 µg/µl) and then extracted once with water-saturated phenol and twice with a 24:1 mixture of chloroform and isoamylalcohol. After ethanol precipitation the DNA was dissolved in 1 ml H_2O.

From blood. DNA from blood cells was isolated as described in Aarts et al. (1991).

DNA analysis

Seven micrograms of chicken genomic DNA was digested to completion with the restriction enzyme *Sst*I (all DNA samples) or *Bam*HI (only DNA samples from the progeny of cross R × *ev*0). The DNA fragments were separated in a 0.6% TAE-agarose gel (TAE buffer: 40 m*M* TRIS-acetate, 2 m*M* EDTA, pH 8.2) and blotted (Southern 1975) to a nylon filter (Hybond-N, Amersham). After overnight hybridization at 65 °C with the appropriate probe, the filters were washed twice with 2 × SSC/0.1% SDS (SSC: 0.15 *M* sodium chloride, 0.015 *M* sodium citrate) at 65 °C for 30 min and twice with 0.1 × SSC/0.1% SDS at 65 °C for 20 min. Lambda-DNA *Hin*dIII fragments were used as markers to estimate the size of the hybridizing fragments.

Probes

The complete RCAS plasmid was used as the principal probe to detect the *ev* loci (Hughes et al. 1987). The 1.4-kb *Bam*HI fragment of the RCAS plasmid (*gag* probe; coding region for p27 and part of p19) and the 1.2-kb *Eco*RI/*Kpn*I fragment of the RCAS plasmid (*env* probe; coding region for gp85) were used as probes to detect *ev*-gene specific sequences. All probes were labeled with ^{32}P-αATP (Multi-prime, Promega) prior to use.

Nomenclature of the ev loci

The nomenclature of the *ev* loci in this study is mainly based on the length of the *SSt*I junction fragment. In most *ev* gene sequences only one *Sst*I recognition site, located near the 5′ end, is present. The length of the *Sst*I proviral-chicken junction fragment is determined by the first *Sst*I recognition site in the chicken genome upstream from the *ev* gene sequences. Hence, the length of this fragment depends on the *ev* location within the chicken genome. The *ev* loci within the R line birds were additionally characterized on the basis of the length of the 3′ *Bam*HI junction fragment. *Bam*HI digestion generally gives two consistently sized internal hybridizing fragments (1.4 and 1.8 kb) and one variable 3′ hybridizing fragment per complete *ev* locus. The length of the 3′ end *Bam*HI proviral-chicken junction fragment, like that of the *Sst*I proviral-chicken junction fragment, also depends on the *ev* location within the chicken genome.

The complex RFLP patterns obtained from the DNA of the line R birds made it difficult to pair the corresponding *Sst*I and *Bam*HI *ev*-locus hybridizing fragments. To circumvent this problem we crossed the line R birds with *ev*0 birds and analysed the *ev*-RFLP patterns of their progeny.

Results and discussion

Characterization of ev loci in the White Plymouth Rock lines cw1 and cw2

In cw1 line 19 and cw2 line 14 different *ev* junction fragments were found, with a mean of 8.4 and 6.8 junction fragments per bird, respectively, 11 *Sst*I fragments had the same size and may be identical. However, we numbered the fragments in the different lines separately because their similarity was not proven. In two cases an

Table 1. Nomenclature of *ev* loci 3′ *Sst*I junction fragments in the White Plymouth Rock cw1 and cw2 lines

cw1 locus	*Sst*I fragment (kb)	n^b	cw2 locus	*Sst*I fragment (kb)	n^b
			ev-cw2-1	23.5	1
ev-cw1-1	23.0	3	*ev-cw2*-2	23.0	2
ev-cw1-2	22.0	1			
ev-cw1-3[a]	20.5	1	*ev-cw2*-3[a]	20.5	2
ev-cw1-4	18.0	1	*ev-cw2*-4	18.0	1
ev-cw1-5	17.2	2			
ev-cw1-6	16.2	3			
ev-cw1-7	14.9	1			
ev-cw1-8	13.5	1			
			ev-cw2-5	12.7	2
ev-cw1-9	12.3	2	*ev-cw2*-6	12.3	1
			ev-cw2-7	11.8	2
ev-cw1-10	11.0	3	*ev-cw2*-8	11.0	1
ev-cw1-11	10.3	2	*ev-cw2*-9	10.3	2
ev-cw1-12	9.5	3	*ev-cw2*-10	9.5	3
ev-cw1-13	9.1	2	*ev-cw2*-11	9.1	5
ev-cw1-14	8.5	3			
ev-cw1-15	8.3	3	*ev-cw2*-12	8.3	5
ev-cw1-16	7.6	5			
ev-cw1-17	7.3	3	*ev-cw2*-13	7.3	4
ev-cw1-18	6.2	1			
ev-cw1-19	4.3	2	*ev-cw2*-14	4.3	2

[a] No hybridization found with *gag* probe

[b] Number of birds showing the *ev* junction fragment out of 5 analysed

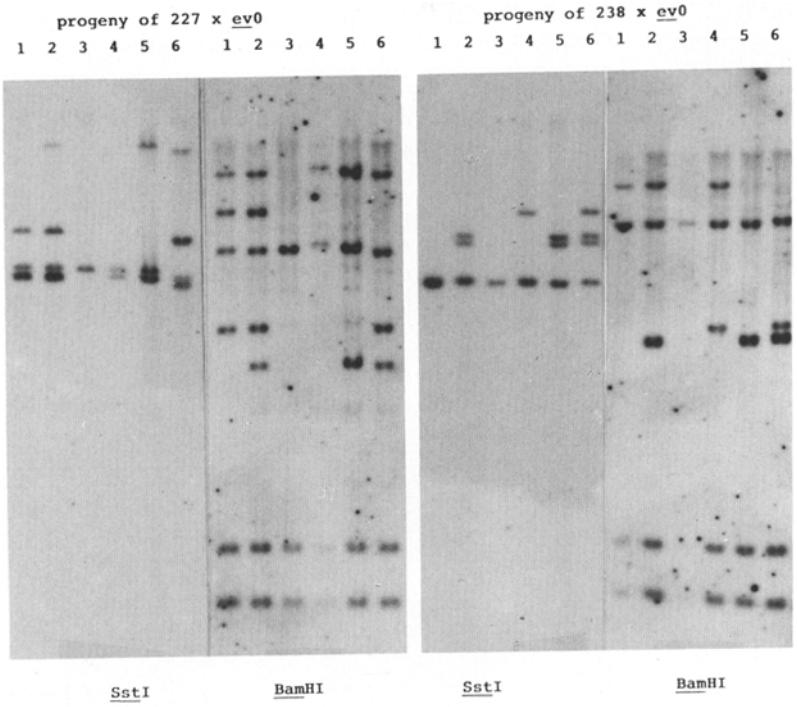

Fig. 1. *Sst*I and *Bam*HI *ev* gene patterns for 6 progeny of the Cornish line R bird 227 × *ev*0 and 6 progeny of the Cornish line R bird 238 × *ev*0 cross. Southern blot analysis and probing with RCAS were performed as described in the Materials and methods. The lengths (in kb) of the bands are: *left panel* (227 × *ev*0) from *top* to *bottom*: *Sst*I 23.0, 10.8, 8.3, 8.0; *Bam*HI 22.0, 13.3, 8.7, 5.2, 3.9 and the two internal bands 1.8 and 1.4; *right panel* (238 × *ev*0) from *top* to *bottom*: *Sst*I 14.0, 10.8, 10.2, 8.0; *Bam*HI 22.0, 13.3, 5.6, 5.2 and the two internal bands 1.8 and 1.4

assumption that two *Sst*I fragments with the same length are identical was strengthened: in both lines the 20.5-kb *Sst*I fragments with the same length are identical was strengthened: in both lines the 20.5-kb *Sst*I *ev* gene fragment (*ev-cw1*-3 and *ev-cw2*-3) did not hybridize to the *gag* subprobe (see Table 1), and interbreeding cross experiments revealed that *ev-cw1*-17 and *ev-cw2*-13 were identical (see below). The Cornish-specific 8.0- and 11.2-kb *Sst*I *ev* gene fragments (Aarts et al. in preparation) were not found in these animals. Our previous investigations on broiler type chickens (Aarts et al. 1991) showed that most of the *ev* loci present within these brids did not have (on Southern blot) visible deletions. Two subprobe fragments that identified the *gag* region and the *env* region, respectively, showed that all of the *Sst*I junction fragments present in the cw birds hybridized equally with the *env* probe and except for the 20.5-kb *Sst*I fragment all equally as strongly to the *gag* probe. Hence, most of the *ev* loci described here do not have severe deletions within their *gag* and *env* sequences.

Characterization of ev loci in the Cornish line R

Upon digestion with the restriction enzyme *Sst*I 15 different 3′ junction fragments were found. *Bam*HI analysis of the *ev* loci within these birds resulted in 23 different 3′ junction fragments. The co-segregating *Sst*I and *Bam*HI fragments were paired by analysing progeny from the cross between the R line hens and *ev*0 cockerels. Figure 1 shows the RFLP results obtained from cross 227 × *ev*0 and 238 × *ev*0. The RFLP patterns indicated that the 8.0-kb *Sst*I band present for both hen 227 and 238 represented 2 different loci, 1 with a 13.3-kb *Bam*HI fragment and 1 with a 23-kb *Bam*HI fragment (compare progeny 1 and 3 of the 238 × *ev*0 cross). Loci *ev-R*-9 and *ev-R*-11, which have different sized *Sst*I fragments (10.8 and 10.2 kb, respectively), contained identically sized *Bam*HI fragments (5.2 kb). As shown in Table 2, 25 different *ev* loci were found among the 8 birds investigated. Most of these loci are putative new. The *Sst*I and *Bam*HI fragment lengths of two loci, *ev-R*-1 and *ev-R*-9, are almost identical to those of *ev*6 and *ev*1, respectively (Smith 1987; Iraqi et al. 1991). The loci *ev-R*-3 and *ev-R*-19 resemble those of *ev-b7* and *ev-b18*, respectively (Boulliou et al. 1991).

Only 1 locus, *ev-R*-1, did not hybridize with the *gag* subprobe and 1, *ev-R*-13, only weakly hybridized with it (see Table 2). Equally strong hybridization signals were found with the *env* probe in all cases. A deletion in the *gag* region of *ev-R*-1 supports the assumed similarity of *ev-R*-1 and *ev6* (Ronfort et al. 1991). The sizes of the *Bam*HI fragments of *ev-R*-2 and *ev-R*-6 are smaller than the expected minimum size of the 3′ *Bam*HI fragment and also the *Sst*I fragments of *ev-cw1*-19 and *ev-cw2*-14 are too small to represent an *ev* gene without structural alteration. As mentioned above, the signal obtained from both subprobes, *gag* and *env*, with the *Sst*I fragments of loci *ev-cw1*-19 and *ev-cw2*-14 was equally strong as those found for all of the other *Sst*I *ev* fragments. However, neither the possibility of deletion within the *pol* region nor sequential alteration resulting in additional recognition sites of the restriction enzymes used can be excluded.

Table 2. Nomenclature of *ev* loci in the Cornish line R

ev locus	*Sst*I fragment (kb)	*Bam*HI fragment (kb)	n[g]
ev-R-1[a,b]	23.0	3.9	2
ev-R-2	18.6	3.3	3
ev-R-3[c]	18.0	5.0	1
ev-R-4	16.8	8.6	1
ev-R-5	16.8	6.7	2
ev-R-6	14.0	5.6	1
ev-R-7	11.2	23.0	5
ev-R-8	11.2	4.8	1
ev-R-9[d]	10.8	5.2	3
ev-R-10	10.2	8.5	1
ev-R-11	10.2	5.2	1
ev-R-12	9.7	4.7	1
ev-R-13[e]	8.5	5.0	3
ev-R-14	8.3	8.7	3
ev-R-15	8.3	6.5	1
ev-R-16	8.1	2.7	1
ev-R-17	8.0	22.0	3
ev-R-18	8.0	13.3	5
ev-R-19[f]	8.0	8.2	2
ev-R-20	7.6	15.5	1
ev-R-21	7.6	5.9	1
ev-R-22	7.6	4.2	2
ev-R-23	7.2	6.2	1
ev-R-24	7.2	4.8	2
ev-R-25	7.2	4.2	2

[a] No hybridization with *gag* probe
[b] Probably similar to *ev6* (Smith 1987; Iraqi et al. 1991; Ronfort et al. 1991)
[c] Probably similar to *ev-b7* (Boulliou et al. 1991)
[d] Probably similar to *ev1* (Smith 1987; Iraqi et al. 1991)
[e] Weak hybridization with *gag* probe
[f] Probably similar to *ev-b18* (Boulliou et al. 1991)

Table 3. Number and the percentage of hemizygous loci in the Cornish R and White Plymouth Rock cw1 and cw2 birds

Line	Bird	Number of progeny[a]	Number of hemizygous loci	Number of homozygous loci	Percentage of hemizygous loci
R	153	12 (6)	3	1	75
	160	6 (6)	7	0	100
	162	6 (6)	7	0	100
	164	6 (6)	7	0	100
	175	6 (6)	6	2	75
	208	6 (6)	6	0	100
	227	6 (6)	4	1	80
	238	6 (6)	4	1	80
			44	5	90
cw1	cw1.1	16 (2)	7	0	100
	cw1.2	11 (3)	9	2	82
	cw1.3	12 (4)	9	1	90
	cw1.4	12 (4)	8	0	100
	cw1.5	9 (3)	5	1	83
			38	4	91
cw2	cw2.1	14 (2)	5	2	71
	cw2.2	11 (2)	1	4	20
	cw2.3	7 (3)	3	4	43
	cw2.4	12 (4)	5	2	71
	cw2.5	16 (3)	3	4	43
			17	16	50

[a] The number of progeny of the cross with *ev*0 are given between brackets

Hemizygosity of ev loci

A large variety of RFLP patterns has been observed between chickens of different types, of different lines and even between chickens of the same line (Aarts et al. 1991). This variety could be the result of either multiple alleles of a few loci or the presence of many different *ev* loci within the populations investigated. In the progeny of crosses between R-, cw1- and cw2-line birds with *ev*0 birds the mutual exclusion of parental *Sst*I *ev* junction fragments was never found. This excludes the possibility of a multiple-allelism of the *ev* loci within the investigated birds. A possible linkage of two *ev* loci was observed, as *ev-R*-9 and *ev-R*-11 were always found together in the progeny of the 238 × *ev*0 cross. However, this may have been the result of a recombination as in bird 227 *ev-R*-9 was found in the absence of *ev-R*-11.

Most of the *ev* loci in the lines investigated here occurred at low frequencies (Table 1 and 2; unpublished results). Consequently, most of the *ev* loci should be hemizygous. However, a Southern blot does not readily discriminate between hemi- or homozygosity. The *Sst*I RFLP patterns of progeny from crosses between birds of the broiler lines and the White Leghorn were used to determine the hemizygosity of the parental *ev* loci. When the results of all line R birds were combined 90% of the *ev* loci were found to be hemizygous (Table 3). In line cw1 at least 91% of the loci were observed to be hemizygous. These frequencies could be an underestimation because one *Sst*I band may represent more than 1 locus. In line cw2 the observed number of hemizygous loci was much lower. Only 50% of the paternal *Sst*I fragments were not found within all progeny. Due to a possible underestimation it is most probable that more than 50% of the loci were hemizygous in line cw2.

Whether the *ev* loci are selectively neutral and usable as indicators of general line heterozygosity, as suggested by Bumstead et al. (1987), is questionable. It could be that their low frequency is due either to negative selection forces, as suggested by Iraqi et al. (1991), or to recent

introduction in these lines. It is of interest that most of the *ev* loci reported here were line specific and did not contain the major deletions reported by Boulliou et al. (1991).

Do different types of chickens share the same ev-loci?

To investigate whether the different chicken types shared *ev* loci, 37 matings were made between cw1 or cw2 males and WLB and R line females. From 130 male progeny 15 were chosen for further analysis. They showed either many parental *ev* fragments and/or a stronger hybridization signal for 1 or more bands present in both parents. To determine whether bands represent 1 (hemizygous) or 2 (homozygous) alleles the 15 males were crossed with *ev*0 hens and their progeny DNA analysed. Only the restriction enzyme *Sst*I was used for this analysis. This introduced the possibility that 2 different loci with the same sized *Sst*I *ev* fragment might be confused. However, the false interpretation of a locus zygosity was diminished by testing a large number of progeny. Table 4 shows the number of progeny analysed and the number of hemi- and homozygous *ev* loci found.

Table 4. The number of hemizygous and homozygous loci in selected male progeny from cw × WLB and cw × R crosses

Parental cross		Number of (selected male × *ev*0) progeny analysed	Number and type of *ev* loci in selected male	
Number	cw × WLB or R		Hemizygous	Homozygous
5	cw1.2 × 115 WLB	25	8	0
8	cw1.4 × 118	20	4	0
10	cw1.5 × 120	12	5	0
12	cw2.1 × 128	25	7	0
13	cw2.1 × 129	27	6	0
15	cw2.2 × 130	10	6	0
16	cw2.3 × 132	28	5	0
23	cw1.2 × 160 R	14	7	0
26	cw1.3 × 250	17	8	0
27	cw1.4 × 164	15	3	1[a]
29	cw1.5 × 170	28	5	1[b]
31	cw2.1 × 252	22	7	1[c]
32	cw2.3 × 208	24	6	1[d]
34	cw2.4 × 227	25	7	1[e]
36	cw2.5 × 152	21	5	1[f]

[a] Alleles *ev-cw1*-17 and *ev-R*-24
[b] Allele *ev-cw1*-16 and an *ev* allele of hen 170 with an *Sst*I fragment of identical size
[c] Allele *ev-cw2*-9 and an *ev* allele of hen 252 with an *Sst*I fragment of identical size
[d] Alleles *ev-cw2*-13 and *ev-R*-24
[e] Alleles *ev-cw2*-12 and *ev-R*-14
[f] Allele *ev-cw2*-13 and an *ev* allele of hen 152 with an *Sst*I fragment of identical size

As we were unable to find any parental band inherited by all of the progeny or any mutual exclusion of 2 differently sized fragments after mating the cw1/WLB or cw2/WLB male progeny with *ev*0 hens, the results suggest that the cw and WL *ev* loci are at different genomic locations to those of the cw *ev* loci. Evidence for shared *ev* loci was only found from the analysis of progeny from cw1 × R and cw2 × R. Six loci were found to be identical in the cw and R line birds (see Table 4). Progeny from *ev*0 parents would be expected to harbour only hemizygous *ev* loci; consequently, identical cw and R *ev* loci would exhibit mutual exclusion in progeny from such an *ev*0 parent. As the mutual exclusion of differently sized fragments was not seen, the few *ev* loci in common contained identically sized alleles. Crosses 27 and 32 revealed that *ev*-cw1-17 and *ev-cw2*-13, both of which displayed a 7.3-kb *Sst*I fragment (see Table 1), are similar. The homozygous locus in the male progeny of cross 27 is occupied by *ev-R*-24 and *ev-cw1*-17 and in the male progeny of cross 32 by *ev-R*-24 and *ev-cw2*-13.

Loci *ev-cw1*-17, *ev-cw2*-13 and *ev-R*-24 as well as loci *ev-R*-14 and *ev-cw2*-12 are probably identical and became integrated within the chicken genome before the White Plymouth Rock and Cornish type chickens were separated. That no identical *ev* loci were found to be shared between WLB and cw chickens might be chance due to the low number of *ev* genes within our WL line or, more probably, is a consequence of the different selection histories of these lines.

Our results suggest that the different RFLP patterns of chickens from cw and R broiler lines is due to the presence of a large number of different *ev* loci within their respective populations rather than a few *ev* loci with multiple alleles.

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