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A polymorphic system related to but genetically independent of the chicken major histocompatibility complex

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Abstract. Analyses of the major histocompatibility complex (*Mhc*) in chickens have shown inconsistencies between serologically defined haplotypes and haplotypes defined by the restriction fragment patterns of *Mhc* class I and class II genes in Southern hybridizations. Often more than one pattern of restriction fragments for *Mhc* class I and/or class II genes has been found among DNA samples collected from birds homozygous for a single serologically defined *B* haplotype. Such findings have been interpreted as evidence for variability within the *Mhc* haplotypes of chickens not detected previously with serological methods. In this study of a fully pedigreed family over three generations, the heterogeneity observed in restriction fragment patterns was found to be the result of the presence of a second, independently segregating polymorphic *Mhc*-like locus, designated *Rfp-Y*. Three alleles (haplotypes) are identified in this new system.

Introduction

The major histocompatibility complex (*Mhc*) is a chromosomal region encompassing a variety of genes, many of which function in the processing and presentation of antigen for the generation of immune responses. An *Mhc* is thought to be carried in one form or another in the genomes of all vertebrates (Kaufman et al. 1990). The complex has been found in all species of mammals so far investigated, with some variation observed particularly in the number and arrangement of the genes providing the antigen-presenting Mhc class I and class II molecules. Recent cloning of chicken *Mhc* genes has allowed further characterization of the *Mhc* in a non-mammalian species in which considerable genetic data are available (Bourlet et al. 1988; Guillemot et al. 1988; Goto et al. 1988). The chicken *Mhc* is designated *B* and the genes corresponding to *Mhc* class I and II genes of mammals are referred to as *B-F* and *B-L*, respectively (Briles et al. 1950; Schierman and Nordskog 1961; Pink et al. 1977). Also within the *B* complex, which maps to a microchromosome (Bloom and Bacon 1985; Dominquez-Steglich et al. 1991; Bitgood and Somes 1990), is a third class of genes, designated *B-G*, which encode highly polymorphic immunoglobulin(*Ig*)-superfamily molecules of unknown function (Pink et al. 1977; Kaufman et al. 1990; Miller et al. 1991). The working hypothesis in the analysis of the chicken *Mhc* has been that all *Mhc*-like genes are located within a single chromosomal region. Little thought has been given to the possibility that *Mhc*-like gene sequences might exist on other chromosomes in chickens, with the possible exception of the non-polymorphic *B-L* α-chain genes (Guillemot et al. 1986; Kroemer et al. 1990). Rather, the diversity of restriction fragment patterns of *B-F* and *B-L* gene sequences within serologically-defined *B* haplotypes has been interpreted as evidence of polymorphism within *B* system genes not detected by serological typing (Chaussé et al. 1989, 1990; Hála et al. 1989; Tilanus et al. 1989; Goto et al. 1988; Miller et al. 1988; Pharr and Bacon, personal communication; Miller, Bloom, and Briles, unpublished data). This study of a fully-pedigreed three-generation family of chickens was carried out in order to determine whether all chicken *Mhc* class I and class II gene sequences are within the same linkage group.

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Materials and methods

Animals. The fully pedigreed family in this study is part of genetic stock that is several generations into the production of congenic lines for eleven *Mhc* recombinant haplotypes. The eleven *Mhc* recombinant haplotypes share a common ancestor which carried a new haplotype derived from an initial recombination event first identified among progeny from crosses between New Hampshire males and White Leghorn females. One of the two *B* haplotypes in the family, B^{R9}, by international haplotype designation B^{24r2}, is serologically identifiable as $B\text{-}F^{24}$ and $B\text{-}G^{23}$ (Briles and Briles 1977, 1980; Briles et al. 1979; Briles, unpublished data). To produce congenic lines, birds carrying B^{R9} (and the other ten recombinant haplotypes) have been backcrossed into birds of the Ancona strain which carry B^{8} and B^{11}. B^{11} is the second *B* haplotype segregating within the family in this study. The restriction fragments indicative of the *Rfp-Y* system alleles, as described below, have been found in earlier generations prior to the production of the congenic lines. Hence, the *Rfp-Y* alleles are contributed by the New Hampshire and/or White Leghorn strains. The association of the *Rfp-Y* system with a recombinant *B* haplotype is almost certainly coincidental.

Serological typing. Serological typing was carried out in hemagglutination assays as described previously (Briles et al. 1950; Briles and Briles 1980, 1982) using alloantisera specific for B^{R9} (B-F24-488-590 and B-G23-489-582) and for B^{11} (B11-640-906).

Southern blot analysis. The cDNA clones used as probes, bg11 [1940 base pairs (bp)] for *B-G* (Miller et al. 1991), F10 (1284 pb) for *B-F* (Guillemot et al. 1988), and a B-L_{β} clone (855 bp) corresponding to *B-L*$_{\beta}$*II* (Zoorob et al. 1990) for *B-L*, were labeled with ^{32}P by random priming and used in Southern hybridizations at 10^{6} cpm/ml. The restriction endonucleases for the three probes were *Pvu* II, *Pst* I, and *Bgl* I, respectively. Genomic DNA was isolated as described by Goto and colleagues (1988). Samples in ten µg aliquots were digested with the respective restriction endonucleases and electrophoresed in 0.8% agarose gels. The size-fractionated DNA was pressure-blotted from the gels into Gene Screen hybridization membranes, and immobilized by baking at 80° C for 1 h and/or UV crosslinking at 0.12 joules/cm^2 for 2 min in a Stratalinker UV crosslinker. After first blocking the filters for 2 h in 10 × Denhardt's solution and 1% sodium dodecyl sulfate (SDS), hybridizations were carried out in 3–5 ml of 5 × saline sodium phosphate-EDTA (SSPE), 5 × Denhardt's solution, 1% SDS, and 100 µg/ml salmon sperm DNA at 65° C overnight in a rotating hybridization tube. The filters were washed at 65° C, in 0.5 × SSC with 1.0% SDS for 1 h and autoradiograms were produced in 3–7 day exposures at –70° C, in the presence of Quanta III intensification screens.

Results

An unpredicted variability in the restriction fragment patterns for *B-F* and *B-L* genes has been found in Southern analyses within a number of serologically defined *B* haplotypes without corresponding diversity in the patterns produced by *B-G* genes (Chaussé et al. 1989, 1990; Hála et al. 1989; Tilanus et al. 1989; Goto et al. 1988; Miller et al. 1988; Pharr and Bacon, personal communication; Miller, Bloom, and Briles unpublished data). Insight into the nature of this unexpected variability was first obtained in a study of four natural *B* haplotypes and 11 *B* recombinant haplotypes derived from them (Briles and Briles 1977, 1980; Goto, Briles, and Miller, unpublished data). In fully pedigreed families, restriction fragment patterns were encountered that could not be accounted for on the basis of normal inheritance of genes from within the *B* complex. To obtain critical genetic data, DNA preparations from a family (C084) of eleven progeny, their half-sib parents and a full brother to the dam were analyzed. The sire, the dam, and the brother were all serologically determined B^{11}/B^{R9} heterozygotes. DNA samples from this family were analyzed in Southern hybridizations at high stringency (65° C washes in 0.5 × SSC, 1.0% SDS), using probes for the three *B* region molecules (Fig. 1). As would be expected from the segregation of two alleles, only three *B-G* restriction fragment patterns are evident in the DNA of the progeny (Fig. 1 A, A′). In contrast, six patterns are present among the progeny in the *B-F* Southern hybridizations (Fig. 1 B, B′). Three of four polymorphic restriction fragments found in the *B-F* hybridizations [bands at 2.3, 2.1, and 1.8 kilobase (kb)] cosegregate with the *B* haplotypes defined by the *B-G* banding patterns and by serological typing. The fourth restriction fragment, designated F4.0, cannot be accounted for by *B*-system segregation, since it is inherited from the dam by seven progeny irrespective of the three restriction fragment patterns produced by the segregation of the $B\text{-}F^{R9}$ and $B\text{-}F^{11}$ genes.

Correspondingly more restriction fragment patterns are obtained in the B-L Southern hybridizations than can be accounted for on the basis of segregation of the two parental *B* haplotypes (see Fig. 1 C,C′ and Table 1). Two unexpected elements were found within these patterns and they are graphically displayed in Figure 1 C′. The first of these is L6.0, inherited from the dam by four progeny independently from the *B* system haplotypes and antithetically to F4.0 defined by the *B-F* probe. The antithetical distribution of the F4.0 and L6.0 fragments is highly unlikely on the assumption of independent genetic assortment ($P < 0.003$, Fisher's exact test). The distribution of the F4.0 and the L6.0 bands among the progeny antithetically to each other and independent of the *B* system haplotypes (see Table 1 for informative gametes) indicates that the DNA responsible for these fragments resides in a limited region distinct from the microchromosome that is characterized by the *B* system and the nucleolar organizer region (NOR; Bloom and Bacon 1985; Dominquez-Steglich et al. 1991; Bitgood and Somes 1990).

The second portion of the *B-L* restriction fragment patterns that shows unanticipated inheritance are the two closely-spaced bands representing restriction fragments L9.0 and L9.5 present in all progeny in the C084 family (Figure 1 C,C′ and Table 1). In the *B-L* pattern of the dam (and her brother) only the L9.5 band is present, in a more intense form suggesting hybridization to more DNA. In the pattern of the sire only the L9.0 band appears, again as a more intense band. Thus,

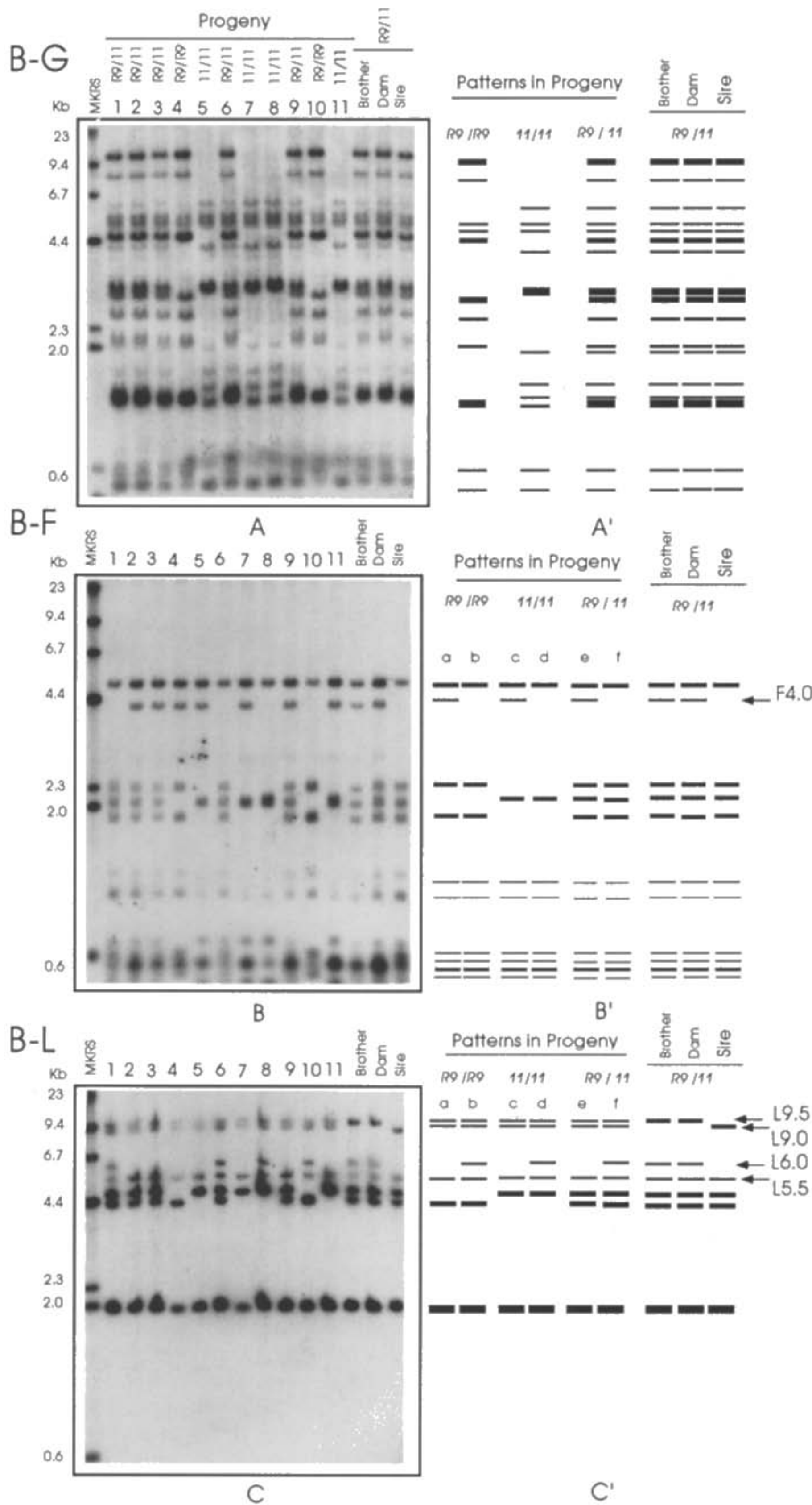

Fig. 1. Southern hybridizations of probes for the (*A*) *B-G*, (*B*) *B-F* (*Mhc* class I), and (*C*) *B-L* (*Mhc* class II) genes of the chicken *Mhc* to the DNA of the C084 family together with corresponding diagrammatic representations (*A′*, *B′* and *C′*) of the patterns found among members of the family. In the progeny, banding pattern *a* (diagramed in *B′* for the B-F probe and *C′* for the B-L probe) was observed in the DNA of number 4, pattern *b* in that of number 10, *c* in that of numbers 5, 7, and 11, *d* in that of number 8, *e* in that of numbers 2, 3, and 9, and *f* in that of numbers 1 and 6. Seven of the progeny carry the F4.0 band, four the F6.0 band.

Table 1. DNA hybridization between probes for chicken *Mhc* class I and class II genes and restriction fragments derived from a second (*Rfp-Y*) genetic system within family C084.

			Restriction fragments not assignable to *B* system alleles ‡						Informative gametes transmitted by dam§	
		B genotype⁺	F4.0	L6.0	L9.0 (kb)	L9.5	L5.5	*Rfp-Y* genotype	*B*	*Rfp-Y*
Progeny	Sex*									
1	M	*R9 11*	–	+	+	+	+	*21*		
2	M	*R9 11*	+	–	+	+	+	*23*		
3	F	*R9 11*	+	–	+	+	+	*23*		
4	M	*R9 R9*	+	–	+	+	+	*23*	*R9*	*3*
5	M	*11 11*	+	–	+	+	+	*23*	*11*	*3*
6	F	*R9 11*	–	+	+	+	+	*21*		
7	F	*11 11*	+	–	+	+	+	*23*	*11*	*3*
8	M	*11 11*	–	+	+	+	+	*21*	*11*	*1*
9	F	*R9 11*	+	–	+	+	+	*23*		
10	F	*R9 R9*	–	+	+	+	+	*21*	*R9*	*1*
11	M	*11 11*	+	–	+	+	+	*23*	*11*	*3*
Brother		*R9 11*	+	+	–	+	+	*13*		
Dam		*R9 11*	+	+	–	+	+	*13*		
Sire		*R9 11*	–	–	+	–	+	*22*		

* The data also prove that the *Rfp-Y* system genes are autosomal, since the dam of genotype Y^1/Y^3, mated to the Y^2/Y^2 sire, produced both Y^1 and Y^3 sons and daughters.

⁺ *R9* designates a *B* system recombinant haplotype derived from B^{23} and B^{24} (Briles and Briles 1977, 1980; Briles et al. 1979; Briles, unpublished data). B^{11}, B^{23}, and B^{24} are classified by serological typing according to Briles and colleagues (1982). *B-G* genotype assignments were made based on serological typing and the restriction fragment patterns revealed by B-G probe, bg11 (Miller et al. 1991).

‡ F4.0 denotes a 4.0 kb band in Southern blots revealed by *Pst* I digestion and a chicken Mhc class I probe, L6.0, L9.0, L9.5, and L5.5 indicate restriction fragments revealed by *Bgl* I digestion and a chicken *Mhc* class II probe.

§ Informative genotypes of gametes are derived from progeny genotypes, e. g., progeny number 4 of $B^{R9}B^{R9}Y^2Y^3$ genotype resulted from fertilization of a $B^{R9}Y^3$ egg cell by a $B^{R9}Y^2$ sperm.

the dam and the sire differ by appearing homozygous for alternate genes represented by the L9.0 (sire) and L9.5 (dam) fragments. The possession of the L9.0 band by the sire and all of the progeny, together with the antithetical relationship of the F4.0 and L6.0 bands transmitted exclusively by the dam, suggests that within this family there exists a three-allele (actually more correctly, haplotype) system identifiable by the inter-related presence of F4.0, L6.0, and L9.0 bands, which at this point we tentatively designate as the locus symbol *Rfp-Y* or *Y* for brevity in discussion. The haplotype producing L9.0 transmitted by the sire to all of the progeny is designated Y^2, while the genes transmitted by the dam producing the L6.0 and F4.0 are designated Y^1 and Y^3, respectively. The *B* and *Rfp-Y* genotypes of individual males and females of the C084 family appear in Table 1.

To learn more about the three restriction fragments already tentatively assigned to *Rfp-Y*, and to determine their relationship with the L9.5 kb inherited from the dam and an additional fragment at L5.5 kb that initially appeared non-polymorphic in the C084 family (Fig. 1C,C′), chickens of selected genotypes were mated to produce informative second-generation families. Two families were produced by mating the full brother of the dam of the C084 family (possessing the same *B* and *Y* genotypes as his sister, see Table 1) to a C084 daughter (number 10 in Table 1) of the genotype B^{R9}/B^{R9}, Y^1/Y^2 to produce family C183 (Table 2), and to a C084 daughter (number 7 in Table 1) of genotype B^{11}/B^{11}, Y^2/Y^3 to produce family D183 (Table 2). Definitive gametes from these two families together with those of the C084 family confirm an independent assortment of the *Y* and *B* loci, resulting in a total ratio of seven B^{R9}-Y^1: eight B^{R9}-Y^3: ten B^{11}-Y^1: seven B^{11}-Y^3 gametes. Further, these two families together with the C084 family allow the assignment of L9.5 and L5.5 fragments (by their presence or absence) to the *Rfp-Y* haplotypes 1, 2, and 3. Preparations from chicks homozygous for Y^1 (Table 2, C183 family) indicate that the chromosomal region represented by the Y^1 haplotype produces the L9.5 fragment, as well as the L6.0 fragment with which it had been identified in the C084 family. Thus, the haplotype Y^1 is characterized as producing two of the five *Rfp-Y* restriction fragments. The full banding spectrum of Y^3 is similarly shown by three

Table 2. Inheritance of restriction fragments assigned to *Rfp-Y* system in second-generation families C183 and D183.

B genotype	*Rfp-Y* phenotype F4.0	L6.0	L9.0 (kb)	L9.5	L5.5	*Rfp-Y* genotype	Progeny of given phenotype (No.)	Informative gametes transmitted by sire *B*	*Rfp-Y*
Family C183 (Sire-B^{R9} B^{11} Y^1 Y^3 × Dam-B^{R9} B^{R9} Y^1 Y^2)*									
R9 R9	+	+	–	+	+	*13*	2	*R9*	*3*
R9 R9	–	+	+	+	+	*12*	1	*R9*	*1*
R9 R9	–	+	–	+	–	*11*	1	*R9*	*1*
R9 11	+	+	–	+	+	*13*	1	*11*	*3*
R9 11	–	+	+	+	+	*12*	3	*11*	*1*
R9 11	–	+	–	+	–	*11*	1	*11*	*1*
R9 11	+	–	+	+	+	*23*	1	*11*	*3*
Family D183 (Sire-B^{R9} B^{11} Y^1 Y^3 × Dam-B^{11} B^{11} Y^2 Y^3)*									
R9 11	+	+	–	+	+	*13*	1	*R9*	*1*
R9 11	–	+	+	+	+	*12*	3	*R9*	*1*
R9 11	+	–	+	+	+	*23*	2	*R9*	*3*
R9 11	+	–	–	+	+	*33*	3	*R9*	*3*
11 11	+	+	–	+	+	*13*	2	*11*	*1*
11 11	–	+	+	+	+	*12*	3	*11*	*1*
11 11	+	–	+	+	+	*23*	2	*11*	*3*

* Matings have a common sire, the brother of the dam in the C084 family (Table 1) was mated to a C084 daughter (number 10) to produce the C183 family and to a second C084 daughter (number 7) to produce the D183 family.

Table 3. *Rfp-Y* system restriction fragment patterns within the A186 family.

Rfp-Y phenotype* F4.0	L6.0	L9.0 (kb)	L9.5	L5.5	*Rfp-Y* genotype	No. of progeny
–	+	–	+	–	*11*	3
–	+	+	+	+	*12*	9
–	–	+	–	+	*22*	4

* Sire is number 1 from progeny in C084 family and dam is number 6. Both are of *Rfp*-Y^1/Y^2 genotype.

Table 4. Restriction fragment patterns defining the *Rfp-Y* haplotypes.

Rfp-Y	F4.0	L6.0	L9.0	L9.5	L5.5
1	–	+	–	+	–
2	–	–	+	–	+
3	+	–	–	+	+

homozygous progeny within the D183 family (Table 2). This haplotype produces fragments F4.0, L9.5, and L5.5 with no indication of the presence of L6.0 or L9.0, in agreement with the previous observation of the fragments segregating within the C084 family.

To clarify further the inheritance of the L9.5, L9.0 and the L5.5 kb bands, an additional mating was made between a brother and a sister in the C084 family (numbers 1 and 6, respectively, in Table 1); both were of genotype Y^1/Y^2. The *Y* genotypes of their 16 progeny (Family A186, Table 3) were three Y^1/Y^1, nine Y^1/Y^2, four Y^2/Y^2, in agreement with the 1 : 2 : 1 ratio expected. The *Rfp-Y* restriction fragment pattern within this family for the Y^2 allele is identical to that of the sire of the C084 family – depicting only L9.0 and L5.5 restriction fragments. The common appearance of L9.0 and L5.5 in thirteen members of the A186 family and the common absence in the remaining three is highly unlikely on the assumption of the independent inheritance of the two bands ($p < 0.0018$, Fisher's exact test). Similarly, the simultaneous inheritance of the L6.0 and L9.5 bands in Y^1 deviates significantly from random assortment ($p < 0.005$, Fisher's exact test). Thus, the patterns of restriction fragments that are observed in these three sire families are most simply attributable to the segregation of three haplotypes in a single system independent of the *B* system and having the band patterns listed in Table 4. At the same time, although current genetic data are compatible with the hypothesis of three *Rfp-Y* haplotypes with the restriction fragments as assigned, the fragments L9.0 and L5.5 could represent yet a third system with three haplotypes (see column L9.0 and L5.5 of Table 4). This interpretation would, however, necessitate a more complex hypothesis in which null haplotypes would have to be assigned in both *Rfp-Y* and the third system. Y^2 would then be a null haplotype assigned by the absence of F4.0, L6.0, and L9.5, while the null allele of the third system would represent the absence of L9.0 and L5.5.

Throughout the analysis of these families, the B-F and B-L probes also identify *B* system haplotypes by revealing additional restriction fragments, as illustrated in Figure 1. The *B*-F^{11} haplotype is associated with a 2.1 kb fragment, B^{R9} with cosegregating fragments of 1.8 and 2.3 kb. Similarly, in the B-L hybridizations the *B*-L^{11} haplotype is associated with a 4.6 kb band, while *B*-L^{R9} is identifiable by the presence of a 4.3 kb fragment. Other, non-polymorphic fragments are present in both hybridizations. These include a fragment at 5 kb and fragments of less than 1 kb in the B-F hybridizations and a prominent non-polymorphic band at 1.9 kb in the B-L hybridizations. Whether these non-polymorphic fragments are associated with the *B* or *Y* systems, or possibly both systems, remains to be determined.

This analysis of fully pedigreed families using recombinant DNA probes for genes within the *Mhc* has demonstrated that in the genome of the chicken there is at least one additional system of *Mhc*-related haplotypes segregating independently from those of the *B* system. Interestingly, the gene sequences have associated with this newly recognized system display

considerable haplotypic polymorphism. In the analysis of a family containing only two *B* system haplotypes we have identified what appear to be three *Y* system haplotypes. Moreover, the three haplotypes may be only a portion of the total number of chickens, since additional non-*B*-associated restriction fragments are apparent in other genetic stocks (Miller, Goto, Bloom, and Briles, unpublished data).

Discussion

While in and of itself the identification of a polymorphic locus separate from the *Mhc* is of interest, the similarity of the *Rfp-Y* genes to the *B-F* (class I) and *B-L* (class II) regions of the *Mhc* increases the significance of these findings. Whether the *Rfp-Y* genes are expressed remains to be determined. Since serology is one of the most sensitive methods for detecting genetic polymorphisms of cellular antigens in chickens, and alleles of none of the known blood groups correlate with segregation of the the *Rfp-Y* alleles within this family, it seems unlikely that the *Rfp-Y* genes are expressed on erythrocytes. Because both *Mhc* class I and class II-like gene sequences appear together in this second location, it seems unlikely that the *Rfp-Y* genes were translocated by the activity of retroviruses. It is perhaps more likely that genes of the *B* system were transferred to a new location by the translocation of a chromosomal segment, and hence it is perhaps likely that at least some portions of the *Rfp-Y* system are expressed. The aneuploidy of the *B* system microchromosome that occurs in chickens (Bloom and Bacon 1985) may have provided an intermediate source of *Mhc*-related DNA that was later incorporated elsewhere in the genome. Thus, the *Rfp-Y* genes could be highly similar to the *B* system genes (as the degree of cross-hybridization suggests), and expressed but undetected until now. For example, if *Rfp-Y* encodes alloantigens, the contribution of the *Rfp-Y* system in tests for tissue compatibility could have been overlooked, particularly in comparisons between *B* haplotypes existing in highly inbred lines. The homozygosity achieved with continued inbreeding during the selection for particular *B* haplotypes would simultaneously fix an *Rfp-Y* haplotype along with the *B* haplotype and make the influences of the two systems indistinguishable in assays for function. Similarly, in lines congenic for *B* haplotypes, the *Rfp-Y* system is presumably represented by a single allele and would exert no discernable influence in tissue compatibility testing.

It is possible that the *Rfp-Y* system genes may have been cloned coincidentally during the molecular mapping of the *B* complex (Guillemot et al. 1988). Among the four clusters of cosmid clones in the present molecular map of the *B* system, two are clearly assignable to the *B* complex. Cosmid cluster I contains the *8.5 B-G* gene and a *B-Lβ* gene which is polymorphic in the CB (B^{12}) and CC (B^{4}) congenic lines, and thus it is located within the *B* complex. Cluster III contains genes of the NOR and is thus linked on the *B* system microchromosome. Clusters II and IV, now known to be virtually contiguous, were previously assigned to the *B* complex on the basis of the polymorphism of *B-F* genes found within them. Restriction fragment length differences associated with the two *B-F* genes within clusters II/IV are evident between the inbred lines carrying B^{13} and B^{14} haplotypes, although absent between the congenic lines CC (B^{4}) and CB (B^{12}; Guillemot et al. 1988). Since these differences in restriction fragment polymorphism would reflect the fixation of *B* and *Y* system alleles in inbred and congenic lines as described above, it is possible that clusters II/IV encompass genes of the newly defined *Rfp-Y* system. This possibility is further supported by the lack of polymorphism in the CC and CB lines at another locus within the II/IV clusters. This locus, the *17.5* gene, located between the two *Mhc* class I-like genes within the clusters, is a member of a highly polymorphic family of lectin-like molecules (Bernot and Auffray, unpublished data). The CC and CB congenic lines show no restriction fragment polymorphism associated with the *17.5* locus, suggesting that, again at this locus too, the same allele maybe shared by the two congenic lines, thus further strengthening the proposal that clusters II/IV originate from outside the microchromosome of the *B* complex.

Whether a gene system analogous to the *Rfp-Y* system exists in mammals remains to be determined. Some evidence is accumulating for the presence of *Mhc*-like sequences outside traditionally defined major histocompatibility complexes. For example, Carter and colleagues (1991) reported the presence of a polymorphic region in the rat termed *RT.DPAxM* which hybridizes with a coding region probe of the *HLA-DPA* gene but which is demonstrably separate from *RT1*, the rat *Mhc*. The strength of hybridization between this newly recognized polymorphic region and the probe suggests a high degree of nucleotide sequence similarity between *HLA-DPA* and *RT.DPAxM*. Similarly, a small family of *Mhc* class I-related genes has been detected outside the *Mhc* in the genomes of man, mouse, and several additional mammals (Calabi and Milstein 1986; Albertson et al. 1988; Bradbury et al. 1991). This family of genes encoding CD1 differentiation antigens shows little or no genetic polymorphism, and the nucleotide sequences are poorly conserved between the *CD1* genes and the genes for classical Mhc I antigens. In neither of these instances have *Mhc* class I and class II genes been found to be commonly associated, a prominent characteristic of *Rfp-Y*.

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