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ABSTRACT

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This study documents effects of the major histocompatibility complex (MHC) on reproductive traits in a flock of Cobb X Cobb broiler breeder hens. The objective was to determine if the MHC affects reproduction of non-congenic broiler breeder hens. Genomic DNA was isolated from 35 hens. Restriction fragment length polymorphism (RFLP) analysis was performed on each sample, using a chicken class II MHC probe (Xu et al. 1989.) Restriction endonuclease digestion using *PvuII* resulted in polymorphisms in all birds. Five polymorphic bands were identified. The largest bands, 7.5 kb and 5.4 kb, were present in one and four hens respectively. The 4.25 kb and 3.4 kb bands were present in 34 and 35 of the hens. The fifth band, 2.4 kb, was observed in only 13 of the animals. Five putative genotypes were observed, based on the combination of RFLP pattern observed. The two most frequent putative genotypes differed only in the absence (A; n=17) or presence (B; n=13) of the fifth band. Egg production records for these two groups were subjected to statistical analyses. Total eggs produced and number of eggs produced during a 45-week fertility study were both significantly higher for hens with genotype A. Hens with genotype B had longer pauses between ovipositions. This study provides evidence, in a non-congenic population of broiler breeder hens, that there are associations of egg production with the MHC.

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REVIEW OF LITERATURE

INTRODUCTION

Genetic selection has been a practical method for improving animal production for many years. Before selection pressures can be instituted, one must identify the traits of interest. Reproductive performance is such a trait that is of major concern in the broiler breeder flock, particularly as measured by rate of lay, fertility, and hatchability of eggs. These variables can be quantified easily but the quantification of productivity of offspring requires 66 weeks for broiler breeder hens. Animal production can be improved faster if the genes responsible for these traits can be identified utilizing analysis of deoxyribonucleic acid. Genes, including those of the major histocompatibility complex (MHC) can now be relatively easily detected and analyzed using molecular biology techniques. Alleles of the MHC that correlate with productivity can be identified and, potentially, used as selection criteria in breeding decisions. The review of literature will describe briefly the chicken MHC and associations of reproductive performance with the chicken MHC.

DESCRIPTION OF THE MAJOR HISTOCOMPATIBILITY COMPLEX

"Histocompatibility genes can be defined as genes that encode cell surface structures that are polymorphic within a species and are sufficiently immunogenic to evoke a rejection reaction" Hansen *et al*. (1993). Histocompatibility genes are located in the genomic region called the major histocompatibility complex (MHC). The mouse MHC, or H2, was the first MHC discovered (Gorer, 1936), followed by the chicken (B complex; Briles *et al*., 1950), and the human (HLA) MHC (J. Dausset 1958). Klein (1986) discussed the presence of an MHC in mammalian, avian, and some reptilian species. Recent workers have demonstrated an MHC in more species, including the turkey (Emara *et al*., 1992), nurse shark (Kasahara, *et al*., 1992), rainbow trout (Juul-Madsen *et al*., 1992), Atlantic salmon (Hordvik *et al*., 1993), zebrafish (Takeuchi, *et al*., 1995), *Xenopus laevis* (Sato *et al*., 1993), and the ring-necked pheasant (Jarvi and Briles, 1992 and Wittzell *et al*., 1994).

Briles *et al*. described the B complex in 1950. Two blood group systems, A and B, were identified. Each caused expression of antigens on erythrocytes and were produced by independently assorting alleles. D. G. Gilmour, working in England, recorded the identification of erythrocyte antigens from four inbred lines of white leghorn chickens maintained there (Gilmour, 1959). The antigen systems documented by Gilmour were the same as the systems that Briles and coworkers were studying in the United States.

The B antigen type became an identification and classification tool for many experiments. Schierman and Nordskog (1961) described an experiment in which tissue grafts were performed between chickens from an inbred White Leghorn line. Erythrocyte antigen

types were determined by hemagglutination. Skin grafts were performed between birds with different A, B, D, and L alleles. Graft rejection did not correlate with A, D, or L type but was affected by B type. In 38 grafts the donor had at least one B antigen which the host animal did not, and 20 of these animals underwent a rejection within 14 days. This experiment established the B complex as the chicken MHC.

The chicken MHC has been the subject of several review articles. Chickens have 39 pairs of chromosomes; five pairs of macrochromosomes and 34 pairs of microchromosomes (Ohno 1961, Owen 1965, Klein 1986, Bloom *et al.* 1987, and Guillemot *et al.* 1989). Pink *et al.* (1977) proposed a model for the chicken MHC. They determined that the B complex has two regions comprised of three loci; the B-F/B-L and B-G regions. The B complex is linked to the nucleolus organizing region (NOR; Bloom and Bacon 1985, Bloom et al. 1987, Guillemot et al. 1989). Bloom and Bacon (1985) also determined that the B complex is located on the sixteenth largest microchromosome (reviewed by Lamont and Dietert 1990). The B-F and B-L loci correspond to mammalian class I and class II, respectively. Guillemot and Auffray (1989) described the cellular expression of MHC molecules in the chicken. Leukocytes and erythrocytes express B-F molecules. B-L antigens are expressed by B cells and macrophages (leukocyte subpopulations). B-G antigens (class IV), which do not have a mammalian homologue, are expressed by erythrocytes and thrombocytes (Kaufman and Salomonsen, 1992).

The structure and function of MHC antigens have been deduced from sequence analysis and crystallographic studies. The known structures were reviewed by Roitt *et al.* (1993). Class I molecules consist of a 45 kDa heavy chain that is noncovalently associated

with $ß_2$-microglobulin. The molecules possess three extracellular domains (α1, α2, and α3), a transmembrane domain, and an intracellular domain. Class I antigens serve to present cytosolic peptides to the T cells. The presented peptides are usually self-derived. Class II molecules exist as heterodimers of heavy (α; 30-34 kDa) and light (ß; 26-29 kDa) glycoprotein chains. They also have single transmembrane and intracellular regions. These antigens are responsible for the presentation of exogenous peptides to immune cells. Kaufman and Salomonsen (1992) speculated on the function of B-G antigens. They proposed that either "B-G molecules are recognized by T cells... recognize antigen... or are recognized by B cells."

Briles *et al.* (1993) identified a system that is related to the MHC but is encoded by an independent MHC-like locus. This work was performed in a three generation family of chickens. Restriction fragment length polymorphism (RFLP) mapping was performed and the new locus (designated restriction fragment pattern-Y, *Rfp-Y*) was found to sort independently from the MHC. The restriction fragments produced by *Rfp-y* are detected with DNA probes for chicken B-F and B-L genes in hybridizations of DNA. Miller *et al.* (1994) performed an experiment to ascertain the homology between *Rfp-y* and B genes. The authors concluded that the "*Rfp-Y* genes may represent a separate isotype of MHC class I genes."

Many methods exist for studying the B complex (reviewed by Lamont, 1993). These methods can be divided into two categories, serological and molecular. Serological protocols require incubation of blood fractions with alloantisera that cause hemagglutination when they encounter antigens. Alloantisera may be used that detect B-G and B-F or are specific to B-F antigens (Briles *et al.*, 1982 and Briles and Briles, 1982). Monoclonal antibodies to B-G

antigens also are available. Molecular methods are based on DNA. B complex genes have been cloned. Restriction fragment length polymorphism analysis of the chicken MHC depends on the availability of cDNA probes for the B complex. Restriction fragment length polymorphism analysis of the MHC has proven useful in determining differences among individuals (Lamont *et al.*, 1987a and Lamont, 1993), as it allows for molecular detection of different alleles for the MHC genes within a defined population of birds. Andersson *et al.* (1987) used probes from the HLA to produce RFLP of chicken B complex. Since chicken probes were produced, numerous workers have used them to study the B complex. Shuman *et al.* (1993) and Heath *et al.* (1994) presented data on the development of assays and new probes for practical MHC typing. They are working to develop a colorimetric assay for B-F type that can be analyzed using a plate reader. Heath *et al.* (1994) reported the successful use of 7 probes in typing congenic and commercial strains of chickens, including both Leghorns and broilers.

EFFECTS OF THE MAJOR HISTOCOMPATIBILITY COMPLEX ON REPRODUCTION

Lerner and Finch (1991) reviewed literature detailing associations of the MHC with reproductive functions in many species. Numerous studies have demonstrated effects of the B complex on productivity traits.

HETEROZYGOSITY

Briles and coworkers published a series of abstracts (Briles, 1953, Briles *et al.* 1953, Briles 1954a, Briles 1954b, Briles and Krueger 1955, Krueger *et al.* 1956, Briles 1956a, Briles 1956b) detailing experiments performed in three lines of chickens at Texas A & M

University. Hens from three related Leghorn inbred lines were mated to either a rooster that was homozygous or a rooster that was heterozygous at the "B locus." Hatchability of eggs from matings to heterozygous males was 66.7%, while matings to homozygous males resulted in 48.5% hatchability, a highly significant difference (Briles 1953). Heterozygous cockerel chicks from two lines (22 and 23) were heavier at nine weeks of age than homozygous cockerels (Briles *et al.* 1953). The heterosis effect on male body weight was not observed in the third line (24). Briles (1954a) combined the hatchability data reported in Briles (1953) with a second year's observations. The hatchabilities of embryos with "0, 50, 75, and 100 percent heterozygosity were 46, 62, 71, and 78 percent, respectively." Heterozygous sires produced 15% higher hatchability than did homozygous sires (69% and 54%). Egg production was higher for heterozygotes than homozygotes in all three inbred lines (Briles 1954b). Briles and Krueger (1955) reported that hatchability of fertile eggs from matings expected to produce 50% heterozygotes was significantly higher than hatchability from matings expected to produce 100% homozygotes ($p < 0.01$). Krueger *et al*. (1956) found evidence that progeny from B complex heterozygous sires had higher juvenile livability and egg production than progeny from homozygous sires. Briles (1957) concluded, on the basis of three seasons' production data, "that the *B* blood group locus (or one or more loci very closely linked to it) is displaying overdominant gene action with regard to hen-day production." Briles (1957) confirmed the data of Briles *et al*. (1953) by demonstrating that heterozygous (B^2B^7) cockerels and pullets, produced by matings between two inbred lines, were heavier than homozygous (B^7B^7) siblings. This series of experiments led Briles *et al*. (1957) to conclude that there is some positive effect of heterozygosity at the B locus on

reproductive traits.

Briles and Allen (1961) studied the effects of B genotype on production traits in seven commercial strains of Hy-Line chickens. They found that there was an effect of genotype on percent hen-day egg production in two white leghorn lines, H1 and H3, and one New Hampshire line, H8. This effect was attributed to a lower rate of lay by one of the homozygous classes in each line. Allen (1962) studied crosses of inbred chickens, New Hampshire and Rhode Island Red. He found that B locus affected hatchability and adult survival ($p < 0.01$). Morton *et al*. (1965) found that progeny B type affected embryonic mortality and hatchability in a Light Sussex strain of chicken.

Allen and Gilmour (1962) compared two heterozygous genotypes in 7047 crossbred white leghorn pullets. They calculated the mean of three percentage traits as a measure of overall fitness. The traits measured were: juvenile mortality, adult mortality, and egg production. B blood type was a significant source of variation in this estimate, with the $B^{21}B^{14}$ pullets performing better than the $B^{21}B^{13}$ pullets. These workers were able to show that the heterozygotic advantage demonstrated in previous experiments also occurred in non-inbred chickens. Heterozygosity at the B locus was preserved after generations of selection for different traits; egg production, egg weight, occurrence of a winter pause, shank length, crooked toes, or normal toes (Schultz and Briles, 1953). These data were from 12 inbred lines from the University of California. Eleven of the 12 lines exhibited the continued heterozygosity, supporting the theory that natural selection favors some general fitness which is conveyed by heterozygosity at the B locus.

Two studies point to the idea that heterozygosity of the B locus, alone, does not

explain all of the effects of the chicken MHC on production traits. Gilmour and Morton (1970) found that genotypic associations varied with generation and environment in two sublines of a moderately inbred Light Sussex strain of birds. In 1973, additional evidence was produced by Nordskog *et al.* that the heterozygotic superiority seen earlier may have been dependent on the population's genetic makeup. They studied two lines of white leghorn chickens (S1 and S2) developed by Iowa State University. These lines had been synthesized to have four B locus haplotypes each; B^1, B^2, B^{19}, and B^{21} (S1) and B^{13}, B^{14}, B^{19}, and B^{21} (S2). They found that both mortality and egg production were negatively affected by the B^1 haplotype in the S1 population. The B^1 haplotype affected only mortality of the S2 birds. The homozygotic B^1 class always had increased mortality and lower egg production in this study. This work confirmed the earlier finding by Briles and Allen (1961) that the B^1 allele was associated with increased adult mortality. After this study, research was focused on the effects of specific haplotypes in specific populations.

SELECTION STUDIES

Simonsen *et al.* (1982) established a white leghorn base population from crossing seven commercial hybrids. The F2 progeny from these birds were placed in four countries and selected for egg number, egg weight, or an index of the two. Each location maintained control and selected lines. Ten haplotypes contributed 95 % of the base population's gene pool. Selection for the reproductive traits, egg number, and egg weight resulted in a significant increase in the frequency of B^{15} genes and a concomitant decrease in the frequency of B^{19} genes. Gavora *et al.* (1986) compared haplotypes and production data from three selected strains: unselected control, high egg production, and Marek's disease and high egg

production. Selection altered haplotype frequency relative to controls. The selected lines had higher egg weight, lower adult mortality, and earlier sexual maturity than the randombred controls. Enhanced production was associated with increases in the frequency of B^{21} and B^{2} haplotypes. Lamont *et al.* (1987) determined the B type of 1095 chicks from four sublines of two lines that had been selected for either a high ratio of egg mass to 32 week body weight or a high ratio of egg mass to feed consumption at 32 week of age. The sublines were either replicate selected or unselected controls. Birds were typed after 6 generations of selection. Two haplotypes (B^{2} and B^{13}) were present in over 75 % of all birds. Selection increased the B^{2} allele at the expense of the B^{13} allele. Selection for egg mass relative to 32 wk body weight increased the B^{14} allele. In another study there were no B type associations with production traits in birds that had been selected for antibody response to sheep erythrocytes (Martin *et al.*, 1990). The same group of researchers (Dunnington *et al.*, 1992), using the same selected lines of birds, found that the MHC affected production in white leghorn chickens, dependent on the birds' genetic background.

Boa-Amponsem *et al.* (1992) utilized White Plymouth Rock chickens that were being selected for high and low juvenile body weight and subpopulations with relaxed selection. Selection had been practiced for 28 weeks, before the sublines were established. The workers recorded hen body weight at 4, 8, and 38 wk of age, age and body weight at first egg, percent of normal eggs laid, and percent normal hen-day egg production. No differences were observed in any of these traits due to B genotype.

Abplanalp *et al.* (1992) and Sato *et al.* (1992) published work performed in congenic and inbred white leghorn hens. Abplanalp *et al.* (1992) created 12 congenic lines of birds by

backcrossing birds to achieve 98.4 % identical genes in the birds. The birds utilized shared the background genome of University of California, Davis line 003. "Fertility, hatchability of fertile eggs, egg production, mortality to 40 wk of age, 40-wk egg weight, and 40-wk body weight" were all determined for the 12 lines. MHC haplotype affected all of the traits studied except fertility. Sato *et al.* (1992) designed a study for comparison of different heterozygotes within a common partially congenic background. Egg production, egg weight, and viability to 40 wk of age were all higher for heterozygotic hens than the average of their respective homozygotic parents. Some heterosis was observed for hatchability, but no general conclusion could be made for this trait. B^2 heterozygotes exhibited heterosis for most traits studied.

Kim *et al.* (1989) studied effects of haplotype on reproduction in the Iowa State University S1 line of white leghorn chickens. Traits measured included: body weight at 8, 20, and 32 wk of age, age at first egg, egg production over the entire experimental period, egg production from 29-32 wk of age, and average egg weight. Of the traits studied, only average egg weight was affected by MHC genotype. Homozygous $B^{19}B^{19}$ birds had higher egg weights than B^1 homozygotes. All heterozygotic classes had egg weights ranging between the two homozygotic classes.

STATEMENT OF THE PROBLEM

The effects of the major histocompatibility complex on reproduction have been studied since 1950 in a variety of animals. The majority of this work, in chickens, has been performed in white leghorns, or chickens used for production of table eggs. Broiler breeders are different chickens than white leghorns. They are the parental strains used to produce broiler chickens, used for meat. Thus, identification of traits that can produce quantifiable increases in production of offspring by the parental strains (broiler breeders) would lead to methods of selection for meat-type chickens. The broiler breeder MHC has not, to date, been examined. Therefore, the objectives of the present study were to: 1) determine if the MHC is polymorphic in broiler breeder hens and 2) determine if the MHC is associated with superior reproductive performance in broiler breeder hens.

MATERIALS AND METHODS

Thirty-five Cobb X Cobb broiler breeder hens were raised using a conventional feed restriction program (Cobb Vantress growing guide). Birds were caged individually at 19 weeks of age and fed 150 g of a commercial breeder-layer ration daily. Egg production was monitored every 2 hours from 0800 h to 1600 h daily for 55 weeks, from 20 August, 1992 until 7 September, 1993. Hens were inseminated with fresh, pooled semen weekly, beginning at 36 wk of age (20 October, 1992), after which eggs were set weekly. Egg weight was determined at the time of collection, 10 d and 19 d of incubation. Fertility and hatchability (day 21 of incubation) of each egg was recorded. Blood (1 milliliter) was obtained from each hen via brachial venipuncture, mixed with EDTA, brought to the laboratory, and stored at -20°C. Genomic deoxyribonucleic acid (DNA) was extracted (Appendix 1) from 50 microliters of frozen blood for each hen. After extraction, DNA was subjected to digestion with the restriction endonuclease *Pvu II* overnight. Digested DNA (8 µg) was separated by electrophoresis for 20-24 h at 35 milliamps in a 0.8% agarose gel in single-strength TBE () containing 0.5 µg/mL ethidium bromide. Each gel was photographed to aid in estimating molecular weights of DNA fragments after hybridization. Electrophoresed DNA was transferred to a nylon membrane (Sambrook, *et al.* 1989), then fixed to the membrane by UV crosslinking (Stratalinker® UV crosslinker, Stratagene, La Jolla, CA.)

Membranes were probed using a chicken class II MHC subclone, CC-II-7-1 (Xu, *et al*. 1989.) Probe DNA (50 ng) was labeled with α^{32}P-dCTP by random priming. Thirty milliliters of hybridization solution (0.263 M Na_2HPO_4, 7% sodium dodecyl sulfate, 1mM EDTA, and 1 % bovine serum albumin) was heated to 65°C and added to a hybridization

tube. The membranes (1-2 per labelling) were placed in the hybridization tubes and prehybridized at 65°C for 20 minutes, with constant rotation. Labeled probe was denatured by boiling for 5 minutes, then placed on ice. Denatured probe was mixed with one milliliter of hybridization solution and added to the hybridization tube, to result in 1 X 10^6 cpm of probe per milliliter of hybridization solution. Hybridization was performed overnight. Washing solutions were warmed to 65°C. Membranes were washed at 65°C in: 1) 0.263 M Na_2HPO_4, 1 % SDS once for 15-30 min, 2) 2 X SSC, 0.1 % SDS twice for 15-30 min each, and 3) 1 X SSC, 0.1 % SDS twice for 15-30 min each. Radioactivity was monitored between each wash and washing was stopped when background was low. Membranes were wrapped in plastic wrap and exposed to X-ray film at -80°C for seven days. Autoradiographs obtained were scored by two independent observers on an individual bird basis, for the presence or absence of the five highest molecular weight bands. Membranes were stripped by soaking in boiled 0.5 % SDS and allowed to cool while shaking.

Statistical analyses were performed using the General Linear Models procedures of SAS. For statistical analyses, only hens in Genotypes A and B were compared, since the single difference between these groups was the presence of the 2.4 kb band in the Genotype B group. Total egg production (over 65 weeks), egg mass, 45 week egg production (during the time of insemination and setting of eggs), percent fertility, percent hatchability, average and maximum clutch lengths, and average and maximum pause lengths were analyzed for the effects of genotype (A vs B). For clutch length and pause length (in which data were not distributed normally), a Wilcoxon rank-sum test (non-parametric procedure) was used to analyze for differences due to genotype.

RESULTS

Broiler breeder DNA digested with *Pvu II* was polymorphic when probed with a chicken MHC class II probe. Representative images of hybridizations giving the 5 observed banding patterns are shown in Figure 1. Five bands were observed with the following molecular weights: 7.5, 5.4, 4.25, 3.4, and 2.4 kilobases (kb). These bands occurred in 1, 4 , 34, 35, and 13 birds, respectively (Table 1). Frequency of occurrence of these banding patterns among the 35 broiler breeder hens studied is summarized in Table 2. Five patterns of polymorphism were observed, and they have been designated as (putative) Genotypes A, B, C, D, and E. The 2.4 kb band was present only in 13 of the 35 hens studied (Genotype B). The other 4 bands were in either the great majority (4.25 and 3.4 kb bands) or 1 or 3 birds (7.5 and 5.4 kb bands). The two major patterns observed (Genotype A, n=17; Genotype B, n=13) only differed in one respect, the presence of the 2.4 kb band in hens with Genotype B. Thus, the analysis of reproductive traits was conducted using only these two groups, based on the presence or absence of the fifth (2.4 kb) band. Age at puberty (first oviposition) was not different between Genotypes A and B ($p>.89$). Egg production for the entire 55 week collection period (Table 3) was affected ($p>.11$) by genotype. Egg mass ($p>.26$), percent fertility of eggs set ($p>.62$), and percent hatchability of fertile eggs ($p>.72$) were not different between groups. Egg production during the 46 wk setting period was significantly ($p < 0.01$) affected by genotype; broiler breeder hens with the 2.4 kb band (113 +/- 7.7) laid more eggs than hens without the 2.4 kb band (82 +/- 8.8). Number of clutches was not affected by genotype ($p>.57$). When average pause lengths were ranked and the ranks analyzed using non-parametric tests, it was determined that the birds without the 2.4 kb band had longer

pauses than the birds with the 2.4 kb band ($p<.08$). This is demonstrated graphically in Figure 2, with the rank for clutch length plotted by the rank for pause length of hens with Genotypes A and B. The distribution of data is such that Genotype A values tend to be distributed in the left portion of the graph, indicating shorter pauses (days between clutches). Coincident with this is the distribution of Genotype A values in the upper portion of the graph relative to Genotype B values, indicating that Genotype A hens had longer clutches (sequence of eggs oviposited on consecutive days.)

TABLE 1. Restriction Fragment Polymorphism Analysis of the Major Histocompatibility Complex in Broiler Breeder Hens: Frequency of Bands Observed.

Fragment Length (kb)	Number (n=35)
7.5	1
5.4	4
4.25	34
3.4	35
2.4	13

TABLE 2. Restriction Fragment Length Polymorphism Analysis of the Major Histocompatibility Complex in Broiler Breeder Hens: Observed Banding Patterns With A Class II Probe.*

Number of		Fragment Length (kb)				
Hens	Genotype	7.5	5.4	4.25	3.4	2.4
17	A	0	0	1	1	0
13	B	0	0	1	1	1
1	C	0	0	1	1	0
1	D	1	1	0	1	0
3	E	0	1	1	1	0

* If a band of the indicated length was detected in the group 1 is recorded, and 0 indicates that the band was absent in that group.

TABLE 3. Summary of Egg Production by Broiler Breeder Hens Bearing Different Major Histocompatibility Complex Genotypes.[*]

Variable	Genotype A	Genotype B
65 Week Production (Number of Eggs)	198 ± 12[c]	169 ± 14[d]
Egg Mass (g)	63.54 ± 0.91	65.15 ± 1.04
45 Week Production (Number of Eggs)	113 ± 7.7[a]	82 ± 8.8[b]
Percent Fertility	76.9 ± 3.5	79.6 ± 4.1
Percent Hatchability	61.0 ± 4.0	63.2 ± 4.6
Average Clutch Length (Days)	2.0 ± 0.1	1.9 ± 0.1
Maximum Clutch Length (Days)	7.8 ± 0.7	6.5 ± 0.7
Average Pause Length (Days)	2.1 ± 0.2	2.3 ± 0.2
Maximum Pause Length (Days)	22.2 ± 5.2[c]	35.2 ± 5.9[d]

[*]Values are least squares means ± standard errors.
[a, b]Numbers within a row with different superscripts differ ($p<.01$).
[c, d]Numbers within a row with different superscripts differ ($p<.11$).

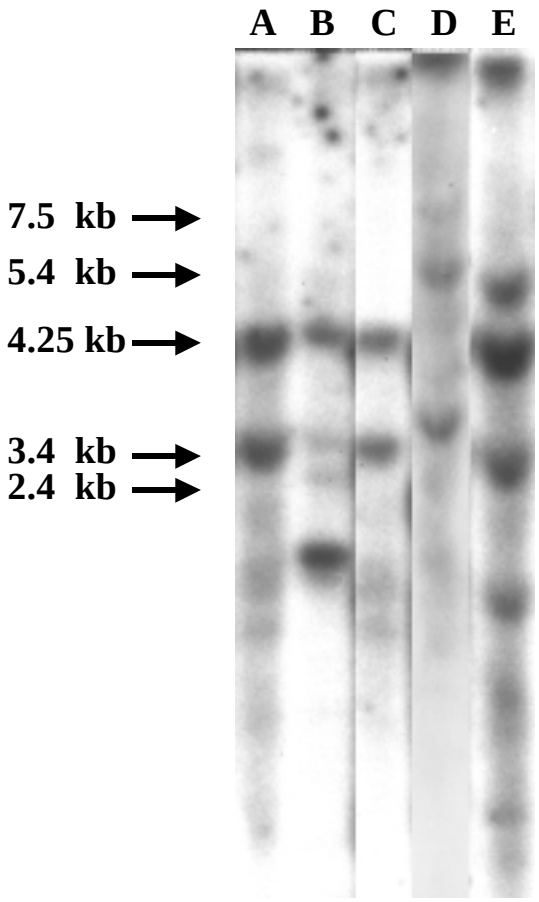

FIGURE 1.Representative autoradiogram depicting results of RFLP analysis of broiler breeder genomic DNA digested with *Pvu*II and hybridized with a chicken MHC Class II cDNA clone, as described in Materials and Methods. Five lanes representing the five (putative) genotypes are presented as listed across the top of the figure. Relative molecular mass of each band is given along the left (in kilobases; kb).

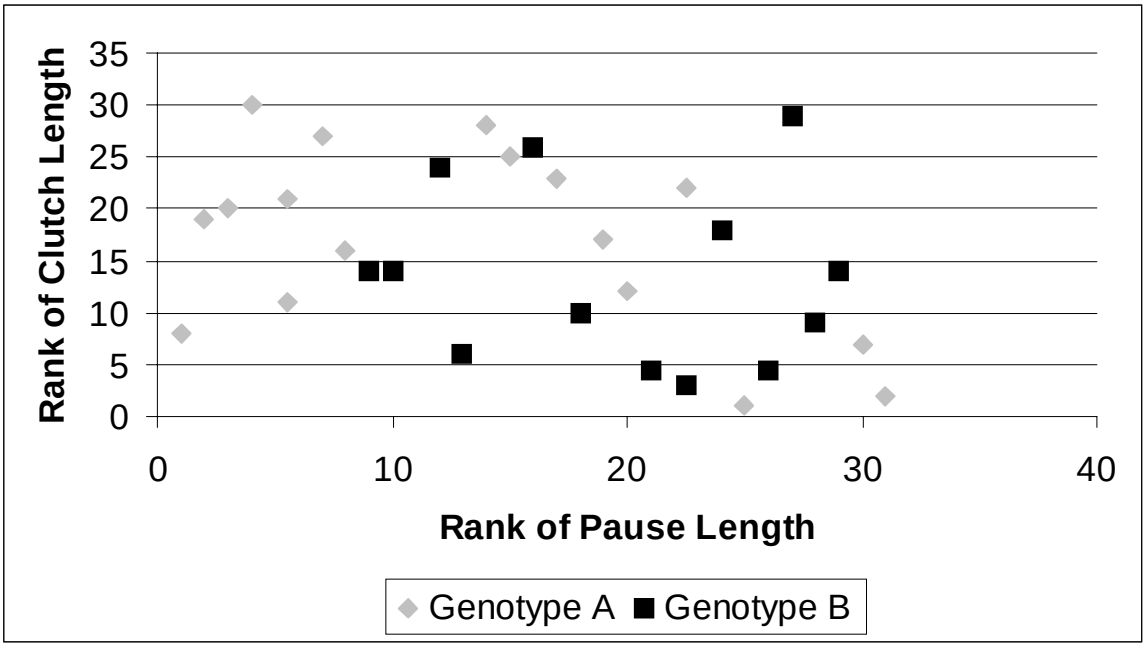

FIGURE 2. Plot of clutch length (replaced by rank) by pause length (replaced by rank) for broiler breeder hens differing in major histocompatibility complex genotype. Using the Wilcoxon rank-sum test for two samples, it was determined that hens with Genotype A (2.4 kb band absent) had longer pause periods between clutches than those hens with Genotype B (2.4 kb band present) at $p<.05$.

DISCUSSION

The broiler breeder MHC is polymorphic, when examined by Class II RFLP mapping. This is consistent with other work performed in white leghorn birds (Chausse *et al.*, 1989 and Lamont *et al.*, 1990). Lamont *et al.* (1990) used Class I and Class II probes to analyze DNA from congenic birds and demonstrated polymorphisms of both loci. Class IV polymorphisms were observed via RFLP mapping by Miller *et al*. (1988) and Uni *et al*. (1993). Emara *et al*. (1992) found that the turkey MHC exhibited Class II polymorphisms, which were further characterized by Zhu *et al*. (1995). Wittzell *et al*. (1994) described three MHC Class II haplotypes in the ring-necked pheasant utilizing RFLP. Thus, the present finding of polymorphisms is not surprising, but expands our knowledge of polymorphisms in different types of birds.

Neither frequency of bands observed with the Class II probe utilized (CCII-7-1; Xu *et al.*, 1989), or banding patterns observed, can be used to assign any known haplotype, since we do not have parent, sire, or offspring genotype data. Parental and offspring information must be obtained and compared to the known generation in order to determine the inheritance of this locus. There are ongoing efforts among avian immunogeneticists to establish an international reference standard for RFLP genotypes as they relate to known serological haplotypes. The serological haplotypes were catalogued by Briles *et al*. (1982) and by Briles and Briles (1989). The reference standards are based primarily on white leghorn chickens. More detailed analysis of the broiler breeder MHC must be performed before the results of this study can be extrapolated to the established haplotypes or to the entire population of broiler breeder hens.

The banding patterns observed in the present study represent five genotypes (A-E). The patterns were observed in 18/35, 13/35, 1/35, 1/35, and 2/35 broiler breeder hens. The 13/35 group (Genotype B) had a 2.4 kb band which was not present in any other group. A 3.4 kb band was observed in 34 birds, as was a 4.25 kb band. The 5.4 kb band was present in two birds. The largest fragment seen, a 7.5 kb band, was observed in only one bird. Since the majority of birds fell into the 18/35 and 13/35 groups, the other four hens were deleted from the statistical analysis, to leave a pure comparison between Genotypes A and B.

Egg production during a 45-week period, when all eggs were set, was different between the two groups. Hens with the 2.4 kb band laid significantly fewer eggs than broiler breeder hens without the 2.4 kb band (Genotype A). Hens with Genotypes A and B laid 0.365 and 0.260 eggs per day respectively. Briles and Allen (1961) also observed lower rates of lay in B homozygous white leghorns and New Hampshires. Both crossbred and inbred white leghorns had similar associations (Allen and Gilmour, 1962; Nordskog *et al*., 1973). Zhu *et al*. (1995) found that selection of turkey lines for egg production resulted in changes in MHC haplotypes observed. The present experiment showed that an association of egg production with the MHC exists in the broiler breeder hen.

Total egg production, average egg mass, percent fertility, and percent hatchability of fertile eggs were not different in this study. The lack of differences for these traits in the current study is not unusual, in relation to the existing body of literature. Although total egg production was relatively higher for the hens without the 2.4 kb band (197 ± 12 vs. 169 ± 14), the values were not significantly different. This may represent a biological difference which is not significant due to sample size. Egg mass was found to be affected by MHC haplotype

in three studies. Kim *et al*. (1989) demonstrated this in inbred white leghorns. Abplanalp *et al*. (1992) and Sato *et al*. (1992) demonstrated the effect in congenic white leghorns, differing only at the MHC. No effect of the B complex on fertility has been shown to date. Hatchability was higher for chicks from heterozygous than from homozygous sires (Briles and Drueger, 1955). Abplanalp *et al*. (1992) observed effects of the B complex on hatchability in 11 lines of congenic white leghorns.

In conclusion, this study establishes the fact that the Class II genes of the MHC are polymorphic in broiler breeder hens. Also, an effect of the MHC on reproduction of broiler breeders was observed during a 45-week hatching period, as evidenced by a higher number of eggs produced by the Genotype A group of hens, lacking a specific restriction fragment of 2.4 kb. The polymorphisms observed here must be characterized further in order to effectively relate the present data to previously published studies. However, the possiblity of utilizing the MHC as a tool in broiler breeder selection seems achievable.

APPENDIX I

DNA Isolation

1. Store 50 µL aliquots of blood in 1.5 mL microcentrifuge tubes containing 100 µL 1X SSC at -20°C.

2. Thaw aliquot quickly at 37°C.

3. Add 400 µL 12X SSC and mix gently.

4. Add SDS to 1% and mix gently for 10 min on a reciprocal shaker.

5. Add 500 µL phenol/chloroform/isoamyl alcohol (25:24:1) and mix 20 min on shaker. Pipette sample until it is homogenized.

6. Centrifuge 5-15 min at 14000 X G.

7. Pipette supernatant to a sterile tube, avoiding the interphase.

8. Repeat phenol/chloroform/isoamyl alcohol extraction.

9. Add 2.5 volumes of 100% ethanol. Precipitate DNA by inverting the tube repeatedly. Carefully discard the ethanol.

10. Add 400 µL H_2O and allow pellet to dissolve.

11. Add 2.5 volumes of 100% ethanol. Precipitate DNA by inverting the tube.

12. Discard the ethanol. Wash the pellet with 500 µL 70% ethanol.

13. (optional) Dry the pellet for 5 min, dissolve it in 400 µL H_2O, and repeat the ethanol precipitations.

14. Invert the tube over Whatman paper to dry the DNA.

15. Dissolve the DNA in 100-200 µL of TE buffer.

NOTE: Always wear gloves when working with phenol.
When using fresh blood, begin at step 3 with the addition of 500 µL 10 X SSC.

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