Analysis of Genetic Polymorphisms in the Major Histocompatibility Complex of Japanese Quail

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ABSTRACT The restriction fragment length polymorphisms (RFLP) of the Japanese quail MHC were assayed in seven lines using PvuII-digested DNA and a chicken Class II probe. The lines of Japanese quail surveyed included a randombred control population (R1) and sublines of R1 divergently selected for 4-wk body weight (HW and LW lines) or plasma yolk precursor as measured by total plasma phosphorus (TPP) (HP and LP lines). In addition, two sublines (HW-HP and HW-LP) of the HW line were included in the analysis. Males of both sublines were selected for increased 4-wk body weight whereas the females were selected for increased (HW-HP) or decreased (HW-LP) TPP. The number of birds surveyed per line ranged from 13 to 16.

The chicken probe used produced discernible bands or fragments using Southern blot analysis. There were 16 different RFLP patterns as well as 7 different heterozygote patterns detected in the various Japanese quail lines. The band or fragment number of each pattern varied from 7 to 15. A total of 28 different bands or fragments were seen in the RFLP patterns and only 2 bands or fragments were common to all 16 patterns.

The distribution of the RFLP patterns differed greatly among the Japanese quail lines. The R1 line differed in frequency of the patterns from all of the selected lines. The divergently selected lines (HW vs LW; HP vs LP; and HW-HP vs HW-LP) also differed in the frequency of the various RFLP patterns. In the comparison of the HW and LW lines, there were no RFLP patterns in common between the two lines. The results of the present study indicated that the Japanese quail MHC Class II genes were highly polymorphic.

(Key words: Japanese quail, major histocompatibility complex, body weight, plasma yolk precursor, restriction fragment length polymorphisms)

INTRODUCTION

The MHC is a chromosomal region originally recognized more than 50 yr ago as controlling the compatibility of tissue grafts (Klein et al., 1993). The chicken MHC was originally described as a blood group system (Briles et al., 1950). Three classes of chicken MHC antigens have been discovered. The Class I and Class II antigens are known to have critical roles in the initial steps of specific immune responses (Dietert et al., 1991). The Class IV antigens (with unknown functions) are distributed on red blood cells. In addition to the MHC genes, Briles et al. (1993) reported the existence of a second, independently segregating polymorphic MHC-like locus, designated Rfp-Y. The MHC and Rfp-Y genes are located on Chromosome 16 but segregate independently (Miller et al., 1994, 1996) and can be detected with a Class II probe (Pharr et al., 1997).

The MHC loci are highly polymorphic. Serological methods have elicited more than 30 haplotypes in chickens (Dietert et al., 1991). The mechanism of maintaining high polymorphism is not fully understood, although a high mutation rate and strong selection are considered possible causes (Kaufman et al., 1990). Haplotypes of the chicken and turkey MHC are associated with genetic resistance to a variety of disease infections (Dietert et al., 1991; Plachy et al., 1992; Nestor et al., 1996b). Heterotic effects have been reported for...
MHC-associated disease resistance (Brown et al., 1982; Collins et al., 1985; Lukacs et al., 1989; Martin et al., 1989), and some haplotypes were resistant to one disease but susceptible to another (Guyre et al., 1982; Clare et al., 1985; Nestor et al., 1992; Zhu et al., 1996b). Genetic changes in body weight and egg production may be associated with changes in frequency of MHC haplotypes (Bacon, 1987; Dietert et al., 1991; Abplanalp et al., 1992; Plachy et al., 1992; Zhu et al., 1995).

Although there are many published studies on the chicken MHC and, to a lesser extent, the turkey MHC, information is lacking on the Japanese quail MHC. In view of the highly conserved characteristics of the MHC between species (Kroemer et al., 1990), the present research examined whether a chicken Class II gene probe would be useful for typing Japanese quail MHC haplotypes and analyzed polymorphisms of the MHC in several Japanese quail lines.

**MATERIALS AND METHODS**

**Japanese Quail Lines**

The lines of Japanese quail used in the present experiment were from a selection study designed to evaluate the genetic relationship between body weight during the growing period and plasma yolk precursor during the early portion of the laying period. The base population for this study was a randombred control population that had been maintained with a large number of breeders (greater than 100 of each sex) for more than 10 generations. Five sublines were developed from the base population. One of these (R1) was maintained as a randombred control. Sublines were selected for high (HW) and low (LW) 4-wk body weight and for high (HP) and low (LP) level of total plasma phosphorus (a measure of yolk precursor) during the beginning of the laying period. Two sublines of the HW line were developed in the ninth generation of selection by continuing to select males for increased 4-wk body weight while selecting females for increased (HW-HP) or decreased (HW-LP) total plasma phosphorus. At the time of the present study, the HW, LW, HP, and LP lines had been selected for 30 generations (21 generations for the HW-HP and HW-LP lines). Selection responses in the lines have been reported by Nestor et al. (1982, 1987, 1996a).

Mating was at random in all lines except that full-sib matings were avoided. With the exception of the HW and LW lines, all lines were reproduced with 36 parental pairs (Nestor, 1977). In the 20th generation, the number of parental pairs was increased to 48 in the HW and LW lines. The number of birds used per line varied from 13 to 16.

**RESULTS**

The chicken MHC Class II probe used in the present experiment hybridized with Japanese quail MHC genes and produced strong signals resulting in bands or fragments that were discernable (Figure 1A, B). Sixteen different RFLP patterns, designated Q1 to Q16, were observed. In addition, seven apparent heterozygotes, designated H1 to H7, were discernable. Of the RFLP patterns, Q1, Q14, and Q15 were similar, differing only in the second (from top of the lane) band or fragment. The second band or fragment of Q1 migrated faster than that of Q15 but both had similar width. The second band or fragment of Q14 was wider than that of Q15 but both had similar width. The second band or fragment of Q15 was wider than that of Q16. The Q10 pattern had two more bands (2.15 and 6.9 kb) than the Q2 pattern. The Q8 and Q11 patterns became two bands upon longer duration of electrophoresis (about 30 h). The Q2 and Q16 (not given in Figure 1) RFLP patterns were very similar except the third band of Q2 migrated more quickly than that of Q16. The Q10 pattern had two more bands (2.15 and 6.9 kb) than the Q2 pattern. The Q8 and Q11 patterns differed in width of the bands from Position 4 through 7. In the Q8 pattern, all of the bands were wider, whereas in the Q11 pattern only the fourth and fifth bands were wider. Each of the wider bands of the Q8 and Q11 patterns became two bands upon longer duration of electrophoresis (about 30 h).

The size distribution of the different RFLP patterns varied greatly (Table 1). Fragment number within patterns varied from 7 to 15. There were 28 different

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**Restriction Fragment Length Polymorphism Analysis**

The restriction fragment length polymorphism (RFLP) analysis method used was reported by Zhu et al. (1995). Briefly, a 2.3-kb fragment from a genomic clone of a chicken MHC Class II β gene (Xu et al., 1989) was utilized to make the probe. The fragment was purified by gel electrophoresis and elution (Maniatis et al., 1986). The fragment was used as a DNA template for probe labeling with a Genius 1 DNA labeling and detection kit. The quail DNA (10 μg) was isolated from blood cells and digested with PvuII restriction enzyme and separated by electrophoresis on a 0.8% agarose gel for 30 h at 1.5 V/cm, then fragments were denatured, neutralized, and blotted to nylon membranes. Prehybridization, hybridization, and color development were based on procedures described in Genius System User’s Guide (Boehringer Mannheim, 1992).

**Statistical Analysis**

The significance of line differences in the frequency of RFLP patterns in the various Japanese quail lines was estimated by chi-square. The R1 line was compared with each of the selected lines. The divergently selected lines (HW vs LW, HP vs LP, and HW-HP vs HW-LP) were also compared. The HW-HP and HW-LP lines were each compared to their base population (HW).
sized fragments included in the 16 RFLP patterns. Of them, only two bands or fragments (2.08 and 4.41 kb) were common in all 16 patterns. Six bands or fragments were pattern specific, two were shared by 2 patterns, and the others were shared by 3 to 12 patterns. The third bands of Q2 and Q16 and the second bands of Q1 and Q15 appeared to be controlled by allelic genes. Some band differences could also be differentiated by band color density. For example, although the 2.74-kb fragment appeared in Q1, Q2, Q5, and Q7 through Q16, the color of this fragment was lighter in Q1, Q5, Q7, Q14, and Q15.

The 16 RFLP patterns were confirmed to be stable after a second digestion, electrophoresis, and hybridization on a different sample of DNA. Based on the RFLP patterns and their distribution in the different Japanese quail lines, H1 was believed to be a heterozygote of Q1 and Q4, H2 of Q1 and Q2, H3 of Q1 and Q16, H4 of Q2 and Q9, H5 of Q2 and Q4, H6 of Q2 and Q16, and H7 of Q1 and Q15. Therefore, the Q1, Q2, Q4, Q9, Q15, and Q16 patterns were haplotypes. It is not known whether the other patterns were heterozygous or homozygous.

The frequency of the different RFLP patterns varied greatly among the Japanese quail lines (Table 2). The R1 line was significantly different \( (P \leq 0.05) \) from each of the selected lines. The HW and LW lines had no pattern in common. The HW-HP and HW-LP lines were significantly different \( (P \leq 0.05) \) from each other and the HW-LP line differed significantly \( (P \leq 0.05) \) from the HW line, which was the base population of both the HW-HP and HW-LP lines. The difference between the HW and HW-HP lines approached significance \( (P = 0.095) \). Of the 16 RFLP patterns, 12 were specific to one line, 2 (Q1 and Q8) were shared by two lines, 1 (Q16) was present in four lines, and 1 (Q2) was present in all lines.

**DISCUSSION**

Information on the serological or RFLP classification of the Japanese quail MHC is not available in the literature. In the present research, individual blood samples obtained from seven Japanese quail lines were tested for MHC Class II polymorphisms using \( \text{PvuII} \) digestion and a chicken Class II \( \beta \)-gene probe. Many distinct fragments and RFLP patterns were observed. With the same method, Emara et al. (1992, 1993)
FIGURE 1. Restriction fragment length polymorphisms of Japanese quail MHC obtained in a Southern blot after hybridization with a chicken MHC Class II probe following digestion of DNA by the PvuII restriction enzyme. A = Patterns Q1 through Q9 and H1 through H3; B = Patterns Q1, Q2, Q10, Q11, Q12, Q13, Q14, Q15, H4, H5, and H6. M = molecular marker.