THE USE OF MAJOR HISTOCOMPATIBILITY COMPLEX CLASS I RESTRICTION FRAGMENT LENGTH POLYMORPHISM ANALYSIS TO PREDICT PERFORMANCE IN THE PIG

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SUMMARY

Restriction fragment length polymorphism (RFLP) analyses of swine leukocyte antigen (SLA) class I genes were performed on 32 litters of Duroc pigs to identify genetic markers associated with performance traits in the pig. Southern blotting and hybridization procedures were performed on genomic DNA isolated from white blood cells, by using Pvu II endonuclease and a swine major histocompatibility complex (MHC) class I probe. Of the 13 different bands observed, 8 were polymorphic. Least squares procedures, using typical animal breeding models, and a mixed animal model with complete relationships were used to analyze the data. Results from the least squares analyses suggested that the polymorphic swine MHC class I restriction fragments accounted for 6% and 4% of the variation for average daily gain and backfat, respectively. When the animal model was used, however, smaller effects were associated with those class I fragments. These results suggest that the swine MHC has a small, but significant effect on performance in the pig.

INTRODUCTION

New technologies may offer opportunities to improve selection efficiency in swine but must be examined both in light of their ease of use and relative to the added genetic improvement they offer. A new class of polymorphisms, RFLPs has been developed. The RFLPs are created by using restriction endonucleases to cleave DNA molecules at specific sites. The fragments of DNA are then identified by using cloned DNA probes that detect specific homologous DNA fragments. RFLP analysis has been suggested as a means to identify varietal strains or parentage, to identify quantitative trait loci (QTL) and to use in genetic improvement programs (Soller and Beckmann, 1983). RFLP analyses could employ a large number of random probes to attempt to cover the entire genome such that QTL could be identified or use loci specific probes to determine if specific loci could serve as markers for performance traits.

The MHC, found in nearly all animals, codes for the predominant cell surface proteins primarily responsible for control of the immune response. The MHC is also involved in many nonimmune functions. The swine MHC is the SLA complex. The SLA complex is located on chromosome 7 (Geffrotin et al., 1984) and is composed of at least three class I, two class II (Vaiman et al., 1979), and three class III loci (Lie et al., 1987). The total span of the SLA region is from 0.8 cM to 1.0 cM (Singer et al., 1983; Vaiman et al., 1988). The number of genes in the SLA class I region has been estimated to be approximately 6 to 15 (Singer et al., 1982; Chardon et al., 1985). An extensive review of the SLA complex and its functions is found in Warner and Rothschild (1990).

The SLA complex also seems to be associated with growth and carcass traits (Capy et al., 1981; Vaiman et al., 1988). Previous research to examine the relationship between the MHC and performance traits has generally relied on serological typing to determine haplotypes. A potentially more powerful tool would be the use of RFLP analysis. Jung et al. (1989) used RFLP analyses to determine if SLA class I genes were associated with growth and performance in a

diverse sample of Duroc and Hampshire pigs. Results suggested that a large number of SLA class I fragments were polymorphic and that some were associated with average daily gain and backfat (Jung et al., 1989). It was suggested that further study was needed using family data so that effects of these fragments could be evaluated more carefully. The purpose of this current research was to determine if SLA class I RFLPs were associated with growth and performance in Duroc pigs and to assess their relative influence on these traits.

MATERIALS AND METHODS

Data and blood samples were obtained from 165 Duroc pigs from 32 litters at the Iowa State University Bilsland Memorial Swine Breeding farm. The litters were the result of crossing lines of pigs which had been involved in a seven-generation selection experiment for leg soundness (Rothschild and Christian, 1988). The 32 litters, born in the fall of 1988, resulted from 13 different line-cross-combinations and 10 different sires. Pigs were weaned at 56 days of age, placed on test in a confinement facility and taken off test at about 100 kg body weight. Backfat (BF) measurements were taken ultrasonically and averaged, and average daily gain (ADG) was computed from weaning to the end of the test. Off-test-weight (OTW) was recorded and used to adjust the data.

Ten ml of sterile blood were obtained from each pig. The isolation of genomic DNA, digestion by Pvu II endonuclease, blotting, and hybridization with an SLA class I gene probe (PD1-A, kindly provided by Dr. Dinah Singer, NIH) were performed as described by Flanagan et al. (1988).

Two approaches were used to analyze the data. The first approach used standard least squares analyses to estimate the effect of various factors, including the polymorphic SLA class I restriction fragments, on BF and ADG. The factors were fit sequentially such that the relative contribution of each factor to the total variation in BF and ADG could be determined. The factors included in the least squares analysis of variance were line, sire, dam(sire), sex of the pig, a covariate for OTW and an effect for the presence or absence of each of the polymorphic SLA class I restriction fragments. All polymorphic restriction fragments were fit simultaneously because they represented the SLA class I genotype of each pig. The R-square (R^2) values were calculated for each model. Tests of significance for each fragment were made by using an F-test.

The second statistical approach used was a mixed model analysis using an animal model (Kennedy et al., 1988) with a complete relationship matrix for the pigs in the experiment. Factors included in the model for the mixed model analyses were line, litter(line), animal(litter), sex of the pig, a covariate for OTW and an effect for the presence or absence of each of the polymorphic SLA class I restriction fragments. All polymorphic restriction fragments were fit simultaneously because they represented the SLA class I genotype of each pig. Litters and animals were assumed random, and heritabilities for BF and ADG were assumed to be .40 and .25 respectively. Each fragment effect was tested by computing a t-test using the elements of the inverse of the coefficient matrix.

RESULTS

The restriction fragments ranged from 1.9 kb to 9.4 kb. Five were monomorphic whereas eight of the restriction fragments were polymorphic with frequencies ranging from 28 to 92%.

In the least squares analyses the models that included all the factors except fragments had R^2 values of .39 and .62 for BF and ADG, respectively. In

Table 1. Effect of the Swine Class I MHC Polymorphic Restriction Fragments on Backfat and Average Daily Gain.

	Backfat (mm)		Average Daily Gain (g/day)	
Fragment(kb)	Model I[a]	Model II[b]	Model I	Model II
7.7	-.77± .87	-.64± .76	-2.85±17.53	-10.43±16.78
6.5	1.10±1.91	.97±1.83	11.60±38.08	-8.17±39.92
5.2	-2.01±1.11*	-1.14± .76	-30.63±22.15	-8.62±16.33
4.2	-.29±1.61	.58±1.50	-37.16±32.20	-28.58±32.66
2.9	.09± .99	-.01± .76	8.27±19.95	-5.44±16.33
2.7	-.13± .71	-.31± .64	-1.27±14.19	-12.70±13.61
2.4	2.37±1.93	1.58±1.14	126.95±38.61***	51.39±23.58**
1.9	-.83±1.65	-.99±1.63	65.49±33.05**	51.03±35.38

* P<.10, ** P<.05, *** P<.01

[a] Least squares analysis, Model = line + sire + dam(sire) + sex + OTW + fragments

[b] Mixed model analysis, Model = Line+ Litter(line) + animal(litter) + sex + OTW + fragments

comparison, the models that included the restriction fragments had R^2 values of .43 and .68, and the restriction fragments accounted for an increase in the R^2 of 4% and 6% for BF and ADG, respectively. The results from the mixed model analyses show a similar pattern with the restriction fragments accounting for approximately 2% and 4% of the variation for BF and ADG, respectively.

Estimates of the effects and their standard errors of the polymorphic SLA class I restriction fragments presented in Table 1 are from the most complete models for both types of analyses. For BF the effect of the 5.2 kb restriction fragment was -2.01±1.11 mm (P<.10) in the least squares analysis and was reduced to -1.14±.76 mm (P<.15) in the mixed model analysis. For ADG, the effects of the 2.4 kb and 1.9 kb restriction fragments were significant (P<.05) in the least squares analysis. In the mixed model analysis of ADG, the effect of the 2.4 kb restriction fragment was reduced to 51.39±23.58 g/day (P<.05) while that 1.9 kb restriction fragment was 51.03±35.38 g/day (P<.15).

DISCUSSION

This sample of Duroc pigs was taken from a seven-generation selection experiment in which no outside germplasm was introduced over the past eight years. Inbreeding within the lines was in excess of .20 before they were crossed to produce most of the 32 litters. Previously, Jung et al.(1989) used a random sample of boars from the 1988 National Duroc test and most of these animals would be generally unrelated to each other and certainly unrelated to the pigs in this present study. In Jung et al.(1989), a greater number of class I restriction fragments were observed and none were monomorphic. It seems logical that fewer SLA class I restriction fragments were seen in this study using family data as compared to the data in Jung et al.(1989).

Previous SLA class I research, which suggested an association of class I genes with performance traits in the pig, had relied on serological methods to detect SLA genotypes (Capy et al.,1981; Vaiman et al.,1988). Recently, Jung et al.(1989) found that two SLA class I restriction fragments seemed to be associated with BF in Duroc pigs. One of these fragments, DP11, is probably the same as the 5.2 kb fragment which was significantly associated with BF in this sample of Duroc pigs. However, the effect on BF of the DP11 fragment in that study was the opposite of the effect of the 5.2 kb fragment from this study.

Jung et al.(1989) also found that four SLA class I restriction fragments were associated with ADG in Durocs. Two of those four fragments, DP26 and DP31, which are probably the same as the 2.4 kb and 1.9 kb restriction fragments, respectively, in this study, were both associated with slower growth rate in the Jung et al.(1989) data. In this study, however, the 2.4 kb and 1.9 restriction fragments were associated with faster growth rate. In both studies, with extremely different samples of the Duroc breed, the same fragments were associated with either BF or ADG. The finding that their effects were in opposite directions suggests that the SLA class I genes associated with these restriction fragments are linked to growth and backfat in Durocs but that the linkage relationship is different in the two populations of pigs. Hence the linkage relationship in a given population would first need to be determined before selection based on these restriction fragments could take place.

In previous studies, MHC data were analyzed by standard least squares analyses. Linkage disequilibrium in field data can lead to apparent associations between quantitative traits and marker genes which may not be repeatable in other studies. A mixed model analysis using an animal model with complete relationships would remove the effects of linkage disequilibrium (Dentine and Cowan, 1989). We analyzed the data using both least squares and mixed model procedures. The effects of the fragments on BF and ADG were less using the mixed model analysis. These side-by-side analyses suggest that the association of SLA class I genes on performance traits is small but significant.

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REFERENCES

CAPY, P., RENARD, C. and SELLIER, P. 1981. Ann. Genet. Sel. Anim. 13: 441-446.
CHARDON, P., VAIMAN, M., KIRSZENBAUM, M., GEFFROTIN, C., RENARD, C. and COHEN, D. 1985. Immunogenetics. 21: 161-171.
DENTINE, M.R. and COWAN, C.M. 1989. Theor. Appl. Genet. (in press)
FLANAGAN, M.P., JUNG, Y.C., ROTHSCHILD, M.F. and WARNER, C.M. 1988. Immunogenetics 27: 465-469.
GEFFROTIN, CL., POPESCU, C.P., CRIBIU, P., BOSCHER, J., RENARD, C., CHARDON, P. and VAIMAN, M. 1984. Anim. Genet. 17: 213-219.
JUNG, Y.C., ROTHSCHILD, M.F., FLANAGAN, M.P., CHRISTIAN, L.L. and WARNER, C.M. 1989. Anim. Genet. 20: 79-81.
KENNEDY, B.W., SCHAEFFER, L.R. and SORENSEN, D.A. 1988. J. Dairy Sci. 71(Suppl. 2): 17-26.
LIE, W.R., ROTHSCHILD, M.F. and WARNER, C.M. 1987. J. Immunol. 139: 3388-3392.
ROTHSCHILD, M.F. and CHRISTIAN, L.L. 1988. Livest. Prod. 19:459-471.
SINGER, D. S., CAMERINI-OTRO, R.D., SATZ, M.L., OSBORNE, B., SACHS, D. and RUDIKOFF, S. 1982. Proc. Natl. Acad. Sci. (USA) 79: 1403-1407.
SINGER, D.S., LIFSHITZ, R., ABELSON, L., NYRJESY, P. and RUDIKOFF, S. 1983. Mol. Cell. Biol. 3: 903-913.
SOLLER, M. and BECKMANN, J.S. 1983. Theor. Appl. Genet. 67: 25-33.
VAIMAN, M., CHARDON, P. and RENARD, C. 1979. Immunogenetics. 9: 353-361.
VAIMAN, M., RENARD, C. and BOURGEAUX, N. 1988. In: Mol. Biol. Major Histo. Complex Domest. Anim. pp 23-38.
WARNER, C.M. and ROTHSCHILD, M.F. 1990. Curr. Concepts Immunogenet. (in press)