Utilizing unique genetic populations to identify molecular markers associated with economic traits in poultry

Susan J. Lamont

Department of Animal Science, Iowa State University, Ames, Iowa 50011 U.S.A.

INTRODUCTION

Any study to identify the molecular genetics underlying important traits in livestock consists of at least five stages. First, the phenotypic trait to be studied and the genetic markers to be analyzed must be carefully defined. Second, an appropriate resource population of animals must be designed and produced. Third, the phenotypic data of the resource population must be collected. Fourth, the molecular genotypic data for candidate genes or linkage disequilibrium analysis must be generated. Fifth, and finally, statistical analysis of the phenotypic and genotypic data must be conducted to identify and interpret the relationships of genetic and phenotypic variation of the resource population. Ultimately, this information can lead to the detection of DNA markers that can be applied for genetic improvement of populations in marker-assisted selection (MAS) schemes of animal breeding.

This paper illustrates the special use of unique genetic populations, and their crosses, to conduct studies of microsatellite molecular markers and candidate genes for important biological traits of chickens. It also shows how specialized population

designs can be used to explore important genetic phenomena such as gene interaction and estimated number of loci controlling various different economic traits.

USE OF INBRED LINES AND CROSSES

Inbred lines to estimate genetic distance by microsatellites

The phenomenon of genetic distance in livestock species is a very important one, in that animal breeders may wish to maximize heterosis in offspring by selecting specific parents or specific lines to cross that are genetically distant from one another. The phenotypic appearance of animals may not be an accurate predictor of their genetic distance. Sometimes the breeding history of populations of interest is not known. *The question, therefore, arises whether there are molecular genetic techniques that may be efficient and accurate for estimating genetic distances between populations.*

We addressed this question by using the unique availability of highly inbred lines of chickens, all with known pedigree and with various levels of genetic relatedness, to explore the efficacy of microsatellite markers as an estimator of genetic distance between lines (Zhou and Lamont, 1999). Twenty-three highly inbred chicken lines derived from Leghorn, Jungle Fowl, Fayoumi and Spanish breeds were analyzed. The role of inbred lines in research is of fundamental importance, because of their ability to provide variation across lines, but constancy within lines over time. Forty-two microsatellite markers were chosen to widely cover the genome (Groenen

et al., 2000). These microsatellites identified alleles that were both breed-specific and line-specific. Using this information on sharing of alleles between lines, the genetic distances were then estimated. The estimated distances were in very good concordance with the known histories of these lines, in that the Fayoumi and Jungle Fowl lines had the greatest estimated distances from other lines, and the MHC-congenic lines were estimated to be genetically very close. A phylogenetic tree grouped the 23 lines into four clusters, which also agreed with their genetic history. These data, therefore, provide support for the use of microsatellites for the study of genetic biodiversity and distance.

Inbred line cross to identify polygenic control of antibody kinetics

Most traits of economic interest in agricultural species are under the control of many genes. Because of this complicated nature, it can be difficult to identify the genes or genomic regions that exert control on traits of interest. *One approach to maximize the opportunity to identify markers and genes for very complex traits is to follow the lead of researchers in mice, and that is to simplify the genetic nature of the resource population under investigation.*

To dissect the polygenic control of antibody kinetics in chickens, we designed a unique F₂ resource population that originated from a cross of diverse, highly inbred lines. The genetic distance between the parental lines maximized the feasibility of finding molecular genetic polymorphisms. Their inbred nature made the entire F₂ resource population as if it was a full-sib family, thereby minimizing genetic “noise” and maximizing the opportunity for detection of marker-trait associations. The

evaluated phenotypic traits were antibody responses to *Salmonella enteritidis*, sheep erythrocytes, and *Brucella abortus*. A step-wise approach was used to identify several microsatellites associated with antibody response. For each of the traits, first, bulked segregant analysis was conducted, then individual genotyping of markers that showed frequency differences between the pooled DNA of the phenotypic extremes and, finally, typing of all F₂ individuals for markers with frequency differences between the two phenotypic extremes. Several candidate genes related to immune function were also tested in a step-wise fashion. The DNA sequence of the parental lines was determined and polymorphisms were identified that could be used to develop simple and economic assays for individual genotyping of the entire F₂ population. Polymorphisms in the promoter regions, which are hypothesized to modulate gene expression levels, of the interferon-gamma, interleukin-2 and immunoglobulin lambda light chain genes were evaluated. Results strongly suggested a role of promoter polymorphisms of interferon-gamma in antibody kinetics of chickens (Zhou et al., in press).

USE OF CROSSES OF OUTBRED BY INBRED LINES

Outbred-inbred F₁ cross to study candidate gene interaction

Many studies to identify markers or genes associated with biological traits evaluate only a single resource cross population. In this situation, accurate information on the effect of the markers can be derived for that specific population, but whether the effect will be expressed at the same level in other populations is not

immediately known. *The extent to which different gene interactions modulate the expression of marker-linked genes across various populations may determine the general applicability of experimental results and, therefore, the effectiveness of application of marker-assisted selection for animal improvement.*

To explore the important phenomenon of gene interaction on the expression of molecular marker effect, we developed a unique F₁ resource population from outbred broiler sires that were each mated to highly inbred dams of multiple diverse lines. Because the dam lines were highly inbred, the effect of alleles segregating from the sires could be evaluated in the F₁ generation. Because the inbred lines were diverse, the differences in the marker effects, as estimated in the different dam-line crosses, could be used as an estimate of the effects of gene interaction. The measured phenotypes were levels of antibody produced after vaccination against *Salmonella enteritidis* and in other, unvaccinated chicks, the bacterial burden after challenge with pathogenic bacteria. We evaluated both microsatellite markers and biological candidate genes in this population, recognizing that the level of linkage disequilibrium of any F₁ population is such that candidate genes may be serving as linked markers to nearby genes. Four microsatellites, previously identified by allelic frequency differences associated with vaccine response in pure broilers, had main effects, or interactions with dam-line or sex, on vaccine response in the F₁ chicks (Kaiser et al., in press). Several candidate genes, selected by biological role (NRAMP1, MHC class 1, and IAP1) or position near these microsatellites (PSAP), were evaluated and generally also showed effects that were modulated by the genetic

background of the dam line (Lamont et al., in press). Thus, we present strong evidence for considering the importance of gene interaction effects that may occur in different populations during the application of marker-assisted selection.

Outbred-inbred F₂ cross to estimate QTL number for biological traits

One of the most fundamental questions that underlies research in the identification of molecular markers for biological traits is: *how many genes control the phenotypic variation in this specific trait in this population?* The answer to this question will help to direct research into the most efficient experimental designs to find markers or genes that may be of maximal value in application for animal population improvement. It is, however, very challenging to estimate the number of genes that control most polygenic, or quantitative, traits, because it is usually very difficult to separate the phenotypic variation that occurs because of environment from the variation that occurs because of genetic differences.

We utilized the unique opportunity of designing an F₂ population that originated from crossing of outbred broiler sires and highly inbred dams to estimate the number of genes controlling several important traits (Lamont and Deeb, 2001). Phenotypic measurements were recorded for a variety of muscling, skeletal, fat, inner organ, and growth characteristics in the F₂ population and in contemporary pure inbred and outbred parental-type lines. Based on the means and distributions of the phenotypic data, and using the inbred line performance as the estimate of genetic variation, the effective number of genes contributing to each trait was estimated. The

results suggest that a low number of genes (approximately 2 to 10) determined the genetic variation in various traits related to fitness, such as skeletal and inner organ measures. In contrast, traits for which broilers have been traditionally selected, such as body weight and breast muscling, appear to be controlled by higher numbers of genes. Combined, these results imply that the traits that have been under very little selection pressure, such as fitness traits, may be controlled by a relatively low number of genes and therefore the identification of such genes or markers may lead to rapid improvements when applied in a selection program. The relatively high number of genes for traits such as body weight suggest that it will be more challenging to identify the genes for these traits and that, generally, each identified gene will account for only a small part of the phenotypic variation.

SUMMARY

This paper has illustrated the use of novel genetic populations and crosses, combined with molecular genetic analyses, to study such phenomena as genetic distance and biodiversity, microsatellite markers and candidate genes for important biological traits, gene interaction, and estimated number of genes controlling various traits in chickens. The important role for using inbred lines, outbred lines, and genetically distant populations in various research designs has been shown.

ACKNOWLEDGMENTS

The author gratefully acknowledges the excellent research work of Nader Deeb, Michael Kaiser, Wei Liu, Huaijun Zhou, Joe McElroy, Lindsey Ingleby, Jenny Lanning, and the staff of the Poultry Science Center, and fruitful discussions with Avigdor Cahaner and Jack Dekkers. Financial support was provided by the State of Iowa, Animal Health, and Multi-state Research Funds, and BARD, the US-Israel Binalational Agricultural Research Fund, postdoctoral fellowship FI-279-98 (to N.D.) and research grant IS-3021-98CR.

REFERENCES

Groenen, M. A. M., H. H. Cheng, N. Bumstead, B. F. Benkel, W. E. Briles, T. Burke, D. W. Burt, L. B. Crittenden, J. Dodgson, J. Hillel, S. Lamont, A. Ponce de Leon, M. Soller, H. Takahashi, and A. Vignal, 2000. A consensus linkage map of the chicken genome. *Genome Research* 10: 137 – 147.

Kaiser, M. G., N. Deeb, and S. J. Lamont, in press. Microsatellite markers linked to *Salmonella enterica* Serovar Enteritidis vaccine response in young F₁ broiler-cross chicks *Poultry Science*.

Lamont, S. J. and N. Deeb, 2001. Genetics of body composition in a novel broiler cross. *Proceedings of the XV European Symposium on the Quality of Poultry Meat*

(eds., R.W. A. Mulder and S.F. Bilgili), WPSA Turkish Branch, Kusadasi Turkey, pages 23 – 28.

Lamont, S. J., M. G. Kaiser, and W. Liu, in press. Candidate genes for resistance to *Salmonella enteritidis* colonization in chickens as detected in a novel genetic cross. *Veterinary Immunology and Immunopathology*.

Zhou, H., and S. J. Lamont, 1999. Genetic characterization of biodiversity in highly inbred chicken lines by microsatellite markers. *Animal Genetics* 30: 256 – 264.

Zhou, H., A. J. Buitenhuis, S. Weigend, and S.J. Lamont, in press. Candidate gene promoter polymorphisms and antibody response kinetics in chickens: interferon-gamma, interleukin-2, and immunoglobulin light chain. *Poultry Science*.