Unique Population Designs Used to Address Molecular Genetics Questions in Poultry

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ABSTRACT The ability to answer some of the questions of fundamental interest in functional genetics depends upon the utilization of appropriate and sometimes quite novel population designs. We will explore examples of how unique population designs of varied complexity can be used to explore questions, such as the following. What is the number of genes controlling different biological traits? Can streamlined genetics at the whole-animal level help to dissect complex genetic traits? Are there beneficial alleles “hiding” in undeveloped lines? To what extent is the expression of a specific gene modified by interactions with other genes in the population? Unique genetic resources, combined with novel experimental designs and contemporary analytical techniques, provide exceptional opportunities to explore both fundamental and practical questions in biological sciences.

(Key words: population design, candidate gene, inbred line, gene interaction, gene number)

INTRODUCTION

Any study to identify the molecular genetics underlying important traits in livestock consists of at least five stages. First, the phenotypic traits to be studied and the genetic analysis approaches must be carefully defined. Second, an appropriate resource population of animals must be designed and produced. The importance of this step cannot be underestimated, because the population design contributes to the efficiency of all subsequent steps and the validity of the study’s conclusions. Third, the phenotypic data of the resource population must be collected. Fourth, the molecular genotypic data, such as candidate genes, microsatellites, or microarray expression profiles, 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 approach can lead to the detection of functional genes and DNA markers that can be applied for genetic improvement of populations in marker-assisted selection schemes of poultry breeding.

This paper illustrates the special use of unique genetic resources, such as gene interaction and estimated number of loci controlling various economic traits.

USE OF INBRED LINES AND CROSSES

Inbred Lines to Estimate Genetic Distance

The phenomenon of genetic distance in livestock species is a very important one, because 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 (Fairfull, 1990). Also, biodiversity preservation will be most effective if based on accurate assessment of the genetic variation and distance of the populations chosen for conservation. 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 molecular genetic techniques may be verified for efficiency and accuracy in estimating genetic distances between populations.

We addressed this question by using the unique availability of many highly inbred lines of chickens, 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. Inbred lines play an important role in research, because of their ability to provide variation among lines but constancy within lines over time and place (Abplanalp, 1992). Twenty-three highly inbred chicken lines derived from Leghorn, jungle fowl, Fayoumi, and Spanish breeds were analyzed (Zhou and Lamont, 1999). Forty-two microsatellite markers were chosen to widely cover the genome (Groenen et al., 2000). These microsatellites identified al-
leles that were both breed-specific and line-specific. Using the information on allele sharing between lines, genetic distances were 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 (Zhou et al., 2001). These data, therefore, provide support for the use of microsatellites for the characterization of genetic biodiversity and distance.

**Inbred Line Cross to Identify Polygenic Control of Complex Traits**

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 individual 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 (named the Iowa Antibody Kinetics Resource Population) that originated from a cross of diverse, highly inbred lines (Zhou et al., 2001). 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*. Several candidate genes related to immune function were tested. 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. As an example, 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., 2001).

**Inbred Line Cross to Study Candidate Gene Interaction**

Many studies to identify markers or genes associated with biological traits evaluate only a single marker or gene in the resource population. In this situation, accurate information on the effect of the gene can be derived for that specific population, but whether the effect is modulated by interaction with alleles of other genes in the population is not immediately known. The extent to which different gene interactions modulate the expression of gene effects may determine the general applicability of experimental results and, therefore, the effectiveness of application of genetic selection for poultry improvement.

To explore the important phenomenon of gene interaction on the expression of allelic effect, we used the same F2 resource population as described in the previous section. Simultaneous examination of multiple genes allowed the opportunity to estimate not only the main effect of each gene but also the interaction effects between genes. As an example, the interaction effects of interferon-gamma and immunoglobulin light chain were more significant than the main effects of each single gene on primary SRBC antibody response and on the time required to reach that level (Zhou et al., 2001). The inheritance of the specific allelic combination of Leghorn alleles for both genes resulted in lower primary antibody level and lowered minimum secondary levels than any of the other eight pairwise combinations of alleles for these two genes. In contrast, F2 offspring inheriting two Leghorn interferon-gamma alleles and two Fayoumi immunoglobulin light chain alleles took a longer time than any other allelic combination to reach the minimum secondary antibody level. Thus, we present evidence for considering the importance of allelic interaction effects that may occur between genes during the application of marker-assisted selection.

**USE OF CROSSES OF OUTBRED BY INBRED LINES**

**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 (the Iowa Growth and Composition Resource Population) that originated from crossing of outbred broiler sires and highly inbred dams to estimate the number of genes controlling several important traits (Deeb and Lamont, 2002). 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 pheno-
typic 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 two to five) determined the genetic variation in various traits related to fitness, such as inner organ weight and total (bone and muscle) drumstick weight, as percent of body weight. Shank ratio (weight to length) was estimated to be controlled by a minimum of about 12 genes. In contrast, traits for which broilers have been traditionally selected, such as total body weight and breast muscling (percent of body weight), appear to be controlled by higher numbers (10 to 80) of genes. Combined, these results imply that the traits that have been under little selection pressure, such as fitness traits, may be controlled by a relatively low number of genes; therefore, the identification of such genes 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 shown the use of novel genetic populations and crosses, combined with molecular genetic analyses, to study such phenomena as genetic distance and biodiversity, molecular markers, and candidate genes for important biological traits, gene allelic interaction, and minimum 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 illustrated.

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