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Use of DNA "Fingerprints" as Genetic Markers in Poultry Breeding

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ABSTRACT

Progress in genetic selection of economically important farm animals is hampered by having to evaluate genotypic worth by measuring phenotypic characteristics. Recent developments in molecular techniques are allowing evaluation of genotype rather than phenotype of individuals. Human minisatellite probes cross hybridize to hypervariable regions (HVR) in the poultry genome, providing extremely polymorphic genetic markers (DNA "fingerprints") which are individual-specific. This BARD project addressed efficacy of DNA fingerprinting as a method of identifying genetically superior individuals for selection programs in animal improvement. Populations of chickens divergently selected for quantitative traits over several decades provided an excellent model for evaluation of the procedures. Two major approaches were evaluated. One was the construction of linear models to identify contributions of specific DNA fingerprint fragments to economically important quantitative traits. Such identification would be especially useful for traits that are expressed late in life, sex-limited, and/or amenable to sib selection and for within-family selection of lowly heritable traits. The second approach used the total scoreable DNA fingerprint information as a tool for either elimination of an unwanted genome or for selection of a desired one. Such genomic selection can enhance breeding programs which include backcrosses such as introgression, upgrading crosses and within-line breeding.

OBJECTIVE

The objective of research described in this proposal is to evaluate the feasibility of using DNA fingerprints (DFP) to estimate genetic worth of individuals for quantitative and qualitative traits. Two major concepts were investigated as possible methods of evaluating these associations: the linear model (LM) and genomic selection (GS)

Linear Model. Within a segregating sire family of chickens, the association between a DFP band and a gene coding for a quantitative trait

(QT) was tested for several growth, immunological and reproductive characteristics.

Genomic Selection. Choice of individuals to reproduce each back-cross generation in an introgression procedure was based on similarity of an individual's DFP to that of its grandparent. Thus, selection for a desired genome or away from an undesirable genome based on DFP information was evaluated.

DESCRIPTION OF RESEARCH RESULTS

Following are summaries of the experiments conducted cooperatively between the researchers in Blacksburg and Rehovot as a result of BARD Research Grant # US-1557-88. These articles include work in the following areas:

- DNA fingerprint techniques
- DFP characterization of selected populations included in the BARD project
- Evaluation of relative contribution of several evolutionary forces to genetic diversity among populations
- Evaluation of the linear model analysis for ascertaining associations between DFP bands and genes coding for quantitative traits
- Several tangential projects which were undertaken during the course of research conducted under the auspices of the BARD grant to characterize particular populations and further test the efficiency of the DFP procedure.

DNA FINGERPRINT TECHNIQUES

"DNA Fingerprints from Blood Mixes in Chickens and in Turkeys" (Hillel et al., Anim. Biotech. 1:201-204, 1990). Although DNA fingerprints are useful in individual identification and genetic linkage studies, expensive and time-consuming laboratory procedures limit their practical application. By mixing blood from individuals within a population, a DNA fingerprint pattern representing the population can be obtained. The pattern was identical to that in which DNA from individuals was mixed, and was not improved by adjusting blood volumes according to hemoglobin levels.

DFP CHARACTERIZATION OF SELECTED POPULATIONS

"DNA Fingerprints of Chickens Selected for High and Low Body Weight for 31 Generations" (Dunnington et al., Anim. Genet. 21:247-257, 1990). Two lines of White Plymouth Rock chickens that were divergently selected for 8-week body weight for 31 generations were compared for patterns of DNA fingerprints. Digestion of DNA with Hinf I and hybridization to Jeffreys' minisatellite probe 33.6 resulted in DFPs that were relatively similar within lines (bandsharing = .50) and less similar between lines (bandsharing = .22). Analyses of scorable DFP bands produced by mixing DNA from individuals within lines indicated that 48% were line-specific. Causes for the differences in DFP patterns between lines and for occurrence of line-specific bands for the two lines divergently selected for body weight were discussed.

"Line-specific DNA Fingerprint Bands in Lines of Chickens Selected for High or Low Antibody Response to Sheep Erythrocytes" (Hillel et al., Arch. Geflugelk. 55:189-191, 1990). DNA fingerprint analyses were employed to ascertain if lines of chickens selected from a random mating, common base population for 16 generations for high (HA) or low (LA) antibody response to sheep erythrocytes were different in DFP patterns. Twenty-five percent of the DFP bands were specific to one of the lines but did not occur in the other. Use of DNA mixed from several individuals within a line to produce DFP bands was shown to be an effective and efficient method of obtaining a "group" DFP pattern.

"DNA Fingerprint Comparisons Between Selected Populations of Chickens" (Dunnington et al., Poult. Sci. 70:463-467, 1991). A pair of lines of White Plymouth Rock chickens selected for high or low juvenile body weight, a pair of White Leghorn chickens selected for high or low antibody response to sheep erythrocytes, and an F1 cross between each pair of lines, were used to produce DNA fingerprints. These DFP were prepared by mixing equal amounts of DNA from several individuals of a particular population, resulting in a DFP characteristic of the population. The populations provided individuals of known genetic relationships and in-

breeding levels to evaluate the sensitivity of the DFP technique with DNA mixing. Levels of bandsharing between breeds were lowest, those between selected lines within a breed were intermediate and those between the selected lines and their F1 crosses were highest. These results show that DFP analysis is sensitive to several levels of genetic relationship.

EVOLUTIONARY FORCES INFLUENCING GENETIC DIVERSITY

"Genetic Factors Accountable for Line-specific DNA Fingerprint Bands in Quail" (Hillel et al., DNA Fingerprinting: Approaches and Applications, 1990). DNA fingerprints, prepared from mixes of DNA of individuals samples from lines of Japanese quail selected for high or low 4-week body weight, were used to evaluate the relative contribution of several evolutionary forces to genetic diversity among populations. Comparisons between lines - two replicates of each selection direction and an unselected control line - were used to determine the frequency of line-specific DNA fingerprint bands produced by each of three major evolutionary forces: 1) mutation; 2) genetic drift; 3) selection. The latter force is expected to generate line-specific bands only if there is linkage disequilibrium between DNA fingerprint loci and quantitative loci (QTL) controlling body weight. Using probes 33.6 and R18.1, an average of 48.4 DNA fingerprint bands in each line were analyzed. On average, 27.8 bands were found to be line-specific among the 96.8 (2×48.4) bands analyzed in an average comparison between pairs of lines. Based on the frequencies of line-specific bands in each particular comparison, it was calculated that 21% of the line-specific bands were due to mutation, 11% due to a single genetic drift event, 21% due to the combined effects of genetic drift and selection, 22% due to double independent events of genetic drift, and 14% due to undefined factors.

ASSOCIATIONS BETWEEN DFP BANDS AND GENES CODING FOR QUANTITATIVE TRAITS

"DNA Fingerprint Bands Linked to Loci Coding for Quantitative Traits in Chickens" (Dunnington et al., Poult. Sci., in press, 1992). Efficacy of DNA fingerprint bands in marker assisted selection programs for quantitative traits in chickens was evaluated. A cross between two populations of White Plymouth Rock chickens that had been selected for 31

generations for high (HW) or low (LW) 8-wk body weight served as the base population for the experiment. Full- and half-sib families were produced over four generations and 400 offspring were measured for body weight at 8 wk (BW8) and shank length at 12 wk of age (SL12). Distributions were constructed for each quantitative trait in offspring of one F_1 sire. The DFP produced from mixed blood of the individuals within each tail of the distribution were compared. From a total of 13 DFP bands that were disparate in intensity between the tails, four bands were chosen for analysis. Matings were made between males and females based on the presence or absence of these bands, but were limited to individuals which were within .5 standard deviation of the mean for the distribution of a particular trait. Quantitative traits of the resulting progeny were analyzed to determine whether parental type (presence or absence of the DFP band) influenced expression of the trait in the offspring. One band out of the four tested was associated with SL12, was an effective predictor of phenotype for both SL12 and BW8, and appeared to be inherited in a dominant fashion.

"Evaluating Linkage between DNA Fingerprint Bands and Quantitative Traits: Appropriate Family Structure" (Dunnington et al., submitted). This study assessed the influence of background genome on expression of genes linked to DNA fingerprint bands in chickens. Two experimental lines of White Plymouth Rocks previously selected for high or low eight-week body weight were crossed to produce two F_1 males that served as heads of two sire families. Each of these sires was mated to three hens from an unrelated White Leghorn population to produce progeny in which quantitative traits of 4-, 8-, and 12-week body weight and shank length at 12 weeks were measured. DFP patterns were produced for all individuals in the study. For the twelve DFP sire-specific bands (common to both F_1 sires but not found in the DFP patterns of the dams), each offspring was classified as having or not having each band. Then, an analysis of variance was conducted for each DFP sire band with sex, hatch, dam family, and presence or absence of the sire band as main effects. Interactions between dam family and presence or absence of sire band were also tested. Of 48 possible analyses of variance (12 sire bands X 4 quantitative traits), five resulted in significant effects due to sire band, and four indicated significant interactions. Thus, associations of a DFP band and

a gene coding for a QTL were present, but, in some cases, the expression of the QTL differed, depending on the dam family in which it occurred. These data suggest that searches for such associations must be conducted within known family structures, preferably full-sib families.

TANGENTIAL PROJECTS

"DNA Fingerprint Analyses of Selected and Relaxed Lines of Chickens" (Haberfeld et al., submitted). Effects of selection and its relaxation on DNA fingerprint patterns were evaluated in experimental lines of White Leghorn chickens. Chickens were included in the study from generations 16, 17 and 18 of divergent selection for antibody response to sheep erythrocytes and from two consecutive generations of relaxation of selection initiated after 16 generations of selection. Comparisons were made of characteristic DNA fingerprint (DFP) patterns for each population to evaluate whether selection or genetic drift was responsible for particular banding patterns. Individual DFP patterns of chickens from the second generation of relaxation in the low-antibody line were analyzed along with the antibody titers of those individuals to ascertain whether any line-specific bands could be attributable to selection. One out of six such DFP bands may have been due to selection for low antibody titer, suggesting that DFP bands may be potential aids in selection programs.

"Genetic Distances Estimated from DNA Fingerprints in Crosses of White Plymouth Rock Chickens" (Haberfeld et al., Anim. Genet. 23:159-165, 1992). Crosses were produced between two lines of White Plymouth Rock chickens, one of which had been selected for low 8-week body weight for 31 generations (L) and the other of which was an bantam population (B). The parental lines, reciprocal F1s, reciprocal F2s and all possible backcrosses to each parental line (total of 16 populations) were available for study. Blood was obtained from ten females within each population. DNA was extracted from blood mixes (equal amounts of blood from each individual) for each population, and from blood samples of each individual in the two parental lines. Fourteen line-specific DNA fingerprint (DFP) bands (those bands present in one parental population, but not in the other parental population) were analyzed (eight from Line L and six from Line B). Regression analyses were conducted to compare the known pro-

portion of genomic contribution from each parental population with values based on relative band intensity obtained with a scanning densitometer. The resulting regression coefficient of 1.004 demonstrated that DFP analysis of relative band intensity is an effective method of estimating the relative proportion of genome contributed by parental populations.

"Jungle Fowl-Domestic Fowl Relationships: A Use of DNA Fingerprinting" (Siegel et al., World's Poult. Sci. J., in press, 1992). There is a long and continuing interest by poultry breeders and scientists in relationships of domestic chickens with their ancestral jungle fowl. Recent commercial practices in poultry breeding have greatly changed and improved the available meat and egg stocks for production of food for human consumption. There is concern, however, that artificial selection for traits of economic importance has caused a narrowing of the genetic base and subsequent reduction in genetic variability. Because continued progress for increased growth or egg production depends heavily on genetic variability, the status of the latter is of interest. Current techniques in molecular biology allow estimations of genetic distance among populations. This paper provides a review of DNA fingerprinting in poultry and uses this technique to survey genetic diversity among jungle fowl, chickens representative of stocks from several decades ago and today's commercial broilers and layers. The genetic distance between wild jungle fowl and layers has been found to be similar to that between wild jungle fowl and broilers. Within the two types of commercial chickens, however, there is less genetic diversity among broiler than layer stocks.

GENOMIC SELECTION

"Genomic Selection: A Use of DNA Fingerprinting in Introgression Procedures" (manuscript in preparation). Two roosters from a commercial layer stock that were heterozygous for the naked neck mutant (donors) were mated with females from a Cornish line (recipients) to produce an F_1 population. Of the resulting F_1 progeny, 22 individuals which were heterozygous for naked neck were selected and backcrossed with chickens from the Cornish (recipient) line, creating the first backcross (BC_1) generation. DNA fingerprints (DFP) were prepared from each 176 Naked Neck BC_1 individuals and compared to DFP of their donor grandsires. Genomic

selection (GS) was conducted in the BC_1 generation for two characteristics: 1) high body weight 2) low band sharing (BS) with the donor, and an unselected control group was also initiated. Individuals from these selection groups as well as the control group were mated with chickens from the Cornish population to produce the BC_2 population as a progeny test. Body weights of the BC_2 generation weight were measured weekly from 3 to 7 weeks of age and the performance was compared to body weights and band sharing levels of their parents. Significant correlation was found in BC_1 between band sharing and body weight ($r=-0.178$) as well as a significant correlation between body weights of BC_2 individuals and their parents ($r=0.18$) and band sharing levels between the two generations ($r=-0.18$). Response to selection was similar in both selection groups, although the BS group performance was slightly higher than the body weight group at the advanced ages. These results are particularly important in view of the significant difference between the average body weight of the BC_1 selected groups. At 7 weeks of age, mean body weight of the BS groups was higher than that of the control. The high bandsharing heritability ($=1$), the high genetic correlation with body weight ($=0.69$) and the other advantages (tagging the entire genome, non sex specific and non tissue or age dependency) make band sharing a successful indirect selection criterion.

COOPERATION

Productivity of this BARD project has been greatly enhanced because of the extremely close cooperation among the researchers. E.A.D. spent two weeks in Rehovot learning the DNA fingerprint technique in December of 1987. Other trips to Rehovot were taken in June of 1989 and June of 1991 to evaluate results, prepare manuscripts and design subsequent experiments. All of the investigators were in Edinburgh in July, 1990 as speakers at the 4th World Congress on Genetics Applied to Livestock Production and were able to interact as a group regarding accomplishments and directions for the project. Additionally, J. H. visited Blacksburg in Summer of 1987, August of 1990, March of 1991 and is currently on sabbatical in Blacksburg for the purpose of interpretation of results of this project and manuscript preparation. From November of 1990 to February of 1992, Alon Haberfeld (who received his Ph. D. under the direction

of J. H. at The Hebrew University of Jerusalem) was a BARD postdoctoral fellow in Blacksburg working in close cooperation on aspects of the BARD project.

In addition to the two projects described in the original BARD proposal, the cooperation between the Rehovot and Blacksburg research groups has resulted in several related projects involving DNA fingerprinting in the various selected populations. Results of these additional projects are described above and are listed in the attached publication list.

EVALUATION OF THE RESEARCH ACHIEVEMENTS

During the three-year course of this BARD Research Grant and the Postdoctoral Fellowship Award, very close cooperation among researchers of the two laboratories has resulted in the following achievements:

- Establishment of a DNA fingerprinting laboratory at Virginia Tech which did not exist prior to this cooperative effort.
- Exchange of ideas and research efforts which resulted in the publication list cited in this report.
- Evaluation of DNA fingerprinting as an indicator of relationship among individuals or among populations
- Determination of relative importance of particular evolutionary forces on occurrence of line-specific DFP bands in selected populations.
- Assessment of the feasibility of using DNA fingerprint bands as genetic markers to aid in classical selection for quantitative traits of economic importance.
- Highlighting difficulties and precautions that need to be addressed when using DNA fingerprinting as a selection tool.
- Evolution of new ideas and concepts concerning DNA fingerprinting that will be used in future cooperative efforts between the two laboratories.

LIST OF PUBLICATIONS

Journal Articles

- Dunnington EA, Gal O, Plotsky Y, Haberfeld A, Kirk T, Goldberg A, Lavi U, Cahaner A, Siegel PB, Hillel J (1990) DNA fingerprints of chickens selected for high and low body weight for 31 generations. *Animal Genetics* 21:247-257
- ** Dunnington EA, Gal O, Siegel PB, Haberfeld A, Cahaner A, Lavi U, Plotsky Y, Hillel J. (1991) Deoxyribonucleic acid fingerprint comparisons between selected populations of chickens. *Poultry Science* 70:463-467
- ** Dunnington EA, Haberfeld A, Stallard LC, Siegel PB, Hillel J (1992) DNA fingerprint bands linked to loci coding for quantitative traits in chickens. *Poultry Science* (In press)
- ** Dunnington EA, Stallard LC, Siegel PB, Hillel J Evaluating linkage between DNA fingerprint bands and quantitative traits: Appropriate family structures. (Submitted May 7, 1992)
- Haberfeld A, Dunnington EA, Siegel PB (1992) Genetic distances estimated from DNA fingerprints in crosses of White Plymouth Rock chickens. *Animal Genetics* 23:159-165
- ** Haberfeld A, Dunnington EA, Siegel PB DNA fingerprint analyses of selected and relaxed lines of chickens. (Submitted May 15, 1992)
- ** Hillel J, Avner R, Baxter-Jones C, Dunnington EA, Cahaner A, Siegel PB. 1990. DNA fingerprints from blood mixes in chickens and turkeys. *Animal Biotechnology* 1:201-204.
- ** Hillel J, Gal O, Siegel PB, Dunnington EA. 1991. Line-specific DNA fingerprint bands in lines of chickens selected for high or low antibody response to sheep erythrocytes. *Archiv fur Geflugelkunde* 55:189-191
- Siegel PB, Haberfeld A, Mukherjee TK, Stallard LC, Marks HL, Anthony NB, Dunnington EA (1992) Jungle fowl-domestic fowl relationships: A use of DNA fingerprinting. *World's Poultry Science Journal* (In press)

Book Chapters

- Hillel J, Gal O, Schaap T, Haberfeld A, Plotsky Y, Marks H, Siegel PB, Dunnington EA, Cahaner A. (1991) Genetic factors accountable for line-specific DNA fingerprint bands in quail. In: DNA Fingerprinting: Approaches and Applications. T Burke, G Dolf, A Jeffreys, R Wolff (editors). Birkhauser, Verlag AG, Basel, Switzerland. pp. 263-273
- Hillel J, Dunnington EA, Haberfeld A, Lavi U, Cahaner A, Gal O, Plotsky Y, Siegel PB. Multi-locus DNA markers: Applications in poultry breeding and genetic analyses. *Proceedings of Manipulation of the Avian Genome*. A. Gibbins, R. Etches, eds. CRC Press, Inc.

Proceedings

Plotsky Y, Cahaner A, Haberfeld A, Lavi U, Hillel J. (1990) Analysis of genetic association between DNA fingerprint bands and quantitative traits using DNA mixes. Proc. 4th World Cong. Genet. Appl. Livestock Prod. XIII:133-136

Abstracts

Plotsky Y, Gal O, Hillel J, Cahaner A (1989) Association between DNA fingerprints and quantitative traits in high-fat and low-fat broiler lines. World's Poult. Sci. Assoc. XXVII Annual Convention, Israel Branch, p. 27.

Hillel J, Gal O, Avner R, Plotsky Y, Haberfeld A, Cahaner A, Siegel PB, Dunnington EA. DNA fingerprints from blood mixes for group identification and linkage studies in poultry. XXII International Conference on Animal Genetics, East Lansing, MI, August 25-31, 1990.

Hillel J, Gal O, Schaap T, Haberfeld A, Plotsky Y, Marks H, Siegel PB, Dunnington EA, Cahaner A. Genetic factors accountable for line-specific DNA fingerprint bands in quail. First International Conference on DNA Fingerprinting. University of Berne, Switzerland, October 1-3 1990.

** indicates publications in which BARD is acknowledged.