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FINAL REPORT
PROJECT NO. US-811-84

**Molecular Approaches to the Improvement
of Economic Traits in Poultry**

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Molecular approaches to the improvement of economic traits in poultry

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C. ABSTRACT

Our studies were aimed at developing methods of inserting avian leukosis viruses (ALV) into the germ line of chickens, and assessing the potential usefulness of these inserts and naturally occurring ALV genes (ev genes) as molecular tags for the identification and cloning of quantitative trait loci (QTL). We showed that recombinant and wild-type replication-competent ALV can be inserted into the germ-line by infecting the blastoderm of fertile eggs just before incubation. Two interesting types of transgenic chickens were generated. One type was defective for complete virus production, but expressed the envelope gene of field type ALV and acted as dominant gene for resistance to ALV. The other type produced complete virus that reinserted at new locations in the genome and represented mobile genetic elements. Theoretical studies explored the numbers of inserts needed for using such elements as insertional mutagens to identify QTL. The numbers needed exceeded practical sized poultry populations and the use of experimental organisms was suggested. The distribution of naturally occurring endogenous ALV (ev) genes in five lines of different breed origin selected for egg production traits was explored. Remarkable conservation and segregation of conserved genes was found suggesting that selection for a common trait favored specific ev genes located at unique chromosomal loci. Association of ev genes with specific egg production traits was confirmed in a segregating F1 cross of two of the lines. Thus, ev genes may represent a selective insertional system that has an enhanced probability of placing molecular markers within or closely linked to QTL and, therefore, could lead to QTL identification and cloning.

D. THE OBJECTIVES--Molecular approaches to the improvement of economic traits in poultry.

- A. Develop biological methods for introducing genes into the chicken germ line via DNA or retroviral vectors (United States).

This research centered around the use of replication-competent avian leukosis viruses. The four attached publications document our success. This objective was pursued as originally planned.

- B. Characterize existing highly productive commercial strains of poultry with respect to ev loci, and examine the effect of these loci on production and pathology (Israel).

This research is described in write ups of two experiments that have been completed and are in the process of analysis and preparation for publication. This objective was pursued as originally planned.

- C. Develop a theoretical framework for applying molecular methods to animal breeding problems and integrate this new theory with presently available quantitative genetic theory (Israel).

A publication giving a theoretical treatment of the use of artificial DNA insertion as a method for the identification and cloning of major genes is attached. This work fits within objective C.

E. BODY OF THE REPORT

Objective A.

The results obtained under this objective are described in four publications.

Transgenic chickens: Insertion of retroviral genes into the chicken germ line.

Segregation, viral phenotype, and proviral structure of 23 avian leukosis virus inserts in the germ line of chickens.

Artificial insertion of a dominant gene for resistance to avian leukosis virus into the germ line of the chicken.

Insertion of retroviral vectors into the avian germ line.

F. COOPERATION

The collaborators in the United States have used molecular methods for the study of endogenous viral (ev) genes for several years and helped the Israeli group develop the methods in their laboratory by providing cDNA probes, samples of genomic DNA with known ev inserts, and maps of ev genes. Fuad Iraqui, a graduate student with Prof. Soller, spent about four months at the Regional Poultry Research Laboratory in East Lansing using our methods and developing new methodology. Prof. Soller met with the PI three separate times during the course of the project to discuss progress and ideas for future research.

Particularly valuable to the PI was the discussions with Prof. Soller on the implications of our germ-line research and the potential use of our families that carry mobile ALV proviruses.

G. EVALUATION OF THE RESEARCH ACHIEVEMENTS

A recent challenge to researchers and companies interested in the improvement of agricultural species, is the development of methodology for utilizing the tremendous potential of molecular biology to enhance research and the genetic improvement of a variety of species. Such integration of the new molecular genetics into classical selection schemes will, in the long run, make large contributions to agricultural productivity.

Our research has implications for two major areas of application of molecular methods to poultry genetics and breeding. First to the production of transgenic chickens, and second to the use of molecular markers for the identification and eventual cloning of major genes influencing economic traits (quantitative trait loci).

First, we have demonstrated that replication-competent avian leukosis virus vectors can be inserted into the germ line of chickens by simply introducing live virus near the blastoderm of the fertile egg just before incubation, resulting in the first transgenic chickens that carry stably inherited transgenes. Other methods have been tried, but this method is the only one that has been successful to date. This approach has now been used by others to introduce replication-defective vectors carrying model genes into the chicken germ line. Such an approach is presently the method of choice for producing transgenic chickens.

We also have shown that a small proportion of the inserts produced by replication-competent viruses are defective for virus production, but express the envelope gene of subgroup A avian leukosis

virus (ALV) that is the most common subgroup in the field. Such expression interferes with ALV infection both in cell culture and in chickens, and acts as a very efficient dominant gene for resistance to infection and oncogenesis by ALV. This model system suggests that such specific resistance could be introduced for other viruses by making animals transgenic for other viral genes. This supports the general hypothesis, proposed by others, that introduction of genes of the pathogen into the host may be a general method of producing resistant hosts. Indeed, work in our laboratory has shown that envelope expression may induce resistance to another avian retrovirus, reticuloendotheliosis virus.

Three families of transgenic chickens that carry inserts coding for complete infectious virus represent mobile genetic elements that can reinsert in the germ line at high frequency. These birds represent a potential system for generating random germ-line insertions that can act as mutagens or molecular marker genes to allow the identification and cloning of mutated or closely linked genes. While these birds have some of the highest insertion rates known in higher animals, our theoretical work has shown that very large numbers of insertions must be evaluated for quantitative traits to find a QTL. Not only must a large number of inserts be evaluated, but at least 30 animals must be tested for each insert. Therefore, a search for QTLs in chickens using random inserts would require very large numbers of animals. Therefore, we propose that preliminary experiments be conducted using experimental organisms such as Arabidopsis.

Endogenous viral (ev) genes have close homology to ALV and have entered the germ line of chickens early in the evolution of the species. They have been inserted at many loci. A least 20 loci have been identified in White Leghorns and many more exist in other breeds. Our studies of five breeds of chickens highly selected for egg production traits have shown surprising conservation of a relatively small number of ev genes. Such conservation of ev genes in these highly selected stocks suggests some selective mechanism to maintain the polymorphism. We have suggested several such mechanisms. Therefore, evolution and selection for a quantitative trait may have given us a naturally occurring selective insertional system that has retained ev genes that have entered into or are closely linked to QTL. Perhaps most ev genes have a detrimental effect on fitness and only those genetically associated with chromosomal regions favorable for fitness or selected traits survive in long-term selected populations. Thus, the study of ev genes in highly selected populations may provide us a method of drastically reducing the number of molecularly tagged inserts necessary for the identification of QTL.

To evaluate the hypothesis that ev genes influence egg production traits, we studied the influence of ev gene polymorphisms on these traits in 250 F1 cross females of two of the originally studied lines. Preliminary analysis of the data supports our hypothesis that ev genes do have an influence on quantitative traits, should be included in on-going studies of the association of RFLP and economic traits, and may help in the eventual identification and cloning of QTL.

D. PUBLICATIONS AND PATENTS

1. Publications:

Salter, D. W., E. J. Smith, S. H. Hughes, S. E. Wright, and L. B. Crittenden. Transgenic chickens: Insertion of retroviral genes into the chicken germ line. *Virol.* 157:236-240. 1987.

Soller, M. and J. S. Beckmann. Cloning quantitative trait loci by insertional mutagenesis. *Theor. Appl. Genet.* 74:369-378. 1987.

Salter, D. W. and L. B. Crittenden. Gene insertion into the avian germ line. *Proc. British Soc. of Animal Prod. and British Poultry Breeders Roundtable*, pp. 32-57. 1988.

Crittenden, L. B. and D. W. Salter. Insertion of retroviral vectors into the avian germ line. In: *Proc. Second Intl. Conf. on Quantitative Genetics*, eds. B.S. Weir, E.J. Eisen, M.M. Goodman, and G. Namkoong. Sinauer, Sunderland, MA. pp. 207-214. 1988.

Salter, D. W. and L. B. Crittenden. Artificial insertion of a dominant gene for resistance to avian leukosis virus into the germ line of the chicken. *Theor. Appl. Genet.* 77:457-461. 1989.

Crittenden, L. B., D. W. Salter, and M. J. Federspiel. Segregation, viral phenotype, and proviral structure of 23 avian leukosis inserts in the germ line of chickens. *Theor. Appl. Genet.* 77:505-515. 1989.

2. Patents and inventions:

Hughes, S. H., D. W. Salter and L. B. Crittenden. Vectors for gene insertion into avian germ line. Patent application in the name of the U. S. Government, P.C. N6948. Filed July 31, 1987.

Salter, D. W. and L. B. Crittenden. Transgenic avian line resistant to avian leukosis virus. Invention Registration in the name of the U. S. Government, P.C. 6982CR. Filed July 12, 1988.