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# Miracles of Genetic Engineering

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It seems the sky is the limit of what molecular geneticist can do these days. Developing new transgenic organisms by inserting genes from one creature to another is becoming common these days and that they draw no special attention anymore. This field is gaining a great importance in control of certain genetically transmitted diseases and disorders in humans (diabetes and Alzheimer's, for example), that it has become one of the leading research areas nowadays.

When it comes to small ruminants inserting genes responsible for certain advantageous effects from one breed of sheep to another or genes from goats or deer to sheep or vice versa, though seems complicated and fascinating, is becoming common. Geneticists did not stop at these achievements, but rather went a step further; they exchanged genetic material from plants to animals. You may wonder how genes from plants can be useful in animals! The whole concept is fascinating and as mentioned before, we are crossing the last frontier between living creatures. I attended an international meeting in 1993 and heard a presentation on the subject. I was so fascinated that I decided to let the readers of *The Shepherd* share this interesting story with me. The article was written by Dr. Ward and his co-researchers in Australia. They presented in a simple and straightforward manner the concept of gene manipulation and the fascinating research they have been undertaking in the field of genetic manipulation for disease control. The following is an abridged version of their article simplified to suit the readers of *The Shepherd*.

Dr. Ward and his co-workers started by presenting the importance of the subject, they wrote: "The ability to control the major diseases of domestic animals is an important factor influencing the productivity of animal farming enterprises and the economic costs involved can be substantial. For example, the costs of controlling the major sheep diseases of the Australian sheep industry in 1990-91 have been estimated at A$450 million and, even with this expenditure, production losses of A$500 million were incurred. It is, therefore, not surprising that disease control is a major preoccupation of all producers. A range of control measures have been adopted over the years, broadly divided into chemical, immunological, and genetic techniques. They have been successful in reducing the incidence and severity of many of the highly-destructive diseases, but each technique has associated drawbacks to its universal or long-term applicability.

Chemical control methods involve both man-made chemicals and naturally-occurring compounds. Both can be associated with problems of residues in the animal and the pasture, variable rates of biodegradation, and some level of toxicity to the animal itself. In addition, the target organisms often develop resistance to the chemicals, resulting in a need for increased dosage and frequency of application in order to maintain control. Chemicals are also recognized as potentially damaging to the environment, particularly if used indiscriminately, and they have frequently been implicated in widespread ecological damage.

Controlling disease by invoking the animal's immune system is an attractive approach because it uses the animal's natural defense system and provides no danger to the surrounding environment. It has proven very successful for some diseases, but may present logistic difficulties. Vaccination requires a suitable antigen, multiple inoculation of each animal to establish immunity and boosting at intervals to maintain antibody titles.

A highly desirable approach to disease control is to breed resistant animals. In this case, the animal requires no specific husbandry and the resistance is passed to succeeding generations. For example, using a conventional selective breeding approach, sheep have been selected that show increased resistance to fleece rot and blowfly strike and to internal parasites. The disadvantage of the method, however, is the slow rate of progress in the desired resistance trait and the difficulties inherent in establishing suitable selection criteria.

Genetic engineering techniques provide a novel way to establish animals genetically resistant to disease. This new technology allows small pieces of highly characterized DNA to be inserted into the genomes (genetic material) of domestic animals in such a way that the DNA becomes an integral part of the genetic repertoire of the recipient animal. One of its major advantages is the ability to transfer genes without regard to interspecific barriers. Thus, genes from diverse sources can be considered for their potential to increase the resistance to specific diseases. The method is potentially faster than conventional selective breeding, although, in practice, the newness of the technology means that progress is currently very slow. In principle, it is also accurate because only the gene sequence conferring the resistance trait is transferred. In addition, multiple resistance factors can be transferred by combining the genetic information from several genes.

***The Technology Involved in Genetic Engineering***

The six steps necessary in any genetic engineering project aimed at the improvement of domestic animal productivity are:

1. Identification of a protein with the properties necessary to establish the desired phenotype (appearance or characteristics).
2. Isolation and characterization of the gene encoding the relevant protein.
3. Modification of the gene for expression as required in the host animal.
4. Transfer of the modified gene to the host.
5. Identification and evaluation of the gene's expression in transgenic animals produced from the gene transfer procedures.
6. Establishment of a breeding program.

The first step in the procedure is one of the most difficult to achieve because of the limited knowledge at the molecular level of the factors that control the physiology of important production characteristics. Furthermore, while some proteins can be clearly associated with important functions, their role in the maintenance of animal homeostasis may be of such importance that any attempt to alter their concentrations would prove deleterious to the animal. In spite of these problems, it is possible to identify a number of ways to improve animal productivity in a manner that is safe for the animal and the consumer. These include the introduction of specific biochemical pathways to supplement rate limiting metabolites, the modification of the structural proteins of wool and milk, the production of foreign hormones and proteins in the mammary glands, the manipulation of the immunoglobulin genes themselves and the production of foreign proteins to act as defense agents against bacterial, fungal, and parasite attack.

Once the appropriate protein has been identified, gene encoding it must be characterized. Depending on the source of the protein and the host species to be modified, the appropriate coding sequence can either be in the form of the complementary DNA (cDNA) or the full genomic copy of the relevant gene. It is then necessary to modify the sequences surrounding the coding information to enable its expression in the new host species. While there are now a number of rules to help guide the construction of genes for transfer (transgenes), much of the experimental work necessary at this stage to achieve adequate levels of expression and the desired tissue specificity remains ad hoc.

The transfer of transgenes to domestic animals is now a well-defined procedure involving the microinjection of single-cell embryos. This technology has evolved from the pioneering studies of gene transfer in the laboratory mouse. In larger animals, the efficiency of the process falls well short of that achieved in the laboratory mouse because of differences in the morphology of the embryos and also, possibly, in the actual mechanism of incorporation of the foreign DNA by the embryo. The dissemination of the transgenic animals to the farming community can be achieved through conventional breeding programs, although no domestic transgenic animal research has yet reached this stage of maturation.

After this general introduction, the authors described their own innovative research on the application of genetic engineering to the control of blowfly strike in sheep in Australia.

One of the most important problems faced by the wool industry in Australia is the attack of sheep by the larvae of the blowfly *Lucilia cuprina*. The adult fly lays its eggs on the skin of the sheep at susceptible sites that include local skin lesions, wool contaminated by feces and urine and wool undergoing attack by bacteria and fungi that cause fleece rot. Larvae hatch from the eggs and initially feed off the local available nutrients. As they grow and develop they become more invasive, penetrate the skin of the sheep, and commence feeding from the living tissue of the animal. The physical trauma caused by the invasion is severe and, together with the accumulation of toxins released by the larvae and the tissue under attack, can result in the death of the animal if left untreated.

Flystrike can be prevented effectively



by the timely application of chemical insecticides, the most effective being those based on the organophosphates and cyromazine. One of the difficulties experienced by the producer, however, is determining whether such treatment is necessary in any particular year, since flystrike occurs only under specific environmental conditions that are hard to predict in advance. If possible, it is to the producer's advantage to avoid the significant labour costs involved in treating sheep.

There are obvious benefits if sheep could be developed with a genetic resistance to blowfly strike. Attempts have been made in previous years to identify such animals and introduce resistance to flocks that inhabit regions of Australia where flystrike is prevalent. However, flystrike resistance is multi-factorial and its selection by conventional breeding is slow and expensive. We believe that it may now be possible, using the techniques of genetic engineering, to introduce a single gene conferring resistance into the sheep genome.

In order to establish resistance in sheep to blowfly strike, it was necessary to identify proteins that would inhibit the growth and development of fly larvae, but at the same time exhibit no toxicity towards the sheep itself. Of the areas of fly larval morphology and biochemistry that might be uniquely sensitive to attack, the larval cuticle presents some attraction because of its high chitin content. This polymer is composed of beta-1, 4-linked N-acetylglucosamine subunits and is one of the most abundant polysaccharides in nature. Chitin is not found in mammals, but forms a vital part of insect cuticle, insect peritrophic membranes, and fungal cell walls.

Chitin is specifically degraded by the chitinase family of enzymes. This family includes enzymes that attack chitin either as exochitinases (largely bacterial in origin) or as endochitinases (largely fungal and plant in origin). Recognizing the link between the chitin component of insects and the degradative activity of chitinases, we examined a range of chitinase enzymes and other chitinbinding proteins for their ability to inhibit the growth of *Lucilia cuprina* larvae and discovered that several members of the endochitinase family possessed significant anti-larval activity. Because of the absence of chitin from mammals, this class of enzymes is, to the best of our knowledge, harmless to all mammals. Therefore, we propose that it may be possible to induce flystrike resistance in sheep if the genetic information for an endochitinase could be inserted into the sheep genome in such a way that it could be expressed as an active enzyme in the skin and skin secretions. The genetic information encoding a chitinase enzyme was isolated from the plant *Nicotiana tomentosiformis* as a cDNA clone.

The authors then went through a highly technical discussion on the procedures they applied to insert the genetic material from the plant to sheep. The results of their research indicated that the transgenic sheep resisted the flystrike. Dr. Ward and his co-workers gave the following general implications of their research.

It is apparent that the expression in transgenic animals of foreign proteins with insecticidal, fungicidal, or bactericidal activities offers the possibility for a novel and powerful approach to the control of disease on the farm. The primary requirement is the identification of a protein possessing appropriate therapeutic properties while invoking no adverse reaction in the host animal. The ability of genetic engineering to canvass the entire rage of nature's genetic resources holds the promise of the identification of many new molecules with such characteristics. A key requirement, however, is a thorough knowledge of the structure and physiology of the target organism and the animal host in order to ensure that the selected proteins are both safe and effective. The selection of a chitinase to attack the larvae of the blowfly provides a good example. In this case, the role of chitin in the structure of the larval cuticle and the peritrophic membrane was known from fundamental studies of insect morphology and physiology. Similar basic scientific investigations had identified the mechanism of action of various chitinases on chitin substrate and the absence of any appropriate substrate in mammalian tissues.

While the attack of sheep by blowfly larvae is a serious and costly management problem, other organisms also cause difficulties to the wool grower and chitinase may also offer some protection in these areas. Lice, for example, are also a major husbandry problem on the sheep and must be removed by chemical treatment. Since lice also have a chitinous cuticle and peritrophic membrane, it is not unreasonable to expect these organisms to be vulnerable to chitinase, although this has yet to be demonstrated experimentally. Another example is that of fleece rot, due to fungal and bacterial attack on the wool fibres themselves. Fungi possess chitin in their cell walls and are known to be sensitive to chitinase action. It has also been demonstrated that endochitinases possess a low but measurable level of lysozyme activity thus providing a method for the attack of the cell wall of grampositive bacteria. Unlike other mammals, sheep secretions are known to be deficient in lysozyme.

Genetic engineering is still such a novel technology that its use engenders considerable uncertainty in the community. The concept that genetic sequences can be moved between the plant and the animal kingdoms is a source of community unease. The driving force behind this disquiet is the concern that nature is being artificially manipulated in a way that may lead to the loss of many of the values currently associated with a naturally-evolving ecosystem. The evolutionary process is considered by some to be the only reliable pathway for genetic change, with an inbuilt mechanism for removing unsuitable phenotypes. There are, however, manfest examples of the limitations and inefficiencies of evolution. For example, it is a simple matter for a gene to be lost from a complex organism, but its restoration is an extremely difficult task for the evolutionary process. It can be argued that, in some instances, genetic capacity lost during a species evolution would be of advantage to the modern form, particularly where man has imposed an additional selection for increased domestic productivity.

Genetic engineering in agriculture offers much more than a technology for the production of rapid genetic change for improved domestic animal production, although over the next decade at least it is in this area that most effort will be concentrated. Its true value lies in its potential to access the genetic resource of the plant. It has been argued on moral grounds that genes should not be moved between species but there is no convincing scientific basis for such a view. It is obvious that each gene considered for such transfer must be evaluated on its merits and the more information that is available about the gene product and its likely interaction with the new host, the more easily can a judgement be made of the likely benefits that might accrue both to the host and society.

A valid criticism of genetic engineering of domestic livestock is that it supports and intensifies the modern "high technology" approach to farming. However, it is not so much the use of modern technology in farming that causes concern as it is the effect such practices can have on the welfare of the farmyard animals. The intrinsic value of animals beyond their productive value to the farmer is now recognized and provides a challenge to genetic engineering to utilize the technology with the welfare of the animal as a primary consideration.

The example provided in this paper of the use of a plant protein to provide protection in animals from the ravages of a specific insect is a useful example of the way genetic engineering can be of value to the animal, the farmer, and society in general. The animal would clearly benefit from resistance to the ravages of blowfly larvae. The farmer benefits from the reduced costs and decreased husbandry involved in the care of sheep in areas susceptible to blowfly strike. Society benefits from the greatly reduced use of chemical insecticides currently used to control blowfly strike in sheep.

***Reference:***

K. A. Ward, A. G. Brownlee, Z. Leish and J. Bonsing (1993). Genetic manipulation for disease control. Proceedings of XII World Conference on Animal Production, Edmonton, Alberta, Canada, pp. 267-280.

## New England Third Annual Fall Sheep Symposium October 28, 1995

The NESWGA is sponsoring their third Fall Sheep Symposium on October 28, 1995, at the UMass Hadley Farm in Hadley, Massachusetts. Another informative program is being offered and includes talks on foot rot, setting up for shearing, intensive pasture management, condition scoring, the "ten commandments" for flock health and well being, and wool handling. Once again, a broad array of commercial displays will be there.

The program begins with registration and coffee at 8:30 a.m. The program is co-sponsored again this year by the NH Sheep and Wool Growers Association, and UNH Cooperative Extension.

The NESWGA Fall Sorted Wool Pool will be held in the livestock barn at the Hadley Farm the same day from 8:00 a.m. to 2:00 p.m. Please make note of these hours . . . they will be strictly adhered to!

Pre-registration is available at reduced rates. For registration information, contact Bruce Clement, UNH Cooperative Extension, 33 West Street, Keene, NH 03431, (603) 352-4550. Registration is also available at the door.