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Impact of biotechnology on animal breeding

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ABSTRACT

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The objective of this review is to consider the techniques for (1) production of embryos, (2) identification of genes, or (3) genetic manipulation and the application of these techniques in animal breeding programmes. Genetic manipulation is the only biotechnology that holds the promise of creating new genetic variation in a species, either increasing the amount available for selection or creating it de novo where none previously existed. Gene mapping and marker assisted selection may enable new genetic variation to be introgressed into one breed from another of the same species. Marker assisted selection has the potential to increase accuracy of selection and also reduce the generation interval, especially if used in conjunction with reproductive technologies that are under development. Gene mapping may also allow the identification and isolation of genes that may be fed back into genetic manipulation programmes. Techniques of embryo production may allow a combination of increased accuracy and intensity of selection at a given level of inbreeding compared to MOET, with reduced generation interval (compared to progeny testing). Additionally, new embryo production, cloning and transfer techniques could revolutionise methods for the dissemination of improvement. As there will be great interdependence between the techniques, the way in which we use a new technique will depend upon which of the other procedures are available. As a result, breeding schemes will have to evolve to take full advantage of each new opportunity. There is a need for research not only to establish the techniques, but also to consider how best to use them in animal breeding and production schemes.

INTRODUCTION

A number of techniques that are being developed at present have the potential to contribute greatly to animal breeding. The objective of this review is to consider their exploitation and their limitations. The techniques can be considered as: (1) production of embryos, (2) identification of genes, or (3) genetic manipulation. In considering potential applications, it is important to distinguish between the initial genetic change, usually in nucleus populations,

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and the dissemination of the change throughout the (inter)national population.

GENETIC BACKGROUND

The potential application of new biotechnologies for the acceleration of genetic change has been an important factor in encouraging their detailed study in livestock and for this reason some general points about the nature of such change may be helpful. Genetic progress depends upon four main factors, its rate increasing with the amount of genetic variation, with the accuracy and intensity of selection and with shorter generation intervals. Each species has its own optimal balance which depends upon variables such as the genetics of the traits involved, the reproductive rate and the technology available to adjust this rate and the particular economics of the situation. Inbreeding cannot be avoided in any livestock population, but in populations under selection this process is usually increased. Too rapid a rate of inbreeding is deleterious to a population. The objective of the animal breeder is to maximise progress with a constraint on inbreeding. Many of the applications for new technologies that have been put forward have the effect of increasing inbreeding alongside genetic progress and thus their benefits are often exaggerated since alternatives would also produce fast progress if they were to be operated at the same rate of inbreeding. Comparisons of different alternatives at the same rate of inbreeding have rarely been made since prediction of inbreeding remains a complex genetic problem.

Before starting to evaluate the gains from new biotechnologies it is necessary to ask what can be achieved routinely at present. In cattle, particularly dairy cattle, AI using frozen semen has played an important role in increasing genetic progress by increasing the reproductive rate of the bull, leading to greater selection pressure and better genetic evaluation through the comparison of widely dispersed half-sibs. While this is the situation in dairy cattle, the starting point for other populations is different. Similar methods can be used in sheep, for example using ram sharing or AI with fresh semen, but the development and use of simpler and more reliable methods for AI, particularly the use of frozen semen, would offer ways to significantly improve genetic progress. In pigs, the use of AI for progeny testing has been largely abandoned in the UK, but AI is now being increasingly used to provide genetic links between different herds within a single breeding company, allowing improved genetic evaluation based on information from contemporary relatives. Gains achievable at present can be very reasonable in all species if standard animal breeding techniques are applied consistently and efficiently. For example, Mitchell et al. (1982) estimated that an improvement rate of almost 2% of the mean per annum had been achieved in a pig breeding programme in the UK for an index of six growth and carcass traits.

PRODUCTION OF EMBRYOS

Cattle breeders have been the first to examine the new embryo technologies because of the greater value of their stock and its intrinsically lower rate of natural reproduction. Their analyses provide a general model of what might be achieved.

Multiple ovulation and embryo transfer (MOET) schemes

Considerable work on developing techniques for superovulation, embryo recovery from donors and embryo transfer to recipients has made it possible to increase the reproductive rate of the female to a significant degree. In several countries over the last decade multiple ovulation and embryo transfer (MOET) has been used in nuclear breeding schemes to accelerate genetic change in dairy cattle. However, problems arise in practice: variation in embryo recovery following superovulation extends generation intervals and can reduce selection differentials; furthermore, evaluation and selection within the nucleus will encourage inbreeding and appropriate comparisons accounting for this have yet to be made. Nevertheless, such schemes are likely to achieve reasonable rates of genetic gain. The nucleus herds established can also serve as a focus for the application of further reproductive technologies.

A more recent and perhaps more exact counterpart to AI than superovulation and embryo transfer are the in vitro techniques of maturation (IVM) and fertilisation (IVF) of oocytes (e.g. Gordon and Lu, 1990). The genetic merit of oocytes recovered from slaughterhouses is not likely to be known and is very unlikely to be outstanding. They may be useful in the production of cross-bred beef embryos for transfer to the dairy herd or as recipient cells during nuclear transfer, but not in selection schemes. However, the potential of these techniques to accelerate genetic gain has been greatly increased by the development of methods to collect oocytes by in vivo aspiration (Pieterse et al., 1991). If the ovaries of cows can be aspirated routinely in vivo without detriment to welfare, to the extent that numerous oocytes can be obtained for maturation and fertilisation, then using this technique with high merit cows will result in the production of embryos of known pedigree and of high expected merit. The key aspects are (i) the ability to obtain oocytes from high genetic merit females and (ii) the introduction of factorial mating designs.

In MOET schemes as envisaged by Nicholas and Smith (1983) a cow produces a family of full-sibs, but such full-sib families proliferate both the parents genes and encourage co-selection of relatives. Factorial mating designs, in which each cow produces the same number of offspring from many sires (i.e. maternal half-sibs), were shown to achieve faster genetic progress at the same rate of inbreeding as the usual hierarchical design (Woolliams, 1989). While this could be achieved to some extent by mating cows to different bulls

for each flush, with IVM and IVF this approach is made very much easier and it can be considerably extended.

The effectiveness of factorial mating schemes was shown to be further improved when the number of cows and bulls used for mating was equal, with each cow mated to each bull (Woolliams and Wilmut, 1989). This is now a realistic prospect using the combination of in vivo aspiration of follicles, IVM and IVF. Nevertheless large numbers of oocytes would be required: 50 oocytes per donor female for a scheme involving 12 bulls and 12 cows in an optimistic estimate. Larger numbers of parents would be an improvement.

An alternative to the in vivo aspiration of follicles that might overcome the problem of numbers is primordial follicle culture; this is not yet feasible, however, in any species. How to obtain these primordial follicles remains a problem. One prospect is a substantial change in culling policy whereby a small proportion of better cows are culled in order to obtain oocytes whilst the less good cows are kept to provide recipients for embryos.

It is within this framework that we should consider what additional genetic progress can be made by other techniques such as the determination of sex or embryo multiplication by splitting or nuclear transfer. Two approaches are being developed to allow selection of the sex of calves born following embryo transfer. Determination of the sex of an embryo by probing for the presence of *Y*-specific DNA is now a routine procedure (Herr and Reed, 1991). By contrast, the separation of *X*- and *Y*-bearing sperm is possible only on a laboratory scale (Johnson et al., 1989). Determination of the sex of an embryo could be used in the nucleus herd to save production of calves of a given sex (usually males); factorial mating, however, firstly reduces the number of male full-sibs and secondly utilises more fully those that are produced. Nevertheless, random fluctuations from an optimum design could be reduced and the sex ratio changed at critical points; these may be important. Thus the applicability of embryo sexing is limited, perhaps allowing up to 10% increases in expected progress.

There are two different approaches to the production of groups of identical embryos: embryo splitting and nuclear transfer. Embryo splitting is a procedure that is commercially available at present, but with the limited potential of producing up to two calves (e.g. Kippax et al., 1991). By contrast, nuclear transfer has the theoretical potential to produce very large numbers of calves, but with present procedures less than 5% of manipulated eggs survived to become calves following the fusion of a donor cell to a secondary oocyte (see Bondioli et al., 1990). In addition, there is evidence that the birth weights of at least some of the calves was abnormal (Willadsen et al., 1991). In the long term, however, it may be possible to routinely transfer nuclei from a stem cell population to enucleated oocytes that have been matured in vitro and in this way to produce very large numbers of calves. Techniques which lead to the production of identical individuals will be less useful than those for the pro-

duction of additional embryos from selected donors. When a fixed number of offspring is considered, each copy of a clone occupies the place of a distinct individual and so accuracy is only gained at the expense of selection intensity, with the net result that genetic progress is reduced, especially when considered in relation to inbreeding (Woolliams, 1989). By contrast, these procedures may be extremely important in the dissemination of improvement from nucleus herds.

Dissemination of genetic improvement

In the wider commercial population the greatest impact of embryo technology would occur if it became economically feasible for cows to become pregnant through the routine use of embryo transfer. This would require a process for freezing and storing embryos so that on farms they can be rapidly thawed and easily transferred to recipients. In addition a cheap source of commercially valuable embryos would need to be developed. Such an application has already been identified commercially with the possibility of obtaining (sexed) twin beef calves from dairy cows. Using oocytes obtained from slaughterhouses IVM and IVF can be used to produce calves with a pedigree 75% or more derived from beef breeds.

Other applications depend on the ability of nuclear transfer from a stem cell population to produce large numbers of clones. It would be possible either to transfer clones from selected animals of high predicted genetic merit or alternatively to introduce a performance test for the clone. Clones would enable the commercial farmer to produce, as desired, a dairy or beef calf of known sex and genotype from any or all cows in his herd. The calves produced would be top performers according to which ever criteria the industry identified as important at the time. Tests could also include information on other management factors such as response to particular environments, disease resistance or temperament. It would also be possible to produce clones from cross-bred embryos and take advantage of heterosis. The benefits are clear: the commercial herds could become genetically superior, on average, to the breeding herds, rather than inferior as at present.

However, much more consideration needs to be given to the costs. Although a large lift in the genetic merit of the commercial herds would occur it has to be remembered that this would be once-and-for-all. Subsequent genetic change will be the same as the rate of genetic progress of the breeding herds. This has a range of implications. The replication of genotypes from the breeding herds will remove potentially many genes from the population that might prove beneficial. The damage to long-term viability could be limited by ensuring that genes that remain in the breeding population come from thorough sampling of the population, and subsequently by constraining the rate of inbreeding within the breeding herds. It is not known, however, what the opti-

mal rate of inbreeding should be. There is also a cause for concern about between-animal genetic diversity in commercial herds: whilst clone families will be more uniform in productivity, temperament and other aspects beneficial to management they will also be more uniform in disease resistance and susceptibility. This problem is difficult to quantify, but before radical changes in breeding structures occur, models are needed to improve our understanding.

Thus whilst cloning may become a commercial proposition the genetic costs as well as the benefits should be considered and a framework should be established to control perceived risks. Freezing of embryos and semen en masse has been suggested as a guard against catastrophes, but there are genetic and economic costs in retreating to these stocks should the need arise. It would seem more prudent to ensure from the outset that the risks are minimised.

MARKER-ASSISTED SELECTION

Traditional selection produces genetic progress by increasing the frequencies of favourable alleles through selection on the phenotype. The mapping of genes controlling traits of value (quantitative trait loci or QTL) provides the possibility of increasing the frequencies of favourable alleles directly by selection of marker alleles known to be linked to the favourable QTL allele. Marker-assisted selection could be used to enhance the introgression of alleles of value from one line to another, as an adjunct to traditional within-line selection or to allow new methods of selection.

Gene introgression

The use of markers to help the introgression of one or a few QTL of value from Line A to Line B is probably where marker-assisted selection will see its greatest initial use in animal breeding. The approach is in essence simple: having mapped a QTL between two markers in an F_2 or back-cross population, animals carrying the alleles from Line A for the flanking markers are chosen and from these are selected animals with as many as possible of the remaining markers from Line B. These animals will thus carry the allele of value from Line A, but have a large proportion of the remainder of their genome from Line B. The animals are crossed to Line B and the process repeated until the desired proportion of genome derives from Line B, at which point the selected animals are crossed inter se and animals homozygous for the desired region are selected.

Selection on flanking markers should be effective in retaining the gene even in the absence of good phenotypic information. In addition, the marker genotype of individuals can be measured in both sexes as soon as they are born, removing the requirement for difficult or costly phenotypic measurements.

Markers which do not flank genes which are to be introgressed can be used

to rapidly increase the proportion of the genome derived from the recurrent parent (Line B in our example). Hillel et al. (1990) have shown that selection on markers under favourable circumstances can result in more than 99% of the genome being from the desired line by only the second back-cross.

Marker-assisted introgression programmes hold some promise for animal breeding where genes of value can be found in genetically less-advanced breeds. The Chinese Meishan pig provides a potential example, for this breed has three to four more piglets than European breeds and reaches puberty at half their age, but it has a slow growth rate and is very fat, making it uncommercial in the west. The mapping and transfer of one or more genes from the Meishan with an appreciable effect on litter size would have a large economic impact and studies aimed at mapping these loci are under way (Haley et al., 1990). Transfer of disease resistance genes could also have a substantial impact on world agriculture because many efficient breeds cannot be used in the Third World because of their susceptibility to diseases to which local, less-efficient, breeds are resistant. Thus an attempt is being made to map alleles responsible for resistance to trypanosomiasis in West African N'Dama cattle for introgression into susceptible zebu breeds (e.g. Soller, 1990).

Georges and Massey (1991) have suggested the use of 'velogenetics' in which the process is further enhanced using technologies which may allow oocytes from prepubertal animals to be harvested, matured and fertilised before transfer to a host animal, thus reducing the generation interval. Experiments to investigate the practicality of this approach have been initiated (Betteridge et al., 1989). Markers would be used to select animals without recourse to phenotypic information. It can be seen that the generation interval would be minimised if embryonic cells could be induced to differentiate and undergo meiosis and gametogenesis in vitro, with marker-assisted selection of embryos in culture reducing the generation interval to a few weeks. Although fanciful at present, this series of steps may become practicable in the future.

Within-population selection

Genetic markers may provide information which can be used as an adjunct to phenotypic information in within-line or breed selection programmes. In an outbreeding population, unlike many line crosses, the phase of linkage between a gene of value and marker is unknown and will vary between individuals making detection and utilisation of QTL in particular very difficult. Lande and Thompson (1990) have suggested selection based upon an index of markers using the overall linkage disequilibrium between markers and QTL detected by multiple regression. But is seems likely that this approach will only be effective for a few generations of selection in a population newly established from a line cross (Zhang and Smith, 1992).

In most circumstances marker-assisted selection in long established outcrossing population will perforce be within family. For example, a dairy sire may be found to be heterozygous for both a marker and a linked QTL by the co-segregation of the marker with the performance of his daughters or the progeny test results of his sons. The marker may thus be used to aid selection, but the information on the association becomes available after his offspring have been selected and thus the marker can only be used to aid selection of his grand-offspring. Kashi et al. (1990) looked at this problem, using marker information to select grand-sons of a sire found to be heterozygous for QTL prior to the normal progeny test. Their conclusion was that genetic response could be increased by between 15 and 30% by the use of marker information Kashi et al. (1990) used parameters favourable for marker-assisted selection (e.g. low heritability of milk yield) and ignored effects such as reductions in the genetic variance due to prior marker-assisted selection and so these gains are likely to be overestimated. An advantage, however, of marker-assisted selection is that it allows within-family selection and thus it may be used to reduce the inbreeding that accompanies best linear unbiased prediction (Smith et al., 1991). Thus there may be value in using marker information even if the short-term gains in genetic progress are modest.

GENETIC MANIPULATION

There are four requirements for an effective scheme which exploits either gene transfer or gene targeting: it must be possible (1) to make the change, (2) to regulate expression of the gene in the desired manner, (3) to identify genes that are able to have a significant effect, and (4) to disseminate the change into the target population.

Procedures for genetic manipulation

There is only one method that has been used repeatably to introduce genetic change in livestock and this depends upon direct injection of a few hundred copies of a gene into a nucleus in early embryos (see Pursel et al., 1989). While this approach does produce transgenic animals, it has serious disadvantages. The efficiency is low, as rather less than 1% of injected eggs become transgenic young. Commonly the transgene has been linked into head-to-tail chains before integration. The site of integration is apparently random and in approximately 8% of mouse transgenic lines insertion of the transgene has damaged an endogenous gene (Palmiter and Brinster, 1986). Direct injection only offers the opportunity to introduce additional genes. The transgene must act in the genetic environment created by the endogenous genome as there is no means of changing endogenous genes. The influence of neighbouring DNA causes very great variation in the pattern of expression of the

transgene and as a result it is necessary to produce several lines with each gene in order to be able to assess the average effect of the gene with acceptable accuracy.

The isolation of embryonic stem cells from livestock species would provide alternative approaches. Following the introduction of any genetic change into a stem cell population it is possible to select cells that carry the desired change. Although this would not necessarily guarantee the desired pattern of gene expression all chimeric offspring would have the change. Secondly, it is possible to make changes to endogenous genes by site-directed mutation. Changes may be made to either the protein encoded by the gene or to the pattern of expression of the gene.

Despite a considerable research effort the mouse remains the only species for which embryonic stem cells have been confirmed by the birth of chimeric offspring following the incorporation of cells into a recipient embryo (see Robertson, 1987). Lines of cells with some morphological resemblance to mouse embryonic stem cells have been isolated from livestock species in at least two different laboratories, but the birth of chimeras has not yet been reported (e.g. Piedrahita et al., 1990). As several hundred ungulate embryos have now been cultured using variations on the procedure used for the isolation of mouse embryonic stem cells it seems probable that radically different approaches will be required.

The greatest limitation to the use of stem cells in the mouse is the need to achieve germline transmission by production of chimeric offspring. In livestock the time required for this additional generation would be a serious disadvantage. However, in these species there is a greater probability that it will be possible to establish successful procedures for nuclear transfer from embryonic stem cells. This suggestion is made on the basis of the observations that mouse embryonic stem cells bear a considerable resemblance to the cells of the inner cells mass (Beddington and Robertson, 1989) and that the nuclei of ruminant inner cell mass cells have been shown to be pluripotent with present methods of nuclear transfer (Smith and Wilmut, 1989). Even if the efficiency of such a process is low it will still provide an extremely effective method for the introduction of genetic change into livestock species as it will reduce the number of animals required for the production of each transgenic line. There is also the possibility of testing the effect of the transgene by producing groups of clones that differ only in the presence or absence of the gene (Woolliams and Wilmut, 1989).

Regulation of gene expression

Normal cell function depends upon the regulation of protein production at several different levels. These ensure transcription of the gene in the right tissue at appropriate stages of development, process and regulate the half-life

of the mRNA, control translation and determine the final site for modification of the protein (see Wilmut et al., 1991). Exploitation of methods for the introduction of genetic change depends upon our gaining a better understanding of these mechanisms.

Transcription is regulated by the binding of *trans*-acting regulatory factors to specific DNA motifs within the coding region of the gene and the flanking region. These DNA sequences are of two classes: those that are common to most genes and those that regulate tissue-specific expression. Identification of specific elements is an essential step in the use of a gene by genetic manipulation. In some cases these elements are closely associated with the promotor region while in others they are some distance from the promoter (e.g. the albumin enhancer is some 10 kb upstream of the transcriptional initiation site). Although such elements will usually direct gene expression in a tissue-specific manner the level of expression of most transgenes has been found to be very variable, apparently as a result of an influence of the neighbouring DNA at the site of integration (see Palmiter and Brinster, 1986). Transcription is believed to depend upon appropriate chromatin structure and in the case of transgenes this may not be formed reliably by fusion genes, which are commonly in tandem arrays, and neighbouring DNA may be able to overrule the influence of the fusion gene. In the few cases for which a suitable dominant control region has been identified (e.g. the globin genes, Grosveld et al., 1987) a transgene may be expected to be transcribed reliably, whereas for the others the site effect remains a considerable problem.

One factor that has been shown to have a very great effect upon the transcription of transgenes is the presence or absence of introns (Brinster et al., 1988). The proportion of lines in which expression occurs and the overall level of expression are both reduced if the transgene has no introns. This limitation is of great practical significance as many genomic clones are too large to transfer by direct injection and until now there has been no alternative to the transfer of clones without introns. However, an alternative strategy has been described which depends upon the occurrence of homologous recombination between overlapping fragments of the same gene injected into nuclei in the normal manner (Shimoda et al., 1991). So far only a fragment 10.6 kbp in length has been incorporated in this way, but there seems no reason to doubt that it will be effective with longer fragments.

The mechanisms that regulate RNA and protein processing are less well understood and accordingly they are not exploited in the design of fusion genes. In the longer term there is the prospect of being able to have more subtle effects, either by the improvement of fusion gene design or by targeting specific changes in endogenous genes. The present procedures will be seen to be crude.

Selection of useful genes

There have been a number of reviews of this subject and it is not the intention here to provide a complete list of projects that are being developed (see Pursel et al., 1989; Müller and Brem, 1991; Ward and Nancarrow, 1991; Clark et al., 1992).

Perhaps the most important step in a genetic manipulation scheme is the imaginative leap in perceiving that a particular protein might have a desirable effect if it were produced in a different way. At present a major limitation is imposed by the small number of genes that have been identified, although gene mapping projects can be expected to identify many other genes. A greater understanding of the mechanisms that regulate expression of endogenous genes will be important. It has been suggested that improvements in reproductive performance may be achieved by introducing changes to the regulatory elements of genes that govern the number of ovulations or the seasonal pattern of reproduction (Wilmut et al., 1990).

Incorporation of transgenic animals into breeding schemes

It is much simpler to use a genetically manipulated animal for the production of a novel product, such as a pharmaceutical protein, than it is to introduce it into the general breeding product. In the former it is only necessary to confirm that the animals are healthy and that the production system is more efficient than any competing system of production. By contrast, if the animal is to be released into the general population rigourous testing is essential.

Several stages are required before a genetic change (be it transgene or targeted change) may be introduced in the general breeding population (Smith et al., 1987). It must be shown that the change has a beneficial effect, without undesirable side effects. The performance of hemizygous and homozygous animals must be measured after back-crossing the new gene into the nucleus population. Haley (1991) estimated that this would cause a delay equivalent to less than two generations and using an estimate of 1.8% improvement in economic merit per generation for the UK pig population concluded that a gene that provided an improvement of 5% might be commercially useful. Similar calculations upon the use of the sex determining gene to increase the proportion of male calves suggests that the costs of including the transgene in a beef breeding scheme would probably outweigh the economic advantages (Bishop and Woolliams, 1991). It is also important that customer acceptability be established for genetically altered products.

GENERAL CONCLUSIONS

The new biotechnologies that we have considered have an impact upon the four main factors that determine genetic progress in different ways. Genetic

manipulation is the only biotechnology that holds the promise of creating new genetic variation in a species beyond natural mutational variance, either increasing the amount available for selection or creating it de novo where none previously existed. Gene mapping and marker-assisted selection may enable new genetic variation to be introgressed into one breed from another of the same species. Marker-assisted selection has the potential to increase accuracy of selection and also reduce the generation interval, especially if used in conjunction with reproductive technologies that are under development. Gene mapping may also allow the identification and isolation of genes that may be fed back into genetic manipulation programmes. Techniques of embryo production may allow a combination of increased accuracy and intensity of selection at a given level of inbreeding compared with MOET, with reduced generation interval (compared with progeny testing). Additionally, new embryo production, cloning and transfer techniques could revolutionise methods for the dissemination of improvement.

A considerable number of procedures have been considered in this review. Very few of them have reached the stage of commercial exploitation and it may well be 20 years before all of the technical advances have been made and we have learned how to use them. As there will be great interdependence between the techniques, the way in which we use a new technique will depend upon which of the other procedures are available. As a result, breeding schemes will have to evolve to take full advantage of each new opportunity. There is a need for research not only to establish the techniques, but also to consider how best to use them in animal breeding and production schemes.

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