USE OF GENETIC MARKERS IN GENETIC IMPROVEMENT PROGRAMMES

M.J. MACKINNON

CSIRO Division of Tropical Animal Production
Molecular Animal Genetics Centre
University of Queensland, St. Lucia, Qld., 4072

SUMMARY

The value and applications of genetic markers in livestock breeding programmes is currently topical because of the availability of large numbers of markers which allow screening of the genome for individual genes affecting traits of economic importance. The chances of major genes existing for most traits of interest and of finding them using genetic markers are high. The realized value of the discovered genes, however, depends on how they are exploited. Selection for major genes within breeds is expected to be more efficient than current within-breed selection procedures, but only worthwhile in a limited number of cases. Greater opportunities for exploiting markers will occur when marker technology is coupled with advanced breeding technologies within specialized breeding nuclei. Examples of how this might occur in practice are the establishment of lines which are homozygous for desirable genes, the development of lines with optimal combinations of both additive and non-additive genes, and the introgression of a desirable major gene from one breed to another. Genetic markers will increase the rates of genetic progress and diversity of genotypes available and the consequent versatility and robustness of livestock industries.

1. INTRODUCTION

Genetic marker technology now allows us to put tags on individual genes, or on small chromosome segments containing genes of interest. Prior to this development, breeders have only been able to manipulate the genetic material available to them by guessing, albeit accurately, the genetic value of animals based on phenotypic information. By providing direct access to the genes themselves, marker technology has opened up a whole new set of possibilities in the way genetic material can be exploited. Soller and Beckmann (1983) reviewed the potential applications of markers in animal and plant breeding. Likewise, this paper explores some of these possibilities in general terms and reviews some of the more recent work on marker-assisted selection (MAS). To set the scene, the kind and value of genetic material which is likely to be exposed and made directly accessible to breeders by genetic marker technology will first be discussed.

2. MAJOR GENES

2.1 Do major genes exist?

For reasons of statistical convenience, breeders have traditionally based their methods on the premise that there are a great many genes each of small effect which contribute to a trait which is observed as continuous. Arguments based on biological, statistical and empirical grounds lead to the conclusion that this theory, although practical, is almost certainly unrealistic. In biological terms, gene products

influence phenotypes through their role as precursors, substrates and catalysts in a series of interdependent biochemical pathways. Because of this interdependence, it is expected that variations in individual genes will have considerable influence on the final outcome of metabolic processes determining phenotype. From a statistical point of view, the observed genetic variation within and between populations is most easily accounted for by the existence of a finite number of major genetic factors. Theory can predict the maximum number of genes determining a given trait (Wright, in Castle (1921); Lande, 1981; Zeng et al., 1990) and it is estimated that on average 5 to 20 genes are expected to influence a quantitative trait (Lande, 1981). Also, it has been shown that continuous variation is equally well explained by a small number of loci than by a large number (Thoday, 1966; Maki-Tanila and Kennedy, 1986). Empirical evidence is mounting to suggest that major genes with, say, allelic effects of > .2 standard deviations (σ) are not uncommon. Table 1 gives examples of major genes which have been discovered in a wide range of species. On the basis of these arguments, then, it is not unreasonable to expect to uncover genes with allelic effects of up to 1 standard deviation for continuous traits exhibiting some degree of heritability.

Table 1. Examples of QTLs found in agricultural species using genetic markers

Species	Trait	Size of gene effect[1]		Reference
		Standard deviation units	Variance explained	
Tomato	Various		4-42%	Paterson et al., 1992
Maize	Various	.3-.8		Kahler et al., 1986
	Various		< 16%	Edwards et al., 1987
Soybean	Various		16-24%	Keim et al., 1990
Dairy cattle	Milk	.2-.5		Geldermann et al., 1985
"	"	1.0		Cowan et al., 1990
Beef cattle	Growth	.2-.6		Beever et al., 1990

[1] Measured as allele substitution effects

2.2 Searching for major genes

Systematic searching for major genes in animals populations is only just beginning. Statistical methods exist for screening populations for major genes without the use of genetic markers (see LeRoy and Elsen (1992) for review of simple tests, and Hoeschele (1988), LeRoy et al. (1989), Knott et al. (1992) and Kinghorn et al. (1992) for more complex tests). However, these tests have rarely been applied in practice because of their complexity or their lack of power and robustness. A more rigorous approach to searching for major genes is to construct experimental populations and conduct a gene mapping exercise. This involves evaluating large numbers of animals for the quantitative trait(s) of interest and genotyping them for a number of markers. Significant associations between marker genotypes and trait

values expose major genes linked to the markers. Soller et al. (1979) estimates that a given marker will have about 10% chance of having an associated linked QTL with effect greater then 0.2σ. Dekkers and Dentine (1991) estimate that by placing a single marker on a chromosome that it will explain approximately 40% of the genetic variation attributable to that chromosome. Thus, in theory at least, the chances of success in detecting some or all useful QTL using this strategy is good. Because of the multiple tests involved with such an exercise, the chances of finding some significant associations which are not due to real marker linked QTL effects must be taken into account.

3. VALUE OF GENETIC MARKERS

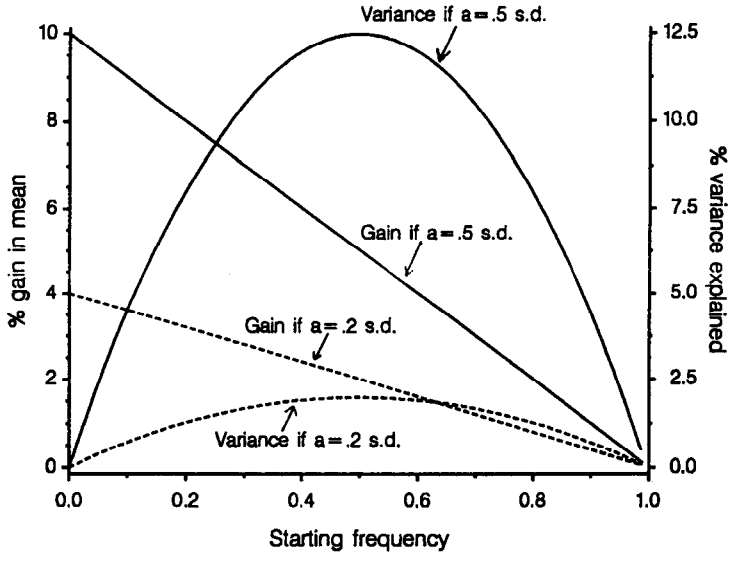
The value of a QTL tagged by genetic markers to a breeder depends first on the value of the QTL itself and second on the efficiency with which it is used via marker-assisted selection. These will now be discussed.

3.1 Value of a QTL

The potential value of a QTL depends on two sets of factors. First, it depends on the characteristics of the gene itself, namely, the magnitude of its individual effect on the trait, its mode of inheritance (level of dominance, autosomal vs sex-linked, nuclear vs mitochondrial) and its population gene frequency. These parameters can be estimated during the experimental phase using maximum likelihood methods (e.g. Mackinnon and Weller, 1992) and used to predict the expected increase in the population mean of the trait by bringing the favourable allele to fixation. Figure 1 shows the percentage increase in mean as a function of starting frequency for co-dominant, dominant or recessive diallelic genes with allelic effects of 0.5σ or 0.2σ. Such genes, if co-dominant, respectively explain a maximum of 12.5% and 3% of the phenotypic variation. For a trait with a heritability of 50% there could be, respectively, up to 4 and 25 similarly sized genes. From Figure 1 it can be seen that the increase in the mean by fixing one of these genes if it was already at a frequency of greater than 0.5 would be less than 5% for a codominant gene with effect of 0.5σ and less than 3% for a gene effect of 0.2σ. These gains would be greater if the genes were recessive. These are not large potential gains considering that a steady rate of improvement of 1% per year is sometimes obtainable using conventional selection programmes (Smith, 1984) and that intense selection may have already brought these genes to high frequencies. These figures will be considerably reduced by the inefficiencies inherent in marker-assisted selection as discussed in the next section. Even if the rate of gain were considerably accelerated by deliberately fixing individual alleles, the long-term scope for exploiting genes in this way is necessarily limited. More imaginative ways of exploiting genes should therefore be explored.

The second set of factors determining the value of a QTL relate to the genetic structure of the population. Some of these factors are the degree of population linkage disequilibrium, the mating structure, the polygenic background for the same trait, and the relationships between the QTL and genes controlling other economically significant traits. All of these determine how the QTL interacts with other factors relevant to selection programmes, and therefore they contribute to the overall value of the QTL. These interactions are complex and have not yet been explored.

Figure 1. Expected increase in mean (left axis) of a trait with a coefficient of variation of 10% by bringing to fixation a QTL with effects of either 0.5σ or 0.2σ, and proportion of total phenotypic variance explained (right axis) by such genes as a function of allele frequency prior to selection



3.2 Efficiency of marker assisted selection

Marker assisted selection can accelerate the rate of genetic improvement in two ways, namely, by increasing selection efficiency and by decreasing generation interval (Smith and Simpson, 1986). Impoved selection efficiency can be applied both in within and across breed genetic improvement strategies.

3.2.1 Within breeds

Within breeds, increased selection efficiency using markers can be achieved in two ways; either by applying index selection across the population based on combined marker and phenotypic information from individuals and relatives, or by within-family selection on the basis of known QTL-marker relationships. Lande and Thompson (1990) thoroughly investigated the impact of index selection using markers on selection efficiency relative to standard methods of index selection. They concluded that gains in efficiency of around 50-200% were expected if at least half of the additive genetic variance could be explained by the markers. Smith and Simpson (1986) estimated gains of the same order. The advantage was greatest for low heritability traits, especially if there was little information from relatives,

and in traits which were sex-limited or expressed late in life. Thus, for traits such as reproduction rate or carcass traits there are considerable potential gains in the rate of genetic improvement, but for conventionally selected traits such as growth rate and milk production the advantages of MAS are modest, especially when considered alongside the cost of scoring marker genotypes. Also, the effectiveness of this approach relies on the existence of substantial population linkage disequilibrium between markers and the QTLs. With the small effective populations sizes and structures characteristic of animal breeding populations, reasonable amounts of disequilibrium, averaged across all loci, can be expected, although this has not been checked empirically. Theoretical studies by Zhang and Smith (1992) and Marko and Soller (pers. comm.) indicate that it will probably not be high enough to explain a useful amount of variation in the quantitative trait. If this is true, then the gain in efficiency predicted by Lande and Thompson (1990) would rarely exceed 50%. The value of an index approach has also been discussed by Soller (1978), Soller and Beckmann (1982 and 1983) and Stam (1986, 1987).

An alternative approach to using an index is to select within families in which a QTL-marker linkage has been fully evaluated (Soller and Beckmann, 1983; Smith and Simpson, 1986). In this way, selection efficiency for the QTL is nearly 100% (providing that haplotypes comprised of markers in the region can discern whether crossovers have occurred, and the estimates of the QTL effects are accurate). This strategy has been evaluated for pre-selection of young dairy bulls for entry into a progeny testing scheme and it has been concluded that increases in rate of genetic gain of 10 to 50% could be obtained (Soller and Beckmann, 1982; Stam, 1986; Kashi et al., 1990). However, this scheme relies on first evaluating sires of these bulls for their QTL status and linkage phase with the marker. This requires considerable effort and in practice will only be done for a small number of sires. Sampling errors on the estimates of QTL effects and insufficient polymorphism will reduce the value of this approach (Smith and Simpson, 1986). This method will have little value in populations where within-family selection is not practical because of small family size.

The basic problem is how to use the information on markers obtained from within families to select for QTLs in the general population. The key is probably to identify haplotypes of linked markers in the vicinity of the QTL, ensure that they exhibit disequilibrium at the population level (markers can be added to the haplotype until this is true) and select on the basis of haplotype. Alternatively, haplotypes could be used with pedigree information to determine animals carrying the identical chromosome segment as the phase-known animals. Ultimately, methods will be required which combine information from both within and between families (Dentine, 1990). The incorporation of major gene and marker information into prediction methods such as BLUP is being developed (Fernando and Grossman, 1989; Cantet and Smith, 1991; Bentsen and Klemetsdal, 1991; Goddard, 1992).

3.2.2 Across breeds

Most consideration of the value of MAS has been its value to within-breed improvement. However, because this technology allows identification and characterization of individual gene effects, it is possible to track and exploit genes across breed boundaries. For example, new genes can be introgressed into breeds to correct a deficiency such as disease resistance (e.g. tick resistance genes could be introduced into Bos taurus cattle (J.E. Frisch, pers. comm.), or trypanotolerance genes into East African cattle (Soller and Beckmann, 1987). Using markers to simultaneously select for the desired QTL but against the rest of the donor genome would rapidly increase the rate of gene introgression compared with traditional backcrossing methods (Soller and Plotkin-Hazan, 1977). Another exciting possibility is that genes which contribute to heterosis through their dominant and epistatic actions could be combined in an optimal way using MAS. This handle on non-additive genes would allow greater

manipulation of the available genetic material than currently possible. In cross-breeding schemes this material is exploited mainly in the form of heterosis in the F-1 and its advantage is sometimes lost in subsequent generations. By using MAS to select for specific gene combinations, a greater proportion of this advantage could be retained. Lande and Thompson (1990) showed that MAS was most effective when it followed crossing of genetically differentiated lines, as applied in plant breeding. This strategy, combined with selection for additive gene effects, may well be a rapid approach to producing populations of genotypes which are optimal with respect to both additive and non-additive effects.

4. INTEGRATED BREEDING PROGRAMMES

While markers can be used by breeders at any level of the breeding industry (i.e. by breeding companies, studs or commercial producers), their greatest benefit is likely to occur when coupled with advanced breeding technologies (artificial insemination, multiple ovulation and embryo transfer, and in vitro fertilization of oocytes (Betteridge et al., 1989)). The benefits of using markers in conjunction with these technologies are twofold. First, these combined technologies allow greater increases in the rate of genetic progress (Georges and Massey, 1991; Kinghorn et al., 1991), and second, they allow better genetic prediction through more accurate information from genotyping. Both of these are key elements in optimising breeding strategies and industry structures (Kinghorn, 1988). More specifically, the benefits of markers are maximized by using advanced breeding strategies for the following reasons: 1) generation intervals can be dramatically reduced using markers to select at the embryo stage, 2) genetic material is more easily manipulated to produce and fix lines of desirable genotypes in small breeding nuclei, 3) linkage phase can be established more readily within such nuclei, 4) genetic material can be disseminated more effectively from such nuclei, and 5) undesirable consequences to selection for QTL can be detected and, if due to linkage, corrected. The advantages of using such schemes to exploit markers can be seen by considering some of the applications markers might have. Some examples of how markers might be used to breed economically valuable animals are: 1) production of specialized lines which are homozygous for desirable trait loci which render them suitable for niche markets, 2) production of high quality animals guaranteed by genotyping to be free of undesirable single genes e.g a genetic disorder 3) production of pairs of lines which are homozygous for loci with large non-additive effects which combine favourably, and 4) rapid introgression of desirable genes from one population to another e.g. tick resistance to Bos taurus breeds. By using markers in intensive breeding schemes in these ways, individual producers will have the ability to target specific market sectors, optimise genotypes for their environment, and change breeding objectives quickly to meet changed consumer demands. Ultimately, the use of markers in breeding programmes will result in a more hierarchical, segmented, and flexible industry which is better able to compete in the markets for livestock products.

REFERENCES

BENTSEN & KLEMETSDAL, (1991). *Genet. Sel. Evol.* **23**:407.
BETTERIDGE, K.J., SMITH, C., STUBBINGS, R.B., XU, K.P., & KING, W.A. (1989) *J. Reprod. Fert. Suppl.* **38**:87.
CANTET, R.J.C. & SMITH, C. (1991). *Genet. Sel. Evol.* **23**:221.
CASTLE, W.E. (1921). *Science* **54**:223.
DEKKERS, J.C.M & DENTINE, M.R. (1991). *Theor. Appl. Genet.* **81**:212.
DENTINE, M.R. (1990) *Proc. 4th World Congr. Genet. Appl. Livest. Prod.* **14**:35
FERNANDO, R. & GROSSMAN, M. (1989). *Genet. Sel. Evol.* **21**:467.
GEORGES, M.A.J. & MASSEY, J.M. (1991). *Theriogenology* **35**:151.
GODDARD, M.E. (1992). *Theor. Appl. Genet.* **83**:878.

HOESCHELE, I. (1988) *Theor. Appl. Genet.* **76**:311.
HOFER, A. & KENNEDY, B.W. (1992). *Genet. Sel. Evol.* (in press)
KASHI, Y., HALLERMAN, E. & SOLLER, M. (1990). *Anim. Prod.* **51**:63.
KINGHORN, B.P. (1988). Proc. AAABG **7**:233.
KINGHORN, B.P., KENNEDY, B.W. & SMITH, C. *Genetics* (in press)
KINGHORN, B.P., SMITH, C. & DEKKERS, J.C.M. (1991). J. Dairy Sci. **74**:611.
KNOTT, S.A., HALEY, C.S. & THOMPSON, R. (1992) *Heredity* **68**:299.
LANDE, R. (1980) *Genetics* **99**:541.
LANDE, R. & THOMPSON, R. (1990) *Genetics* **124**:743.
LEROY, P. & ELSEN, J.M. (1992) *Theor. Appl. Genet.* **83**:635.
LEROY, P., ELSEN, J.M. & KNOTT, S.A. (1989) *Genet. Sel. Evol.* **21**:341.
MACKINNON, M.J. & WELLER, J.I. (1992) *Genet. Sel. Evol.* (submitted)
MAKI-TANILA, A. & KENNEDY, B.W. (1986). *Proc. 3rd World Congr. Genet. Appl. Livest. Prod.* **12**:443.
SMITH, C. (1984) *Research and Development in Agriculture* **1**:79.
SMITH, C. & SIMPSON, S.P. (1986) *J. Anim. Breed. Genet.* **103**:205.
SOLLER, M. (1978) *Anim. Prod.* **27**:133.
SOLLER, M. & BECKMANN, J.S. (1982) *Proc. 2nd World Congr. Genet. Appl. Livest. Prod.* **6**:396.
SOLLER, M. & BECKMANN, J.S. (1983) *Theor. Appl. Genet.* **67**:25.
SOLLER, M. & BECKMANN, J.S. (1987) "Toward an understanding of the genetic basis of trypanotolerance in the N'Dama cattle of West Africa" FAO Consultation Report, Rome, Italy.
SOLLER, M. & PLOTKIN-HAZAN, J. (1977) *Theor. Appl. Genet.* **51**:133.
SOLLER, M., BRODY, T. & GENIZI, A. (1979) *Heredity* **43**:179.
STAM, P. (1986) In "Exploiting New Technologies in Livestock Improvement: Animal Breeding" p.170. Editors C. Smith, J.W.B. King & T.F. McKay, Oxford University Press.
STAM, P. (1987) *Animal Genetics* **18**: *Suppl. 1.* 97.
THODAY, J.M., (1966) *Proc. 3rd Int. Congr. Hum. Genet.* Sept. 5-10, 1966, Chicago, Illinois p.339.
ZENG, Z., HOULE, D. & COCKERHAM, C.C. (1990) *Genetics* **126**:235.
ZHANG, & SMITH, C. (1992) *Theor. Appl. Genet.* **83**:813