Black-and-white spots in the application of genetics to dairy cattle breeding

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Abstract

We have (briefly) discussed the various statistical and genetic assumptions underlying the evaluation and analysis of data on production and functional traits, with the emphasis that researchers should at least be aware of those assumptions. However, it is right to ask what impact violations or ignorance of these assumptions have on genetic progress. Most likely, the answer is "not very much". This is because the milk production traits are so important in determining efficiency of production, and we believe that the present day genetic evaluation methods are efficient in identifying animals of high genetic merit. However, there is always scope for small improvements, particularly in the analysis of the increasingly important health and welfare traits. Areas identified in which more research efforts could be beneficial include:

i.   Asking the scientific questions to determine what data needs to be collected.
ii. Avoidance of heterozygosity loss in the world-wide dairy population.
iii. Providing more realistic genetic models, including several QTL and polygons, for analysis of data.

Future developments are likely to be in the areas of statistical modelling and analysis. For example, it seems logical to perform a joint statistical analysis of all milk recording data simultaneously, i.e. a multi-variate multi-lactation test-day model. To ensure the robustness of such estimation and prediction procedures with many parameters, methods which take into account the uncertainty in estimated covariance components, e.g. Bayesian analysis, may be needed. More sophisticated analyses using realistic genetic models (e.g. a geometric series of QTL effects) will also be needed, so that phenotypic and genotypic information is used most efficiently.

1. Introduction

Animal improvement is simply the identification of breeding objectives, the identification of individuals likely to breed superior offspring, effective selection of these offspring, and making appropriate matings among them. In practice we make a lot of assumptions at each stage, and some of these we wish to review.

Whilst we shall focus here primarily on the assumptions used in analysing and interpreting data and in reaching decisions as to selection and mating, in practice we also need to stand further back, and consider why data are to be collected, and how they are to be used. Thus milk records, in the UK and many other countries, are collected from monthly visits by recorders. These were set as a standard long ago, largely to get reliable information on phenotype of cows in official records for both the farmers' own use and for marketing purposes with some stamp of authenticity. They were not collected in this way to be a cost-effective part of the genetic evaluation process, although indeed they might be; and

because of the current expectations of monthly recordings, the incorporation of records from automatic equipment is inhibited. When we consider functional traits, the situation is often quite unsystematic and opportunistic at best. In the UK, for example, the AI companies collect insemination records, but we have not been able to tie these up to production data collected by the milk recording organisations to use in analysis of genetic variation in fertility, for example. The Holstein Friesian Society collects data on abnormalities (e.g., undershot jaw, roach back) when doing type evaluation, but there is no demand by breeders for genetic analyses of these traits. The questions which need to be asked first are: What information is needed, and how is that to be obtained; rather than: what do we do with the data we have?

The standard model used for parameter estimation and breeding value prediction for production or type traits is of the additive linear form $Y = Xb + Za + e$, where in many analyses the Y, a and e are assumed to be multivariate normal with homogeneous variances. Yet we know that the distribution is not exactly normal, and certainly can not be over generations of selection unless the infinitesimal model holds or there is stabilising selection with a normal-shaped fitness function; and we know enough about the genotype to know that the infinitesimal model does not hold. If we assume the distribution is random, we ignore any special treatment based on pedigree for example. When we consider several generations we assume any selection practised is on the records included in the analysis; but not all cows that fail to have a second lactation do so because they were culled for milk. In addition, breeding values for milk, fat and protein are often calculated separately, so that even if cows were culled for milk only, breeding values for the other production traits may still be biased. The assumption of homogeneous variances and covariances does not, for example, accord with the general observation for production traits of heterogeneity of variances over herds; although heterogeneity is fitted in analyses of production traits in several countries, the subject has been little studied for other traits. We have no particular justification for assuming the additivity of fixed and random effects, but it is rarely tested. In summary, we have no blind spot if we appreciate all these assumptions and can demonstrate that they do not matter. We certainly do have a blind spot if we are ignorant that we have made them, or if we believe them to be facts.

2. Statistical issues

2.1 Indirect selection

Under certain circumstances (this is not the place to debate Henderson's $L'X$ matrix), BLUP, REML and related Bayesian mixed model methods can take account of selection in previous generations and culling in the present generation to obtain estimates of genetic parameters and predictors of breeding value that are free of the effects of selection. The essential requirement is that all the information (data) on which selection is practised is included in the analysis. Thus, if culling and selection are only on milk yield, unbiased estimates (formally, not quite unbiased) can be obtained for second lactation parameters and for comparisons among individuals in different generations. A trivariate analysis of milk, fat and protein yield can give unbiased estimates for fat yield, but a univariate analysis on fat alone will not, except under very restricted relationships between phenotypic and genetic correlations. This we all know. Yet analyses of many traits are univariate or multivariate, but ignoring traits which are obviously associated with the trait(s) under analysis. Clearly univariate estimates on one yield trait, e.g. fat, are biased by the selection on e.g. protein

content or yield. More subtly, culling on fertility leads to biases in trivariate analyses of milk, fat and protein; but we know culling on fertility occurs. Analyses of functional traits such as udder depth are undertaken with inclusion of, at most, data on other type traits, but not on yield of milk (or its constituents), which is correlated with udder depth and known to be under strong selection. Not only do the combined analyses enable biases to be avoided, they permit more precise breeding value prediction for the lowly inherited, often less frequently recorded functional traits.

Although these theoretical arguments point towards the use of multivariate evaluations, it is important to note that these do not come without problems. One is the increased computational cost, in terms of software, hardware and potential lack of timeliness; the other is the problem of getting sufficiently good estimates of variances and covariances to lead to improved accuracy of breeding value predictions in the multivariate case, a problem which rises with the dimensionality of the problem. There is obviously a trade-off between what may be small or large benefits and small or large costs. The point being made is merely that this should be investigated and univariate analyses not used simply through laziness or ignorance, for almost always the data cost a lot more to collect than to analyse.

2.2. Repeated records

Partly for computational reasons and partly through ignorance, it has been customary to analyse data recorded on the same trait at different ages or stages of lactation either as repeat records, i.e. assuming a unit genetic correlation, and homogeneous or heterogeneous variances between test days or lactations as appropriate, or as a series of separate traits, describing their covariances or correlations on a pairwise basis without any underlying structure. This is now changing in the analysis of milk production data from test day records, where random regression methods are being introduced for the analysis of such longitudinal records (Jamrozik *et al.*, 1997) and where the covariances can then be described as a continuous covariance function of time (Kirkpatrick *et al.*, 1994). For many functional traits, repeated records are collected on animals, for example on somatic cell count, mastitis incidence or fertility. For others, such as udder traits, chest width or condition score, animals may be recorded only once, but members of sire families provide observations at different stages of lactation, so covariance functions and sire PTAs for change in condition score can be obtained by developing the random regression methods (Jones *et al.*, 1998). It will then be possible to relate this to fertility, for example, and milk yield. Probably the more important applications to functional traits will, however, be across lactations, particularly in relation to milk yield, fertility and longevity.

2.3 Special treatment

The problems of special treatment are neither new nor vanishing. The fundamental problem is that, in a statistical sense, the error variances are not random and homoscedastic, and, perhaps more problematically, may be correlated with the genetic effects, for example in special treatment of the daughter of a good cow, who is thereby a potential bull dam, or in the differential treatment of animals got from very expensive imported semen from fashionable sires. There has been much recent discussion and analysis of the problem of differential use of BST, but this seems no different from non-random differential feeding, say of concentrates, to individual cows. Important steps have been taken to minimise effects at the statistical and management level. Included in the former category are, for example ignoring the cow's own records when

choosing bull dams, putting imported animals into separate contemporary groups from non-imported herd-mates, including sire x herd interaction terms in the model, allowing for heterogeneity of variance in the model. New robust methods are being developed (Strandén and Gianola, personal communication) to reduce effects of outliers, such as might be introduced by special treatment, for example by fitting non-normal (e.g. t) distributions to such data. Included in the category of minimising management effects are progeny testing stations, the requirement that sires have progeny distributed over many herds, (second) lactation testing stations as in Osnabruck, and nucleus herds for MOET schemes.

The concerns and steps taken have largely focused on production traits, quite properly as they are of most economic impact, but the more important functional traits become, the more the likelihood of abusing the data by special treatment, so problems of special treatment may become more serious. An obvious case is that of longevity, whereby favoured cows may get more effort paid to treat mastitis or get in calf. The latter may be not only by more attempts to achieve an effective service, but the use of special feeding to minimise loss of condition and possible infertility at peak lactation. There are not obvious solutions beyond those being practised in the design of breeding programmes and statistical analysis.

3. Genetic models

3.1. *Sources of variation*

In dairy cattle breeding we have traditionally used very simple models, the random effects typically being only breeding value, permanent environment effect (in multi-lactation or test-day analyses) and residual error. Computing opportunities and fashion have led to various other terms being added. These include: cytoplasmic (e.g., mitochondrial lineage) effects, maternal genetic effects, sire x herd interactions, and dominance effects.

Cytoplasmic (e.g. mitochondrial lineage) effects. These have waxed and waned in perceived importance, for example in our recent analysis effects were mostly non-significant (Roughsedge *et al.*, 1998). Meanwhile dairy farmers still believe in 'cow family' differences, whereas MOET based nucleus breeding programmes ignore them. Perhaps when cloning (by nuclear transfer) becomes economically feasible, the interest in cytoplasmic effects will resurface.

Maternal genetic effects. These are more commonly fitted in analyses of data on growth traits in beef cattle and other species than in dairy cattle. Their frequent inclusion shows the impact of computer packages on the models fitted, for it followed the inclusion of maternal genetic terms in Karin Meyer's DFREML program.

Sire x herd interactions. These are confounded with *environmental covariances of half sibs* and are fitted in models for genetic evaluation of dairy cattle used in the UK, US and elsewhere. Oddly, they have not been included in most analyses of beef cattle data; and it has transpired from recent analyses that inclusion of such interactions can reduce the substantial, consistent and puzzling negative genetic correlations which have been found between direct and maternal genetic effects.

Dominance effects. Whilst inbreeding depression effects have been included as a covariate in analyses, even though it is hard to square this with the infinitesimal model, dominance covariances could not be fitted until methods became available from Hoeschele and VanRaden (1991) for inversion of the dominance relationship matrix and computational methods from Misztal (1997). Now it has become fashionable, not just in the dairy cattle but in pig and poultry data analyses, even though in the typical hierarchical structure

of full within half sibs, the environmental covariance (c^2) and dominance effects are almost completely confounded.

Which is the 'correct' model? Obviously we do not know. The point we wish to make is that we should be open-minded: if in the full and half sib case, for example, we include only a c^2 term, we are likely to conclude there is indeed environmental covariance of sibs. If we include only a dominance term, we are likely to conclude there is dominance. Only if we fit models sequentially, for example dominance after c^2, can we be surer about its presence; even so the 'significant' effect may be something else we had ignored, say genetic maternal effects. This argument can, of course, be continued indefinitely so no solution is reached, which is not a practicable state. But we must always rule out at least the obvious alternative explanations before drawing conclusions about the magnitude of effects or variances and modifying breeding value prediction methods or the design of breeding programmes accordingly, and not be seduced by the most recent technological developments.

3.2 Infinitesimal models and QTL

The genotype comprises chromosomes with a finite number of genes, so clearly the infinitesimal model can be no more than a first approximation to describing the inheritance of a quantitative trait. In mapping experiments for QTL in both livestock and laboratory animals quite narrow regions of the genome with large effects on the trait have been identified (for example by Georges *et al*. (1995) in dairy cattle). Further, when analyses of selection experiments in mice spanning several generations has been undertaken using REML, estimates of heritability changed when referred back to different numbers of generations, even when selection and reduction in heterozygosity due to finite population size were taken into account (e.g., Meyer and Hill, 1991).

Formally this means that many of the important properties of the BLUP model fail, for example that the distribution of segregation variance within families is independent of parental breeding value. The rise of QTL mapping has led to some sophistication, most notably the inclusion of one QTL of large effect, perhaps identified by markers, but this QTL is assumed to be unlinked to all others. It is not clear that, if there are problems in using the infinitesimal model, they are alleviated substantially by adding just one QTL. What mapping experiments usually show is that there are several regions of the genome to which significant evidence of a QTL can be ascribed. For example, Cheverud *et al*. (1997) found QTL activity for morphological traits in mice on a majority of chromosomes, and Georges *et al*. (1995) detected QTL on five different chromosomes from an analysis of 14 dairy cattle families. In any plausible model of the genetic determination of a quantitative trait it is assumed that there are many genes (QTL) affecting it, but that their effects and frequencies differ: perhaps there are increasingly many genes (QTL) of increasingly small effect.

3.3. Non-linearity

Under the infinitesimal model, genotypes and phenotypes of individuals and their relatives are multivariate normally distributed, and therefore regressions of performance of individuals on that of their relatives are linear. Even though the infinitesimal model fails, for example if there are one or two QTL of non-negligible effect, it does not follow, that there will be either a significant or substantial departure from linearity; but the assumption ought always to be questioned even when phenotypic distributions appear normal in form. Whilst linearity is questioned as a matter of course when considering traits with obviously non-normal distributions, for example discrete traits such as twinning rate, it is liable to be ignored in others, for

example in the analysis of calving interval. Some analyses of even apparently normally distributed traits in laboratory animals have revealed substantial non-linearity of regression of offspring on parent (Gimmelfarb and Willis, 1994), and analyses of clearly non-normal continuous data such as egg number in poultry have revealed non-linearity of regressions among relatives, which can be reduced by appropriate transformations (Koerhuis, 1996). Transformation of data can, in general, achieve linearity only when the distributions of the different random effects, e.g. breeding value and residuals, have the same distribution.

In the standard selection index calculations, it is assumed that the regression of value on performance is linear. There has been a considerable amount of work considering how to incorporate non-linear profit relationships. It is debatable, however, in view of the small amounts of genetic change to which a population is subjected over one generation, and since overall merit is largely a function of its mean performance, if much improvement derives from these non-linear profit considerations, especially for lowly heritable traits.

4. Breeding structure

4.1. Inbreeding, heterozygosity and crossbreeding

With dairy cattle we are in the unusual situation among commercial livestock that most commercial animals are pure-breds, particularly as the Holstein has come to dominate production. Furthermore, with increased globalisation of dairy cattle breeding and intense selection on the bull-bull pathway, inbreeding rates in the Holstein population are beginning to rise. This has two potential consequences: an increase in inbreeding depression, which is likely to have a greater proportional

effect on traits of fitness such as fertility than on milk production, composition or growth; and a reduction in heterozygosity and thus potential for further response. Consequently there has been a substantial amount of recent work on optimisation of population structure (mating systems, family size) to maximise response and minimise rates of increase in inbreeding and loss of heterozygosity. The relevance of some of these analyses are moot, however, in that the usual assumption is of a closed breeding population. This assumption may be valid for breed conservation programmes, but not necessarily for livestock populations. In practice, there is some migration: in pigs and poultry this is likely to be between the 'closed' lines maintained by individual breeders; in dairy cattle there has long been some inter-breed introgression (now there may be some flow again of black-and whites from Europe to America). Perhaps some more theoretical analysis combining the ideas of migration from analysis of natural populations should be introduced into the analysis of livestock populations.

Inbreeding depression and heterozygosity loss in commercial animals increase at the same rate only when they are randomly mated. The ability to manipulate the dominance covariance in BLUP referred to above has also led to procedures to optimise matings within a population. These seem potentially to offer only a marginal improvement while there remains overall a single interbreeding population, where inevitably heterozygosity falls. The obvious way to avoid this is to maintain subdivided populations with as high a proportion as possible of commercial animals being two or higher way crosses among populations. (Alan Robertson pointed out the problem and solution many years ago.) The obvious problem is to effect such a structure in the freely competitive environment that exists, and where individual sires turn up with such high

PTAs that they are hard for any breeder to ignore in choosing bull sires. Ideally the population should be subdivided, so bulls are bred only from within a sub population, and then commercial breeders could practice some form of rotational/criss-cross breeding structure. Sires would be progeny tested across the population as a whole, in preference with animals to which they are lowly related, so in effect a cross-testing programme operates. It would be simple enough to fit population additive and interaction effects in the model. As so few sires are required, a large population is not needed for intense selection on the cow to breed bull pathway. Therefore only a small part of the total population would need to be strictly 'pure-bred'. Perhaps this sort of structure will evolve as there is a constant search for high PTA sires unrelated to those most widely used, but some positive action might help effect the process of breed subdivision, for it simultaneously minimises long-term inbreeding and maximises accumulation of useful genetic interactions, whether due to dominance or epistasis.

4.2. Genotype x environment interaction

For production traits, GxE is taken into account at a farm level by the inclusion of a sire x herd interaction term, and in international evaluations (by INTERBULL) by incorporation of a genetic correlation between countries. It is, however, the case that these correlations are very high; and indeed when estimates have been obtained of the correlation between defined environments, for example high/low temperature regions in the USA, high/low producing herds, and intensive /extensive management systems, rather small interactions have been found (e.g., Carabano *et al*., 1990). Thus the assumptions of unit correlations for milk production within countries seems justified.

For functional traits the information is much more scanty. Inter-country analyses of type traits generally show high correlations, and departures from one may reflect rather more differences in criteria rather than differences in response of the conformation of animals to different management systems or climates. Thus, Robert-Granie *et al.* (1997) found heterogeneity of residual and genetic variances but homogeneity of intra-class correlations in analyses of type traits recorded in different environments. For traits such as fertility or disease resistance, there is no prior reason to believe that the correlations across environments will be as high as for production traits, however. Nevertheless, recent analyses of somatic cell count evaluations in the US and UK suggest high correlations, and preliminary analyses for what must be potentially the most country/management dependent trait, herd life, also show quite high correlations. Rogers *et al.* (1998) reported genetic correlations of 0.3-0.6 between length of productive life in the USA and clinical mastitis in Denmark and Sweden. Genetic correlations between somatic cell count measures in these countries were close to unity. These figures are encouraging, and suggest that GxE effects may also be small for traits other than production.

At this stage we need to be open-minded about the magnitude of interactions among functional traits, and consider the issues in data analyses where opportunities to categorise them by environment arise.

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