

WHERE TO FROM HERE? THE TECHNICAL LANDSCAPE AHEAD

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"As we know, there are known knowns. There are things we know we know. We also know there are known unknowns. That is to say we know there are some things we do not know. But there are also unknown unknowns, the ones we don't know we don't know."

Donald Rumsfeld (2002)

SUMMARY

Advanced genetic and reproductive technologies have been slow to develop practical implementation, but prospects for high impact still prevail. Improved design and delivery technologies deserve high priority. Better supply chain signals will stimulate uptake of both old and new technologies, and impact favourably on directions for genetic change.

DAYS OF FUTURE PAST

The dramatic picture of a giant mouse on the December 1982 cover of *Nature* (Palmiter *et al.* 1982) led to quite widespread prediction that quantitative geneticists would soon constitute a threatened breed. In fact the genomic revolution took a different turn in animal breeding. The challenges and opportunities have continued to increase, not decrease, and the ongoing promise of dramatic outcomes has continued to fuel strong investment in the chase for Quantitative Trait Loci.

It could be argued that this ongoing flirtation has caused us to take our eyes off the ball, just as the infatuation with BLUP did over an even longer period. The real game is in the technology of design and implementation of industry-relevant breeding programs, in which QTL and BLUP are facilitating components. Of course these words represent a highly polarized view, but most of us would recognize some degree of truth. The genomic honey-pot is all too easy to fall into – so we must take care to balance its undoubtedly high potential with other technologies that impact on stated objectives.

In 1996 the author wrote a paper "Future developments in animal breeding" (Kinghorn, 1998). Few of the predictions have come true. But all of the predictions are still predicted to come true. We seem to be consistently optimistic about the timeframe to implement new technologies, and their impact. However, optimism drives action, and action has led to some high impact and a generally strong reputation for progressiveness in most of the Australian and New Zealand animal breeding industries.

GENOMIC TECHNOLOGIES

There is a growing movement to abandon the hunt for individual QTL, in favour of using genome-wide marker assisted selection, or Genomic Selection, GS (Meuwissen *et al.*, 2001). This uses thousands of genetic markers to test all genome regions for genetic variation in phenotypic merit, with all traits being candidates for improvement (van der Steen, 2007). We then evaluate individuals by integrating our evaluations of the components of genetic material that they have inherited.

There are two changes of focus here:

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1. From the viewpoint of exploiting genetic mutations: We no longer hunt for specific mutations (“magic bullets” or “needles in haystacks”) – a gamble for success, where the risks are largely technical. We now aim to capture all that our genomic information can offer, where the risks are largely in costs. But of course the truth is that we can do both!
2. From the viewpoint of classic genetic evaluation: The primary focus is to evaluate segments of genome, rather than to evaluate presumed polygenic merit, as in classic BLUP. But of course the truth is that we can do both!

When we evaluate an individual using a classic BLUP analysis, we make use of information from relatives. For example, to evaluate an animal we can use information from a distant relative with a coancestry of 0.03. This is a weak connection and it adds little value. However, if we have sufficiently dense marker information, this coancestry figure will polarize very close to 0 or 1, depending on which genome location is used. The two animals will be identical by descent, or not, at a given location, and we will have a pretty good handle on which of these is the case.

This is like changing the off-diagonals in the Numerator Relationship Matrix to 0 or 1 (but up to 2 with inbreeding), giving a great deal more certainty (power) in use of information from relatives. Integrating evaluations of genome segments leads to animal-level EBVs that are reportedly indicating to be highly accurate.

The key task in GS is to detect common ancestry through shared haplotypes that are highly likely to be identical by descent. There are two approaches to this, and they can overlap in practice. They both aim to exploit the same underlying QTL, but they get a handle on these in different ways:

Population approach: Identify haplotypes that have persisted across the wider population and are likely to be identical by descent. Only very small regions of genome are likely to remain intact as haplotypes across a large population, and we need several markers to characterize each of them, and so this approach initially requires many genetic markers, say 30,000 to 300,000. First analysis on a dataset of ideally some thousands of genotyped animals is used for calibration – to evaluate the haplotypes. GS can then proceed on these animals, and/or on other genotyped animals, by integrating the evaluations of the haplotypes carried by each animal. Paradoxically this evaluation can be seen as smaller than classic BLUP – we might end up with evaluations on only a few thousand haplotypes, compared to a million animals in a classic dairy BLUP.

Pedigree approach: Identify haplotypes that are segregating within the pedigreed part of the population that we are dealing with. In this case we can aim to infer inheritance states for all alleles, identify where recombinations have taken place, and infer essentially full haplotype information. In this way we can access the new linkage disequilibrium generated over the last several generations – and rather large regions of genome are likely to remain intact as haplotypes, such that we need many fewer genetic markers, say 3,000 to 10,000, enough to sufficiently narrow down the predictions of recombination locations within the pedigree, such that we end up with identity-by-descent probabilities that are well polarized for moderately distant relatives. In this approach, calibration and evaluation sit much closer to each other, and some ongoing calibration is required to track evolving haplotypes.

The pedigree approach requires fewer markers and uses all available information, but it requires genotyping animals in some depth of the pedigree. Moreover, the ongoing pattern of genotyping needs to be designed to maintain adequate information for tracking the evolving haplotypes. Both

methods need some ongoing calibration to accommodate shifting QTL effects and interactions, but the pedigree approach would probably require more animals genotyped overall. It is also likely to be computationally demanding.

With few markers the pedigree approach largely misses out on inference of haplotype sharing among founders, which is more important if the pedigree is shallow. On the other hand, a QTL arising by mutation within a “host haplotype” will be missed by the population approach, but picked up by the pedigree approach due the larger and more recently constituted haplotypes that it tracks.

When it comes to implementing the MAS phase, the population approach has the advantage that it can be used on animals in the population that are not closely related to the dataset used for calibration. For example, we could carry out GS in distantly related animals in the same breed/population, or genotype unpedigreed animals in the production system to help evaluate them for management purposes. This is particularly valuable for traits that are difficult/costly to measure.

Where to from here? GS is now highly fashionable, and it is indeed likely to have high impact as genomic information increases and genotyping costs come down. The ever-reducing costs of genotyping may proceed at a sufficiently rapid pace that we aim directly for exploiting population-wide LD. Early steps are taking this direction. However, we may be able to increase power by also exploiting larger haplotypes generated locally in the prevailing pedigree, especially in largely closed herds.

Detection and exploitation of individual QTL/mutations is not a thing of the past – it may suffer a dip in attention, but it is likely to enjoy a revival as we get on top of the biology behind our key traits, and begin to *understand* what mutations will be useful, how they interact with other components, and how to exploit them for their effects beyond our simple breeding objective traits. We may eventually move to biologically mechanistic prediction of multi-locus genotypes to target, as opposed to the current statistical extrapolation methods, but that will be far in the future.

REPRODUCTIVE TECHNOLOGIES

The most useful reproductive technologies for improving the rates of genetic gain in livestock are those that boost reproductive rates: AI, MOET and oocyte harvesting with IVF, especially in juveniles (“JIVET”).

AI is now commonplace at the seedstock level wherever it is practical and effective to carry out. In many cases its impact on connection among breeding farms and management units rivals its value through increased selection intensities. Its use should continue to increase.

MOET may be proving to be more viable than IVF due to lower costs, and the diminishing returns for ever-increasing female fecundity. However, the ability to make cross-classified matings under IVF has yet to be fully exploited for the information-rich designs it gives and the potential to exploit non-additive effects.

Cloning and sexing of semen/embryos have great potential to immediately lift genetic levels and to give flexibility of production in commercial populations. On the other hand, these technologies have limited potential to increase the ongoing *rates* of genetic gain in breeding programs. Widespread implementation in either setting will require considerably improved technology and reduced costs.

The holy grail of reproductive technologies is to close the life cycle *in vitro*. This might ideally involve cloned lines of haploid and diploid cells, rather than only mixed lines, in order to facilitate genotyping for evaluation, most powerfully in the haploid phase. The main challenge in this is likely

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to be induction of meiosis in diploid cells. This technology would bring generation intervals down to periods of days, but growth of some animals to phenotypic stages would be required for genetic marker calibrations and other forms of evaluation. This technology will not appear anytime soon for animals

INFORMATION TECHNOLOGIES

Constraints to the development and implementation of better information technologies are largely business constraints. This is natural, as the custodians of information require that information to conduct business. This in itself can bring good industry benefit, as the centralization of information and its downstream services means that we have unambiguous industry-wide genetic evaluation services, with strong technical backing.

However, the diversification of types of information and services means that we have different agencies delivering interacting services to the same breeders. This brings mixed messages and lack of coordination for the driving of breeding programs. A challenge for the future is to make these services work in an integrated fashion.

The classical BLUP backbone for genetic evaluations will remain for the foreseeable future, with some ongoing development to accommodate new traits and designs. However, there will be increased need to integrate this with genetic marker information, both technical integration, as the information sources are correlated, and delivery integration, as breeders need to exploit all information.

This latter point highlights the need to provide proper service and training to help breeders turn information delivered into actions taken.

BREEDING PROGRAM IMPLEMENTATION

We have come a long way in two decades. Naïve scientists and dogmatic practitioners have met somewhere in the middle, resulting in many breeding program implementations that are both technically progressive and industrially relevant. But there is a long way to go.

Scientists still tend to provide information and leave practitioners to juggle this through to implementation. Extensive breeding industries usually involve too many decision makers. This provides some useful diversity, but it leaves them in the dust of the highly focused and effective intensive breeding industries. This situation is exacerbated by poor signal in supply chains, providing insufficient incentive for breeders to do what is best for the whole industry. However, increasing communication and vertical alignment in supply chains are beginning to have impact on progressive breeding program implementation.

Where to go – Breeding objectives. In the old days, the scientists would preach total focus on fleece weight and fibre diameter. Animal breeding practitioners would listen politely, but deviate considerably because sheep have to walk to water, and so on. Scientists and economists can help in the process of setting breeding objectives, but there is a need to keep in touch with the wide range of issues in animal production – many of them relating to traits that are not yet a formal part of the breeding objective.

This has been highlighted by Rauw (2007), who discusses the generally unfavourable responses in key physiological traits that are often outside the breeding objective, such as resistance to pathogens, longevity, and heart and leg problems. In some industries we have come a long way in making our

breeding objectives more reflective of true production efficiency, with increasing emphasis on traits related to longevity and health. This trend should continue, albeit challenged by the costs of measuring appropriate traits. There will be increasing emphasis on developing more holistic breeding objectives that tune animal genotypes to all aspects of production systems, and thus decrease the relative emphasis on extreme performance in the animals that grow the product.

To a large extent, such comprehensive breeding objectives will benefit animal welfare. A key exception relates to highly fecund meat species, notably pigs, where reproduction and longevity have a lower relative impact on overall profitability. However, in such cases, unfavorable correlated responses under commercial production conditions, including increased mortality, are forcing breeders to place more emphasis on robustness (Knap, 2005). This is largely a business reaction. The breeding company that goes even further to improve animal welfare at the cost of commercial productivity will suffer in the marketplace. Commercial reality means a need for incentive – signals to breeders from the commercial level. However, welfare traits are not as simple as growth rate and litter size (and yet many would argue the latter are a good proxy for the former), and generating clear signal will be a challenge. But this is a challenge we may have to face.

How to get there - Design strategies. A well-designed breeding program can be twice as effective as a poorly designed program. For many industries this is a little-exploited low-hanging fruit. Key opportunities relate to how we can generate, identify and exploit exceptional merit wherever it occurs in a population. The high fecundity of males means that this works quite well in the dairy industry, and the relatively low level of cooperative competition in the beef and sheep industries means that opportunities tend to be unexploited. Special opportunities are being highlighted where appropriate design can make extra genetic progress by focusing attention on the difference between domestic and natural environments, and by selection for group performance instead of individual performance (Bijma *et al.*, 2007).

Communication and cooperation between the breeder and players further down the supply chain give opportunity for more relevant design. This is particularly true for cases in which GxE interactions are important in different production environments, and in which feedback on fitness traits and product performance through to the processed level can have real impact.

Getting there – Design tactics. The sharp end of implementing breeding programs is simply selection of parents and mate allocation. The best selections depend on what allocations will be made, and vice-versa, and so we ideally make these decisions simultaneously, using *mate selection*. Tools to invoke mate selection decisions have appeared over the last few years, including Ani-Mate (Amer, www.abacusbio.co.nz/products.html), TGRM and X' Aim (X'Prime, www.xprime.com.au). Recent developments give much increased computation speed, automatic control of outcomes, and flexibility to handle complex breeding structures involving many breeding units.

Delivering this technology to industry has been costly, as it requires a proper handling of the many technical, cost and logistical issues that motivate a breeding program – this is not the same as delivering EBVs. It has perhaps been easier to implement such programs in breeding companies, which have strong focus for directions to take and use of technology. However, low-cost routine implementation in conjunction with genetic evaluation services is becoming realistic, given standard recommendations for key issues such as the balance between genetic gain and genetic diversity.

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CONCLUSION

The impact of both old and new genetic technologies will increase in concert with more effective delivery. Improved supply chain signals will stimulate uptake of these technologies, and impact on directions for genetic change.

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REFERENCES

- Bijma, P., Mulder, H.A. and Ellen, E.D. (2007) *Proc. Assoc. Advmt. Anim. Breed. Genet.* **17**: 235.
Kinghorn, B.P. (1998) *Acta Agric. Scand., Sect. A, Animal Sci. Suppl.* **28**: 27.
Knap, P. W. (2005) *Australian Journal of Experimental Agriculture* **45**: 763.
Palmiter RD, Brinster RL, et al. (1982) *Nature* **300**: 611.
Meuwissen, T.H.E., Hayes, B.J. and Goddard, M.E. (2001) *Genetics* **157**: 1819
Rauw, W.M. (2007) *Proc. Assoc. Advmt. Anim. Breed. Genet.* **17**: 240.
van der Steen, H.A.M. (2007) *Proc. Assoc. Advmt. Anim. Breed. Genet.* **17**: 1.