

New Opportunities for Genetic Change in Pigs

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▪ Introduction

The revolution in biotechnology from genetically modified food to Dolly the cloned sheep is brought to our notice almost daily. Certainly these technologies are very expensive and controversial. Yet to the pig industry they represent an opportunity as well as a threat: the opportunity for dramatically cheaper pork which is healthier, safer, and produced with better animal welfare.

For more than thirty years conventional selective breeding, increasingly assisted by computers, has been highly successful in improving lean yield and feed efficiency. It has the advantage of being proven, safe, acceptable to the public, and highly cost-effective. In the difficult trading conditions of today, the pig industry cannot afford to fall behind on technology. What then is the role for the new technologies, what is the right mix of investment in present versus future technology, and indeed what is the role of genetics in the food chain of tomorrow?

▪ Genetic objectives

The pig industry has usually competed on the basis of low cost per kilo of lean meat. The genetic objectives have therefore been to increase lean growth rate and pigs produced per sow per year. However, the structure of the industry is shifting towards larger more integrated pyramids serving specific needs of retailers. As well as production efficiency, value-added aspects such as quality, uniformity and differentiated products are becoming more important. The way society views animal production now carries more weight, including issues of naturalness, traceability, the environment, sustainability, ethics and animal welfare.

It is no longer enough just to increase genetic potential. Even today some 20-30% of the potential that already exists is not realised on commercial farms. The reasons are: poor housing and management, incomplete knowledge or application of the nutritional needs of the modern genotype, and poor herd health. Pig disease is becoming more complex, with multifactorial conditions such as "Aporcine respiratory syndrome" which embraces PRRS, flu= and EP. As well as increasing potential, genetics must therefore now be deployed to *increase the probability that this potential can be realised on the commercial farm.*

▪ **Statistical selection**

For over ten years cheap desktop computer power has allowed the application of BLUP (best linear unbiased prediction) in pig selection. By using family records this gives more accurate prediction of genetic merit for traits of low heritability. Together with artificial insemination (AI), it allows direct comparisons of genetic merit across herds, opening the way for larger more geographically diverse nucleus populations, greater selection differentials and faster improvement.

Shipping breeding stock from a nucleus in one country for production in another is costly in terms of transport and health security, and runs the risk that market requirements may be very different. The solution is to establish separate nucleus populations in key countries and select for local objectives. Where necessary BLUP calculations can easily now be conducted on data transmitted over the Internet using centralised statistical expertise. New genotypes can be introduced via frozen semen or embryo transfer.

This decentralisation also brings the challenge to reduce overhead costs by reducing the size of each nucleus population. Conversely the more accurate the selection the faster the rate of inbreeding. Procedures are therefore now being developed to combine a BLUP prediction of merit with a measure of inbreeding to give a single selection criterion, which balances the two (Grundy *et al* 1998). Nevertheless BLUP does not overcome the problem that traits such as meat quality or disease resistance are difficult to measure in the live animal.

▪ **Breed resources**

Since changing to a different a breed or line will always be a faster method of genetic change than selection, the best use of existing breeds has to be kept under active review. Particular attention needs to be paid to the Meishan for prolificacy, the rapidly improving German Pietrain for leanness, and the Duroc for meat quality and hardiness. After 30 years of intense selection, some

populations of the traditional Large White and Landrace are becoming very homozygous. The dilemma is that unless populations of sufficient size can be maintained, the minority breeds will quickly fall behind, however the cost may be prohibitive.

One solution adopted by Cotswold has been to introduce 25% of its White Duroc line into each of Large White and Landrace type dam lines to give new composite lines. When crossed together these give a parent gilt containing 25% Duroc from two rather than three nucleus lines. The two larger nucleus populations result in faster selection. They are also cheaper to maintain with better physical condition than pure breeds due to residual heterosis from the Duroc. Multipliers also benefit from heterosis because they now use crossbred rather than purebred lines.

Since 1987 Cotswold has been developing a 50%Meishan composite dam line by selection for lean growth. Trials at Cotswold's UK R & D Centre, in alliance with London University's Wye College, show an advantage for the resulting 25% Meishan parent females over non-Meishan parents of 3.8 piglets weaned per sow per year. Backfat of the resulting 12.5% Meishan progeny was increased by 0.7 mm on ad lib feeding to 95 kg live weight, feed efficiency was 1% worse and there was no difference in growth rate. Work continues to improve uniformity and lean distribution. In Germany, Cotswold has developed a very lean Pietrain-type composite sire line which is approaching homozygosity for the absence of the halothane gene.

▪ Gene mapping

The new science of molecular biology is starting to provide an understanding of the mechanism of inheritance at the level of DNA. In the pig the DNA is distributed over 19 pairs of chromosomes and organised into some 100,000 functional genes. The DNA code is made up of sequences of the four bases (A, C, G, T) and a typical gene would be some 5-20 thousand bases in length. Once the sequence for a gene is known, its presence can be detected using a DNA test, as in the case of the halothane gene.

Research teams from the USA and Sweden as well as the EU-funded Pig Genome Mapping Project (PiGMap) are collaborating to map the genome of the pig (Visscher and Haley, 1998). By locating genes on the chromosomes, the objective is to understand how genes are organised and interact with each other. To date some 2000 DNA sequences showing genetic variation have been placed on the maps. These maps are freely accessible on the Internet.

▪ Marker genes

Most quantitative traits such as growth rate are controlled by many hundreds of genes, each with a small effect. A gene with a large effect such as the halothane gene is very much the exception. Nevertheless much research is now under way to identify possible genes with useful effects on performance. The function of most of the genes so far detected is unknown. They may however be situated on the chromosome close to a gene that does affect performance, for example growth rate, but for which no DNA test exists. Due to genetic linkage the gene which can be detected will then show an association with growth rate, which is actually caused by its neighbour which cannot be detected.

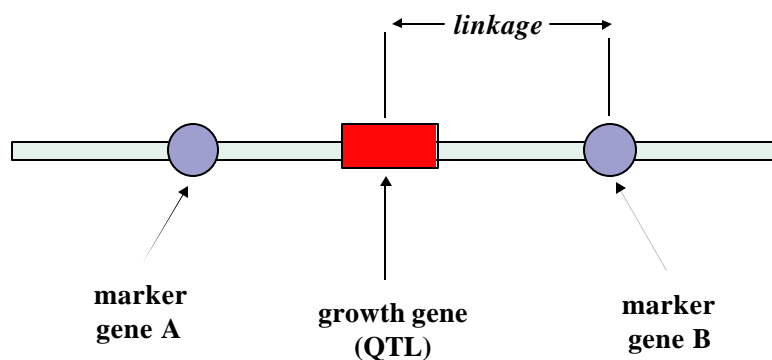


Figure 1. Flanking marker genes A and B used to predict the presence of a growth gene or QTL (*quantitative trait locus*).

In this case the DNA tested gene is known as a *marker*, because it marks a section of chromosome affecting performance. The gene whose presence it detects is known as a *quantitative trait locus* (QTL), with linkage between the marker and the QTL (Figure 1). Possible markers have been reported for all the important traits, and many have been mapped. The Ahot spots≡ from worldwide research are shown in Figure 2. Hot spots for litter size exist on chromosomes 1,8 and 16; lean growth on 4 and 7; and meat quality on 1, 6 and 15.

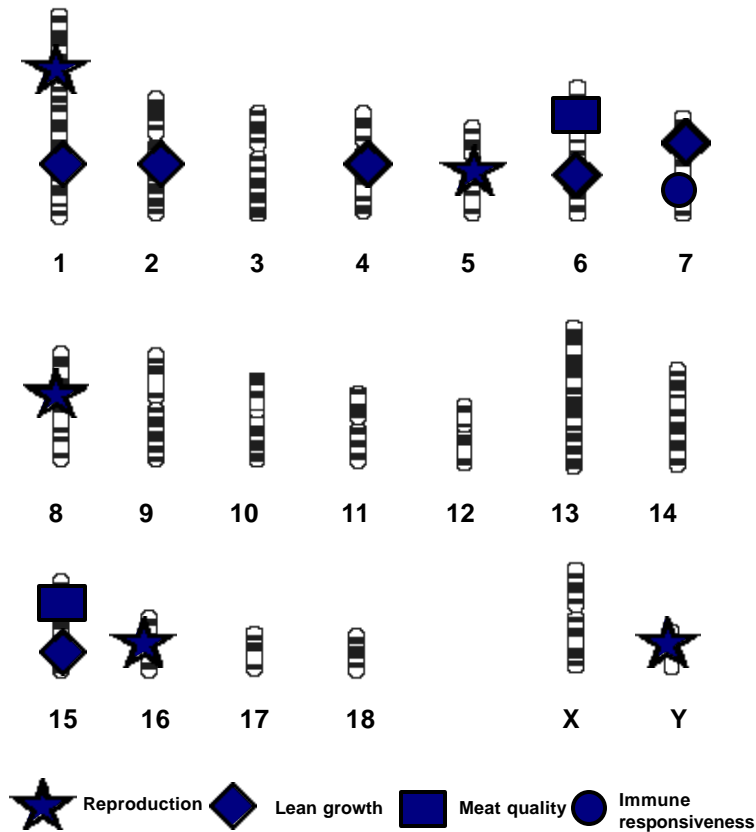


Figure 2. Hot spots on pig chromosomes affecting reproduction (*Courtesy of P R Bampton*).

▪ Marker assisted selection

In the process of *marker assisted selection*, DNA testing for the marker can be used to increase the frequency of the QTL and lead to an improvement in growth rate. The main benefit would be in traits such as meat quality or disease resistance which are difficult or expensive to measure in the live pig, or reproduction which occurs late in life. There are however a number of problems:

- ▶ DNA testing is still relatively expensive in relation to the small benefits in performance for most markers.
- ▶ There is no further benefit after the marker has been made homozygous.
- ▶ Marker effects are often inconsistent between lines and even families.
- ▶ Due to the high number of candidates and traits, there is a statistically high chance of false positive markers. Already the number of markers reported would explain more than 100% of the genetic variation for some traits.
- ▶ Selecting on markers causes a loss of selection on other traits.
- ▶ Markers may have unknown harmful as well as beneficial effects. There may therefore be good reasons why selection has not fixed apparently favourable QTLs at 100% in these populations.

Current thinking is that the interaction of genes with each other is probably more important than originally recognised, so the implications of changing the frequency of any gene with a large effect may be difficult to predict. A further difficulty is that the information from hundreds of markers of small effect may be difficult to collate. At this stage the use of markers is therefore risky, whereas BLUP selection is already proven and cost-effective.

▪ **Marker assisted introgression**

As an alternative to selection within a line, a marker can be used to introduce a QTL from one line into another by *marker assisted introgression*. Suppose for example that a single gene for prolificacy is to be introduced from Meishan into Landrace. An F1 cross of the two is then backcrossed to Landrace over several generations, gradually increasing the proportion of Landrace while selecting for the desirable gene. Without the need for DNA testing this is the method by which Cotswold introduced the dominant white coat colour gene from the Large White into its White Duroc.

By the same principle other markers can be used to reduce the proportion of background genotype from the undesirable line, for example fatness from Meishan, hastening a commercially viable result. A further application might be the use of markers to retain maximum heterozygosity in any closed population. The risk of introgression is that it takes several generations. During this time the linkage with the marker could break down, selection on other traits may be lost, and the intermediate Meishan crosses could be over-fat and costly. The benefit of the QTL would therefore need to be large.

▪ New types of markers

The main disadvantage of existing markers is their high cost and low accuracy. The majority are random segments of DNA of the form CACACA (*microsatellites*) that show genetic variation in the number of repeats. The inaccuracy stems from the weakness of the linkage in predicting the presence of the QTL. Either closer markers are needed or ideally a method of detecting the QTL directly. Several new options are now appearing:

- **AFLPs** Amplified fragment polymorphisms can be generated by enzymes which cut the chromosomes only at specific sequences. The presence of different genes results in DNA fragments of different length which can be correlated with performance traits. Patented by KeyGene NV in the Netherlands and applied in plants, this has the advantage of producing a set of markers specific to one line. It also overcomes any patents on published markers.
- **SNPs** Single nucleotide polymorphisms are changes in a single specific coding unit of the genetic code. They are easy to detect and usually occur within the functional gene. Unlike microsatellites SNP tests could be performed on a microchip array when this technology shortly becomes available.
- **ESTs** Expressed sequence tags allow genes to be detected when they are switched on. This would allow selection for animals expressing rapid early growth, earlier puberty, or perhaps on immune response. ESTs will provide the key to how genes are organised and controlled.

Using AFLPs performed by KeyGene, Cotswold found six markers each with an effect of 2 mm on backfat. Unfortunately a second trial on the same population failed to confirm these effects, illustrating very well the inconsistency of markers.

▪ Candidate genes

Rather than searching at random for markers, the candidate gene approach uses knowledge of physiology to identify likely QTLs with a major effect. Some examples of mapped and published markers obtained largely via the candidate gene approach are given in Table 1. Patents have been filed on some markers, but can often be overcome using others which are near to the QTL.

The halothane gene (RYR1) appears to be the functional gene or QTL responsible for all the effects on lean growth and stress susceptibility. The oestrogen receptor (ESR), which affects litter size in some populations but not

others appears to be a marker rather than the QTL responsible. H-FABP was discovered to affect intramuscular fat in Dutch Durocs and is currently being tested under licence in other populations (Gerbens *et al*, 1998). Candidate genes to control boar taint from skatole and androstenone are being investigated by several groups (Davis and Squires, 1999).

Table 1. Some published mapped markers for performance in pigs with chromosome number (Chr).

Trait	Marker	Name	Chr	Patent
Litter size	Oestrogen receptor	ESR	1	yes
	Osteopontin	OPN	8	yes
	Prolactin receptor	PRLR	16	yes
Lean growth	Marker for fat	SO112	1	--
	Insulin like growth factor	IGF2	2	--
	Myogenic factor 3	MYF3	2	--
	Marker for fat	SO107	4	--
	Leptin receptor	LEPR	6	--
	Marker for fat	SO102	7	--
	Myostatin	GDF8	15	--
Meat quality	Skeletal muscle calpain	CAPN	1	--
	Calpastatin	CAST	2	--
	Halothane gene	RYR1	6	yes
	Rendement Napole	RN ¹	15	--
Intramuscular fat	Heart fatty acid binding protein	H-FABP	6	yes
Immunity	Tumour necrosis factor	TNFB	7	--
	Histocompatibility	SLA-1	7	--
Coat colour	Dominant white	KIT	8	yes

As an example, Cotswold is co-sponsoring a study at Glasgow University in which a knowledge of myosin heavy chain muscle protein polymorphisms is being used to deduce likely sequences of DNA that could act as markers within the genes affecting eating quality (Beuzen *et al*, 2000). Certainly markers represent an important opportunity to accelerate genetic improvement, and Cotswold is very actively continuing its programme of in-house evaluation with exploratory selection on a combination of markers and BLUP.

▪ Gene transfer

Gene transfer between individuals and species has been possible for some years. The method of micro-injection of DNA into the fertilised egg had a low success rate and could not control where and how many copies of the DNA sequence were incorporated. Dolly type cloning would make gene transfer much easier and cheaper allowing DNA to be incorporated into cloned cells before transfer into the embryo. Much research effort is therefore now aimed at cloning pigs.

First attempts to add extra copies of pig or human growth hormone genes brought adverse publicity due to undesirable effects on fertility and physical soundness. Methods are now being developed to control the number of copies, site of insertion, and the degree of expression. The technology of gene transfer is being driven by the use of the pig as a donor of hearts and other organs for humans (*xenotransplantation*). Human genes are added and pig genes “knocked out” to avoid rejection of the heart as “foreign”.

▪ Genes for transfer

What are the opportunities for gene transfer in pigs? Take as an example the *myostatin* gene, a naturally occurring mutation which is the cause of double-muscling in Belgian Blue Cattle. When this gene is knocked out[≡] of laboratory mice, lean growth rate is doubled and ham weight tripled (McPherron *et al*, 1997). In a pure Meishan line with 9 extra pigs per sow per year, a similar knockout might restore the very fat carcass to normal with dramatic consequences for productivity. Other opportunities might include:

- ▶ Lean growth (*eg leptin, IGF*)
- ▶ Boar taint (*eg androstenone, skatole*)
- ▶ Meat quality (*muscle proteins*)
- ▶ Disease resistance
- ▶ Gender determination (*SRY on Y-chromosome*)
- ▶ Pollution control (*eg phytase*)

Genes for androstenone and skatole might be knocked out to control boar taint. The SRY region has a major role in determining maleness. Transfer of this onto one of the non-sex chromosomes might allow sires which produce only male or female offspring.

Guelph University has produced pigs transgenic for phytase, which emit less phosphate pollution. In rats attempts have been made to introduce cellulase genes to improve digestion of plant material. This raises the issue of which genotype should be chosen for manipulation: the animal, the fodder plant, or the gut flora. The animal itself should probably be the last choice. An even better long term solution will be to control the expression of the existing genes and avoid gene transfer altogether.

▪ **Disease resistance**

This major source of loss in pig production has attracted strangely little genetic research. The existence of genetic variation in immune responsiveness within and between breeds has only recently been demonstrated. At the University of Guelph, selection on a BLUP index for high or low immune responsiveness was successful in creating a genetic difference (Mallard *et al*, 1992). At Iowa State, Durocs showed greater resistance to PRRS virus than other breeds (Halbur *et al*, 1998).

However a line with higher immune responsiveness would probably show a correlated reduction in lean growth every time the immune system was triggered. This is a natural defence in response to infection, and is mediated by the cytokines such as interferon and interleukin. One challenge for molecular genetics would be to break this association so that high immune responders could continue to grow normally.

▪ **Reproductive Technologies**

Improvements can be expected in the reproductive technologies such as frozen semen, frozen embryos, and non-surgical embryo transfer (ET). As pigs already have large litters the main benefit of ET will be in obtaining 100% of the desired genotype with minimum health risk. Cloning the slaughter generation would give 100% uniformity from top pigs in the nucleus, but cloning would need to be repeated each year to keep pace with genetic improvement.

Cotswold, through an investment by its parent company Ridley Inc in Gensel Biotechnologies Inc, is actively involved in a novel approach to gender selection by raising antibodies to X or Y sperm. The unwanted sperm can then be made to clump together, and can be filtered off through glass wool even for on-farm collection. The benefits are increased production efficiency and uniformity without the complication of split-sex feeding.

In vitro fertilisation together with ET will be an enabling technology for gene transfer. In vitro meiosis to produce sperm and eggs would be the final step

which would allow marker assisted selection to be performed using IVF to produce cloned embryos in successive generations without the need for any live pigs at all (Haley and Visscher, 1998).

▪ **Putting the Technology Together**

The new technologies could be brought together to revolutionise the pig industry. Suppose for example that a Meishan type containing the *myostatin* knockout could give single line production with an acceptable carcass and 32 pigs per sow per year. Semen sexing could be used to give 100% entire male offspring, and androstenone/skatoles knockouts could remove boar taint. This would immediately improve production efficiency by some 30%.

Another possibility would be to produce surrogate mothers from an F1 cross of say Meishan with Fengjing. These would receive cloned and frozen embryos from a dedicated sire line containing *myostatin*. Dam lines would be bred only for uterine capacity and the sire lines only for slaughter pig attributes.

Of course no one is advocating these methods, but their adoption by rival industries could pose a threat in terms of lower production cost. On the other hand there could be a huge benefit for the animal and human by manipulating genes for disease resistance or environmental sustainability.

▪ **Future Genetic Strategy**

So what strategy should be adopted for future genetic improvement and research? There are three components:

- Maintain maximum rates of improvement using existing BLUP methods.
- Close the gap between genetic potential and actual performance on farm.
- Secure access to the new technologies and be prepared to quickly deploy them if required to defend the competitive position of the industry.

To close the achievement gap Cotswold has a large investment in better understanding the nutrient requirements of modern genotypes, especially of the young growing pig where energy intake may be limiting lean growth. There is also a need to increase research on the immune system of the pig.

To access the new technologies Cotswold has developed an international science base run by its four full-time PhD geneticists. This consists of research

fellowships and alliances, collaborative research agreements and research contracts with biotech companies, and shareholdings in semen sexing company Gensel and DNA typing company Rosgen. The aim is to be closely informed on new ideas and technology with the opportunity for early experimentation where appropriate.

▪ **Conclusions**

Beyond 2000 the goal for the pig industry must be to produce high quality lean meat at minimum cost, with the proviso that this is done with public approval, with good welfare, and at maximum safety. The industry must be seen to care for the animals and the environment. So what will be the role of the breeding organisations?

Breeding companies will be integrators rather than primary developers of technology, providing a package of genetic services (and rather few live pigs) to the food chain. While not advocating genetic manipulation, and at this stage without the knowledge to assess the risks, the industry should ensure that it has access to whatever new technology will be needed to compete.

Meanwhile, present methods of genetic change are safe, non-controversial and highly cost-effective, and can be expected to continue to deliver improvement for many years to come. Breeding objectives can be expected to shift from production efficiency to consumer appreciation and added value for the end-product.

The safest strategy to ensure that the industry can quickly respond to whatever the future may bring will be to understand the biology of the pig through continuing support for fundamental research.

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