PiGMaP: GENE MAPPING AND ITS CONTRIBUTION TO MEAT PRODUCTION AND MEAT QUALITY PARAMETERS

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SUMMARY

Mapping of genetic markers and genes rapidly provides a basis to study the nature of genetic variation for performance traits in pigs like growth, efficiency, leanness, meat quality and litter size. Combining maps developed in Europe and America leads to over 500 mapped - mostly microsattelite- markers and some 80 genes. These maps offer the possibility to carry out association studies to really identify chromosomal regions and later on genes which are responsible for differences in performance. For this purpose at present e.g. in Europa experiments are being carried out crossing the Chinese Meishan breed with European pig breeds. Data collection is underway while typing animals for marker genotypes is to be started. Already an association study has been analyzed using a cross of wild pigs with Large White. Though the experiment was of relatively small size two different chromosomal areas were shown to be linked to genes affecting growth rate and one region was shown to be linked to genes affecting backfat and abdominal fat percentage (Andersson *et al.*, 1994). The prospects for successful association studies for meat quality in pigs are supported by the fact that recently major genes have been identified statistically, which is less powerful a technique than using markers. Le Roy *et al.* identified a major gene for yield of cured meat and Janss *et al.* (1994) found a major gene with a difference of over 2% intramuscular fat between both homozygotes. It seems that this major gene does not affect overall fatness. Once markers linked to relevant traits are identified, these may be used to support introgression of alleles of interesting genes e.g. from Meishan into commercial pig populations. Markers may also be used to increase the precision of estimation of breeding values.

INTRODUCTION

Genetic improvement of meat production traits and meat quality in pigs generally is achieved by selection based on measurements of traits for growth, efficiency, leanness and meat quality. These traits in part are determined genetically. In addition environmental effects like feeding level, housing system and disease status will affect meat production traits, while quality traits are influenced by practices of pig transportation and slaughterhouse management as well.

Genetic variation between animals for those traits is thought to be governed by many genes, nearly all of which are not yet identified. The halothane gene (Eikelenboom and Minkema, 1974) is the most important one which has been identified. The halothane gene is perhaps rather exceptional in that its two known alleles cause differences in very many traits (Carden *et al.*, 1985; Simpson *et al.*, 1986). Though this gene has been known for over 20 years, selection initially was carried out on the phenotypic level, measuring the pig's reaction on the administration of the halothane gas (Eikelenboom and Minkema, 1974). Ten years later, selection could be based on identifiable marker alleles which were closely linked to the halothane gene (Cepica *et al.*, 1984). Only recently has the exact difference between the allele causing the status of Halothane susceptibility and the allele causing non-susceptibility been found (Fujii *et al.*, 1991) and selection now can be based typing individual animals at the DNA-

level. The large advantage of this is that all three genotypic states (two homozygotes and one heterozygote) can be distinguished, which was not the case with the Halothane test, and not completely the case using markers.

At present, various research projects are underlay to identify genes affecting production and quality traits. Perhaps the most massive approach followed is gene mapping as exemplified by the Pig Gene Mapping project or PiGMaP (Haley *et al.*, 1990). Firstly a large number of genetic markers is developed and mapped on the chromosomes. These markers then serve as aides to the identification ofchromosomal regions and later on genes which are important in causing genetic variation in traits of interest. Obviously, it is easier to detect such regions and genes if the effects of genes involved is large. This has renewed the interest in the search for such major genes by statistical means, because it may support decisions to set up high cost searching projects which use genetic markers.

The outline of this paper is as follows. First, genome mapping technology and genome maps will be briefly discussed. Secondly, the possibilities of identifying relevant genetic markers and relevant chromosomal segments will be illustrated. Thirdly, an example of recently detected major genes in meat quality will be examined. Finally, the potential of genetic-marker technology to enhance genetic improvement in pigs will be considered.

GENOME MAPPING TECHNOLOGY AND GENOME MAPS

The ultimate goal of genome mapping is to identify genes affecting performance of animals. This involves both location of these genes to chromosomes and also understanding of the full scope of effects of such genes. The latter is needed because genes may influence various traits. A first step is to identify genetic markers on the chromosomes which not necessarily themselves affect levels of performance, but which are located closely enough to interesting genes to serve as a marking point. These markers then can be used to detect chromosomal segments important for trait expression and subsequently to come closer to the actual genes. To serve this goal, genetic markers should meet the following properties.

1. Markers should be easy to develop.
2. Markers should be plentiful and distributed randomly over chromosomes. They should not only occur in specific chromosomal areas.
3. Markers should be polymorphic, i.e. markers should have various distinguishable alleles, in general, the more the better.
4. Markers should be based on genotyping of DNA and should be codominant (i.e. both alleles distinguishable), so that all genotypes can be readily determined from virtually any sample at any age.
5. Markers should be technically tractable and portable, i.e. it should be easy to type them in any laboratory with few typing errors.
6. Typing of animals for marker genotypes should be cheap and relatively rapid, and should therefore have properties which enable automated typing.

In recent years various types of markers have been developed. At present microsatellites (Litt and Luly, 1989) are the most valuable ones and are being used in various research projects. Microsatellites are repeats of one, two ormore DNA bases[1], with the different alleles

having different numbers of the repeated sequence (e.g. $(TG)_n$, i.e. sequences of variable numbers of the two bases TG). The occurrence of microsatellites on the genome is abundant, and each microsatellite generally shows variation in the number of repeats. Microsatellites meet the second to fifth requirements mentioned above, and also are relatively easy to develop. Also the last requirement is met, due to the possibility to use PCR (polymerase chain reaction) and automated DNA-sequencers.

The PiGMaP project has started in 1990 and is a concerted effort of 18 laboratories in the EU and Nordic countries to develop microsatellites and other types of marker and to map these markers to the genome. Since the start of the project additional laboratories in Europe, Australia and the U.S.A. have joined the collaboration. The aim of the project was to reach a coverage of the entire genome such that some 150 markers could be identified which should be evenly distributed over the genome for future association studies (see below). This goal now has been reached (Archibald *et al.*, 1994). The PiGMaP genome map contains 239 markers of which about 80 correspond to functional genes. Examples of the latter are GH1 (the growth hormone gene), FGA (fibrogen gene alpha) and CRS (the calcium release channel which is the site of the halothane mutation). Simultaneously in the United States a microsatellite map has been developed containing 383, mostly microsatellite, markers (Rohrer *et al.*, 1994). As an example the map of chromosome 4 based on Archibald *et al.* (1994) is given (Fig 1). This map shows 14 microsatellite markers, covering 165 cM in total[1]. As indicated before, around 150 evenly distributed markers is thought to be sufficient for a first association study. In that case, the distance between flanking markers will be less than 20 cM and the maximal distance between a marker and a gene less than 10 cM. This means that each generation a link between alleles of marker and gene will be broken with the rate of up to 10%. Using both flanking markers to catch an allele of a bracketed gene the linkage will be much tighter.

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1. The entire genome is a string built up of four nucleotide bases, C,G,A and T. Genes consist of sequences of groups of three bases, where particular groups of three bases code for a particular amino acid. As a consequence a gene codes for a protein, e.g. an enzyme. The pig genome consists of some 3×10^9 bases and perhaps some 100.000 genes. Only about 10% of the genome actually is coding DNA. The function of the remainder is not known. Microsatellites generally are located in the non-coding regions, but nevertheless can be located closely enough to genes to be relevant marking spots.
1. A unit of one centiMorgan (cM) can be explained as follows. Suppose an animal is of the genotype AB/ab. This notation means that the animal has one gene with alleles A and a and also one gene with alleles B and b. The A and B alleles are located on one of two sister chromosomes, a and b on the other. If the animal produces gametes (ova or spermatozoa) these contain one half of the sister chromosomal material and may be either of the type AB or ab (the parental type) or Ab or aB (non-parental type). The production of new types is caused by crossing over, the phenomenon that parts of sister chromosomes exchange material during the process of meiosis. If genes are as close as 1 cM, only in 1% of the cases we expect the new types of gametes.

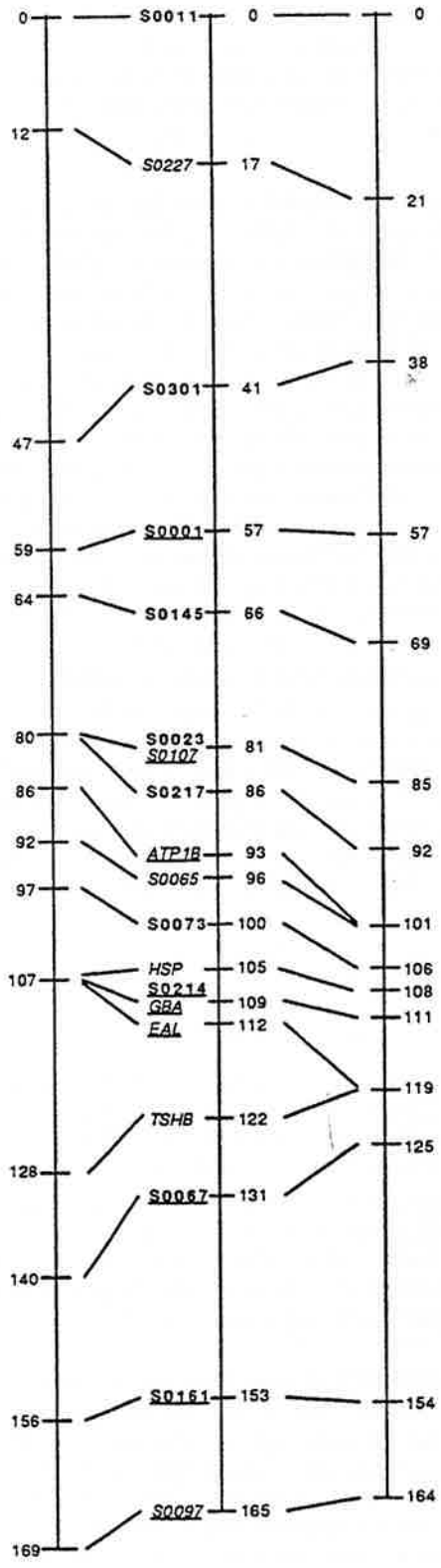

Fig. 1. Map of chromosome 4 (archibald et al., 1994). The location starting with S are microsatellite markers. Others are genes. The left map is for females, right for males and combined in the middle. The figures at marker and gene locations are cM.

ASSOCIATION STUDIES

The general idea of association studies is to identify genetic markers for which different alleles coincide with different levels of performance for traits of interest (e.g. growth rat, fatness, meat quality and litter size). Such associations are caused by the fact that the marker alleles are linked to alleles of genes affecting the traits of interest.

Consequently, the identified markers will be close enough to the gene on the same chromosome to virtually appear in an experiment as though it were itself affecting the level of performance. A powerful manner to set up association studies is the production of crosses of extreme lines to produce F_1's and to intercross the F_1's to produce F_2's. This can be explained for a simplified case as follows. Suppose that for an interesting gene (affecting some performance trait) the one line is homozygous of the type AA and the other of the type aa. Suppose further that linked to that gene exists a marker which is homozygous in the one line of type MM and in the other of the type mm.

If one type F_1's for the marker one will find that all are of the heterozygous type Mm, and those animals will be all of the type AM/am. Intercrossing of the F_1's will produce AM/AM animals, AM/am animals and am/am animals in case the distance between gene and is zero (so no crossing over between them occurs). If the distance between the marker and the gene is not zero the situation will be more complicated due to crossing over, but this example illustrates the case. If AA- animals show a better performance than Aa, and these again better than aa, typing F_2-animals will show that animals of the marker genotype MM are better than Mm and these again better than mm. In that case the experiment will result into the conclusion that the marker concerned with its two alleles M and m is useful for the trait concerned. Although the situation is more complicated in practice, where lines used generally will not be homozygous and also the distances between genes and markers will not be zero, the design described is a powerful one to identify interesting markers. A set up like that has been reported by Andersson *et al.* (1994).

The experiment involved the crossing of two European wild boars with eight Large white sows to produce F_1's. Subsequently four F_1-boars were mated to 22 F_1-gilts to produce 200 F_2-offspring. The parents and offspring were typed for 105 different type of markers including microsatellites and genes which where distributed over 15 of the 19 chromosomes of the pig. On the 200 offspring traits were recorded including growth rate from birth to 70 kg, average back fat depth, and abdominal fat percentage. Though the analysis was more complicated in reality than the simple example described above, it essentially involved a comparison of performance of F_2's typed to be MM, Mm or mm for each of the markers. Markers in two different chromosomal regions were shown to be linked to genes affecting growth rate significantly, while markers in one region were shown to be linked to genes affecting backfat and abdominal fat percentage (table 1).

The two genes significantly affecting growth rate are located on chromosomes 4 and 13. For the example of chromosome 4, the two flanking microsatellite markers are located at positions 24 and 68. Between these two markers four genes are also mapped. The mapped genes are unlikely to be responsible for the differences in growth, and further research is needed to identify potential candidate genes for the effect. The genes for the two fat traits are estimated to be located nearly on the same position, and actually very well may concern the same gene.

Trait	Chromosome	Map Position	Allelic difference
Growth from birth to 70 kg (g/d)	4	58	-53.5 ± 4.9
	13	53	-13.5 ± 3.6
Average backfat depth (mm)	4	3	2.32 ± 0.42
Abdominal fat percentage (%)	4	7	0.38 ± 0.06

Table 1. The estimated location[1] of genes significantly affecting growth and fat deposition traits and the estimated difference of allelic effects of the genes.

1) cM distance from the proximal end of the chromosome

In thatcase flanking microsatellites are located at positions 0 and 16, with no genes mapped in between.

These markers have been identified in a fairly small experiment, and the result can be considered to be caused by the fact that the difference between the pig breeds crossed were very extreme, indeed. From a practical point of view it may well be that the results are not immediately useful because the negative allele probably occurs in the wild pig and the positive one in Large White. However, the experiment identifies a chromosomal region which contributes to variation in economically important traits. Studies in plants suggest that such regions may contain alleles of lesser effect which may still be of importance within and between commercial breeds (e.g. Edwards *et al.*, 1992). Furthermore, if the same region were responsible for some of the fatness differences between the Meishan and European breed, which *a priori* seems quite likely, the markers may be of use in ensuring the *exclusion* of this region from any cross involving the Meishan. Finally if it were possible to identify and clone the gene loci involved in the wild boar cross this would greatly contribute to our understanding of the control of these traits and hence may contribute to maintaining genetic progress in the long term.

Some crosses between extreme lines could be considered to be immediately useful also for practical purposes if both lines exhibit interesting traits. In that context, in the framework of PiGMaP three extensive experiments have been carried out in France, The Netherlands and The United Kingdom of a set up similar to the one described with the wild boar. In these cases it concerns crosses of the Chinese Meishan breed and the white breeds Large White and Landrace. Within PiGMaP, data recording in the experiments is underway, but typing of animals for markergenotypes is to be started. Such a cross actually may lead to the identification of useful trait markers as the example of the oestrogen receptor gene claimed to result in a difference of 1.5 piglet in litter size (Rothschild *et al.* 1994).

SEARCH FOR MAJOR GENES

Janss *et al.* (1994) have reported the search for major genes utilising the same Dutch experiment which will be used to carry out the marker typing described above (i.e. a Meishan x European breed cross). The phenotypic data on its own have been analyzed using a statistical approach called segregation analysis to look for the existence of major genes. It should be noted that the proof of the existence of such a major

gene virtually guarantees the identification of useful markers for such a gene because association studies are more powerful a technique than segregation analysis. Janss' results for intramuscular fat are given in Table 2.

parameter			
a	d	σ^2_α	σ^2_u
1,16 ± 0,082	-1,10 ± 0,125	0,057 ± 0,052	0,128 ± 0,039

Table 2. Characteristics of a major gene for intramuscular fat detected in a F_2-cross between Meishan and European breeds (Janss et al., 1994). Parameters given are a (half the difference between homozygotes carrying or not carrying the major gene), d (the difference between the heterozygote and the average of both homozygotes), σ^2_α (the additive genetic variance due to the major gene) and σ^2_u (the additive genetic variance due to all other genes affecting intramuscular fat).

The results in the table show a considerable effect of the major gene. The percentage intramuscular fat of homozygote carrying the major gene is 3,32 higher than the other homozygote. The "lean" allele turns out to be about dominant and as a consequence the percentage intramuscular fat of a heterozygote about equals that of the homozygote not carrying the major gene. The difference of 3,32% is considerable given the mean percentage intramuscular fat in this experiment of 1.84 with a standard deviation of about 0.80. Current analysis indicates that the major gene affecting intramuscular fat does not affect overall fatness (Janss, personal communication). By using this gene it may therefore be possible to further decrease pigs' backfat without leading to too low degrees of intramuscular fat. Le Roy *et al.* (1990) have used a similar statistical approach to identify a gene which affects the yield of cured meat after a standard pickling and cooking procedure. Again it should be possible to find markers linked to this gene relatively easy.

PROSPECTS OF GENETIC MARKERS FOR GENETIC IMPROVEMENT

In principle, there are two ways to utilise genetic markers in a selection programme. One is to utilise markers themselves to select for the desired alleles of genes which are linked to them. The second possibility is to use genes, after really identifying their properties at the DNA-level[1]. For the time being the most realistic question is how to utilise genetic markers themselves, because after the detection of the existence of an interesting gene, it may take quite a long time before the gene is characterized on the DNA-level. There are two possibilities to utilise genetic markers. These may be listed as follows:

1. Support the introgression of an interesting chromosomal area from one breed into another.

1. In this context it is worth noting that the estimated location of a gene is rather imprecise, even if the knowledge that the gene is between two flanking markers is firm. If a distance between two flanking markers is 20cM, then the number of bases between these markers is in the order of 20×10^6. The number of genes in such a region is of the order of 500.

2. Increase the precision of the estimation of breeding values adding marker information to phenotypic measurements.

Marker assisted introgression

Examples of this may be the introgression of the area containing the oestrogen receptor gene discussed above from Meishan into a white breed and also the introgression of the intramuscular fat gene (unlinked with backfat!) once a suited marker for this gene is identified. The process of introgression works as follows: Firstly cross the breed with the interesting allele with the commercial line into which the allele should be introgressed. Then the resulting crosses are backcrossed to the commercial line and this backcrossing is repeated with the progeny of this cross and so on a number of times. The goals of this process are to introgress the new allele into the commercial line, without loosing the positive overall performance of the commercial line. In principle, genetic markers can be used to follow the introgression process, and to make sure that the interesting chromosomal area is not lost. This is particularly useful if the trait concerned is difficult to measure or cannot be measured early in life.

For intramuscular fat, for example, measurement requires slaughtering of sibs of candidates for selection. Additionally, markers can be used to speed up the process with which the original commercial genotype is reattained. Hillel *et al.* (1990) concluded that the process of introgression (which traditionally takes about 6 generations of backcrossing) could be reduced to two generations. Hospital *et al.* (1992) showed that this was too optimistic. Groen and Smith (1994) looked into the situation where they compared the process of reattaining the commercial genotype using markers with the situation of usual selection, supplemented with marker information. They concluded that additional utilisation of markers not really speeds up the process and that the large advantage of utilising markers really is to be sure that the interesting chromosomal segment is introgressed and is not lost in the process of simultaneous selection. However, further research looking at alternative scenarios and optimising the balance of selection on markers and the phenotype is needed in this area. An alternative to the backcross introgression outlined above is to start selection based upon phenotypic performance and marker genotypes in a synthetic population derived from intercrossing the F_1-crosses (and a number of intermediate scenarios are possible where the synthetic is produced after one or more backcrosses). Lande and Thompson (1990) first looked at this problem theoretically and predicted that substantial additional progress could be obtained from selection on markers compared to selection solely on the phenotype. Subsequent simulation studies have shown less additional progress using markers than originally predicted (Shang and Smith, 1992; Gimmelfarb and Lande, 1994). However, these studies have been based upon rather simple models (e.g. a crossbetween inbred lines) and studies based upon models which mimic more realistically animal populations are needed.

Marker assisted estimation of breeding values

The principle of this is that not only phenotypic measurements are used to estimate breeding values, but on top of that knowledge of marker typing. The principle of the method initially is given by Fernando and Grossman (1989) and has been extended by Van Arendonk *et al.* (1994). In dairy cattle, increased genetic improvement utilising markers is found up to 20% (Meuwissen and Van Arendonk, 1992), if markers are used to increase the precision of breeding value estimation early in life. In particular, using markers to preselect bulls to

be progeny tested improves genetic change partly due to shortened generation intervals, while utilising markers to improve the selection of progeny tested bulls doesn't add much because breeding values then are estimated rather precisely without markers, anyway. Brascamp *et al.* (1993) showed that in the case of dairy cattle, there is a potential financial benefit to really utilise markers. It should be kept in mind that the value of enhancing the probability to breed a top rating bull for extensive international use has a real economic value. In pigs this is not the case, and the value of markers should be based upon enhance genetic change at nucleus level increasing the overall competitiveness of the programme. Based upon work of Van der Beek *et al.* (1994) with laying chickens it seems that the increase in genetic change in pig breeding programmes will be smaller than in dairy cattle breeding programmes and may be some 5% at most. Looking at sex-limited traits such as litter size, pig nucleus populations have a structure similar to a dairy MOET. It is therefore likely that markers could be used in a similar way to improve the selection response for traits such as litter size.

DISCUSSION

Genome mapping in farm animals is a very interesting way to better understand the genetic basis of genetic variation of traits. It will give a first insight in the number of chromosomal segments containing genes with considerable effects. For the time being it will not be possible to conclude if a significant effect associated with a chromosomal segment represents the effects of a single gene or a cluster of relevant genes. The steps discussed in the paper (develop markers, carry out association studies, study chromosomal segments more in detail in an attempt to identify a gene) is however a very powerful way to approach the problem. One of the ways to perform the third step is comparative genome mapping. Both the DNA-sequence of individual genes and the linkage relationships between groups of adjacent genes tend to be conserved between species and the knowledge of genes in human and mice is accumulating very fast. Parts of chromosomes of these species show similarities with other chromosomal parts in e.g. pigs. Oncean interesting chromosomal segment in pigs is located, comparison with the corresponding chromosomal area in human or mouse (where many more genes will have been identified, cloned and sequenced) allow us to hypothesise as to which genes really are involved in the causation of differences in performance.

In the short term it is expected that markers for genes with fairly large effects will be detected. Probably initially the route of marker assisted introgression will have direct practical impact. Lines in hybrid programmes will be additionally improved by introgressing interesting genes from in itself non-commercial breeds like the Meishan. On the long run, the research efforts may lead to routine application or markers in breeding programmes, but probably will be more fruitful in producing knowledge of genes, characterised on the DNA-level, which will support further pig improvement.

Summarising, mapping markers and genes to the chromosomes is a very promising as a basis for further understanding genetic variation. In the short run, applications in breeding programmes especially may be found in the introgression of valuable alleles into commercial populations.

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