

## Perspectives of genetics and breeding to prevent boar taint

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Male piglets are castrated primarily to prevent the development of the objectionable sensory perceived odour or flavour of boar taint in their carcasses. A combination of measures is probably required to achieve an efficient and effective way of fattening entire boars. Which precise combination of measures is the most effective and efficient is not known yet. The aim of this quick scan, based on best professional judgement, is to explore recent developments in the advance of genetics and breeding technology to prevent or significantly reduce boar taint of male pigs with a carcass weight varying from 85 to 100 kg. The quick scan is commissioned by the Dutch Ministry of Agriculture, Nature and Food Quality, the VION Food Group, and the Dutch Farmers Organisation (LTO) to the first author and is the result of an intensive discussion during one day (June 13, 2007) with the input of a breadth of expertise in the area of boar taint, quantitative and molecular genetics and the practice of breeding programmes in pigs.

### Introduction

According to "Welfare aspects of the castration of piglets" (EFSA, 2004, p 45) boar taint is a distinctive and unpleasant taint perceived through a combination of sensory odour, flavour and taste in pork and pork products during cooking and eating. It has been described as 'animal', 'urine', 'faecal' and/or 'sweat' like in character. At commercial slaughter weights the incidence of boar taint is highly variable, ranging from 10 to 75% according to different studies. It is considered that two compounds are largely causal in boar taint namely, androstenone and skatole".

It may be noted that not all humans detect boar taint. Some 30% of humans cannot detect androstenone unless the concentration is extremely high (See EFSA, 2004, p 48). Skatole is perceived by a majority of consumers (EFSA, 2004, p 48). Though it is unclear which of the compounds has a stronger contribution to boar taint (EFSA, 2004, p 47) it seems that also some 30% of humans doesn't detect boar taint.

Boar taint is not caused by androstenone and skatole alone, but these are the major compounds. Boar taint does not, however, occur in females or castrated males, unless kept in environments heavily contaminated with own urine and faeces or fed special diets (EFSA, 2004, p 45). In that case it is caused by skatole.

Though the topic will be addressed in a bit more detail later on, we here briefly summarize some genetic aspects of boar taint. The incidence of boar taint is heritable, as are the levels of androstenone and skatole (somewhat higher for the first with a heritability value of 0.50 than the second, 0.30, EFSA, 2004, pp 57-58). Genetic selection against boar taint therefore is expected to be successful. When selecting for androstenone and skatole it is useful to look into the biological function of these substances in order to anticipate side effects, including female reproduction.

Androstenone is produced in pig testes, along with the sex steroids, androgens and estrogens (EFSA, 2004, p 57), which implies that selection against high androstenone levels, may affect reproductive traits negatively, both in males and in females. See, for example, for Dutch data, Engelsma *et al.* (2007). Androstenone causes boar taint by its occurrence in fatty tissue. It is interesting to note that the level of androstenone in fat is correlated to the level of one of the two isoforms of cytochrome b5 protein (EFSA, 2004 p 58, see Davis and Squires, 1999); this gene will be discussed in more detail in the paragraph 'the single gene case'. This may offer a

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possibility to decrease the level of androstenone in fat without decreasing the production of androstenone as such and therefore without the risk to affect biological functions related to it. In contrast to the situation with androstenone, levels of skatole are not associated with biological functions.

Skatole is produced in the lower gut by bacterial flora (EFSA, 2004, p 57), as a metabolite of tryptophan. Its production can be prevented by special diets (e.g. Lösel *et al.*, 2006). Skatole is fat soluble and metabolized in the liver.

The purpose of this paper is to discuss the potential of genetic measures to significantly reduce or even prevent boar taint. For this, we look into the genetic determination of the level of androstenone and skatole on the one hand and possibilities to affect these by the incorporation of the selection against higher levels of both substances in breeding programmes on the other hand. Because boar taint is practically restricted to entire males, preventing the conception of males by 'sperm sexing' (Brascamp and Haley, 1993) may offer a solution. In the foreseeable future this is not promising, however, because the capacity of sperm sexing equipment (maximum yield is 2 million sorted cells for each sex per hour) is far insufficient for routine production of boar semen, even for intra-uterine insemination (in excess of 1 billion cells per dose). Hence we don't pay further attention to this option.

### **Approach**

For the discussion of the potential of genetic selection it is useful first of all to determine whether levels of androstenone or skatole are primarily affected by a very limited number of genes or that these should be considered as affected by many genes. For the sake of argument we consider the situation with a very limited number of genes as 'the single gene case'.

#### *The single gene case*

If the inheritance of boar taint is simple, pre-selection of potential breeding animals is likely to be a useful option, in particular when pre-selection can take place early in life. With the current status of molecular genetics, this is generally feasible because the development of a genetic test in such a case is a lot easier and faster than before. The situation in the past will be illustrated with the example of the halothane gene. In homozygous recessive form the halothane gene causes the malignant hyperthermia syndrome and higher stress susceptibility in pigs –and also higher lean meat percent, lower feed intake under ad lib, lower litter sizes, etc. It was eliminated successfully in the nineties of the previous century from the vast majority of breeding populations. At that time, however, it took some 25 years after the discovery of the phenomenon and the realisation that it concerned a single gene before the causal mutation was discovered (Fujii, *et al.*, 1991) and a simple genetic test could be developed. With advances in knowledge of the pig genome it would now be possible to reduce this interval to 5-10 years. In the seventies and eighties selection was carried out but using for example test matings instead of using a genetic test.

Particularly if the number of genes is very low, or if there is only one gene for each of the compounds androstenone and skatole, pre-selection of candidate breeding animals early in life can be very fast and effective. Because it is pre-selection, performance testing of positive and hence unselectable animals can be avoided. The selection intensity for other traits under selection, like reproductive performance, robustness, growth and efficiency, and carcass traits, is only somewhat reduced, depending on the number of rejected animals.

#### *The polygenic case*

If, however, the level of the traits are affected by many, probably interacting, genes, then it is still very well possible to select against higher levels of androstenone and skatole, but the selection process is likely to be integrated in the general selection process for many traits in a selection index type of manner. In that case the response to selection is bound to be fairly slow because in that case selection for these traits clearly goes at the expense of the selection for other traits. Even if selection were for androstenone and skatole alone, the response to selection would be lower than in the single gene case.

So, the approach taken in this paper is firstly discuss the likelihood of the single gene case and the polygenic case and after that discuss the consequences for breeding programmes and the prospects of reduction of boar taint by genetic means.

### Intermezzo

Before going into the single gene case and the polygenic case we offer proposals for the re-analysis of existing data and for the set up of a relatively simple experiment. In fact these proposals are useful irrespective whether inheritance involves single genes or is polygenic. They may provide fast results and if affirmative would lead to immediate prospects for application in breeding programmes.

Some 10 years ago, Keller *et al.* (1997) published the results of an experiment where they compared the levels of androstenone in progeny of five AI-boars, three with a low level of androstenone in a biopsy of back fat and two with a very high level. They found some differences in progeny group means for androstenone, but in particular the percentage of progeny with androstenone levels below the sensory threshold (0.5 µg/g) looked promising. These percentages were over eighty percent for the progeny of the low level AI-boars and 64% for the progeny of the AI-boars with a very high androstenone level. The experiment was very small (only five AI-boars chosen out of 10 with only 71 progeny in total. Whether the experiment was repeated on a larger scale is not known, but a possible follow up seems not to have been published. We feel, however, that it is worth the effort. This would also provide a good opportunity to study the effect of androstenone being high or normal on libido, sperm count and sperm quality of AI boars. It is likely that in The Netherlands routinely sampling of fat probes from live boars is not allowed; In that case fat probes may be replaced by estimated breeding values (Merks, 2007), which in combination with the suggestion of Ten Napel (2007) –see below- may be a good alternative.

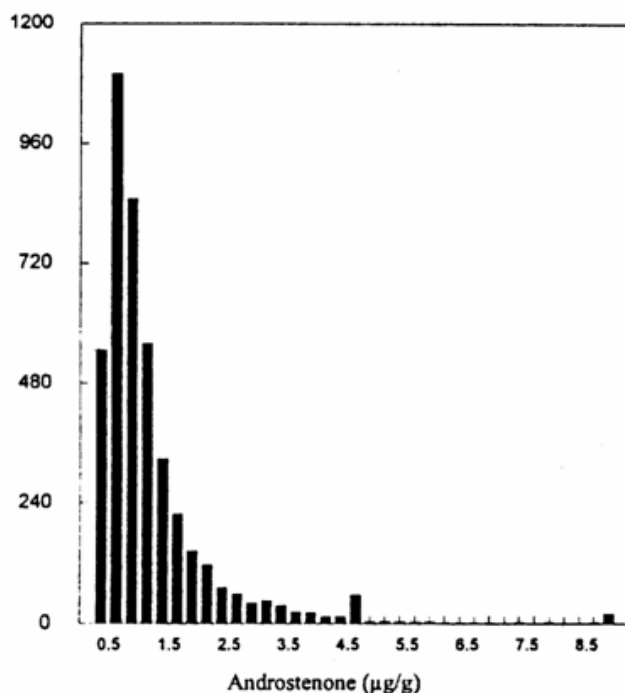


Figure 1. Numbers of animals with various levels of androstenone. Source: Walstra *et al.* (1999)

As a contributor to this paper, Jan ten Napel offered the possibility that the distribution of the levels of androstenone and skatole should not be interpreted as the result of one *single* distribution as jointly affected by genetic and environmental factors. In contrast, it might be looked at as consisting of *two* distributions, one symmetric distribution with a low mean level of androstenone or skatole, and one asymmetric distribution with a tail with values with low frequency but very high levels. See Figure 1 (Walstra *et al.*, 1999) for the distribution of androstenone. The distribution of skatole is similar to this. Ten Napel *et al.* (1995) used a similar approach in the analysis of interval of weaning-to-oestrus in gilts and found in a selection experiment that selection for a shorter interval did not change the mean of the symmetric distribution, but increased the incidence of animals with observations in the symmetric

distribution and decreased the incidence of animals with observations belonging to the distribution with the long tail.

If this situation holds true for levels of androstenone and skatole, selection against high levels of androstenone looks very appealing if it does not affect the mean of the normal distribution because in that case it may be expected that correlated effects on reproductive traits may stay away. It requires, however, that data analysis –and the estimation of breeding values– accommodates this statistical property of the distribution of androstenone. This actually is the case for the estimation of breeding values for the interval weaning-to-oestrus as currently carried out by Topigs (Ten Napel, 2007)

Also in this case (like the experiment of Keller *et al.*, 1997), it is very worthwhile to re-analyse existing data on androstenone and skatole to determine whether there is sufficient evidence for the existence of two different distributions. If affirmative, it seems also worthwhile to re-analyse other existing data sets where associations are sought between genetic markers and the incidence of alleviated levels of androstenone and skatole.

### The single gene case

For traits which are not only affected by genetic causes but also by the environment (where environment is the conglomerate of external effects affecting the performance of an animal, but also animal related effects like development, reaching puberty, etc) it generally is not easy to distinguish between the single gene case, the case with a few genes or the case with many genes. Taking the distribution of androstenone above for example, it can easily be interpreted as the mixture of two groups, the one consisting of two genotypes (AA and Aa, where AA and Aa have a similar phenotype) and the other of one genotype (aa) where the latter causes long tailed distribution.

More advanced analysis, taking pedigrees into account may reveal single gene inheritance although the distribution of the trait as such hides it. Fouilloux *et al.* (1997) analyzed a four-generation selection experiment in a Large White x Landrace crossbred population using mixed statistical models assuming that androstenone levels are affected both by polygenes and a major gene. In this analysis they found evidence for a major gene. The model which fitted the data best showed a major gene with a dominant allele for low values of androstenone, with a difference of three phenotypic standard deviations between the extreme genotypes.

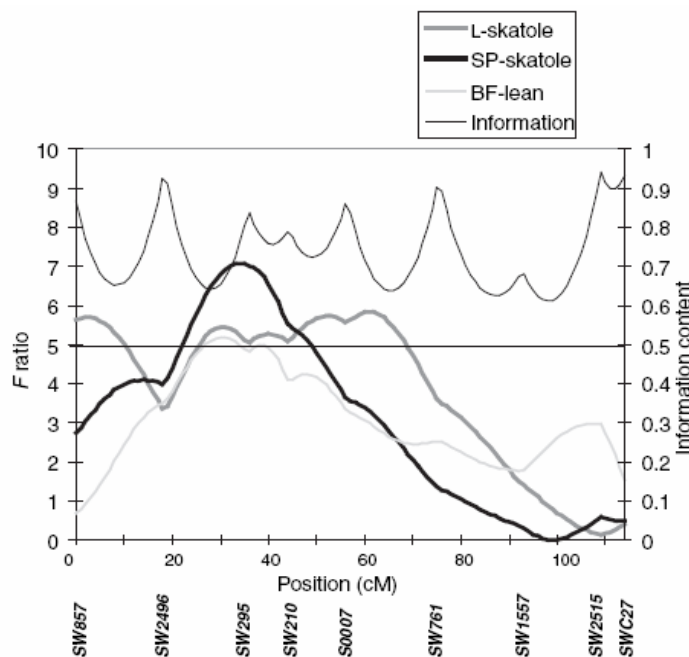


Figure 2. Chromosome 14 – F-values for L-skatole, sensory panel (SP)-skatoel and boar flavour (BF)-lean assuming alternative alleles fixed in the two breeds. Marker information content is shown on the second axis. A dotted horizontal line marks the chromosomal 5% significance threshold.

location of genes on the different chromosomes of the organisms under study. Figure 2 (Lee *et al.*, 2005) illustrates a typical result from such an experiment. They looked into androstenone, skatole and boar taint in a Scottish experiment with Large White x Meishan crosses). The picture

shows on the horizontal axis chromosome 14 of the pig (perhaps containing 1000 genes with a variety of biological functions) where animals were genotyped for 9 genetic markers (SW857 to SWC27). The picture shows the likelihood that in the neighbourhood of such a genetic marker a gene is located which affects –in this case- the level of skatole. The result shows that it is likely that a relevant gene is located in the vicinity of SW295. Making the transition from identifying a chromosomal region (QTL) harbouring gene(s) influencing a trait of interest to identification of the gene and a causal genetic variant remains very challenging notwithstanding advance in knowledge of genomes, whether human or pig. The principle limitation is having DNA samples from sufficient animals for which the trait has been recorded.

From a DNA-point-of-view, the area in which the gene may be located, around SW295, is still very large. Technology advances, however, and re-analysis of data for a much larger number of genetic markers is currently feasible and may result in the precise location –and identification- of the gene which biologically affects the alleviated level of –in this case- skatole but certainly results in a toolkit for genomic selection. We will come to this issue of genomic selection later. A very relevant experiment, which is currently in progress using large numbers of genetic markers (in this case SNPs), is the SABRE experiment<sup>2</sup>. It aims to find the genes with strongest effect on the level of skatole. As a matter of fact, samples will be analyzed for androstenone as well (Archibald, 2007), but for this the experiment is not well-designed because animals are selected to differ specifically for skatole.

Also a French experiment (a three generation experimental cross with Large White and Meishan) detected a large number of possible locations for genes relevant for boar taint (Quintanilla *et al.*, 2003).

Increasingly not only genetic markers are developed and located on specific chromosomes, but also genes with biological functions are positioned. Bits of information on these genes are not at all limited to the pig, but become also known for other species like humans, mice, cattle, etc. Because the genomes of different species resemble each other to a high degree candidate genes can be hypothesized combining the knowledge of biological functions of these genes on the one hand and location on genomes of various species on the other hand, in such way that candidates are looked for in chromosomal areas in pigs in which candidates are expected on the basis of experiments described above.

An example of that are the already mentioned cytochrome 5b. This gene is located on chromosome 1 of the pig and was analyzed by a Canadian group (Zhihong Lin *et al.*, 2005) where they studied allelic effects in boars of various breeds and crosses on levels of androstenone. The alleles differed for only one nucleotide (SNP) and the group identified an effect for a particular SNP. This effect was not present in the genome scan of Quintanilla *et al.* (2003). In another paper Zhihong Lin *et al.* (2003) showed the effect of the polymorphism in *SULT1A1* on the clearance of skatole in the liver. This gene is located at chromosome 3 of the pig (Skinner *et al.*, 2006). In a fairly small experiment Varona *et al.* (2005) did not find associations of genetic markers on skatole levels in an outbred Landrace population –taking the gene to be located on chromosome 3 on the basis of human analogy- neither did Lee *et al.* (2005) in the Large White Meishan crosses. Skinner *et al.* (2006) did not find segregation of the polymorphism in the Danish commercial pig population.

The polymorphisms found by the Canadian group are patented and available under licence. As it seems until now this has not provided increased evidence for the usefulness of these markers for selection purposes.

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<sup>2</sup> This is an experiment in the framework of the EU-project SABRE (Cutting edge genomics for sustainable animal breeding) in which 500 high skatole progeny are matched with 500 full sibs with low skatole level. These animals are currently being typed for 7300 SNP's. International efforts are underway to develop tools to type up to 50,000 SNPs per animal. Once such tools are available the SABRE samples could be typed again, if funding were available. Thus, again the key is DNA from sufficient animals recorded for the trait. Sufficient numbers are in the range of 1,000 – 10,000 depending on the trait of interest and the number of genes controlling the trait.

### The polygenic case

Current breeding programmes selecting for traits like litter size and growth and efficiency are based on the assumption that the performance of animals for these traits are the result of the action of many genes all affecting various biological pathways jointly causing the observed variation in performance. There is still little understanding in what way these genes act, or how many there are, but selection generally is successful as shown by numerous selection experiments and also in practice. A key tool in these breeding programmes is the method used to estimate breeding values of individual animals on the basis of observations on the animals themselves and relatives. Currently the statistical methods underlying breeding value estimation are very advanced and also feasible because of increasing computing power.

As pointed out in the introduction of this paper there is ample evidence that levels of androstenone and skatole are heritable to a fairly high degree such that in principle incorporation in a breeding programme is possible. Also experiments in which selection was carried out on the basis of levels of androstenone confirmed this point (Willeke *et al.*, 1987; Sellier *et al.*, 2000).

In general it is not possible to predict the response to selection for a specific trait without defining fairly precise the set up of the breeding programme (how many selection candidates, what breeding goals, etc.). To get some idea about possible responses to selection, Ducro-Steверink

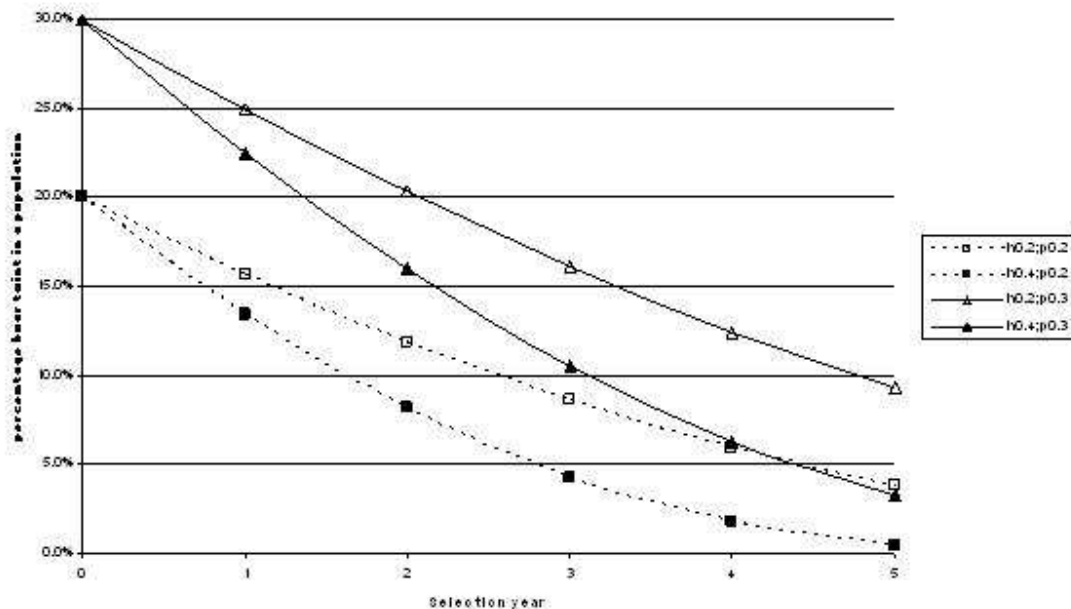


Figure 3. The percentage boar taint in five successive years in a population of pigs when selection is against boar taint, for two starting values (20 and 30%) and two levels of heritability (0,20 and 0,30). (Ducro-Steверink, 2006)

(2006) simulated five generations of selection in one single population. Selection against boar taint was combined with selection for growth rate and back fat thickness. Figure 3 illustrates the results for two levels of heritability (0.2 and 0.4) and two initial incidence levels of boar taint (20% or 30%).

There are a number of issues which deserve attention when planning to select against boar taint.

1. The measurement of traits should be realistically possible. Boar taint can not be measured on live animals, but levels of androstenone and skatole can be measured in fat probes of selection candidates. Before doing so, it is useful to carry out an experiment as suggested above (intermezzo) to verify the relationship between these measurements and the incidence of boar taint in entire male progeny and the levels of androstenone and skatole in this progeny. As indicated, results may suggest to chose for pre-selection of candidates, but if

that turns out not to be a logical way to proceed, the information can be used to decide how to utilize the information in the integrated system of breeding value estimation.

2. In case of pre-selection it may be effective just to pre-select AI-sires of the slaughter generation. If that turns out not to be useful (because pre-selection is insufficiently effective) genetic selection has to be carried out in each of the parental dam and sire lines which jointly are the ancestry of the slaughter generation. In that way a gradual improvement with respect to boar taint is envisaged.
3. In general, for a breeding organization to incorporate boar taint in its breeding programme there are two cases to be distinguished. The first is that it provides competitive advantage because future clients prefer breeding stock with lower incidence of boar taint. Alternatively, compensation should be provided for the fact that selection against boar taint goes at the expense of the improvement of other traits and therefore goes at the expense of the competitive position of the breeding company. In the situation slaughter houses in one way or another have a payment system in place which stimulates pig producers to deliver slaughter pigs with lower incidence of boar taint the first case may be realistic. If that is not the case, it seems that an arrangement has to be made between slaughter houses and breeding companies.

In this specific case of selection against boar taint it may be that a breeding organization sees sufficient competitive advantage in the fact that from their breeding stock boars can be grown for slaughter in stead of castrates (Merks, 2007) because boars grow more efficiently with a higher lean percentage than castrates. It should be kept in mind, however, that growing of boars is only feasible after a number of years of selection when the incidence of boar taint has reached an acceptable, low, level.

### **Genomic selection**

In a way the difference between selection in the single-gene case and in the polygenic case essentially is whether genomic information is used or not. In the single-gene case pre-selection can be envisaged on the basis of genotyping of candidates for selection, while in the polygenic case selection completely is on the basis of the measurement of relevant traits. It is expected that increasingly breeding programmes are a mixture of both, which means that genomic information is combined with measurements of traits and optimized in a method of breeding value estimation using both sources of information. To some extent this probably already is in use in some breeding programmes in dairy cattle and pigs.

Meuwissen *et al.* (2001) envisaged a breeding programme in which the polygenic variation is captured on the genomic level using a very large number of markers, like the 50.000 SNPs mentioned in the footnote on page 4. In such a set up traits are measured in order to estimate the effects of this vast amount of genetic markers, the results of which can be used for a couple of generations, but have to be re-estimated regularly to remain useful. Such applications are expected to be in place within –say- 10 years and will of course incorporate all relevant traits. It is not logical to develop such a system for androstenone or skatole alone. In general the association between genomic variation and expression of traits will be population specific and can not be transferred from one population to another.

In a way genomic selection very much resembles the polygenic case in that we select without understanding the underlying biological or physiological details of the process of genetic change. Application of it may well change the optimal set up of the breeding programme in terms of generation intervals or allocation of testing facilities. In a pig breeding programme with four lines, for example, it may be feasible to limit the test capacity to test candidate breeding boars of each line only once every four years. Van der Beek (1996) may be seen as an example of such a study, where he looked into the consequences of genomics-related technologies for the structure of a breeding programme for broilers.

### **Summary and conclusions**

1. Selection against high levels of boar taint is possible, in particular against high levels of the most important substances causing it: androstenone and skatole.
2. It is useful to re-analyse data sets with androstenone and skatole in order to look into the hypothesis that the distribution of the levels of androstenone or skatole in fact is made up of

two distributions. If this is the case, it offers potential for the re-analysis of datasets with genomic data and observations of androstenone and skatole, and it also offers potential for improved breeding value estimation.

3. It useful to set up an experiment to test the results of the experiment of Keller *et al.* (1991) in which androstenone, skatole levels in entire male slaughter pigs is compared with those in fat probes of their AI-sires. In such an experiment it is essential to check for boar taint in the slaughter pigs.
4. On the basis of results of conclusions 2 and 3 it can be decided whether testing of AI-sires of the slaughter generation and the elimination of some of them is useful as a tool to considerably reduce the incidence of boar taint in slaughter pigs. Also, it provides a basis as to how to include these traits in regular breeding value estimation and selection in pure lines.
5. Considerable experimentation, genotyping and analysis of data are underway which may give rise to the identification of a very limited number of genes which explain most variation in androstenone and skatole. If these results are affirmative and in the experiments useful genes are detected, it offers clear opportunities for selection of AI-sires of the slaughter generation and for pre-selection of candidates for further testing and breeding in the pure lines. Once the new higher density SNP typing tools are available additional funding would provide the opportunity to address the genetic control of boar taint unambiguously given sufficient trait recorded animals.
6. For breeding companies it is useful to anticipate on genomic selection.

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