The future of molecular genetics in poultry breeding

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Effectively, poultry breeding started when the chicken was domesticated thousands of years ago. Through human intervention many different breeds evolved over the centuries, but breeders began to use scientifically based selection methods less than a century ago. Mendel discovered the working of single genes and the chicken was one of the first species in which this basic concept was applied, e.g. in the selection methods for plumage colour. Industrial poultry breeding for production traits, however, is mostly based on quantitative genetics theory, which was largely developed for selective breeding of large farm animals such as cattle and pigs. Nevertheless, poultry breeding always led in the exploitation of knowledge of single genes such as those for plumage colour and for sex linked traits that can be used for sexing of day old chicks.

Quantitative genetic methods effectively regard the animal as a black box with many genes contributing to the expression of all traits under selection. Molecular genetics is now opening this black box by elucidating the effect of single genes on the phenotypic expression of traits. As breeding deals with identifying and exploiting the genetic basis of phenotypes, there is no doubt whatsoever that the use in breeding of knowledge of molecular genetics, i.e. molecular breeding, will totally change our current practices of selective breeding in poultry breeding. In addition, it is likely to also affect the role of the poultry breeding industry in poultry production.

The only relevant qualification of this prediction is on the time scale. We predict that methods of assessing genetic variability at the genome level (DNA) rather than at the phenotypic level (measuring traits in animals) will be the basis of selective breeding within ten years. This will affect the structure of breeding programmes and also impact the integration of breeding in the poultry production system. The new knowledge of the molecular basis of poultry phenotypes that is generated along the way will be used to engineer and re-design the poultry genome with novel technologies, and genetically engineered poultry breeds will be marketed within twenty years from today.

Keywords: poultry; breeding; genetics; molecular; genomics;

Introduction

The domestication process of wild jungle fowl also marked the start of selective breeding of poultry, albeit inadvertently. Over the millennia many different breeds were developed and by the end of the nineteenth century AD many breeds were in existence. These showed marked differences between breeds and homogeneity within them for a range of traits, including production traits such as body size, musculature, egg production and egg colour. Some of these breeds were the basis of industrial poultry breeding as we know it today: White Leghorn, Rhode Island, Cornish Game and Plymouth Rock, to name the most important ones.

Industrial breeding commenced with the hybridization of selected pure breeding lines sampled from these base breeds and continued with more and more intense further selection of the pure lines. As time progressed and competition between breeding companies intensified, the methods used in the genetic selection process applied to the pure lines were increasingly based on science.
This paper will give a short overview of the nature and impact of the methods introduced until now. It will then focus on the impact of breeding methods derived from molecular genetics on the future of poultry breeding.

**Historical developments**

The first scientific notion to have an impact on genetic selection was the discovery by Gregor Mendel of heredity through single gene effects on the phenotype of living organisms. Already before the start of ‘industrial’ selective breeding programmes in the 1940s, these principles were applied in poultry breeding: breeding for plumage colour used the same principles as noted by Mendel and selective breeding from superior parents to produce improved offspring was commonly applied by poultry breeders. At the same time reproductive technologies such as artificial incubation and hatching, lighting programmes to enable year round reproduction, and artificial insemination were being developed and introduced into poultry breeding programmes.

Another milestone in genetic selection was the development and application of the theory of quantitative genetics. This theory regards the animal as a black box with many genes contributing to the expression of all traits that can be measured. For every gene two copies are inherited from the parents, one from the mother and one from the father, and for the total genetic value this means that every offspring effectively samples fifty percent of its value from each of its parents. The impact of the genetic value of an animal on its phenotype is quantified by the heritability of a trait, i.e. the percentage of variance of a trait that is attributable to genetic origin rather than to environmental impacts. Around these two simple mathematical descriptions of genetic principles, i.e. 1. how genetic potential shows in the phenotype of an animal, and 2. how genetic potential transfers from one to the next generation, the whole theory of quantitative genetics has been built.

The first steps towards this mathematical-statistical approach to genetics and, consequently, to genetic selection were taken by R.A. Fisher in 1918. A real breakthrough came through the work of C.R. Henderson (1953), who developed methodology to combine all information about each individual’s breeding value into an index of merit. In his seminal paper he introduced what we now know as BLUP (Best Linear Unbiased Prediction) breeding value estimation. It was not until machines could provide the computing capacity that was required to do the extensive matrix calculations that this technology was put to use in animal breeding.

Today’s poultry breeding programmes all apply full pedigree-ing of all birds and exploit BLUP-breeding value estimations to obtain the best possible identification of superior breeding candidates. Obviously, the parents of the next generation have to be identified as early as possible (to minimize the generation interval) and genetic selection has to be devoted with the right emphasis to the right traits as demanded by the market. Competitive forces have assured that only those breeding programmes that have done this in the best possible way have survived until today!

**The next shake up in poultry breeding: the exploitation of genomics**

Now we are on the brink of a new technological development in genetic improvement of poultry: molecular breeding. The following paragraphs describe the steps towards two separate applications: genomic selection and genetic modification.

**Markers and maps**

Traditional breeding techniques, inspired by quantitative genetics, treat the animal as a black box with an indefinite number of genes influencing the expression of all characteristics of the animal. Since the 1980s we have been slowly opening this black box. The first development that had an impact on poultry breeding was the discovery of genetic markers. Genetic markers that form the most widely used category were small anonymous repeat sequences of DNA (microsatellites) that are scattered across the entire genome and can be used as landmarks to construct a map of the genome. After initial,
The ‘practical’ use of genetic markers was in the establishment of linkages between these landmarks on the chromosomes and the genetic variability of traits of interest. Linkages were established through extensive “QTL mapping” experiments. Essentially these experiments quantified the co-inheritance of genetic markers with variance of traits of interest and thereby were able to localize the sources of significant amounts of genetic variability to a certain region of a chromosome, called a Quantitative Trait Locus. The first study of this type was done on Hybro birds and was reported by Van Kaam et al. (1998). A review of all QTL mapping experiments in chicken by Hocking (2005) shows that up to the end of 2004 well over 100 statistically significant QTL were discovered and these covered all major production traits.

Although this result was better than expected by pessimists, who tended to believe that major genes with a large effect either do not exist or have been fixed during many years of selection, practical breeders mostly chose not to exploit QTL findings. Practical selection for QTL would involve the genotyping of many animals for many markers as the linkage phase between QTL and marker has to be established in every family. Moreover, the cost of such marker assays is prohibitive as the procedure for microsatellite markers is not suitable for high throughput genotyping systems.

Gene hunting

QTLs however do indicate the presence of one or more major genes on a marked position in the genome. This information can be used in attempts to identify a major gene by what is called positional cloning. This has proven a major hurdle to most research groups. The confidence intervals for the locations of QTL in the studies reviewed by Hocking (2005) ranged from 30 to 150 centiMorgans and therefore contain hundreds of genes. Indeed there are very few examples of successful attempts to identify the causative sequence for major gene effects mapped as a QTL. One of the best is that of the research groups of Georges and Andersson at the Universities of Liège and Uppsala who found a mutation in the IGF2 gene to be responsible for major variation in muscle growth in pigs (Van Laere et al, 2003). The cost of such projects amounts to several millions of euros and, if the probability of success is only limited, this is not an attractive proposition to a breeding company.

Information on QTLs may however be used in what is called the “candidate gene” approach. This approach uses prior and external information to hypothesize that a certain gene (the candidate) may be responsible for a known major genetic effect. Research then focuses on identifying that gene through prior information on its sequence (e.g. through information from other species, such as man or mouse), identifying sequence variation in that gene in poultry, and finally associating the various alleles with phenotypic variation of the trait in question. A recent example of success with this approach in chicken may be found in a publication by Gunnarsson et al. (2006) on plumage colour.

The full genome sequence of the chicken

The use of comparative genomics is extremely useful in the candidate gene approach, but the added value of the genome assembly of the chicken that resulted from the efforts of the International Chicken Genome Sequencing Consortium (2004) cannot be overstated. Since March 2004 we have the first draft of the chicken genome assembly and its quality is constantly being upgraded. The latest version is available through www.ensembl.org and predicts some 24,000 genes in the chicken genome. Although more than 50% of these have a known homologue in another species, only a minority of these (around 1,000) have been studied in any significant detail (see for this e.g. www.thearkdb.org). Nevertheless, the genome assembly of the chicken is an enormously rich resource and it is the major opening to the “black box” that contains all information on the genetic basis of poultry traits that are relevant to commercial poultry breeding. The only limitations to the full opening of the black box are time and money. Fortunately, the chicken is regarded as the main avian species for general and medical research and, therefore, the investments in poultry genomics do not depend on the poultry industry alone. In fact the sequencing of the chicken genome was fully funded by the United States National Institutes of Health (NIH), who provided 50 million US dollars for this effort that led to the
chicken being the first agricultural species to be fully sequenced. Nevertheless, the challenge now to poultry researchers is to exploit this rich resource for breeding purposes.

One way of using the genome information on the chicken is to study candidate genes in the way described above. Another way could be to use an intermediate (“gray box”) approach by exploiting the information on huge numbers of genetic markers that was indirectly derived from the sequencing effort.

**SNP markers**

The same issue of Nature that contained the report on the genome assembly of the chicken featured an accompanying article on Single Nucleotide Polymorphisms (SNPs) in the chicken genome (International Chicken Genome Sequencing Consortium, 2004). An SNP is a DNA sequence variation occurring when a single nucleotide – A, T, C or G – in the genome differs between members of the species. In this case partial sequences of a broiler chicken, a layer chicken and a Chinese Silkie chicken were compared with the full sequence of the Red Jungle Fowl. Millions of SNPs were detected in this exercise and, by themselves, these are a rich resource for genomics applications in poultry breeding.

The value of SNPs for breeding is mainly in their use as genetic markers. Genetic markers can be used because of their linkage to genomic locations that explain a significant share of genetic variance. However, the practical usefulness of a marker depends on its degree of linkage to the relevant genomic location and on the effort and cost that is associated with the marker assay. On both criteria SNP markers score particularly well: firstly, SNP markers are so abundant (1 in every 200 base pairs of DNA) that, theoretically, an SNP marker can be found for every unique location, and, secondly, the cost of SNP marker assays is relatively low. The cost may be as low as cents per marker per sample in novel high throughput genotyping technologies. Therefore experiments can now be done in the human field where as many as 500,000 SNP markers are being used to identify those that are closely associated with genetic variance of a trait of interest.

In poultry breeding research, the previous QTL mapping exercises can now be greatly improved by using SNP markers and major genes may be much more precisely localized by a relatively small set of markers. In fact, this new generation of QTLs may be mapped down to such narrow genomic areas that a few markers can be safely used for further selection for the major genes involved without the need to re-establish the linkage phase on a regular basis.

Finally, a whole genome marker approach is now slowly becoming a realistic option. This approach was first proposed by Meuwissen et al. (2001) and its rationale is that the genetic value of an individual animal can be obtained by estimating the effects of all genes or chromosomal positions simultaneously. To do this, we would require a number of genetic markers that is of the order of magnitude of the number of genes and these would have to be assayed in all breeder candidates. This is an enormous effort and the computational problems to handle all the resulting data are not trivial, but the latest technical developments do bring Meuwissen’s visionary option within reach.

**The future**

**Genomic selection**

We expect that over the coming years we will see a growing number of major genes being directly selected for in poultry breeding programmes. We also see aspects of genome-wide marker coverage by SNPs being applied for selected purposes. Taken together, we predict that within ten years from now selection procedures based on genomic information will be an essential part of every poultry breeding programme. We believe that such selection procedures could well turn into the core of the breeding programme. After all, the genome is the core of genetic variability, the livelihood of breeding companies.

A further speculation would be that, if indeed the core of the selection programme changes over time, the structure of the programme, which is very much connected to the selection system, will also change. This will increase genetic progress and, indeed, maintenance costs of the programme, but may
also provide means of specifically directing the flow of genes to poultry production companies. Through that route molecular breeding techniques will eventually impact on the working relationship between breeding companies and production companies. Thus, molecular breeding technology contains all aspects of what is called a breakthrough development.

**Genetic modification**

With the assembly of the chicken genome a major step towards the full elucidation of all gene structures of the chicken has been made. The black box will however not be fully opened until we walk the full path — from gene structure through gene function, gene expression, protein interactions, biochemical and signaling pathways, cellular function and cell-cell communication — towards a complete understanding of how phenotypic performance of the chicken is regulated. To do this takes an effort that cannot be imagined today. Nevertheless, through current and future research efforts in proteomics, metabolomics and all the other “–omics” areas, knowledge of this entire field will increase exponentially as new technologies become available. Therefore, it is safe to predict that over the coming years many isolated, critical pathways from gene structure all the way to phenotype will be understood. Once such knowledge is available, its exploitation through directed manipulation of gene structure and function is a natural next step.

For directed gene manipulation to be workable, we also need effective and efficient technology for genetic modification of birds. This has proven to be a major hurdle in avian systems and especially the delivery of a transgene or gene construct to an avian embryo is much more complicated than in a mammalian system (Mozdziak and Petitte, 2004). However, perseverance for more than twenty years in this area by several research groups (reviewed by Naito, 2003) has created definite progress and recently breakthrough-like successes have been claimed by at least two academic-private partnerships (Zhu et al., 2005, Viragen, 2006). Although current transgenics are focusing on applications in the pharmaceutical domain, these achievements do open the way to exploitation in poultry breeding for agricultural purposes.

However, this will take a lot of time. Firstly, much more knowledge on gene action in the chicken is needed to come up with a sound proposal for genetic modification of a chicken for agricultural use. Secondly, genetic modification systems for the chicken still need major improvements. After these two steps have been taken, the establishment of a genetically modified breed, from idea to introduction, takes at least another five years. Therefore, we expect the first genetically modified chicken with commercial potential in agricultural production to be on the market in fifteen to twenty years from now.

**References**


