Applications of biotechnology in the poultry industry

Reviews

Applications of biotechnology in the poultry industry

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Progress in the selection of production traits in the poultry industry has been dramatic over the past 75 years. Genetic progress has its biological limits and these may be reached in the next 20 years. The poultry industry will need to adapt with more emphasis on the needs of the consumer (e.g. product quality, health and animal welfare), as well as the needs of the breeders/producers (e.g. lower feed costs and higher fertility). These new traits are difficult and costly to measure by conventional genetic selection methods - the application of genomics is a possible solution. Poultry genomics has benefited from the rapid technological advances in the genetics of model organisms and human. A number of resources and approaches are now well established, including genetic markers and maps (both genetic and physical), QTL mapping, comparative mapping, EST and BAC resources. In addition, the next phase of gene discovery - *Functional Genomics* is underway. How the poultry industry can benefit and get access to these new technologies is discussed.

Keywords: Poultry; genome mapping; quantitative trait loci; genomics; ESTs; functional genomics; bio informatics; database

Abbreviation Key: BAC = bacterial artificial chromosomes; cM = centiMorgans; EST = Expressed Sequence Tag; FISH = fluorescence in situ hybridisation; Kb = thousand base pairs; Mb = million base pairs; PCR = polymerase chain reaction; QTL = quantitative trait loci.

Introduction

Applications in biotechnology have and will continue to have a major impact on agriculture: in nutrition (vitamins, nutriceuticals), animal health (vaccines and antibiotics), transgenic animals (therapeutic proteins) and genomics (breeding). During the past 15 years there have been rapid advances in genomics with the key driving force being the human genome project (Lander and Weinberg, 2000). Research on livestock genetics has benefited from these developments, with the creation of detailed genetic marker maps and mapping of trait-genes (Georges and Andersson, 1996). In this paper, I will review genomic applications in poultry and how they can be applied to the industry.

Genomics: meeting the needs of the industry and the consumer

During the last century, the poultry industry has been very effective at applying new technologies when needed (Albers, 1998). In particular, during the past 75 years modern selective breeding has made spectacular progress in both layer and broiler traits. Egg production (number of eggs per hen per year) has increased three-fold and growth rate (days to 1.5 kg live weight) four-fold, during this period. Associated with this success have been a number of undesirable correlated traits (*Figure 1*). In broiler or meat-type chickens there has been an increase in the incidence of metabolic disorders (e.g. ascites and lameness), reduced fertility, poor vaccine response and reduced resistance to infectious disease. In layer or egg-type chickens there has been an increase in the incidence of osteoporosis associated with increased egg production. Therefore genetic progress has its biological limits and these may be reached in the next 20 years (Albers, 1998). So with this end in sight, what are the prospects for the poultry industry?

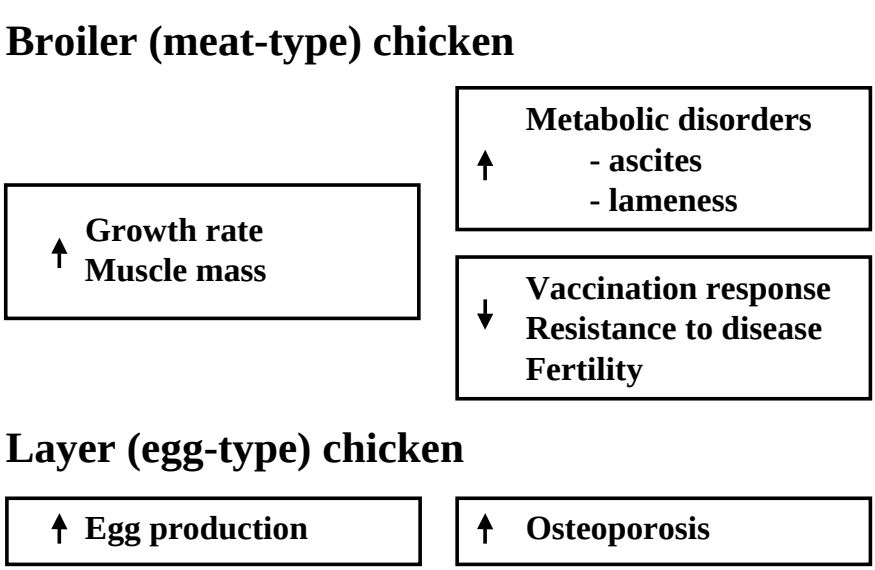

Figure 1 Traits associated with selection for broiler and layer production traits.

Given the likelihood that genetic gains in egg and meat production will reach their limit within two decades, priorities in the poultry industry will be to reduce costs and capture new markets. A major cost is feed and so improvements in feed conversion will be needed. Losses and reduced production due to poor fertility, lameness, stress-induced poor meat quality, ascites and osteoporosis need to be reduced or eliminated. These traits also affect animal welfare, which will become more important in the future, due to consumer demands and EC regulations. The reduction in resistance to infectious pathogens, in particular in broilers, is a problem that needs attention. The cost of vaccines and antibiotics is great and could be eliminated by genetic selection. This is desirable to save costs but also because vaccines and antibiotics are becoming less effective. Also, one of the potential causes for an increase in multi-drug resistance in bacteria has been the overuse use of antibiotics as growth promoters in livestock (Ferber, 2000). This will soon be phased out and so alternatives must be sought - genetic resistance is a possibility.

With the limits on egg and meat production in sight, the needs of the consumer will be more important to poultry breeders, producers and retailers. The consumer wants high quality products this requires greater uniformity and predictability in production. For example, increased eggshell strength and meat quality will be more important to increase market share. With an increased awareness of food safety, there will be a need to reduce the use of chemicals, antibiotics and other additives. Genetic resistance to pathogens will be an important trait. These new traits are difficult and costly to measure by conventional genetic selection methods. One solution to these problems may be through the application of *Genomics* in the poultry industry.

The principles of genome mapping and gene discovery

In a few cases, the genes that control genetic variation between animals have a large enough effect to be individually recognisable, such as the sex-linked *dwarf* gene in the chicken. Usually this is not the case for traits such as growth and fertility, which are traits controlled by many genes. The trait-genes that control these quantitative traits are located at quantitative trait loci (QTL). The chicken genome contains approximately 30,000 genes distributed over thirty-nine pairs of chromosomes. QTL mapping is the first stage in the discovery process of identifying the trait-genes at these loci (*Figure 2*). QTL can be located in the genome through associations between performance and the inheritance of genetic markers in a suitable pedigree (Hillel, 1997). The key to this process is a map of genetic markers evenly spaced throughout the genome (Georges and Andersson, 1996).

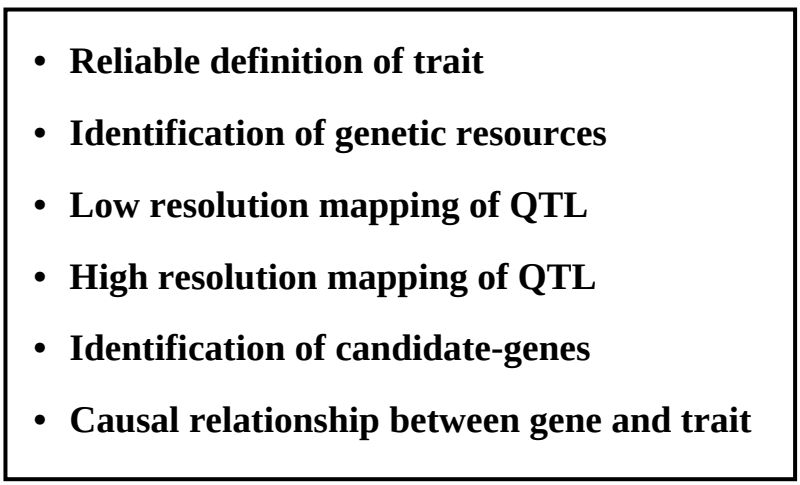

Figure 2 Gene discovery: a multi-stage process from a trait to a causal link with a gene.

Recent advances in poultry genomics

Genomics is the science of whole genome studies applied to biological systems (Lander and Weinberg, 2000). In poultry genomics there are four areas of interest: (a) isolation and mapping of genetic markers (crucial for whole genome mapping), (b) QTL mapping (the process of using marker maps to map QTL), (c) candidate-gene identification and (d) gene discovery.

MARKERS AND MAPS

The chicken has been the target for most studies and currently there is a genetic linkage map of over 2000 loci, covering most of the genome of 1200-Mb and 4000 cM (Schmid *et al.*, 2001). This map is the product of a collaboration of over twenty laboratories throughout the world. Over 1000 microsatellite markers have been mapped; these are crucial for genomic mapping. The chicken karyotype comprises thirty-nine pairs of chromosomes, which are divided into eight pairs of cytologically distinct macrochromosomes along with the Z and W sex chromosomes and thirty pairs of small, cytologically indistinguishable 'microchromosomes'. Genetic and physical maps of all macrochromosomes have been produced (Smith *et al.*, 2000a). Since they cannot be distinguished individually, the microchromosomes are ordered arbitrarily by decreasing size and only an estimate of the chromosome number can be given. BAC and PAC clones (Zoorob *et al.*, 1996; Crooijmans *et al.*, 2000) were used as tags for identification of microchromosomes in two-colour FISH experiments (Morisson *et al.*, 1998) from which twenty-two individual chromosome pairs were identified. A nomenclature based on the estimated size of each labelled microchromosome pair has been proposed. The genetic marker-containing clones led to the integration of genetic and cytogenetic maps for sixteen linkage groups (Morisson *et al.*, 1998; Schmid *et al.*, 2001). Recently, a genetic linkage map of the turkey based on microsatellite markers has been initiated with over 100 microsatellite markers (Bentley, 1997; Burt, *unpublished results*). All data on markers and maps is being made available through the *Arkdb* genome databases (http://www.thearkdb.org/).

QTL MAPPING AND MARKER-ASSISTED-SELECTION

QTL can be located in the genome through associations between performance and the inheritance of genetic markers in a suitable pedigree. The key to this process is a map of genetic markers evenly spaced throughout the genome; in particular the use of microsatellite markers has been very successful (Georges and Andersson, 1996). Microsatellites are abundant, highly polymorphic and assays based on PCR can be automated for high-throughput genotyping. Developments in robotics and thermostable enzymes have increased the throughput and reliability of microsatellite assays. In addition, the use of DNA fragment analysis on 96 lane automated DNA sequencers and resource databases (http://www.resSpecies.org/ Law and Archibald, 2000) have been crucial in the production and analysis of these large datasets.

A number of experiments are underway at the Roslin Institute mapping QTL in poultry: (a) broiler x layer cross (broiler male-line used was 15 standard deviations heavier and produced only one-tenth of the eggs laid by the layer line) and (b) fat x lean cross (lines differed three-fold in abdominal fat and triglyceride content). A QTL for muscling, an important broiler trait is shown in *Figure 3*, as an example of QTL mapping. So far, a number of QTL have been mapped in the chicken for a range of traits including body weight (Groenen *et al.*, 1997), muscling and body composition (Table 1), resistance to *Salmonellosis* (Bumstead, *personal communication*) and susceptibility to Marek's disease (Yonash *et al.*, 1999). These QTL are now available for exploitation by marker-assisted-selection (Spelman and Bovenhuis, 1998) and are under investigation by several poultry breeding companies.

CANDIDATE-GENE IDENTIFICATION

Once a QTL has been defined, the final step is to formally find a causal relationship between a candidate-gene and the genetic trait. This is a difficult and very labour intensive step. Understanding the fundamental biological mechanisms behind quantitative traits provides new opportunities for exploitation. Definition of allelic variation at the causative

Table 1 Summary of QTL for traits mapped in the Roslin broiler x layer cross. Indicated for each trait is the number of putative QTL detected for that trait and the fraction of the phenotypic variation explained by each trait. Traits were measured on 9-week-old broiler (average weight 2 kg).

Trait	No. of QTL	F statistic (%)*	% Phenotypic Variation
Abdominal fat pad (g)	2	14 (1)	6
		16 (1)	5
Body weight, 3 wk (g)	2	10 (1)	4
		10 (1)	4
Body weight, 6 wk (g)	4	8 (5)	4
		9 (5)	4
		10 (1)	5
		13 (1)	5
Body weight, 9 wk (g)	1	10 (1)	4
Breast muscle (g)	1	10 (1)	3
Thigh muscle (g)	2	11 (1)	3
		12 (1)	2
Leg muscle (g)	1	9 (5)	3
Spleen (g)	1	8 (5)	5
Intestine length (mm)	1	8 (5)	3

*, 1% and 5% genome-wise level of significance (Lander and Kruglyak, 1995).

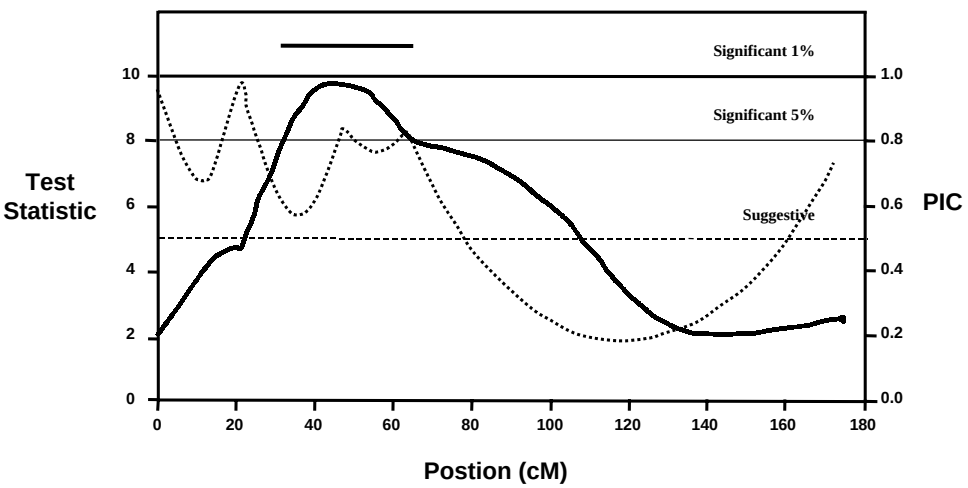

Figure 3 QTL for a muscling trait mapped in a broiler x layer cross. The solid line is a plot of the test statistic for the presence of a QTL. The dotted line is a plot of the polymorphic information content (PIC). The significant and suggestive linkage thresholds are included, as proposed by Lander and Kruglyak (1995).

gene provides the means for direct selection for the trait of interest, without family data. Also, in depth knowledge of the biological pathway affected may suggest other approaches, for example, for overcoming fertility problems by specific dietary modifications. This would be particularly important if there was a conflict between the desired production trait and a negatively correlated trait, controlled by the same trait-gene.

Examination of the genome map may identify candidate-genes, which have been mapped to the region of interest with a function appropriate to the trait under investigation. Given that less than 500 genes have been mapped in the chicken, so far (Schmid *et al.*,

2001), makes this approach unlikely, until a dense gene map is constructed. *A future goal will be a complete gene map of the chicken genome.* Comparative gene mapping is a possible solution, where the maps of chicken and human are compared using genes that have been mapped in the chicken as anchor loci. From these comparisons the gene content of a chicken QTL can be predicted. In *Figure 4* chicken chromosome 3 is compared with homologous chromosomes in mouse and human (Burt and Cheng, 1998). The chicken and human chromosomes look more like each other than that between the two mammals. Comparing the gene maps of chicken, mouse and human allows us to estimate the number of conserved segments between these species (Waddington *et al.*, 2000). The conclusion at first was a surprise - the genomes of chicken and human are more alike than that of mouse and human (Burt *et al.*, 1999). The number of conserved segments between chicken-human was only 154, of which 100 have already been defined (Schmid *et al.*, 2001).

Once candidate-genes have been defined the next step is to obtain chicken DNA sequences for the development of genetic markers. The simplest way to do this is to screen a database of chicken expressed sequence tags (ESTs); these are simply a partial sequence of the transcribed portion of these genes (Gerhold and Caskey, 1996). Currently in

Gene	Chicken		Human		Mouse	
ADPRT	**3**	**75**	720	1q41-q42	1	98.6
TGFB2	**3**	**81**	704	1q41	1	101.5
ACTN2	**3**	**135**	742	1q42-q43	13	7.0
HMX1	**3**	**163**		4p16.1	5	18.0
T	**3**	**163**	459	6q27	17	4.0
TCP1	**3**	**164**	620	6q25-q27	17	7.5
IGF2R	**3**	**167**	620	6q25.3	17	7.3
VIP	**3**	**182**	603	6q24-q27	10	S
ESR1	**3**	**182**		6q25.1	10	12.0
MYB	**3**	**196**	545	6q23.3-q24	10	16.0
PLN	**3**	**200**	495	6q22.1	10	S
FYN	**3**	**210**	468	6q21	10	25.0
CCNC	**3**	**218**	430	6q21	10	S
ME1	**3**	**232**	367	6q12	9	48.0
EEF1A1	**3**	**250**		6q14	4	S
BMP5	**3**	**250**	218	6q12-q13	9	42.0
GSTA2	**3**	**252**	199	6p12	9	43.0
ODC1	**3**	**279**	39	2p25	12	6.0
MYCN	**3**	**290**	56	2p24.3	12	4.0
RASGRF1	**3**	**310**		15q24	9	50.0

Figure 4 Location of genes on chicken, human and mouse chromosomes showing conservation of gene content and gene order. The chromosome numbers are shown together with the location on the genetic maps in chicken and mouse or on the radiation hybrid map of human.

collaboration with other Universities and Institutes (University of Delaware, University of Hamburg, Institute of Animal Health and Roslin Institute), 24,000 chicken ESTs have been produced (http://www.ri.bbsrc.ac.uk/). *A future goal will be to have a complete gene catalogue of the chicken genome.*

A problem may arise early on in this approach; there may not be any genes mapped to the QTL of interest to allow a comparison to be made with the human genome. Flanking genetic markers can be used to isolate genomic DNA (cloned in cosmid or BACs; Burt and Cheng, 1998) and sequence sampling of these genomic clones followed by searches for homologies with chicken/human ESTs will identify homologous genes for comparative mapping (Smith *et al.*, 2000b). *A future goal will be a complete physical map of the chicken genome based on overlapping BAC clones, onto which all chicken genes will be mapped.*

GENE DISCOVERY - FROM TRAIT TO GENE

Testing candidate-genes will be difficult for quantitative traits when compared to genetic diseases, since the effect is likely to be subtle, for example a quantitative change in how a receptor responds. Evidence in favour of a candidate-gene is likely to be an accumulation of evidence, such as: (a) bio informatics (e.g. sequence homologies and literature searches), (b) sequence variants and (c) population wide association studies, and (d) changes in gene expression patterns. Through the use of cDNA microarrays (Brown and Botstein, 1999) and other gene expression platforms it may be possible to associate changes in gene expression between selected lines and genes that map to the QTL. If this fails, it may be possible to identify an affected signalling pathway (using gene expression profiles as sensors), which involves a gene at the QTL.

The focus of research is moving from the genetic mapping of regions of the genome controlling quantitative traits to studies on the trait-genes themselves. This new era of *functional genomics* (Lander and Weinberg, 2000) requires access to large resources (arrayed cDNA and BAC libraries, EST databases, etc.) and high-throughput technologies (sequencing, microarrays, etc.). *ARK-Genomics* (http://www.ark-genomics.org/), the UK Centre for Functional Genomics in Farm Animals, is an initiative funded by the UK Biotechnology and Biological Sciences Research Council (BBSRC) that aims to fill this gap. The aim of this project is to build strong links between genomics, physiology, immunology and developmental biology to identify genes controlling traits of interest in agriculture and human health.

Technology transfer and exploitation

The principles for mapping QTL and identifying trait-genes are available now to the poultry industry, but there are obstacles to use and exploitation of these new technologies (*Figure 5*). The costs of gene discovery and exploitation are high in terms of infrastructure, resources, technology and skills. The solutions are here: gene discovery is a research phase, as described above, and is possible in a few universities and research institutes, with opportunities for Biotechnology and the Service sectors. Direct interest has increased in recent years, with a number of collaborations between universities/research institutes and breeders in the fields of QTL mapping (e.g. Nutreco, Cobb, BUT and Nicholas Turkey Breeding Farms) and DNA profiles (Ross Breeders, Hubbard-ISA, Cherry Valley Farms and Hy-Line International).

Recently, there has been considerable public concern over the use of biotechnology in plant and animal breeding (Trewavas, 1999). It is our responsibility to explain to the public that poultry genomics is a science that does not create genetic variation but is a precise tool that detects natural genetic variation in breeding populations. These genetic markers then provide more information to the breeder on which to base future breeding and selection strategies for the benefit of the consumer and poultry.

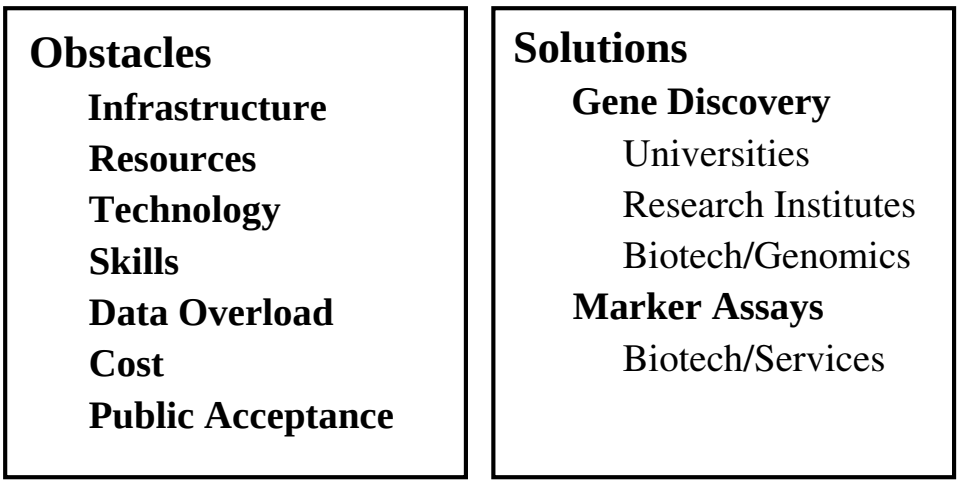

Figure 5 Obstacles to the transfer of genomic technologies to the poultry industry.

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