Contributions and perspectives of chicken genomics in Brazil: from biological model to export commodity

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Chicken is one of the most important sources of animal protein for human consumption, and breeding programmes have been responsible for constant improvements in production efficiency and product quality. Furthermore, chicken has largely contributed to fundamental discoveries in biology for the last 100 years. In this article we review recent developments in poultry genomics and their contribution to adding functional information to the already existing structural genomics, including the availability of the complete genome sequence, a comprehensive collection of mRNA sequences (ESTs), microarray platforms, and their use to complement QTL mapping strategies in the identification of genes that underlie complex traits. Efforts of the Brazilian Poultry Genomics Programme in this area resulted in generation of a resource population, which was used for identification of Quantitative Trait Loci (QTL) regions, generation of ESTs and candidate gene studies that contributed to furthering our understanding of the complex biological processes involved in growth and muscular development in chicken.

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Introduction

The use of chicken (*Gallus gallus*) as a model system in biology has largely contributed to fundamental discoveries and scientific progresses. The importance of some of these scientific developments, such as the demonstration that B cells are a distinct class of small lymphocytes and the discovery that a virus can cause tumour formation, might be regarded as great historical scientific milestones (Brown *et al.*, 2003). Chick-based experimental systems have continued to lead to important scientific breakthroughs, such as the discovery of gene conversion as a mechanism for antibody diversification (Reynaud *et al.*, 1987); the identification of DT40 cell line (Buerstedde; Takeda, 1991); and the revelation of alternative strategies of molecular and genomic evolution in the vertebrate immune system (Kaufmann, 1999).

Chicken has extensively been used as model organism for the last 100 years for several reasons. Birds are the evolutionary connection between mammals and other vertebrates, and chicken serves as the main laboratory model for ~9,600 extant avian species (Hillier *et al.*, 2004). Years of selection transformed chicken into an excellent model for understanding the genetic basis of phenotypic characteristics, due to its remarkable diversity among domesticated species (Andersson; Georges, 2004; Burt, 2005). Chicken embryos are also ideal for studying vertebrate development, because embryos develop in morphologically similar ways to mammals, yet, unlike mammalian embryos, are accessible to study with an *in ovo* development instead of *in utero.* Most of the knowledge about human limb formation has been uncovered through studies of chicken (Schmutz; Grimwood, 2004).

Further, chicken meat is one of the most important sources of animal protein for humans, without any major dietary restriction, such as swine (avoided by Muslims and Jewish) or beef cattle (not consumed by Hindus), and is therefore a common denominator for coexistence. In Brazil, chicken meat production represents an important component of agribusiness. The realized yield reached during the last few years has placed Brazil among the main world producers and exporters.

Research on chicken biology promotes basic sciences, with potential impact in other areas, including human health, food production and environmental conservation. Recent developments in poultry genomics offer new insights on avian biology, which facilitate applications in agriculture through increased efficiency in breeding and knowledge of animal physiology. Hence, the objective of this work was to review the current progress of Poultry Genomics and its future perspectives in breeding and biological research, highlighting developments in Brazil.

Background on poultry genetics and genomics

Poultry breeding programmes have relied on phenotype to select superior individuals as parents for the future generations, employing a classical breeding approach. This approach has been improved during the 20th century by the introduction of advanced statistical genetic procedures to better assess heritability of traits and breeding values of animals. Many years of selection resulted in enormous advances in chicken characteristics, associated with yield and adaptation. The live weight of broiler chicken is one of the best examples of the progress achieved throughout years of selection and breeding. In 1930, for instance, 105 days were needed for chickens to reach 1,500 g live weight (UBA Annual Report, 2005/2006). Nowadays, commercial broilers reach 2,300 g in only 42 days, decreasing the slaughter age by two months, with feed conversion rates decreasing proportionally, from 3.5 in 1930 to 1.8 in 2005 (UBA Annual Report, 2005/2006).

Despite clear successful gains obtained because of selection, the biological limits for genetic progress in egg and meat production were theoretically estimated to be achieved within the next twenty years (Burt, 2002). Conversely, small random molecular changes in genes (Hill, 2005) and selection-induced genetic variation (Carlborg *et al.*, 2006; Siegel *et al.*, 2006) were suggested as potential mechanisms responsible for widening genetic variability, which may enable the surpass of these theoretical plateaus. Consequently, the progress, or even the maintenance, of current yield constantly requires changes in the poultry industry's priorities. New changes may involve cost reduction or the development of new products (*e.g.* nutraceuticals), together with offering novel solutions for problems associated with a number of undesirable traits resulting from years of selection (Burt, 2005). For instance, there has been an increase in the incidence of diseases, such as ascites and lameness; reduced fertility; and a decrease in resistance to infectious diseases in broiler chicken (Burt, 2005).

To surpass the current genetic bottleneck, incorporation of new alleles by hybridization and/or by direct manipulation would be required. Gene introgression might provide a solution for some of these genetic limitations. New alleles could be introduced into commercial populations by directed crossings with wild populations, which may have conserved a wider set of alleles. Introgressed wild alleles would warrant continuous genetic progress in these lines by transgressive segregation. On the other hand, limitations of introgression include linkage drag and shortage of recognized or defined wild species.

Marker-assisted selection (MAS) was developed as an alternative to reduce linkage drag, promoting direct changes in DNA frequencies by selecting individuals that carry favourable polymorphisms at genetic markers and/or genes associated with economically important traits. DNA-based molecular markers, originally developed by Botstein *et al.* (1980) offer the potential to assist breeders by directly evaluating the genetics of a trait with minimal environmental influences. Selection of superior individuals was supposed to become a faster and more efficient process. However, the potential of molecular markers in animal selection has not been fully developed, except for mutations affecting monogenic traits in certain target populations. There are many examples of mutations listed at OMIA database (Online Mendelian Inheritance in Animals, http://omia.angis.org.au) that can be screened using molecular tools in livestock breeding. Among the chicken mutations listed at OMIA, those linked to sex have been exploited for sexing day-old chickens in specific populations and/or breeds, including mutants for the Feathering gene (Chambers *et al.*, 1993; OMIA380). Dwarf mutations (Agarwal *et al.*, 1994; OMIA309) can be exploited for selection of animals for reduced feed consumption. However, to our knowledge, there is no report about the use of mutations in poultry breeding programmes as genetic markers to improve production or meat quality. Nevertheless, there is extensive use of molecular tests in swine and cattle (Traas *et al.*, 2006; Dekkers, 2004). Characterization of mutations with important effects on relevant phenotypic traits remains a critical step to develop useful markers for selection. A large majority of important economic traits exhibit a polygenic pattern of inheritance, controlled by several genes with small individual effects, each largely influenced by the environment. Identification of genomic regions containing loci associated with quantitative traits (Quantitative Trait Loci or QTL) became possible with the development of saturated genetic maps using molecular markers.

The development of high-density genetic maps was initially limited by the availability of sufficiently polymorphic genetic markers, but microsatellites or simple sequence repeats (SSR; Tauz, 1989) and single nucleotide polymorphism (SNPs; Collins *et al.*, 1998) have enabled efficient mapping. Highly saturated genetic maps together with powerful statistical methods have allowed the dissection of complex traits into Mendelian units with identification of specific alleles.

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The use of segregation analysis in informative families or experimental crosses to map QTLs is now well established in animal genetics, including poultry. Currently, there are chicken genetic maps with various levels of resolution, based either on genetic or physical linkage, including FISH (Fluorescence *in situ* Hybridization) and radiation hybrid mapping (Morisson *et al.*, 2002). The consensus chicken genetic linkage map includes 2,261 genetic loci covering a total of ~4,200cM on 53 linkage groups (Schmid *et al.*, 2005). QTLs have been mapped along the genome for the most important economic traits, affecting animal growth (van Kaam *et al.*, 1998; 1999; Tatsuda, Fujinaka, 2001); feed efficiency (van Kaam *et al.*, 1999); carcass traits (van Kaam *et al.*, 1999); resistance to Marek's disease (Vallejo *et al.*, 1998; Yonash *et al.*, 1999; Lipkin *et al.*, 2002); body fat (Ikeobi *et al.*, 2002); and egg quality (Tuiskula-Haavisto *et al.*, 2002; Wardecka *et al.*, 2002) (reviewed by Romanov *et al.*, 2004; Hocking, 2005 and Abasht *et al.*, 2006). A chicken QTL database was recently organized at the site http://www.animalgenome.org/QTLdb/chicken.html, integrating available public chicken QTL data in a chicken consensus linkage map (Schmid *et al.*, 2005).

However, the adoption of QTLs in MAS schemes in animal breeding has not been completely realized so far, due to various technical limitations to identify the molecular basis of a QTL. First, it has been difficult to precisely locate a QTL in a chromosome. QTLs are determined statistically, and precision localization demands genotyping and phenotyping a large number of individuals, with the simultaneous reduction of environmental effects. Imprecise QTL location hinders the definition of the exact chromosomal fragment to be analyzed to identify the responsible genes and potential mutations that might influence the trait. Second, QTL chromosomal regions are mapped in experimental populations, developed in specific linkage disequilibrium (LD). Consequently, the QTLs mapped using experimental populations are not straightforwardly transferred to commercial lines, and require validation (Andersson, 2001; Harlizius *et al.*, 2004). Nevertheless, segregation of QTLs associated with production traits, mapped in extreme crosses scheme, have been verified in commercial lines of chicken (De Koning *et al.*, 2003; 2004). Despite all difficulties, examples of identification and use of QTLs have been shown to be possible in animal breeding. Two QTLs controlling milk composition and production were associated with mutations on dyacilglycerol O-acetyltransferase gene (*DGAT1*) (Grisart *et al.*, 2002), and on the growth factor hormone receptor gene (Blott *et al.*, 2003). In swine, QTLs influencing muscle mass and body fat deposition were identified near the IGF2 gene locus (Jeon *et al.*, 1999; Nezer *et al.*, 1999), and a single mutation was identified by sequencing a fragment of 30 Kb of this gene (van Laere *et al.*, 2003). Considering the incomplete knowledge of biological mechanisms controlling complex economic traits, the potential use of markers associated with QTLs in assisted selection is even more troublesome. For example, growth largely involves additive genetic effects, with a few reports of epistasis for early growth (Hocking, 2005; Carlborg *et al.*, 2003). Further, different QTLs control early and late growth, with very few affecting the entire process, indicating distinct regulatory control (Carlborg *et al.*, 2003).

An ideal situation for quantitative genetic selection is a trait with high heritability and a phenotype that can be observed in all individuals before reproductive age. This ideal is hardly ever achieved, which limits the effectiveness of quantitative genetics selection (Dekkers; Hospital, 2002). These limitations can be alleviated by the "–omics" technologies, which attempt to give a comprehensive description of all the components from a species genome (genomics); expressed transcripts (mRNA) of the genome (transcriptomics); translated products of the transcripts (proteomics); and the complete set of metabolic components of the cell (metabolomics). These technologies can fundamentally change the practice of animal breeding from a basically "black-box" approach towards to one that considers the regulatory networks and pathways underlying

the expression of important phenotypes (Kadarmideen *et al.*, 2006). The "–omics" technologies provide an initial framework for breeders, who want to understand how genetic variation influences traits important for production of domestic animals, including chickens, by allowing the traits to be mapped back at a precise genomic location (Schmutz; Grimwood, 2004). Saturated genetic maps, containing many microsatellite and SNP markers, and possible haplotype maps containing QTLs, will theoretically have direct correspondence with the genome sequence in commercial or experimental populations. Identification of candidate genes and casual mutations will also be enabled by the direct access to genome sequences and to the transcriptome, by expressed sequence tags (ESTs, partial sequences obtained from one of the ends of a complementary DNA); and the re-sequencing of QTL intervals will be an attractive approach for positional cloning.

Chickens were the first livestock species to have their genome sequence published in 2004 (Hillier *et al.*, 2004), based on DNA of a female of *Gallus gallus gallus* ('Red jungle fowl'), the ancestor of the domestic chicken. A comprehensive collection of ESTs has also been established for the most important tissues and embryonic developmental stages of chicken. More than 599,000 ESTs are available for *Gallus gallus*, deposited into the ESTs database (NCBI, dbEST, accessed in April, 2007). Abdrakhmanov *et al.*, (2000) were the first to obtain chicken ESTs, selecting bursa as the target tissue, because of its importance to generate B and DT40 cell lines. The Biotechnology and Biological Sciences Research Council (BBSRC) has sequenced more than 340,000 ESTs (Boardman *et al.*, 2002). The EST database provided evidence to identify 18,989 genes of the chicken genome, and for 19,626 full-sequence cDNA, which were essential for the genome annotation and a direct source to functional studies (Hubbard *et al.*, 2005).

More than 2.8 million SNPs were identified comparing the genome sequence of the ancestor (*Gallus gallus gallus*) with those from domesticated lines: a broiler male ('White Cornish'), a layer female ('White Leghorn') and an ornamental female ('Silkie Chinese'), with an average substitution rate of five SNPs Kb^{-1} (Wong *et al.*, 2004). The SNP map developed will be mainly used to increase marker density of linkage maps for QTL analysis and to identify genes and gene combinations that produce desirable traits in chicken breeding populations.

The collection of chicken sequences has also allowed the construction of array platforms for expression pattern analysis of thousands of transcripts simultaneously. A number of tissue-specific arrays has already been developed based on databases previously mentioned, including an intestine-specific array with 3,072 transcripts (Van Hemert *et al.*, 2003); a macrophage-specific array containing 4,906 transcripts (Bliss *et al.*, 2005); a lymphocyte-specific array with 3,011 clones (Neiman *et al.*, 2001); an immune response-specific array with 5,000 genes (Smith *et al.*, 2006); a heart precursor cell-specific array with 11,000 genes (Afrakhte; Schultheiss, 2004); among others. There are also microarrays representing 24 chicken embryonic and adult tissues (Burnside *et al.*, 2005); and one containing 32,773 transcripts corresponding to over 28,000 chicken genes (http://www.affymetrix.com/products/arrays/specific/chicken.affx). These arrays represent fundamental tools to search for biological functions of genes using the tissue-specific expression pattern and, consequently, useful for a number of unsolved molecular puzzles that were not previously revealed using QTL mapping and high-throughput sequencing of the chicken genome. Further, expression analysis might turn out to be useful complement to QTL mapping for identifying genes that underlie complex traits (Andersson; Georges, 2004).

Brazilian poultry genomics initiative

The surge of poultry as a worldwide attractive business began during the Second World War. Food scarcity forced USA growers to start breeding programmes using 'New Hampshire' and 'Barred Plymouth Rock' breeds as progenitors, followed by 'Red Cornish' and 'White Cornish' with 'White Rock' (Souza; Michelan-Filho, 2004). In Brazil, before 1920-1930s, producers were stimulated to breed ornamental chicken lines for meat production. Later, American breeds were imported as genitors of the National Breeding Programme (Souza; Michelan-Filho, 2004), so all commercial broiler lines on the market nowadays have these breeds in their genetic background.

In the private sector the Aviagen group (Scotland, UK) has a breeding programme to develop broiler lines, with two commercial hybrids, called AgRoss 308 and AgRoss 508, already available for producers in Brazil. In the public sector, the breeding programme developed by 'Embrapa Suínos e Aves' (Concórdia, SC, Brazil) was established in the late 1980s, and several commercial broiler and layer lines have been recently launched, such as 'Embrapa 011', 'Embrapa 021' and 'Embrapa 031'. 'Embrapa Suínos e Aves' and the 'Universidade de São Paulo' ('Escola Superior de Agricultura Luiz de Queiroz', ESALQ-USP, Piracicaba, SP, Brazil) had the strategic view to develop an experimental population specific for genomic studies, named 'EMBRAPA F2 Chicken Resource Population', segregating for main industrial and production traits, derived from crosses between a broiler and a layer line. Parental lines used presented distinct genetic composition, with the broiler line derived from crosses between 'Cornish', 'Hampshire' and 'White Plymouth Rock', whereas the layer line had the 'White Leghorn' breed as its background.

The broiler line, called TT, is a male line, and has been selected since 1992 for body weight, feed conversion, carcass and parts yields, chick viability, fertility, hatchability of fertile eggs, and reduced abdominal fat and metabolic disorders. The layer line, called CC, has been selected since 1989 for traits such as egg production, egg weight; feed conversion, chick viability, sexual maturity, fertility, hatchability of fertile eggs, egg quality and decreased body weight. The resource population was created from reciprocal crosses between both lines, in the proportion of one male for each female. The F_1 generation was obtained from crosses between seven males and seven females from each line, resulting in seven families from crosses between broiler males and layer females (TC), and seven families from reciprocal crosses between layer males and broiler females (CT). The F_2 generation was derived from crosses between one male and three females from different F_1 families, randomly selected. A total of seven males and 21 F_1 females from each cross (CT and TC) generated around 100 F_2 chickens per F_1 family, in 17 hatches, in a total of approximately 4,000 F_2 chickens, being half of each sex and half of each cross – TC and CT. Phenotypic traits, mainly associated with performance (body weight at 1, 35, 41 and 42 days of age, feed consumption and feed efficiency), carcass yield (breast, drums and thighs, and wings weight), carcass composition (water, crude protein, fat content, ash) and physiology (organs weight, triglyceride and cholesterol levels) were evaluated and recorded for these animals.

Structural and functional genomic analyses of this experimental population were started, firstly developing genetic maps for QTL identification, followed by searching for genes associated with skeletal muscle development to be used as candidate genes for selection of highly muscular animals. Genetic maps were developed using microsatellite markers, which allowed the identification of QTLs. For example, QTLs for performance and carcass traits were mapped on chromosome 1 (Nones *et al.*, 2006), co-localized with QTLs affecting body, carcass, legs and wings weight, abdominal fat, and feed intake, previously mapped in other populations (van Kaam *et al.*, 1999; Sewalem *et al.*, 2002; Ikeobi *et al.*, 2004). The overlap of QTLs mapped by independent experiments in distinct

populations is rare, but highly important because they validate genomic regions as truly associated with the analyzed traits. Novel QTLs were also mapped on chromosome 1 for liver, gizzard, lungs, heart and feet weight, intestine length, and feed conversion (Nones *et al.*, 2006). QTLs for feed conversion, abdominal fat, and for lung and heart weights are of great interest to the poultry industry, since these are difficult traits to measure and to select independently from body weight. The QTL mapping approach is still a recurrent theme, with the genetic maps currently being saturated to allow QTL mapping in other chromosomes, including the poorly represented micro-chromosomes, as well as the detection and estimation of epistatic QTL effects. QTL-defined regions have to be subsequently selected for a fine mapping approach, searching for effective linked markers or genes that can be used in MAS.

Phenotypic differences in muscle mass between TT and CC chicken lines can be detected from embryonic stages until adulthood. For example, after 17 days of embryonic development, the TT *pectoralis* muscle weighed 0.280 g, while the one from CC weighed 0,190 g. At twenty one-days post-hatch, a fivefold difference can be observed between TT and CC *pectoralis* muscle weight, with TT weighing 42 g and CC, 7.7 g (unpublished results). At slaughter age (41 days), the same five-fold difference can be observed, with TT weighing 2,395 g and CC, when reared as broilers, 513 g, (Ledur *et al.*, 2000a,b; Nones *et al.*, 2006). These evidently contrasting phenotypic differences can also be observed in different broiler and layer lines, among different anatomical muscles (Scheuermann *et al.*, 2004; Remignon *et al.*, 1995; Burke; Henry, 1997; Tesseraud *et al.*, 2000), and suggest that they are determined during early development. Coutinho *et al.*, (1993) described a delay in somite formation (embryonic muscle precursor structures), in expression of transcription factors that determine the fate of the myogenic cells (called myogenic factors), and of myosin heavy chains in quail lines selected for growth in relation to controls. The delay in myogenic factors expression could be responsible for a superior presumptive myoblast and proliferation rate of myoblasts in the growth selected line, which occurs immediately before muscular differentiation initiation. Embryonic proliferation stages of myogenic precursors practically determine the number of mature muscle fibres in the adult animal (Christ; Brand-Saberi, 2002). The majority of the muscular growth observed after birth is caused by hypertrophy (Moss, 1968). Consequently, knowledge of the intricate molecular events that occur during myogenesis would allow revealing candidate genes capable of affecting muscle mass deposition potential in domestic animals.

Biological functions of the myogenic transcription factors during embryogenesis have already been well-established. MyoD/Myf5 double-null mutants were not able to form skeletal muscle cells, due to an absence of myogenic precursor cells (Rudnick *et al.*, 1993). Absence of these factors changes the location of the precursor cells to abnormal sites of myogenesis, causing other cell fates (Tajbakhsh *et al.*, 1996). MRF4, myogenin, and MyoD act later during myogenesis as differentiation factors (Hasty *et al.*, 1993; Braun; Arnold, 1995). Based on these findings, the expression levels of the genes encoding known myogenic factors (MyoD, Myf5, myogenin and MRF4) and growth factors (IGF I and II, and TGF-β).were investigated in samples of *pectoralis* muscle dissected from the Embrapa broiler and layer chicken lines. Transcription analysis by quantitative RT-PCR revealed significantly lower levels of MyoD, Myf5 and myostatin in the broiler line (unpublished results), in agreement with the myogenesis delay previously observed in quail lines selected for growth (Coutinho *et al.*, 1993).

The demonstration that genes encoding myogenic factors were differentially expressed between the lines suggested that MyoD, Myf5, myogenin, MRF4 and myostatin could be used to select animals with higher skeletal muscle deposition potential. Polymorphisms identified on the sequence of the myostatin gene have been exploited to be used in animal

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selection for muscular potential. Myostatin is a known negative regulator of skeletal muscle growth. A loss-of-function mutation in the myostatin gene causes double muscling phenotype in 'Belgian Blue' and 'Piedmontese' cattle breeds (McPherron; Lee, 1997). Muscle weight of mice lacking the myostatin gene are about two or three times greater than those of wild type (McPherron *et al.*, 1997). The generalized muscular hypertrophy caused by the loss of function of this gene has been positively selected in some cattle breeds. The effect of myostatin sequence polymorphisms were evaluated for important traits in three commercial broiler chicken lines (Ye *et al.*, 2007), and significant associations (P < 0.031) were found with growth, mortality, blood oxygen, and hen antibody titre to infectious bursal disease virus vaccine, suggesting a pleiotropic effect of the myostatin gene on broiler performance (Ye *et al.*, 2007).

Simultaneously, ESTs have been generated from skeletal muscle precursors and adult tissues, and from hypophysis and hypothalamus, to identify new candidate genes associated with the myogenic programme and growth process, and also to generate a resource for gene expression studies. A total of eight cDNA libraries were constructed from somites (one library, stage HH15; Hamburger; Hamilton, 1951), limb buds in three development stages (one library, HH21, HH24 and HH26), whole embryo (one library, HH26) (Jorge *et al.*, 2004), *pectoralis* muscle in different developmental stages for broiler and layer lines (pool of HH35 and HH43, for broiler and layer lines; and pool of one and 21 days post-hatch, just for the broiler line, in a total of three cDNA libraries), and the hypophysis and hypothalamus cDNA libraries from chickens with 21 days post-hatch, one for broiler and another for layer line (unpublished results). A total of 13,664 ESTs were generated from these cDNA libraries, which after clustering and assembly (CAP3, Huang; Madan, 1999), revealed 6,774 putative chicken genes or unique sequences. The ESTs were annotated based on the chicken and human genome, and ESTs obtained for chicken, human and mouse. All ESTs were deposited into the dbEST (NCBI, http://www.ncbi.nlm.nih.gov), identified as CD760792 to CD765430, C0502869 to C0507803 and CO419474 to CO423759. These ESTs represent an important source of novel genes in chicken, and they have been used to construct the first muscle-associated microarray platform for chicken, with 4,534 clones spotted in duplicate (unpublished results). This platform corresponds to an important tool for functional investigation of new genes, determining the expression pattern according to developmental stages, evaluating differences in muscular growth potential between lines, and for the identification of tissue-specific genes.

Perspectives on the use of genomic approaches in breeding programmes and biological research

QTLs underlying variation in economically important traits have been successfully identified in chicken (reviewed by Hocking, 2005; Abasht *et al.*, 2006). The ultimate goals of these studies were to identify either genetic markers closely linked to QTLs (linkage disequilibrium markers) or the gene(s) responsible for the QTL (direct marker) to be used in MAS in breeding schemes (Abasht *et al.*, 2006). These objectives have seldom been achieved because of the intrinsic limitations of the quantitative approach, but it still offers great potential by compiling results across independent studies with the identification of consistent QTLs (Hocking, 2005; Abasht *et al.*, 2006).

The availability of the chicken genome sequence (Hillier *et al.*, 2004) and the SNP genetic map (Wong *et al.*, 2004) opened new possibilities for identifying QTLs (Abasht *et al.*, 2006). Quantitative trait analysis has been facilitated by the adoption of SNPs as markers for genotyping (instead of microsatellites and RFLPs), as well as the

implementation of association or admixture mapping based on LD between markers and important traits (Soller *et al.*, 2006; Abasht *et al.*, 2006). The main contribution will be the direct application of QTL-associated markers in commercial breeding programmes (Soller *et al.*, 2006; Abasht *et al.*, 2006). The large number of SNPs identified in the chicken genome will also eventually be available onto genomic oligonucleotide microarrays for applications in quantitative genetics, allowing genotyping with a dense SNP-set, in large sample sizes, quickly, accurately, with minimal optimization and with a feasible unit cost (Meaburn *et al.*, 2005).

Further, expression microarrays and segregating populations available for chicken can also be used to identify expression QTLs (e-QTLs), in a novel approach named 'Genetical Genomics' (Jansen; Nap, 2001). A segregating pedigreed or resource population is mapped with genetic markers to identify QTLs, and simultaneously used for expression profiling for each individual. By treating the expression level of each gene on a microarray as a quantitative trait, these data allow the identification of genomic regions that affect or regulate gene expression phenotypes, known as e-QTL (Jansen; Nap, 2001; de Koning; Haley, 2005). The meaning of these e-QTL regions is that they might contain genes with significant effect on transcriptional regulation of some of the genomic regions associated with QTLs for the analyzed traits (Kadarmideen *et al.*, 2006). Genetical genomics combining SNPs mapping data and QTL identification with microarray profiling on the same population would reveal *cis* and *trans*-e-QTL, and also SNP markers associated with phenotypic expression differences (Jansen; Nap, 2001; de Koning; Haley, 2005; Kadarmideen *et al.*, 2006). This approach would enable functional identification of uncharacterized genes, and unravel gene and gene products involved in metabolic and regulatory networks, finally allowing the identification and elucidation of epistatic effects. e-QTL have been successfully associated with variation in disease phenotype in mice (Schadt *et al.*, 2003) and rats (Hubner *et al.*, 2005). Genetical genomics offers a unique potential to be used in chicken, because microarrays, SNP map and mapped resource populations are already available.

The availability of high-throughput measurements of gene products (*e.g.* proteome and metabolome) has enabled the collection of comprehensive data-sets on whole systems performance, which can be added to the complexity of metabolic and regulatory networks in association with phenotype evaluation under various ontogenetic and/or environmental conditions. Because a biological entity is not just a linear flow of information, a small scale analysis of gene products is not sufficient to predict the behaviour of the whole organism (Kitano, 2002). Thus, an integrative approach is emerging in this post-genomic era, called Systems Genetics or Systems Biology, depending on the development of statistical and analytical methods to link all the information obtained from genomics, transcriptomics, proteomics, metabolomics, computational biology, chemistry, protein biochemistry and mass spectrometry, to be incorporated into the already existing approaches of phenotypic selection, selection on DNA polymorphisms, and combination of both, for a more efficient application in animal breeding (Kitano, 2002; Cassman, 2005; Kadarmideen *et al.*, 2006; Bogyo; Cravatt, 2007; Srivastava; Varner, 2007).

In a complementary approach, transient transgenesis has been used to study unknown, uncharacterized and/or 'bizarre' genes revealed by functional genomic analysis. Transient transgenesis is required to determine gene function *in vivo*, allowing the evaluation of phenotypic and genotypic effects of exogenous expression of the target gene. Chicken embryos are easily accessed and manipulated, and are consequently an excellent model system for functional studies. Methods of delivering expression constructs into chicken-living cells, such as retroviral vectors and electroporation, have resulted in sufficient amount of transfected cells after transformation (Krull, 2004; Bourikas, Stoeckli, 2003; Pekarik *et al.*, 2003; Alvares *et al.*, 2003). Further, chicken stable transgenesis has been

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long envisioned with the description of the primary conceptual requirements for inserting designed genetic alterations into the chicken genome. Recently, studies using germline transmission of primordial germ cells to transfect chicken embryos have turned chicken transgenesis into a more efficient and unlimited process (van de Lavoir *et al.*, 2006). The development of a facile cell-based system for introducing precise genetic modifications in the genome has opened new doors for chicken to resume a leading role in developmental biology and to gain a new role in the manufacture of biologics and therapeutics (Etches, 2006; Ivarie, 2003).

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