INTEGRATIVE POULTRY BIOLOGY: CURRENT APPROACHES AND PROSPECTS FOR FRANCE-TAIWAN COLLABORATION

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ABSTRACT

Few results are yet available for integrative biology in chickens. The purpose of this paper is to present some of the studies done in the France-Taiwan collaborative programmes in the framework of integrating data on population history, DNA polymorphisms and performance. The first programme involves the characterization of genetic diversity of chicken breeds using molecular markers, the second programme focuses on the molecular study of a feather colour gene in connection with variability of plumage colour, and the third programme deals with the study of genotype x environment interactions on egg production. Then the general properties of network analysis are briefly described in order to open the discussion towards the potential usefulness of this approach for animal breeding.

KEY WORDS: Chicken, Egg production, Feather colour, Gene diversity, Systems biology, Tropical climate.

INTRODUCTION

The concept of integrative biology has emerged in the last 10 years, based upon the evidence that most biological characteristics arise from complex interactions at the levels of cells, tissues, organs, whole organisms, populations and ecological systems. The progress in genomics leads to the production of many data (genome sequence, gene expression, protein structure ..) which make possible to study these interactions at a more global level than was previously possible. A short definition of 'systems biology' is the integration of experimental and computational research (Kitano, 2002). This definition looks like that of animal breeding, where performance data are analysed with specific computer programmes in order to infer the genetic value of candidates to selection. Yet, the biological pathways involved between the statistical genetic value and the observed performance have not been identified. The challenge of integrative biology is to extract the most relevant information for a particular question from the huge amount of data that may be obtained with genomics at the different levels of organisation, from cell to population. Because the chicken is both a species of economic interest and a model species for embryo development, it has benefited from a large international effort to decipher the genome sequence and produce tools to study gene expression on a large scale. Yet, few studies have reached a global level of integration.

The aim of this paper is to analyse a few examples taken from the France-Taiwan collaborative programmes as preliminary steps of what could become an integrative approach.

Then the general properties of network analysis will be briefly described in order to open the discussion towards the potential usefulness of this approach for animal breeding.

INTEGRATING DATA AT THE POPULATION LEVEL: GENETIC DIVERSITY OF LOCAL CHICKEN BREEDS

The European research projet, 'AvianDiv' has made possible, for the first time, to compare populations of different origins and selection histories at the level of genome variability, using 25 anonymous molecular markers, i.e. microsatellites (Rosenberg *et al*., 2001; Hillel *et al*., 2003). One of the 'take-home' messages was the ranking of populations on the basis of gene diversity: the highest level of gene diversity was found in the wild ancestor red jungle fowl and in a few local breeds (0.6 to 0.65), white-egg layers exhibited the lowest level of gene diversity of all commercial lines (0.35 to 0.4), brown-egg layers showed a slightly higher level (0.45) and broilers exhibited a higher level of gene diversity (0.5 to 0.6); local breeds exhibited a wide range of gene diversity, from 0.2 to 0.65.

The same approach was applied to the study of six local breeds maintained in a conservation programme at Chung-Hsing University. Gene diversity was studied on a sample of 50 animals per breed with the same set of 25 microsatellite markers that was used in the AvianDiv project. Results showed that gene diversity varied from 0.42 to 0.57 (Chen *et al.*, 2004a). The most diverse breed (Hua-Tung) originated from the east coast of Taiwan whereas the lowest value of gene diversity was found in the population (Shek-Ki) originating from a defined area on mainland China. The Hua-Tung breed appeared to be rather central in a factorial analysis, suggesting it could be the main reservoir of gene diversity, whereas the 2 most distant breeds, also isolated from each other, were also the most distant on the geographic scale, one from mainland China and one from Japan.

Thus, some consistency appears between the genome variability, revealed at a limited scale by a set of 25 microsatellite markers, and the population history. Individual performance data, which have been recorded on the same individuals, have still to be analysed. It is expected that merging phenotypic data with molecular data and population history will provide an integrated analysis of the genetic diversity of these populations.

BRIDGING THE WAY BETWEEN A 'SIMPLE PHENOTYPE' AND A CANDIDATE GENE: POLYMORPHISMS OF *MC1R* GENE IN LOCAL CHICKEN BREEDS

The same set of breeds used previously for the global gene diversity study was included in a comparison of the diversity of a specific gene, *MC1R*, between French and Taiwanese chickens. *MC1R* gene is the molecular counterpart for the *Extension* locus, *E*, which controls the distribution of black and yellow-red pigment on the body (Kerje *et al.*, 2003). Compiling literature data and unpublished data from INRA leads to the identification of 9 nucleotide changes in the *MC1R* coding region, associated with 7 amino-acid changes. These SNP are associated into haplotypes, the correspondence of which with plumage colour is not always straight forward.

The six populations of the conservation programme at Chung-Hsing University differed also in plumage colour with a continuous range from almost full yellow to full black: two of them (Nagoya and Shek-Ki) exhibited a yellow-red plumage with black tail feathers (*E*Y* or *E*WH* alleles), the Hsin-Yi breed exhibited a darker red colour with black tail feathers (*E*B* allele), the Quemoy breed had black feathers not only on the tail but also on the belly and sides (*E*R* allele) the Ju-Chi breed was almost black with some yellow feathers (*E*E*) and the Hua-Tung was fully black (*E*E*). A major fragment of 700 bp of the coding sequence of the MC1R gene was sequenced in all the 300 animals previously studied with anonymous markers (Chen *et al.*, 2005). At the same time, a set of 248 animals from label-type chicken lines was studied in France with the sequencing of the same fragment of 700 bp (Tixier-Boichard *et al.*, 2006).

Results showed the presence of 8 SNP in the French populations and 6 SNP in the Taiwanese populations. As compared to the wild-type sequence (H0), the 8 SNP could be associated in 4 mutant haplotypes (H1 to H4): H1 was found in full black animals, H3 and H4 were found in yellow and red animals, respectively, H2 was found in animals showing a mixture of red and black, H2 has been associated either to the *E*BC* or the *E*B* allele, depending on the study. Heterozygous carriers of these haplotypes exhibited variable phenotypes, which were not always easy to analyse in terms of additive actions of each haplotype.

The Taiwanese populations carried 3 of these 4 haplotypes. H1 was found in the black feathered breeds, Hua-Tung and Ju-Chi (*E*E*), but also in the Quemoy breed (*E*R*). It was found previously that breeds classified as either *E*E* and *E*R* could harbour the same *MC1R* genotype, the explanation would be that some black breeds are actually carrying *E*R* in addition to an eumelanising mutation, possibly *ML* (melanotic mutation). H2 was found in the Hsin-Yi and Shek-Ki breeds, and H3 in the Nagoya, which would suggest that Shek-Ki was not carrying the *E*Y* allele as the Nagoya does. Curiously, the Shek-Ki breed exhibited the highest proportion (50%) of heterozygous genotypes (mainly H2/H3) birds, although this breed had shown the lowest gene diversity with anonymous markers.

From these results, it can be concluded that the *E/MC1R* locus exhibits a high level of polymorphism, which results in a polymorphism of the amino-acid structure of the receptor. The functional consequences of these changes have been studied by Ling *et al.* (2003) and support the role of H1 haplotype in the enhanced activity of the receptor, leading to an increased synthesis of eumelanin, the black pigment. Yet, the genotype x phenotype relationship is not fully explained by the variability of the *MC1R* gene. Other genes are most probably interacting with *MC1R* gene to regulate the balance between red and black pigment, as illustrated in mice (Lamoreux *et al.*, 2001). The identification of these interacting genes should progress as a result of the knowledge of the chicken genome sequence.

In the future, this type of study could be extended to other genes of known function, such as the major histocompatibility complex, known to be highly variable and involved in immune function.

STUDYING GENOTYPE X ENVIRONMENT INTERACTIONS AT THE POPULATION LEVEL

Studies on experimental lines in France have shown that the association of the dwarf gene, *DW*, and the naked neck gene, *NA*, was improving heat tolerance in laying hens, and this could be combined with a genetic improvement of laying intensity by selection on clutch

length (Chen and Tixier-Boichard, 2003; Chen *et al*., 2004b). The purpose of the collaborative project was to estimate the effects of this selection and the effect of the *NA* gene in the tropical conditions of Taiwan. To achieve that aim, a set of 800 chicks was shipped from France to Taiwan, from six genotypes : control line, carrying or not carrying the *NA* gene in heterozygous or homozygous conditions (C, CNA, CNANA) selected line, carrying or not carrying the *NA* gene in heterozygous or homozygous conditions (S, SNA, SNANA). Egg production of the same genotypes obtained from the same families, (11 in the control line, 12 in the selected line) was measured at the same time in France on 280 layers..

The results were quite unexpected, since a marked decrease in performance of survivor hens (-30 to -35% on egg number) was observed in Taiwan as compared to France. The selected line performed significantly better than the control line, but the homozygous naked neck genotype was not at an advantage in Taiwan, although performance was recorded also during the hot season. This could be due to the fact that several environmental factors differed between France and Taiwan, such as diet composition, water quality, health conditions. Furthermore, the high ambient temperature did not start from the onset of lay, which was the case in the experimental design done in conditioned rooms in France. The comparison between this 'in situ' test and the experimental conditions tested in France suggests that the main difference between the two environments (France/Taiwan) was probably not the ambient temperature, but rather the health conditions. More interestingly, sire rank correlations, calculated for a similar trait recorded on progeny in the two environments, differed between the lines, and were low and non significant for the productivity traits (clutch length, egg number, laying rate) in the selected line only. These rank correlations were higher and significantly positive for egg weight and body weight, in both lines.

Thus, this suggests that some sire families were less affected by the change in environments and that genetic variability may take place for this robustness to a major change in environment. The way to investigate this robustness would require the identification of metabolic pathways that were particularly involved in the adaptation to this new environment. A gene expression approach would be a possibility, provided that the appropriate tissue to be sampled can be chosen according to physiological knowledge.

POTENTIAL USEFULNESS OF NETWORK ANALYSIS

This approach is entirely prospective for chickens, but appears quite promising from the studies published on the human and on model species, as reviewed by Barabasi and Oltvai (2004). The production of biological data in large numbers has triggered the development of mathematical models to analyse the interactions taking place between the cell's numerous constituents. Interactions are best described by networks and several networks are involved in the regulation of cellular activity. Thus, the theory of complex networks has been developed in the past years. A striking result was the finding of some universality in the set-up of networks, going from the cell machinery to the internet organisation. Briefly, a network is a set of nodes connected by links, the degree of a node is the number of links it has with other nodes. The probability function giving the number of links for a given node is a power function in the so-called 'scale-free' network. Those few nodes which have a high number of links are called hubs. A subset of highly interconnected nodes represents a module within the network.

Interestingly, 'scale-free' networks have been shown to fit biochemical reactions, regulatory gene networks, protein motifs, but also social networks. Such a network evolves by a growing process, with preferential attachment of new nodes to hubs. A very important feature of these networks is their robustness: there is a high number of nodes with few links and a small number of hubs. The probability that a random error affects a hub is all the more small than the network is large and includes many nodes with few links. This model explains very well the redundancy which is known to occur in biology and is a source of robustness.

Application of this model to gene expression data has been done to build a yeast transcriptional network (Ihmels *et al.,* 2002). The analysis of 1000 gene expression profiles has made possible to identify experimental conditions where gene expression changes, and to identify sets of co-regulated genes showing consistent changes under these experimental conditions. Furthermore, it is possible to retrieve genes of unknown function because of their identification within the network corresponding to a known metabolic pathway. Finally, it is possible to quantify the degree of co-regulation by the design of experiments aimed at modifying the expression of a single gene. The resulting matrix of correlation coefficients would be most useful to identify key regulating genes. As an example, conserved gene-co-expression networks for major biological functions have been identified across human, fruifly, nematode and yeast micro-array data (Stuart *et al*., 2003).

From the view point of animal breeding, several questions could be raised: is a QTL a hub in a given network? Are all hubs potential QTLs? Could we identify gene co-expression networks involved in selection response by the comparison of data obtained on pairs of selected/control lines from different genetic backgrounds? In the case of the GxE interaction for egg production between France and Taiwan, it would be most interesting to compare the matrix of correlation coefficients between gene expression levels in the different 'conditions x genotypes' combinations. Yet, a major difficulty remains: which type of biological material to sample and which kinetics of sampling? Lessons from model organisms will be most useful in order to set up suitable experimental designs that could help us to understand the molecular basis for genotype x environment interactions. For the moment, the knowledge of the chicken genome sequence is being improved and major progress in the understanding of poultry biology can be expected in the coming years. One specific issue of animal breeding will still be that questions are addressed at the population level, and variability is needed to make decisions in animal breeding.

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