Proceedings of the

8th European Symposium on Poultry Genetics

World's Poultry Science Association (WPSA)

Working Group 3 "Breeding and Genetics"

25-27 September 2013

San Servolo Conference Centre

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Welcome address
Italian Branch of WPSA

The Italian Branch of the World's Poultry Science Association is glad to welcome the participants to the 8th European Symposium on Poultry Genetics. Genetic research is in large part responsible for the great success achieved worldwide by the poultry industry during the last decades, of course, together with the cooperation of the 10 disciplines involved in the other Working Groups of the European Federation. Despite that my research activities have been focused on different topics, I recognize that genetic research has a sort of primacy in the progresses achieved by the poultry sector. For this reason, I am particularly grateful that finally colleagues have chosen Italy to organize their Symposium and special thanks go to Steffen Weigend, who for many years has promoted the activities and successes of the WG3, and Martino Cassandro who has spent so much time on the organization of this event, which will have the success they both deserve. Venice is a unique location in the world where the participants will be surely able to alternate work and discussion with times devoted to visiting this amazing city. This is my wish and my greetings.

Prof. Achille Franchini
President of the Italian Branch of the World´s Poultry Science Association (WPSA)

Welcome address
European Federation of WPSA

On behalf of the European Federation of the World's Poultry Science Association, it is my great pleasure to welcome in Venice all participants to the 8th European Symposium on Poultry Genetics. This meeting is organised by the Italian Branch of the WPSA chaired by Prof. A. Franchini and by the local Organising Committee with active participation of the Working Group 3 on Poultry Genetics. I really do congratulate the Italian committee, the international scientific committee and the Working Group 3, and in particular Prof. Martino Cassandro and Dr. Steffen Weigend, for spending time and effort for the benefit of the community. Such a gathering of experts in one area is a major factor in achieving the main objectives of the WPSA to promote avian research education and disseminate knowledge from avian biologists to the poultry industry. Genetics had a tremendous impact on the development of poultry production over 50 years. The genomic sequencing of *Gallus gallus* and other domestic birds at low cost is currently providing opportunities to identify a myriad of novel components in poultry products and tools to understand the regulation of tissues and to accelerate the process of selection. Maintaining diversity and adaptation of birds to diverse environmental conditions requires reinforcing collaboration with experts in physiology, nutrition, welfare, environment and pathology to develop sustainable systems of production. The WPSA network should favour these interdisciplinary exchanges. That is also the main challenge to attendees of the 8th European Symposium on Poultry Genetics, and this event should contribute to reinforce your collaboration for developing new projects, to get novel information and help solving your burning questions.

Dr. Yves Nys
President of European Federation of the World's Poultry Science Association

Welcome address
Working Group 3 "Breeding and Genetics" of WPSA

It is my great pleasure to welcome all participants to the 8th European Symposium on Poultry Genetics. The Symposia are a common activity of Working Group 3 and the European poultry breeding industry, and have a long and successful history resulting from the merger in 1999 of the Poultry Breeders Roundtable in Western Europe and the AVIAGEN conference in Eastern Europe. As in the past, the aim of the 8th Symposium on Poultry Genetics is to present on-going research activities and provide an update of current knowledge for people from both industry and research institutes. The program includes invited talks on up-to-date topics in the field of poultry genetics, short communications and poster presentations, and provides time for personal interactions between old friends and new contacts. We hope you will be delighted by both an attractive scientific program and the lovely Venice scenery, and will take home positive memories of the meeting.

Dr. Steffen Weigend
(on behalf of Working Group 3 and the Scientific Committee)

Welcome to the
8th European Poultry Genetics Symposium

The local Organising Committee is pleased to welcome you to the 8th European Symposium on Poultry Genetics which will be held at San Servolo Conference Centre, in the attractive island of Venice – Italy, on September 25 – 27, 2013. The Symposium is an opportunity of meeting for the international scientific and research community and a major opportunity to exchange new ideas and valuable information on the matters of poultry genetics and for trading professionals. The cooperation among the members of the WPSA Working Group 3 on poultry genetics will stimulate the drawing-up of a varied scientific program presenting the updates on this sector. The popular Italian hospitality, the historical environment, and the beauties of the surroundings will contribute to make the Symposium an unforgettable event.

Prof. Martino Cassandro
Chair of the Organising Committee

Editorial

The 8th European Symposium on Poultry Genetics is the current symposium of a series of meetings that are held every two years for researchers and specialists in poultry breeding and related topics. This year is the first time it has been held in Italy and as before it is promoted by WPSA Working Group 3.

The history of the Symposium started in 1999 and the venue is alternated between "West" and "East" European areas. So far the symposia have been held at:
- Mariensee, Germany (1st Symposium) in 1999
- Gödöllö, Hungary (2nd Symposium) in 2001
- Wageningen, The Netherlands (3rd Symposium) in 2003
- Dubrovnik, Croatia (4th Symposium) in 2005
- Braedstrup, Denmark (5th Symposium) in 2007
- Bedlewo, Poland (6th Symposium) in 2009
- Edinburgh, Scotland (7th Symposium) in 2011

The aims of the symposia are to exchange scientific knowledge on poultry breeding and conservation and to create a "scientific osmotic flow" between private and public researchers involved in the improvement of the poultry sectors around the world.

This year the symposium is located in Venice, a unique and historic city in northeast Italy sited on a group of 118 small islands separated by canals and linked by bridges. It is located in the marshy Venetian Lagoon which stretches along the shoreline between the mouths of the Po and the Piave Rivers. The Symposium will be held from 25th to 27th September, 2013.

Around 100 participants from 18 countries of 4 continents who are working in more than 55 public and private research centres are enrolled in the 8th European Symposium on Poultry Genetics. The invited speakers are among the most important specialists in the poultry and genetics sectors. The scientific presentations have been organized into the following sessions: 1) Genomics and contemporary resources and methodologies; 2) Disease resistance: genetics and breeding; 3) Molecular tools and genetic diversity; 4) Other poultry species; 5) Genetic parameters and phenotypic tools; 6) Enforcement of rules and its impact on breeding. Two poster sessions have been also organized. Moreover, we will have two special presentations: the invited lecture by Paul Siegel and the art event lecture by Koen Vanmechelen. Original scientific articles and abstracts will be included in a special book of Proceedings of the 8th European Poultry Genetics Symposium, edited by the Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE) of the University of Padova.

All members of the Organizing and Scientific Committees wish to express cordial gratitude to everyone that participated in the programme with posters or oral presentations and to the reviewers. Finally, special thanks to the Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE) and everyone who has helped to make this a successful symposium.

Dr. Mauro Penasa
Member of the Organising Committee

Prof. Martino Cassandro
Chair of the Organising Committee

PROGRAM

Wednesday, 25th September

10.00-12.00	**Registration of participants**
12.00-13.00	**Welcome cocktail**
13.00-13.15	**Opening the Symposium**
13.15-13.45	**Invited Lecture** - Using long-term selection experiments in the molecular era (*P.B. Siegel*)
13.45-15.45	**Session 1** - Genomics and contemporary resources and methodologies Chair: *M. Tixier-Boichard and P. Hocking*
I.C. Dunn	Functional long range *cis* acting QTL: examples from growth and development
G. Haberer	Bioinformatics of chicken genome varieties in the SYNBREED project
	Focused presentations (15 minutes each, including discussion)
15.45-16.15	**Coffee break**
16.15-17.00	**Poster session 1** - Brief presentations (2 minutes each poster)
17.00-18.30	**Poster viewing while tasting typical Italian products**

Thursday, 26th September

09.00-10.45	**Session 2** - Disease resistance: genetics and breeding Chair: *P. van As*
M.-H. Pinard-van der Laan	Breeding for disease resistance
H.H. Cheng	The genetic architecture of genetic resistance to Marek's disease
	Focused presentations (15 minutes each, including discussion)
10.45-11.15	**Coffee break**
11.15-11.45	**Poster session 2** - Brief presentations (2 minutes each poster)
11.45-12.30	**Poster viewing**
12.30-13.30	**Lunch**
13.30-15.00	**Session 3** - Molecular tools and genetic diversity Chair: *S. Weigend*
H. Simianer	Linking phenotypic with genomic diversity in the Synbreed Chicken Diversity Panel
D. Laloë	Landscape genomics and multivariate analyses: examples and prospects for poultry
C. Castellini	The importance of poultry biodiversity on rural poultry production
15.00-15.30	**Coffee break**
15.30-16.30	**Session 4** - Other poultry species Chair: *D. Guemene*
D.W. Burt	Structural aspects of genomes across species
A. Vignal	Genomics in minor species: the example of duck
16.30-17.30	**ART Event Lecture** – "This is not a chicken" *(K. Vanmechelen*)
17.30-19.00	**Business meeting of WG3**
19.00-22.00	**Gala dinner**

Friday, 27th September

09.00-10.30	**Session 5** - Genetic parameters and phenotypic tools Chair: *M. Cassandro*
W. Icken	Phenotyping for layers using different systems
M. De Marchi	Near infrared spectroscopy: an innovative phenotyping technique
N. Gengler	Genetic parameters using phenomics
10.30-11.00	**Coffee break**
11.00-12.30	**Session 6** - Enforcement of rules and its impact on breeding Chair: *D. Cavero and Y. Jego*
E.D. Ellen	The consequence of selection on social genetic effects for survival in laying hens
A.-M. Neeteson	A European perspective on meat poultry breeding
G.A.A. Albers	Impact of current and future European welfare regulations on animal breeding
12.30-13.00	**Closing the symposium**
13.00-14.30	**Lunch**

INVITED CONTRIBUTIONS AND FOCUSED PRESENTATIONS

ABSTRACTS OF POSTERS

ART EVENT Lecture

Using long-term selection experiments in the molecular era

P.B. Siegel and C.F. Honaker*

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The poultry and genetics literature has a long history of selection experiments for quantitatively inherited traits. Selection experiments involving chickens were mostly short term, and at the beginning of the molecular era there was a dearth of selection experiments that were long-term (>30 generations). This void remains and is unfortunate because, especially with the sequencing of the chicken genome, such populations provide unique resources for explaining mechanistically phenotypic variation, particularly those that are age and organ related. Once not feasible, studies can now be conducted via whole genome resequencing at the DNA level, real time PCR, RNA sequencing as a method of whole transcriptome analysis, microarrays, bisulfite sequencing for comparing DNA methylation, western blot, and mass spectrometry for proteomics.

At Virginia Tech, we have conducted two long-term bidirectional selection experiments, which have been used in molecular studies. One, currently in its 56th generation, is for high or low 56-day body weight. The other, currently in its 40th generation, is for high or low antibody response to a single intravenous injection of sheep red blood cells. The weight lines originated from a common founder population consisting of crosses of seven moderately inbred lines of White Plymouth Rocks. The founder population for the antibody lines was the Cornell Randombred White Leghorn line. During the course of selection, sublines were produced by relaxing selection in the mainlines, as well as first and advanced generation crosses. In both selection experiments, correlated responses were measured in unselected behaviors, reproduction, and metabolic traits. These correlated responses, along with endocrine and neurophysiological factors, are documented in the scientific literature.

During the past decade, these lines have been used in numerous experiments involving laboratories whose focus is on molecular approaches. These experiments have yielded results not only on mechanistic aspects in these populations per se, but also in the context of the value of the chicken as a model organism in biological research. Presented here is an overview of the phenotypic responses of these lines across generations, with examples of results from experiments conducted involving them during the molecular era. For those wishing additional details of the molecular studies, a sampling of references follows.

The selection experiments per se

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Some examples from the molecular era

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Functional long range *cis* acting QTL: examples from growth and development

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Understanding the genetic and genomic basis of important commercial traits has been a major aim of animal scientists with the view that it will improve our understanding of how selection has acted to improve production traits. This will ultimately lead to greater understanding of how selection has changed the physiology of modern strains of chicken and improve strategies for their management. However, with a couple of exceptions most progress in identifying the genes responsible has been for Mendelian traits associated with ornamentation. These are mostly due to changes in the genome that act close to the affected gene. These include examples of effects which alter the translated protein such as the *GHR* and dwarfism (Tixier-Boichard, 2002), *PMEL17* and plumage colour (Kerje et al., 2004), α-Keratin and frizzled feathers (Ng et al., 2012) or *FMO3* and fishy taint (Honkatukia et al., 2005), and duplications associated with retroviral insertions which cause increased expression of genes such as the k locus affecting early feathering (Elferink et al., 2008). There are also examples of relatively short range *cis* acting elements where a duplication in intron 1 alters expression of *SOX5* causing pea comb (Wright et al., 2009), the control of the *BCDO3* gene responsible for yellow skin (Eriksson et al., 2008) or the effect of an endogenous retroviral insertion immediately upstream of the biliverdin transporter *SLCO1B3* to produce blue eggs (Wang et al., 2013). Recently there have been examples of traits which have their origin in the effects of long range *cis* acting QTL, notably the naked neck phenotype which is thought to be due to a large insertion 260 kb downstream of the BMP2 gene which increases expression of this gene in the naked neck birds (Mou et al., 2011).

We have been fortunate enough to work on two traits, one quantitative and the other Mendelian, which have proven to be relatively tractable in terms of their biology, one of them concerns the QTL with the greatest effect on growth of poultry (Dunn et al., 2013) and the other the number of limb digits (polydactyly) (Dunn et al., 2011). However, although the loci have proven tractable in terms of identification of the genes whose expression was affected and the phenotype they cause, the causative genetic differences underlying them are more complex, also involving long range effects acting in *cis* on gene expression.

Polydactyly would not appear to be of commercial interest but we believe the genetic locus also underlies differences in limb size. The locus for polydactyly was fine mapped in a segregating F2 population of a White Leghorn x Silkie cross, aided by studies that had previously located the region on chromosome 2 (Pitel et al., 2000). The locus was found to contain a single base pair difference 1 Mb upstream of the sonic hedgehog gene (*SHH*), the temporal and positional expression of which it affects (Dunn et al., 2011). This was proven in part from the imbalance observed in the expression of *SHH* from alleles of heterozygotes chicks derived from the different founder lines. This was confirmed by observation of the link between the alleles and both ectopic expression and increased expression of the Silkie allele in the developing limb. The SNP causing the effect is in intron 5 of the *LMBR1* gene: this area is known in many species to control digit formation and the region has become known as the zone of polarising activity regulatory sequence (ZRS) due to its role in determining digit formation in the developing limb. Studies have shown that this region is not only associated with the Mendelian inheritance of digit number but also with adult limb size (Huang et al., 2007) which we believe is initiated early in embryonic limb development. This emphasises that developmental differences in embryonic gene expression may affect carcase composition in the hatched chick.

The presence of a QTL on chromosome 4 which explains the largest amount of the variation in growth and body weight in chickens has been reported in multiple studies (e.g., Sewalem et al., 2002; Nadaf et al., 2009). We have established that the effect of this locus is due to ~50% reduction in the

expression of the cholecystokinin A receptor (*CCKAR*) gene in high growth chickens. *CCKAR* mediates food intake through the secretion of the receptor ligand, cholecystokinin (*CCK*), from the intestine in response to food intake. Animals carrying the high growth allele do not show the expected reduction in food intake in response to injections of *CCK* and are therefore refractory to its effect on food intake. The release of *CCK* signals through *CCKAR* in the afferent vagal nerve and directly through the circulation to *CCKAR* in the feeding centre located in the hypothalamus of the brain. Here it alters the expression of agouti related peptide (*AGRP*) which increases the orexigenic or food intake drive. High growth animals have higher *AGRP* expression because of the reduced effect of *CCK* due to decreased *CCKAR* expression. The size of the effect of this locus represents around a 12% difference in body weight between the high and low growth allele in the advanced inter-cross population used in the study. Although functional studies clearly implicate *CCKAR* in the aetiology of differences in growth and body weight, fine mapping in the broiler layer advanced intercross suggests that the loci most strongly associated with the trait is between 1 and 2 Mb downstream of the *CCKAR* gene. We established that the effect was a *cis* acting effect and not due to an intermediate factor such as a downstream transcription factor by demonstrating that there was allelic imbalance with around 3.5 fold higher expression of the *CCKAR* gene inherited from low growth animals in animals heterozygotic at the locus for the high and low growth alleles.

These examples extend our knowledge of how growth and development are controlled in the chicken. Although most, but perhaps not all of the variation at the *CCKAR* locus has been fixed in modern meat type birds it may be valuable for improvement of dual purpose breeds. This is because although the locus appears to be correlated with age at first egg (Podisi et al., 2011) it may not affect follicle recruitment (Hocking et al., 2008) despite effects on numerous other traits. Both these loci which change the growth and body composition of chickens have been shown to be either due to or strongly suggested to be due to long range *cis* acting effects acting as enhancers on the target gene. As more results emerge from genome wide association studies in species including the chicken it would be wise to remember that the location of the associations are not necessarily a guide to the gene which is responsible for the difference in phenotype. In the case of the chicken with its relatively compact genome we have a better chance of making the link between phenotype and gene than in mammals, but as these examples demonstrate, it will still not be easy unless we have good annotation and knowledge of gene function.

Acknowledgements

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Bioinformatics of chicken genome varieties in the SYNBREED project

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Next generation sequencing (NGS) has revolutionized our view on populations and their variation both for basic and applied science. The massive amount of primary data, however, requires powerful computational infrastructures as well as efficient bioinformatics analysis. NGS data are one of the cornerstones for the analysis of plant and animal European breeding populations in the SYNBREED project (www.synbreed.tum.de), a national cooperation between industrial and academic partners funded by the German Federal Ministry of Education and Research (BMBF). SYNBREED targets the genetic variation in European varieties of three, economically highly important species, maize, cattle and chicken, to develop advanced knowledge and technologies for a sustainable agricultural production.

In this framework, high coverage NGS data from three chicken lines of the Lohmann Tierzucht GmbH, representing a White Layer (WL), White Rock (WR) and Rhode Island Red (RIR) line, were sequenced from DNA of individual roosters and of 15 pooled individuals using the Illumina platform and two different library sizes. Read alignments to the new red Jungle Fowl reference genome (build4) generated a high density SNP map of about 10 million genomic positions with variation in at least one of the chicken lines. Complementary to the identification of small scale allelic variation, structural variants (SV) with sizes ≥50 bp were detected by deviations in reference guided assembly paths using multicolor deBruijn graphs. In addition, contigs and scaffolds of *de novo* genome assemblies of the three chicken lines were ordered along the Red Jungle Fowl genomic sequence to construct in combination with the allelic variation data reference genomes of domesticated chicken varieties. This high resolution map of genetic variation allowed identifying candidate regions and functional genetic elements of domestication in the three lines at a high level of detail. Predicted functional impact of the genomic variation will be illustrated by several examples. Additionally, the session hopefully provides a basis for the discussion about advantages and limitations of NGS applications to breeding programs and how programs may benefit from such analysis.

Genomic selection experiment implemented in layers

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Genomic selection has revolutionized animal breeding by promising increased selection accuracy and shortening generation interval. This study compared response to selection in two sub-lines derived from a common population of brown-egg layers. One sub-line was selected based on own performance and pedigree information with an approximate one-year generation interval, whereas the other was selected based on genomic information with halved generation interval. To reduce costs, the size of the genomic sub-line was reduced five-fold and cross-classified mating was introduced to compensate for the decrease in effective population size. Selection was on an index combining 16 traits. GBLUP and BayesB were used to estimate genomic breeding values. Selected parents from four generations preceding the base population were genotyped with a 42K Illumina SNP chip to provide information about marker effects as were all selection candidates. Retraining was performed every round of selection. The accuracy of predictions varied between traits and generations, but was on average higher for genomic than for pedigree-based breeding values. By the end of the 3-year experiment, the genomic sub-line outperformed the pedigree sub-line for the majority of traits, but with higher inbreeding. The experiment has demonstrated that genomic selection can be successfully implemented in an egg-type chicken breeding program under a typical evaluation condition.

Selective sweeps using hapFLK combined with genome re-sequencing data reveals strong candidate mutations in QTL regions in divergent chicken lines

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In the context of genome wide re-sequencing using NGS technologies, we analyzed selective sweeps in two divergent chicken lines selected for abdominal fat weight (AF). We applied a new statistic, hapFLK, to analyze the 9.4 M of SNP observed for the whole genome of 20 chickens. This genome scan revealed 129 significant selective sweeps with a size of 86 kb that contained 839 SNP and 2.2 genes, on average. These selective sweeps colocalize with different QTL responsible for abdominal fat and muscle weight, and drastically reduce their size. We then performed three complementary analyses for positional genes in these regions: 1) analysis of their biological functions to select functional candidate genes; 2) analysis in different tissues of their differential expression between both lines to argue in favor of a candidate mutation in regulatory or coding regions; 3) analysis of their polymorphism from DNA-seq and RNA-seq data to identify candidate mutations. The combination of these approaches reveals several strong candidate genes and mutations associated with the traits of interest.

Mapping the *sc* gene by contrasting high-density SNP genotyping of two pooled DNA samples: featherless chickens versus feathered sibs

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A cost-effective, labour-efficient approach was used to map the *sc* mutation responsible for featherless phenotype in chickens. Two blood pools were prepared from 86 featherless (*sc/sc*) individuals and 120 feathered (*+/sc*) sibs, progeny of *+/sc* X *sc/sc* matings. The two extracted DNA samples were genotyped with the Illumina 57k SNP array. Relative allelic frequency (RAF) in each SNP was calculated in the two DNA samples, and absolute RAF differences (absRAFdif) between them were plotted over the genomic location of each SNP. The plot revealed genomic region on Chr. 4 with exceptionally high values of absRAFdif; the 3 SNPs with absRAFdif>0.45 were located within 1.25Mb, suggesting it as the location of *sc* mutation. Indeed, a nonsense mutation completely and exclusively associated with *sc/sc* phenotype was found in the *FGF20* gene (one of 11 in the region) that controls embryonic feather follicle development (Wells et al., 2012), confirming the accuracy of the mapping approach. This approach was applied two generations later; two pooled DNA samples from 80 *sc/sc* individuals and 80 *+/sc* sibs were genotyped with Affymetrix 580k SNP array. Genomic plots revealed the same region in Chr. 4, with absRAFdif=0.53 for the most indicative SNP, located only 5k bases from the *sc* mutation in *FGF20*. These results demonstrate the mapping power of genome-wide SNP genotyping of pooled DNA from genetically-contrasting groups. This approach is currently used to contrast DNA-genotyping of several pairs of extreme-phenotype groups.

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Analysis of genomic regions affecting eggshell color in layers

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For consumers, eggshell color is one of the most important selection criteria, although it is not associated with the nutritional content of the egg. Consumers' preferences for eggshell color differ according to cultural and geographical background, but uniformity of the color is often preferred. Eggshell color varies from pure white to very dark brown and speckled depending on the breed or line in question. Eggshell color is determined by pigments produced by protoporphyrin, biliverdin and Zinc chelate, mainly regulated by genes belonging to pigment pathway. Additionally, other factors are tuning the shell color during the pigmenting process. To study genomic regions affecting egg shell color, we conducted QTL analysis in an F_2 cross between White Rock and Rhode Island Red. The phenotype was evaluated in the F_2 generation of 1599 hens by Minolta colorimeter at the age of 40 weeks. First QTL analysis of major chromosomes covered with 162 microsatellite markers revealed five chromosomal areas affecting shell color. Chromosomes 3 and 6 were selected for fine-mapping first with a low density SNP marker panel in commercial lines and then sequencing the parental lines (pooled DNA-samples). Preliminary results indicate that the QTL regions contain 96 exonic SNPs of which 17 are non-synonymous. Effects of the detected SNPs will be assessed in pure lines.

Breeding for disease resistance

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In the context of intensification and specialization of poultry production, next to welfare regulation on animal breeding, animal health issues are of increasing importance to the breeding sector because of the huge related production losses. But animal health and welfare issues are also of importance to the consumers because of potential effects on their own health and their lifestyle choices. Most effective disease control strategies should be developed in an integrated animal health management approach, including prevention, cure, environment control and breeding for disease resistance. Until recently, selective breeding approaches have been applied successfully by poultry breeders to enhance production and reproduction traits; but the inclusion of animal health related traits are scarcely considered because of a clear lack of easy measurable and relevant phenotypes and associated genetic markers which could be integrated in running breeding programs. Though, there have been numerous studies in the past showing evidence of genetic variability of responses to various diseases of economic interest, like parasitic, bacterial or viral diseases. New opportunities have been arising thanks to major advances in animal genomics and related technologies. Most research strategies are now developed, combining structural, population and functional genomics approaches. The objectives are the identification of genes, gene products and regulatory networks involved in host pathogen interactions which could be used in selection and a better understanding of their functions and the underlying mechanisms. Also, there is a growing interest in deciphering genetic parameters underlying the immune response (innate and adaptive) of chickens and how this can be applied to breeding in combination with vaccination. Thus, research and development aiming at understanding and implementing host genetic variation in disease resistance require large use of field data, collaborative multidisciplinary programs and can only be hopefully applied if efficient technology transfer between research and industry is occurring.

The genetic architecture of genetic resistance to Marek's disease

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Marek's disease (MD), a T cell lymphoma induced by the oncogenic Marek's disease virus (MDV), is one of the most serious disease problems for the poultry industry. While MD is controlled through vaccination and biosecurity, it still costs more than $2 billion worldwide annually due to meat condemnation and reduced egg production. Combined with the emergence of more virulent MDV field strains, there is a need for alternate MD control strategies. Increasing genetic resistance to MD is an attractive solution as it has a proven track record and will also enhance animal welfare. To identify the underlying genes, over the past 15 years, we have employed and integrated various genomic screens at the DNA (e.g., QTL scans), RNA (e.g., microarray), and protein (e.g., two-hybrid screen) levels, which identified three MD resistance genes and many more candidates along with greater knowledge of the associated biological pathways, as reviewed in Cheng et al. (2012). More recently, we have incorporated allele-specific expression (ASE) screens in response to MDV infection, a simple yet powerful approach that identifies SNPs and genes that show variation in transcriptional response to virus challenge (MacEachern et al., 2012; Perumbakkam et al., 2013). Using these ASE SNPs for genomic selection in experimental lines, we found that they accounted for the majority of the observed genetic variance. This result strongly suggests that differences in MD genetic resistance are due to variation in transcriptional regulation and not other classes of polymorphisms (e.g., nonsynonymous amino acid changes, CNVs). This conclusion is further supported by some of our other efforts, e.g., we have identified SNPs that can lead to alternative splicing following MDV infection or binding of the MDV Meq oncoprotein, a bZIP transcription factor (Subramaniam et al., 2013). Currently, experimental and commercial layers are being selected with ASE SNPs and progeny tested to validate this approach. If our genomic predictions are confirmed, then this would suggest that the ASE approach can be applied to other complex traits. Furthermore, for specific traits, ASE SNPs should be incorporated in SNP chips for genomic selection as they provide added value compared to randomly selected SNPs.

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Comparing resistance to colibacillosis of six chicken lines

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Colibacillosis, caused by avian pathogenic *Escherichia coli* strains, is one of the main causes of economic losses in the poultry industry. We infected two medium-growing broiler lines (DP and DM), one fast-growing broiler line (pHU), and three experimental inbred layer lines (6, 15 and N) known for their distinct levels of resistance to *Salmonella* carriage. The lethal doses 50% (LD50) were determined by assessing survival after the infection of one-day-old chicks with *E. coli* strain BEN2908. Colibacillosis was mimicked by injecting the same *E. coli* strain into air sacs of 23 days-old-chickens. Bacteraemia was determined 24h and 48h post-infection (pi), and bacterial load in lung, liver and spleen was determined 48 hours pi. LD50 of BEN2908 was very low in all lines. The line DP was always the less susceptible among the broiler lines, while the line N was the less susceptible among the layer lines. These results indicate that there might be a genetic control of the resistance to colibacillosis and thus that genetic selection could be an alternative to the use of antibiotics.

Variation in the MX gene in commercial egg layer elite lines

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The MX protein has been reported to have anti-viral properties in the chicken. Studies have indicated that a specific variant within exon 13 of this gene (amino acid S631N) influences resistance to avian influenza. Sequencing of genomic DNA from 9 elite egg layer lines identified 29 SNP variants within the coding region and upstream putative promoter region. Focusing on non-synonymous changes only, these SNPs result in a total of 12 haplotypes, yielding 12 different MX proteins. Each elite line contained from 1 to 4 haplotypes, with many of these haplotypes being found in only one line. The putative resistance variant N631 was found in 5 of the 12 haplotypes. Significant changes in haplotype frequency over 14 generations were found in 6 of the 8 lines that were segregating, suggesting some selective advantage for certain haplotypes.

Identification of SNP markers for resistance to coccidiosis in chickens

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Coccidiosis in poultry, caused by the protozoan parasite *Eimeria*, is a serious intestinal disease with a global economic impact estimated at >£2 bn due to production losses and costs associated with prevention (vaccination or drugs) and treatment. Breeding for resistance to coccidiosis provides a complementary approach. An F2 intercross experimental design between two inbred lines, C.B12 (resistant) and 15I (susceptible), with different susceptibility to *Eimeria maxima* was used to map resistance. Phenotype was measured as total oocyst output following oral challenge and 76 birds representing the extreme phenotypes were genotyped using a high density whole genome SNP array (620K, Affymetrix). The genome-wide scan revealed 23 SNP located on chromosomes 1, 2, 3 and 5 with genome-wide significant association with resistance based on the Wald test and a Bonferroni correction. Many chromosome-wide significant SNP were also detected on chromosomes 6, 18 and 23. Some of the significant SNP are located close to annotated genes that are known to impact immune response, lipid transport and metabolism. Results of this study confirmed previously identified loci for coccidiosis resistance and revealed new interesting markers.

Linking phenotypic with genomic diversity in the Synbreed Chicken Diversity Panel

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Within the framework of the SYNBREED project, a large panel of breeds was characterised, comprising more than 2000 individuals from more than 120 breeds and colour variants, covering a large fraction of breeds kept by fancy breeders as well as most diverse geographical origins, wild ancestors and commercial lines. In this Synbreed Chicken Diversity Panel (SCDP) all animals were genotyped with the newly developed Affymetrix chicken 600k Axiom-SNP-array, and a large proportion of animals was phenotyped according to a standard phenotyping protocol. For the present study we used a subset of the SCDP comprising 1810 adult individuals from 116 populations. After quality control (call rate per SNP >99%, call rate per individual >95%, MAF >5%), 311,006 polymorphic SNPs were used. The objective of this study was to use this diverse set to map SNPs, genes and pathways associated with phenotypic variability in the sample by applying appropriate statistical strategies.

We illustrate our approach with a subset of phenotypes representing the trait complex body size. For all animals, the following phenotypic measures were available: WL: mean of wing length left and right in cm; SL: mean of shank length left and right in cm; ST: mean of shank thickness left and right in mm; KL: keel length in cm; LW: live weight of chicken in g. Since all measured phenotypes reflect some aspects of the size of a bird, we derived a combined measure based on a principle component analysis with all five phenotypic traits and used the first principle component which accounts for 88% of the total variation as additional combined phenotype, which was termed PC.

Genome wide association studies (GWAS) were performed for SL, KL, LW and PC. To account for the population structure of the studied sample, a principal component analysis with the genotype data was performed revealing the first 221 principal components as significant ($p<0.01$) in a Tracy-Widom test. These 221 principal components as well as the sex of the birds were included as covariates in all models. We compared two alternative GWAS strategies:

a) a conventional single marker regression (SMR), in which each of the 311,006 SNPs or 141,425 intragenic SNPs, respectively, was tested;
b) a gene-based score test (GBST) based on a list of all known genes for the species *Gallus gallus* from Ensembl Genes (www.ensembl.org, release 72). SNPs were assigned to genes according to the physical transcription start and end positions. In total, 15,068 genes were available with 11,701 genes containing between one and 252 SNPs. In GBST, a single weighted score statistic for each gene combining all SNPs in that gene was calculated and tested following the approach suggested by Pan (2009).

With both tests, a Bonferroni correction was applied to account for multiple testing. Note that with the SMR each single test is by more than an order of magnitude more conservative than in the GBST, due to the difference in the number of tests (311,006 SNPs *vs* 11,701 genes). We also applied the False Discovery Rate (FDR) approach by Benjamini and Hochberg (1995) which controls the proportion of false positive signals among all positive signals and is known to be less conservative than the Bonferroni correction.

Table 1 shows the number of significant genes that were obtained with the variable combination of approaches. In the SMR, both the total number of SNPs and the subset of SNPs located in known genes were used. The gene counts reflect the number of genes that were found significant with at least one of the analysed phenotypes.

Table 1. Number of significant genes detected with different mapping approaches (SMR *vs* GBST), different testing principles (Bonferroni correction *vs* FDR) and different sets of SNPs and genes used.

	Number of SNPs/genes	α<0.05 after Bonferroni-correction	FDR<0.05
SMR	all 311,006 SNPs	2	3
	all 141,425 SNPs in genes	2	12
GBST		7	76

The results show that the GBST unveils a much larger number of genes associated with the analysed genotypes, especially with the less conservative FDR criterion. The advantage of GBST is caused by two mechanisms: (i) it combines all signals within a gene and thus is able to detect a gene in which, say, several SNPs in a SMR are almost significant, while the combined signal exceeds the significance threshold, and (ii) due to the smaller number of genes compared to the number of SNPs, the Bonferroni or FDR correction is less conservative. On the other hand the GBST has the disadvantage that it cannot detect signals in intergenic regions, although it is well known that polymorphisms in regulatory sequences may have a large impact on the functionality of genes. Among the genes found to be significant are CDKAL1 (CDK5 regulatory subunit associated protein 1-like 1, for LW) and UQCC (ubiquinol-cytochrome c reductase complex chaperone), which both can be functionally linked to growth performance in various species.

With the p-values for all genes obtained with the GBST in all four traits (SL, KL, LW and PC), a gene set enrichment analysis was conducted following the idea of Subramanian et al. (2005) which was shown to be efficient compared to other approaches according to results of Hung et al. (2011). This approach is based on a ranked list (ordered by increasing p-value) of all genes from which an enrichment score for a given pathway is calculated. The empirical null distribution of the enrichment score was determined through an extensive permutation of the phenotypes (n=5000 permutations for each of the 141 described KEGG pathways and phenotypes, respectively). For KL, the most significant pathway (p=0.00099) is pathway *gga00040: Pentose and glucuronate interconversions*, which plays a central role in the carbohydrate metabolism where a number of genes at various positions in the pathway (marked in yellow in Figure 1) have contributed to the significance. In general, the majority of identified pathways are linked to the carbohydrate metabolism or to steroid hormone synthesis, both of which have obvious links to growth and body size.

The Synbreed Chicken Diversity Panel represents a valuable resource for the high resolution analysis of phenotypic variability in the entire within species diversity of Gallus gallus. The suggested analytical approach is an efficient way of retrieving relevant genes and pathways in this complex data set.

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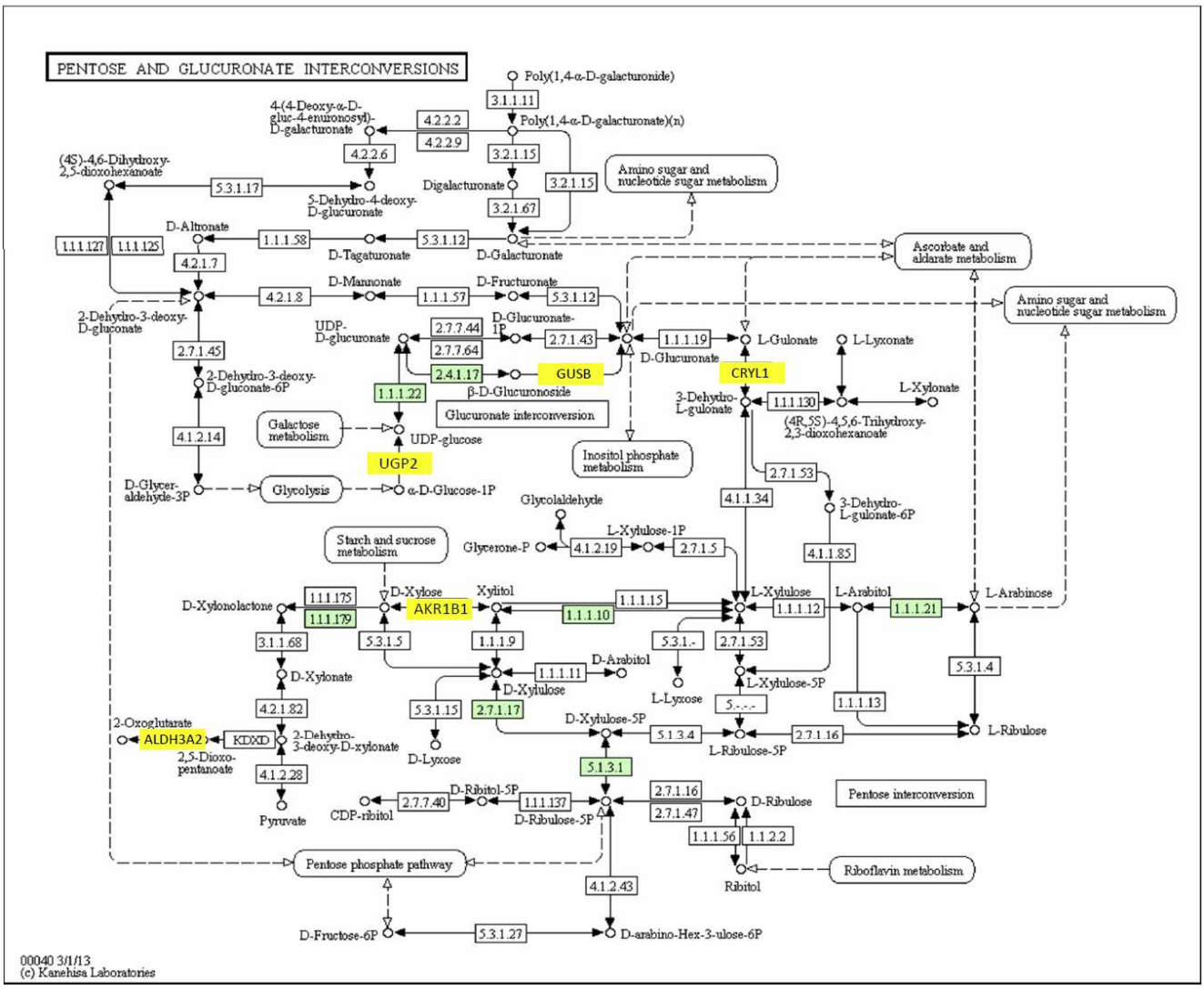
Figure 1. Kegg-pathway *gga00040*: *Pentose and glucuronate interconversions*, the most significant obtained for the trait KL, and the genes (marked in yellow) that contribute most to its significance (Pathway downloaded from http://www.genome.jp/kegg/kegg2.html).



Landscape genomics and multivariate analyses: examples and prospects for poultry

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Multivariate Analysis or, more specifically, Geometric Data Analysis, is a statistical approach that represents multivariate datasets as a cloud of points in n-dimensional space and bases the interpretation of data on these clouds (Le Roux and Rouanet, 2004). It may be traced back to Karl Pearson, whose Principal Components Analysis (Pearson, 1901) is based on a geometric display of data. This geometric modeling allows us to consider a data table as a cloud of individuals (points representing individuals), or as a cloud of variables (points representing variables). This dual approach has been formalized by the so-called duality diagram (Cailliez and Pages, 1976; De la Cruz and Holmes, 2011), which provides a simple way to put many multivariate methods in the same framework.

Landscape genetics may be defined as an approach for describing how geographical and environmental features structure genetic variation at both the population and individual levels, highlighting the relationship existing between spatial genetic structure and the structure of landscapes. In the context of multivariate analysis, this problem may be addressed through specific methods, namely spatial multivariate analyses and redundancy analyses (a.k.a analyses with instrumental variables).

Spatial multivariate analysis

Briefly, while in the principal component analysis (PCA), the optimization criterion only deals with genetic variance (with the eigenvalue decomposition of **X'X**, where **X** is the matrix of allelic frequencies), the spatial PCA (sPCA) aims at finding independent synthetic variables that maximize the product of the genetic variance and the spatial autocorrelation. This is accomplished by the eigenvalue decomposition of the matrix **X'(L+L')X** where **L** synthesizes spatial structure among populations via a neighboring graph connecting the populations on the geographical map to model spatial structure among breeds. The resulting eigenvalues can be either positive or negative reflecting respectively a global or local spatial pattern (Jombart et al., 2008). Calculations were carried out using the *adegenet package* (Jombart, 2008) of the R software (http://www.R-project.org).

Redundancy analysis

Redundancy analysis (RDA), also known as "Principal Components Analysis with instrumental variables" (Rao, 1964) is an analysis that seeks how much of the variation in one set of variables, say **X** (e.g., landscape or climate variables) explains the variation in another set of variables, say **Y** (e.g., genetic data). RDA produces principal components that are constrained to be linear combinations of **X**. This analysis is appropriate when the number of variables in **X** is lower than the number of variables in **Y**. RDA permits variance partitioning to measure the variance explained by different sets of instrumental variables and then to sequentially test the significance of variables by an ANOVA-like permutation test (Liu, 1997; Legendre and Legendre, 2012). Calculations were carried out using the R packages *vegan* (Oksanen et al., 2013) and *ade4* (Chessel et al., 2004).

Data

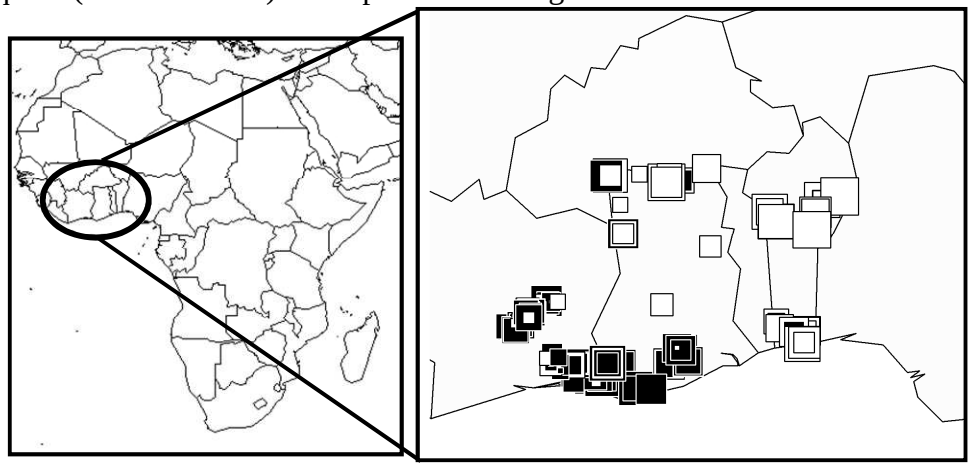
Features of these methods are illustrated through the analysis of published data (Leroy et al., 2012) consisting of 317 local African chickens sampled in an area including Ghana, Benin and the Ivory Coast, and genotyped with a set of 22 microsatellites. Geographic coordinates for each animal

were recorded, and climatic data (elevation, temperatures and rainfall) were obtained from the FAOCLIM database.

Results

The information contained inside a sPCA object can be displayed in several ways, but a frequent practice in spatial genetics is mapping the first principal components (PCs) onto the geographic space because it offers an interesting visual result of multivariate analyses. The sPCA for the African chickens is summarized in Figure 1 where individual scores are plotted on the geographical map of origin. Individuals are represented by squares. The areas of the squares are proportional to the absolute value of the score. The color of the square (black or white) corresponds to the sign of the score. Figure 1 shows the existence of a clear genetic structure opposing animals from the south-west to animals from the north-east regions. No clear geographical physical barriers separate the two areas, but they differ climatically.

Figure 1. Projection of the individual scores of the first spatial principal component onto the geographical map. The areas of the squares are proportional to the absolute value of the score. The color of the square (black or white) corresponds to the sign of the score.

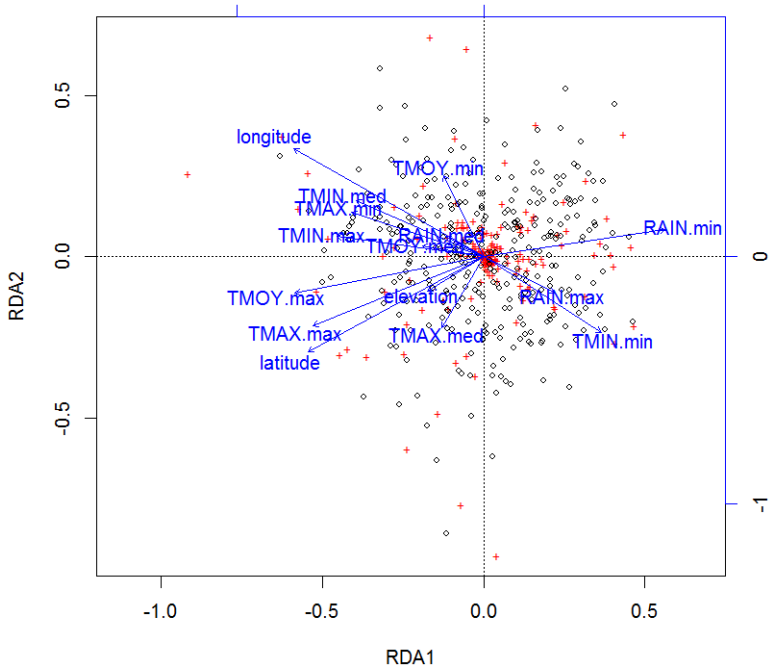


To quantify how much of the genetic variation is explained by geography and/or climatic conditions we used the RDA approach. The results are summarized in the biplot of Figure 2 that represents the correlation of each geographical or climatic variable with the two first components. Particularly, the first component (RDA1) is associated to geographical coordinates, longitude and latitude, and to a rainfall variable *RAIN.min* (minimum monthly rainfall over a year). ANOVA-like tests indicate that the effects of the geographical and climatic variables on the genetic variation are significant ($p<0.01$). Climate conditions, independent of the geography, account for 33% of the constrained total variance, while the geography, independently of the climate, accounts for 13% of it. The rest is due to a combined effect of the two.

Perspectives

The landscape genomic multivariate analysis is certainly a very promising approach for understanding the geographic effect in shaping population genetic structure. The field of landscape genomics has been largely employed in conservation and ecological studies. On the contrary, there have only been a few studies involving domesticated species (Laloë et al., 2010; Gautier et al., 2010) but the growing amount of genomic data available for domesticated animals makes this approach particularly suitable to understand the evolutionary processes and adaptive events that have shaped the genetic diversity of domesticated animals.

Figure 2. Redundancy analysis: biplot corresponding to the first two components. Points are the projection of individuals onto the components; blue arrows represent the correlations of geographical and climatic variables with the components. Climatic data gathered in form of twelve monthly averages of precipitation (RAIN), minimal temperature (TMIN), mean temperature (TMOY), maximal temperature (TMAX) were used to obtain the maximum (.max), the median (.med) and the minimum (.min) values then used in the analysis. For example, TMIN.max is the maximal value among the monthly minimal temperatures (i.e., the minimal temperature of the warmest month).



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The importance of poultry biodiversity on rural poultry production

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FAO and other International agencies have pointed to the extinction of many domestic breeds in the last years. In Italy there are numerous chicken breeds and the current situation is critical because about 60% of the Italian chicken breeds are extinct and most of the others are endangered. This negative trend is more relevant in poultry than in other species due to the industrial chicken breeding that employs only few chicken strains. Intensive poultry meat production uses few lines selected on the basis of productive performance (fast-growing) resulting in a loss of genetic diversity. As a consequence, many local breeds are at the risk of being lost and their preservation largely relies on hobby farmers. These local breeds may, however, represent a resource of genes for future breeding strategies and research purposes. Many traits of economic importance, that are waiting to be promoted, are disappearing every day. Different *in situ* and *ex situ* conservation programmes have been planned in Europe; but, one of the more effective strategies for the preservation of biodiversity consists of including such local strains, as purebred or cross, in the commercial chain of production. Fortunately, the expectation of European consumers evolved toward a demand of local and traditional products more attentive to the environmental impact and to the animal welfare widening the opportunity of rural poultry production.

According to the resource allocation theory, animals less selected for productive traits have more resources available for immune response and kinetic activity. Therefore, the lower productive performance of local strains (slow-growing), is generally associated with resistance to disease, higher capability of food foraging and different meat traits. National and international organizations promote the use of slow-growing strains for rural poultry farming (organic, free range, traditional products) on the basis of their better adaptation to natural environments and disease resistance. There is also clear evidence of the effect of bird behaviour on animal welfare and metabolism, notably in experiments conducted using GPS technology to measure the distances travelled by birds in outdoor runs. The high kinetic and foraging behaviour of slow-growing chickens determines the ingestion of large quantities of grass, insects and worms which in turn increases many bioactive substances such as fatty acids, carotenoids, tocopherols, and polyfenols in the eggs and meat. Pilot studies suggest a differential capability of slow-growing breeds to metabolise and store in their tissues essential fatty acids (C18:3n-3, C20:5n-3 and C22.6n-3) and vitamins and require further research.

Structural aspects of genomes across species

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Our knowledge of poultry and other avian genomes has increased dramatically over the past 10 years. In 2004 we reported the sequence of the chicken genome (Hillier et al., 2004). The current (Galgal4) assembly covers 1.03 GB (96% of genome) assigned to chromosomes GGA1-28, 32, two linkage groups and the sex chromosomes Z and W. The task is not complete however with GGA16 and W poorly covered and contigs have not been assigned to the smallest microchromosomes GGA29-30, 33-38. Plans to complete the chicken genome will be discussed further.

Recent advances in next generation sequencing (NGS) now make it possible to produce draft sequences of any vertebrate genome, quickly and cheaply. Recently, NGS has been used to sequence 42 avian genomes, as part of the Genome 10K project (Genome 10K Consortium, 2009). Together with other projects including Zebra finch (Warren et al., 2010), turkey (Dalloul et al., 2010) and Mallard duck (Huang et al., 2013) there are now 56 avian genomes sequenced. These provide new opportunities for comparisons within species (sequence variation and genetic selection) and between species (sequence divergence and genome evolution).

These new sequencing technologies have revolutionized genetic studies within populations by allowing rapid sequencing of many individuals for detection of genetic variants. For example, to apply Genome Selection to broilers and layers, we developed a 600K SNP Axiom Affymetrix genotyping array (Kranis et al., 2013). This chip is based on SNPs discovered from the genome sequences of 24 chicken lines, including broilers and layers, and experimental and traditional lines. Genomic DNA pools of 10 individuals per line were sequenced to a depth of about 15-fold coverage. Sequence reads were aligned to Galgal4 using BWA (Li and Durbin, 2009) and SNPs called using SAMtools (Li et al., 2009) with mapping quality score >20, base quality score >20, SNP quality scores >40 and read depths >5. Using these criteria we defined almost 15 million high quality SNPs with an estimated false positive rate of 9% and false negative rate of 8%, based on random fragments sequenced by the traditional Sanger method. The functional effect of these SNPs, if any, has been explored further, such as their potential effects on protein coding sequences and other genome characteristics (gene location, selective constraints, coding and non-coding genes, etc.).

In these studies the annotation of the chicken genome is critical and has also been under continuous improvement. The latest revision (Ensembl 72) has taken advantage of huge volumes of transcriptome data generated by the Chicken RNA Sequencing Consortium, in addition to the conventional comparative approaches (Flicek et al., 2011). These analyses also benefit greatly from the comparison of the genomes, genes and proteins predicted from the draft sequences of the many avian genomes within the Avian Phylogenetics Project in collaboration with our partners at BGI and around the world. There are plans within the consortium to sequence the genomes of all 40 orders, 231 families, 2268 genera and 10,476 species of birds. These developments provide many new opportunities, but also raise a number of challenges for the future.

- How will we assemble and annotate so many genomes?
- Do we need a new data access model?
- Will we continue with model species databases (chicken, zebra finch…).
- What about ancestral species databases, one per order?
- What will we do for other species; only provide access to genome sequences, gene annotations and tools?
- We need to create a new model of parallel annotation for 100's species.

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Genomics in minor species: the example of duck

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Introduction

Duck is the common name for a number of species divided between several subfamilies in the Anatidae family of Anseriforms. Most domestic ducks raised worldwide as an agricultural resource for meat, eggs and down, are of green-headed wild mallard *Anas platyrhynchos* descent and are known as common ducks. The most usual breed used is the Pekin duck. The other duck species used is the Muscovy duck *Cairina moschata*. The mallard is believed to have been domesticated 4000 years ago in China, whereas the Muskovy was domesticated by South American Indians long before Europeans arrived on the continent (Brun et al., 2005).

The Muskovy duck is raised because of its ability for producing lean meat, but lays fewer eggs than the common duck. To overcome this, common duck females are crossed with Muskovy drakes to produce sterile offspring known as mule ducks, taking advantage of the characteristics of both species. Production of mule ducks has taken place in Taïwan for more than 200 years and is now commonplace in France for the production of foie-gras. Although natural mating of Muskovy drakes with common duck dams is possible, the fertility rate is usually very low and the production of mule ducks on a large scale requires artificial insemination. The phylogenetic distance between the two species is estimated to be between 13.5 and 20.2 million years (Gonzalez et al., 2009).

Thus, although both the common duck and the Muskovy duck species are reared as farm animals, the common duck *Anas platyrhynchos* is by far the most widely used. Moreover, duck is also studied for its role as a natural reservoir of all influenza A viruses, as it can carry the infection with no sign of disease and can thus potentially propagate the virus to other bird species and potentially also to mammals. There again, the Mallard, as a well known migratory species, has been widely used to study the molecular genetics (Magor, 2011) and population genetics (Kraus et al., 2013) aspects of the problem.

Genome characteristics and sequence assembly

The duck genome presents most of the characteristics encountered in birds, which are: (i) a more compact genome, one third the size of a typical mammalian one, (ii) a large number of chromosomes (2n=80), (iii) the presence of macrochromosomes and microchromosomes, the latter being as small as a few Mb, and (iv) the females are the heterogametic sex (ZW) and males the homogametic one (ZZ). The main difference between the duck and chicken karyotypes are the short arm of chicken chromosome 4 corresponding to one extra microchromosome in duck, with GGA4 being equivalent to APL4 and APL10.

Genomics is a discipline of biology working at the scale of genomes, whose entire sequencing is therefore considered as a peak for the study of a given species. The sequence of the duck genome was very recently published (Huang et al., 2013). Briefly, the sequence assembly is 1105 Mb long, in the form of >78,000 scaffolds, the largest of which is 5.9 Mb in size, whereas the estimated size of the largest chromosome is around 200 Mb. The scaffolds N50 is 1.2 Mb, meaning that 50% of the genome is covered by scaffolds of size 1.2 Mb or greater.

Building the sequence into chromosomes by mapping

The first generation genetic (Huang et al., 2006) and cytogenetic (Skinner et al., 2009) maps were used to assign 225 scaffolds, covering a total of 289 Mb to chromosomes. Thus, this first effort does not yet allow for chromosome-wide analyses of events such as evolutionary breakpoints between species or for choosing genetic markers on the basis of position in the genome.

The two genetic maps actually published contain 115 and 91 microsatellite markers in 19 and 16 linkage groups (Huang et al., 2006; Kileh-Wais et al., 2013), and are therefore far from covering the 40 chromosome pairs of the genome. These maps cover 1353 and 778 cM, whereas the chicken genetic map is 3228 cM long (Groenen et al., 2009), for a genome of very similar structure. A new generation genetic map including several hundred SNP chosen along the genome, based on the alignment of the duck scaffolds on the chicken genome and previous knowledge of chicken-duck synteny conservation, is under construction. BAC (Yuan et al., 2006) and fosmid (Moon and Magor, 2004) libraries have been constructed, but no physical map has been produced using them yet.

Another approach undertaken for assembling the duck scaffolds into chromosomes is RH mapping. A duck RH panel was built and its utility for ordering scaffolds and building maps was demonstrated for a first set of two chromosomes (Rao et al., 2012).

Structural and functional annotation

Once the raw sequence has been obtained and assembled into scaffolds, biological meaning must be drawn out of it. This implies detecting gene structures and as much as possible on function. One major source of data for annotation is the sequencing of transcripts from various tissues, whose sequence can be aligned to the assembly for gene detection. Brain, muscle, liver, lung, spleen, intestine were sequenced, in some cases from influenza-infected birds. Other source of data for annotation include published duck transcripts, protein sequence data from other bird, mammals and other vertebrate species and bioinformatics gene predictions. According to the annotation pipeline used, 19,144 or 15,634 genes were detected. Sequence similarity search in databases was used to identify gene function. Seventy-five percent of the 19,144 predicted genes had orthologs in the KEGG or Uniprot databases and 50 could be mapped to Gene Ontology categories.

Inter-individual variability, genetic maps and QTL analyses

The first two published genetic maps were entirely composed of a limited number of microsatellite markers, respectively 115 and 91, allowing QTL detection on an estimated half of the genome. One map was used to detect body weight, conformation and meat quality traits in Pekin duck (Huang et al., 2007a,b), whereas the other was used to detect QTL segregating also in the common duck, but influencing metabolism, meat quality and liver quality traits in their overfed interspecific mule duck offspring (Kileh-Wais et al., 2013). The genetic maps used clearly need completing and new markers must be developed to this end. As a first source, 150,000 SNP were detected by re-sequencing Mallard ducks (Kraus et al., 2011). However, only a small subset of 384 markers could be tested for informativity in the mapping populations and only a few of these were informative. As a first option towards the improvement of the genetic maps and QTL detection, a new set of several hundred SNP markers will be developed specifically by sequencing F1 individuals.

However, a more satisfactory approach would be to design a high density SNP chip, containing at least several thousands of SNP. This requires a consortium of participants interested, to allow for reasonable pricing of the SNP chip through its marketing in sufficiently large quantities.

Functional genomics

Until recently, gene expression level analyses in duck could only be done on a gene-per-gene basis by qRT-PCR, as for instance in Herault et al. (2008 and 2010). This was done by designing assays based on sequence data from other species, usually chicken, with the risk of failure due to sequence divergence with duck. Primer design can now be based directly on the genome sequence, allowing for higher success rate. But, with the genome sequence at disposal, functional genomics can take a new turn in duck, with the possibility of building comprehensive gene expression chips or by using RNA-Seq and the genome assembly as a reference for sequence alignments. The choice between the two approaches will be dependent on the experimental design and the scientific question. The expression chips will be better suited in the case of experiments involving large sample numbers,

whereas the RNA-Seq approach will allow for the discovery of new gene and/or splicing variants. This latter approach will thus aid in the continuous process of annotating the genome.

The proteomics approach has also been used in duck by two-dimensional gel electrophoresis. However, the identification success by mass spectrometry of the spots of interest was low, probably in part for lack of duck sequences in the databases (Theron et al., 2013).

Specific questions related to the interspecific mule duck

The mule duck is an inter-specific cross between a female common duck and a Muskovy drake and the Hinny duck is a result of the reverse inter-specific cross: a female Muskovy crossed with a common duck drake. These two hybrids are sterile, but show specific phenotypic traits; some of which are of interest to agriculture. They represent an interesting model to study the interaction between two diverging genomes in birds, with all questions related to heterosis. The sequencing of the Muskovy duck would allow performing RNA-Seq analyses in the hybrids, thus allowing the analysis of orthologous gene expression levels and interactions between the two genomes. The mule and Hinny ducks are also a good model to study mechanisms related to sterility and speciation in birds.

Conclusion

Less than 10 years ago, having a vertebrate genome sequenced was quite an event and the chicken genome, published in 2004, was amongst the very first just after human, mouse, rat, fugu and tetraodon. It was also the first bird genome sequenced. Since then, with the advent of parallel sequencing, or NGS (Next Generation Sequencing), costs have dropped and throughput risen greatly. Thus, species of minor importance can now benefit from what used to be the exception. Concerning birds of importance to agriculture, chicken, turkey and common duck are now sequenced. Other species, such as guinea fowl or quail will follow shortly. One major challenge to face will be to deal with the data and experiments in the post-genomic era: transcriptomics, proteomics and metabolomics.

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Phenotyping for layers using different systems

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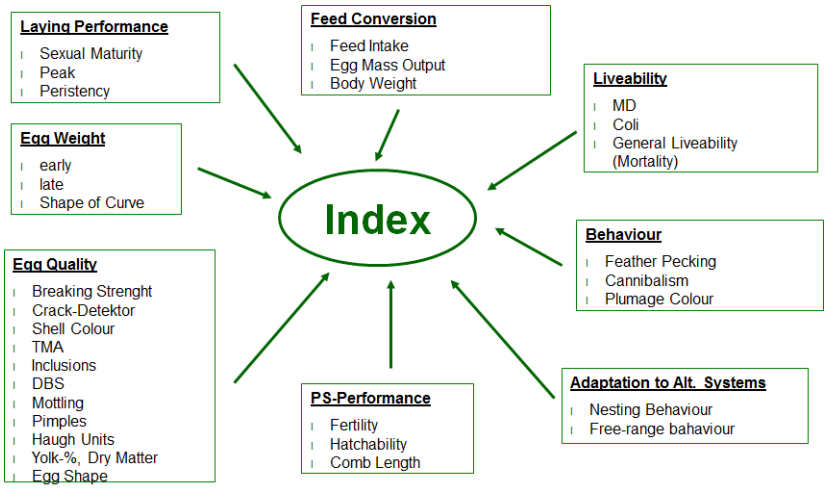
For further genetic improvement in relevant egg production traits, it is important to consider changes in the global structure of the egg industry. In accordance to Besbes et al. (2002), the big challenge for breeding companies is to provide commercial layers that are able to express their full genetic potential under a large variety of field conditions such as high density cages, deep litter and free-range systems. Due to these varying field conditions, the testing environment of pure lines should also be adapted to minimise the negative effect of genotype-environment interactions and maximise genetic progress. Therefore, breeding companies have extended their laying hen testing stations to non-cage layer systems.

The controlled housing in single bird and group cages has been considered as the most favourable environment for testing birds and lines which are dedicated for cage production units. Performance testing in single bird cages provides exact hen specific data of the most important selection traits (Figure 1). Thus, daily egg numbers, feed intake and egg quality characteristics are captured with justifiable efforts and in sufficient amounts for very reliable genetic analyses. Group cages, housed with full and half sib hens, enable phenotypic data recording for general behaviour and the hens' plumage condition as well as their extent of production in one family. Other behaviour related traits such as nest acceptance, which is for welfare and for economic reasons of big interest, cannot be captured in cage systems. Therefore, hens have to be tested under floor housing conditions and preferably individually. For realisation purposes, a special nest, the Weihenstephan Funnel Nest Box (FNB) was developed. Furthermore, a second recording method, the Electronic Pop Hole (EPH) was created to analyse the ranging behaviour.

Figure 1. Selection traits for Lohmann Layers.



The main breeding target for layers is still to maximise the number of saleable eggs that represents a high proportion of the economic efficiency of the egg producer in many markets. In alternative housing systems, this overall target has to be adjusted according to nest acceptance. This goal is therefore adapted to the number of saleable nest eggs which is already recorded in the FNB when capturing the nest acceptance data. The simultaneous recording of two important traits, nest acceptance and egg number, is of additional benefit to the selection process. However, a larger number

of selection criteria may reduce the power of each single criterion in the selection index, especially if the traits are negatively genetically correlated.

In terms of potential genotype x environment interactions, full sibs in single bird cages are tested at the same time as their siblings in the FNB. Estimated genetic correlations between the data of sisters in single bird cages and nest boxes, displayed a moderately close genetic correlation for egg number at the beginning of lay. The genetic correlations for the average egg number in the later production period are lower. The low correlations for egg number during the main laying period as well as the important trait of nest acceptance, enhance the importance of the FNB as a performance testing method in future layer breeding.

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Near infrared spectroscopy: an innovative phenotyping technique

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Poultry products are often considered by consumers as an alternative to red meat because of their healthy characteristics. This perception, combined with low price, has been responsible for significant increase of poultry consumption in the last 30 years. Contextually, consumers have become increasingly demanding in terms of meat quality, which is mainly influenced by appearance, palatability, nutritional value, and safety. Nevertheless, analytical techniques for the assessment of most physical, sensory and chemical traits are destructive, costly and unsuitable for on-line or at-line applications. Near infrared spectroscopy (NIRS) can overcome these limits as it is a fast, easy to use, non-invasive and cost-effective technique. All these aspects make this tool useful for routine application in field conditions to collect phenotypes at population level and to use this information for labeling and genetic improvement. Although NIRS has demonstrated its potential to accurately predict some physical, sensory, and chemical traits, and groups of fatty acid composition related to chicken meat, it has often shown limits in predicting accurately some minor components of the meat (e.g., single fatty acids). Major efforts should be made to improve the robustness and accuracy of prediction equations and to find practical solutions for the poultry industry. In the near future, many challenges for a modern meat processing could be accomplished using NIRS:

- at-line and on-line prediction of poultry meat directly on the carcass surface, by implementing NIRS in the abattoir;
- assessment of meat for secure high quality, which is essential for gaining market share in the competitive retail market;
- determine new quality phenotypes to improve food information to consumers.

Genetic parameters using phenomics

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Until recently the concept of phenomics was relatively unknown in animal breeding as it originates from evolutionary biology. It can be defined as the acquisition of high-dimensional phenotypic data on an organism-wide scale. Associated to this is often the concept of high-throughput data acquisition. Phenomics is also a formalized reply to new challenges as putting phenotypes and genotypes on the same level of detailed knowledge, identifying the genetic basis of complex traits, trying to add a causal explanation of the observed phenotypes and getting hold of pleiotropic effects. With always more complex breeding goals trying to address economic but also social and ethical challenges of the future, selection indexes need to be developed having the complex interrelationships between these traits in mind. There are essentially three challenges associated with phenomics: comprehensive trait ontology, trait measurements and data analysis including estimation of genetic parameters. Two of the most important issues are in this context the high-dimensionality and the repeated, potentially longitudinal nature of the phenotypes. Advanced methods will be presented to estimate parameters reducing dimensionality taking into account the longitudinal nature. This type of data already exists at least partially in poultry when considering animal growth and egg production curves. Phenomics in dairy cattle is, with linear description of type and recent advances in detailed milk phenotypes, rather advanced. In the near future multiple sensors are becoming available generating on farm high-throughput data.

The consequence of selection on social genetic effects for survival in laying hens

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Negative social interactions between individuals living in a group can harm welfare, productivity, and health of the individuals in that group. In domestic laying hens, social interactions can result in feather pecking, which can cause mortality due to cannibalism. Mortality due to cannibalism is a worldwide economic, health and welfare problem, occurring in all types of commercial poultry housing systems (Blokhuis and Wiepkema, 1998). Several studies have shown that social interactions can contribute to the genetic variation in a trait (Moore, 1990; Muir, 2005; Bijma et al., 2007). In cannibalistic laying hens, social interactions contribute 33 to 87% of the total genetic variation in survival time (Ellen et al., 2008; Peeters et al., 2012). Genetic improvement of socially affected traits, such as mortality due to cannibalism, however, requires adapting selection strategies. Selection methods currently used in animal breeding, such as individual selection, neglect the effect of an individual on the trait values of its group mates. Using these traditional selection methods can result in selection responses opposite to expectation (Craig and Muir, 1996). There is an urgent need to reduce feather pecking and to improve survival in laying hens, because beak-treatments, currently used to prevent or diminish the effect of feather pecking, is or will be prohibited in several (North-West) European countries (Van Horne and Achterbosch, 2008). Further, since conventional cages are prohibited in the EU since 2012, laying hens are now kept in much larger groups in furnished cages or non-cage systems, resulting in an increased risk of feather pecking and cannibalism (Rodenburg et al., 2011).

Improving socially-affected traits requires a selection method that takes into account both the direct genetic effect of an individual on its own phenotype and the social genetic effect of an individual on the phenotype of its group members. Muir (1996) showed that group selection can be used to improve survival in laying hens. Unfortunately, group selection can only be used when selection candidates (SC) are housed in groups. In the poultry breeding industry, SC are housed individually to record individual egg production traits. Therefore, a selection method is needed that takes into account social interactions between group members, while keeping SC individually. Ellen et al. (2007) proposed a selection method where SC are housed individually and selected based on survival of sibs and/or offspring kept in family groups. Here, we present the results of a selection experiment in laying hens where we applied this selection method. We hypothesize that selection for socially-affected traits, using a selection method that targets both the direct and social effect, will improve survival of animals kept in groups.

In 2004, a selection experiment was started to select against mortality due to cannibalism in laying hens, using selection based on relatives kept in family groups (Ellen et al., 2007). For this selection experiment, a purebred White Leghorn layer line from Institut de Sélection Animale B.V. (ISA), a Hendrix Genetics company, was used. Individually housed SC were selected based on the survival time of their sibs and/or offspring kept in family groups. Sibs and/or offspring of the SC were kept in 4 or 5 bird cages (depending on the generation) and had intact beaks. Keeping sibs in family groups guarantees that both direct and social effects are captured in the selection index, even when social effects are ignored in the breeding value estimation (because genetic parameters are unknown). In total, six generations of laying hens were selected according to this method. A description of the selection method for the first four generations is given in Ellen et al. (2010). In the first generation, SC were selected in two directions, high survival and low survival, and also a control group was present (Table 1). The control group were the remainder SC, with an average breeding value (BV). In

generation 2 through 4, SC were selected only for high survival. Furthermore, in generation 2 through 4 there was not a true control line available, though the founding purebred line of ISA was kept in the same facilities. Ancestors of this ISA line had been used as base generation of the selection line. Therefore, the selection line and the ISA line originated from the same ancestors. In generation 1 through 4 high BV sires where randomly mated with high BV dams. Due to small expected responses in generation 2 through 4 (Ellen et al., 2010), it was decided to make some changes in the selection program for generation 5 and 6. First, SC were again selected in two directions, high survival and low survival, and also a control group and the ISA line were present (like generation 1 except for the ISA line, which was not present in generation 1). Second, a two-step procedure was used, SC were selected at two time points. The first selection step took place when the hens, used for collecting survival data, were 56 weeks of age. Based on the survival data, BV were estimated for the SC and SC were selected. For the high survival line, twice as many SC were selected than needed. The second selection step took place when the hens, used for collecting survival data, were at the end of the laying period. Again BV were estimated for the SC. Based on the second step, it was decided if offspring of the SC were used for the high survival line, low survival line or control. Third, positive assortative mating was used to create more extreme offspring. Highest BV sires were mated with highest BV dams for the high survival line and the opposite for the low survival line. In all generations, mating of sibs was avoided.

Table 1. Number of birds per generation and per direction of selection.

Generation	Location[1]	Group size	High survival	Control	Low survival	ISA
1	NL-S	4	422	408	382	-
2	NL-S	4	61[2]	-	-	385[2]
3	NL-J	4	537	-	-	579
4	NL-J	4	592	-	-	584
	CA-O	5/10	965	-	-	924
5	CA-O	5/10	754	805	339	400
6	CA-O	4/8	703	711	317	320

[1]NL = The Netherlands; CA = Canada; -S, -J and -O = farm name.
[2]Only hens from the same hatch were used.

In each generation, mortality was recorded to calculate the survival time of each hen and the survival (percentage of laying hens survived at the end of the laying period). Furthermore, in each generation plumage condition was recorded at 40 weeks of age and egg production was recorded during the entire laying period. In generations 2 and 6, a novel object test and a manual restraint test were applied to measure the fearfulness and stress sensitivity of the hens. Here, we will present the survival of the different generations and the directions of selection with a group size of 4 or 5 birds per cage (Table 2).

Table 2. Survival (%)[1] per generation and per direction of selection.

Generation	Group size	High survival	Control	Low survival	ISA
1-NL-S	4	78±2.1	70±2.3	60±2.4	-
2-NL-S	4	71±6.4	-	-	72±2.5
3-NL-J	4	82±1.9	-	-	80±1.8
4-NL-J	4	90±1.3	-	-	91±1.3
4-CA-O	5	69±2.2	-	-	70±2.4
5-CA-O[2]	5	75±2.7	78±2.5	64±2.8	68±2.6
6-CA-O[3]	4	96±1.8	91±1.8	88±1.8	91±1.8

[1]Survival = percentage of laying hens still alive at the end of the laying period (~76 weeks of age).
[2]Survival was collected until 74 weeks of age.
[3]Survival was collected until 48 weeks of age, this generation is still on-going.

In the first generation, the high survival line yielded a significantly higher survival than the control and the low survival line ($p<0.02$). In generation 2 through 4, compared to the ISA line, there was no significant difference in survival between the high survival line and the ISA line ($p>0.40$). In generation 5, the high survival line yielded a clearly higher survival than the low survival line and the ISA line, whereas there was no significant difference in survival between the high survival line and the control ($p=0.39$). In generation 6, until 48 weeks of age, the high survival line yielded the highest survival. The low survival line yielded the lowest survival, whereas the control line and ISA line were in between. Especially results of generation 1, 5 and 6 (until 48 weeks of age) suggest that selection for improved survival using selection based on relatives kept in family groups is possible.

Comparing the survival between the different generations is difficult, because during the selection experiment locations of the barns, used for the hens kept in groups, changed. Generation 1 and 2, generation 3 and 4, and generation 4, 5, and 6 were kept in three different locations (Table 1). The three locations differed in environmental conditions, such as light intensity, group size, and diet type. In location NL-J, light intensity was lower than in the locations NL-S and CA-O. In the locations NL-S and NL-J, hens were kept with four hens in a cage, whereas in location CA-O hens were kept with five hens in a cage (except for generation 6). Furthermore, the diet type differs between The Netherlands and Canada. These environmental conditions can have an effect on the survival. When comparing the survival of the different generations within a location, it was found that average survival increased for generation 3 and 4 (the Netherlands), and for generation 4 and 5 (Canada; Table 2). When comparing generation 1 and 2, there was a decrease in average survival. In generation 2, high survival hens were hatched in different batches. Hatching in different batches had a large impact on survival. Furthermore, dams used to breed the high survival line were much older than dams used to breed the ISA line and dams used to breed generation 1 (Ellen, 2009). This makes it difficult to draw conclusions from comparing generation 1 and 2. Comparing the survival between generation 3 and 4 in the Netherlands, and between 4, 5, and 6 in Canada suggests that using selection based on relatives kept in family groups indeed results in an increase in survival over generations.

Besides comparing survival, hens within generation 2 and 6 were also compared using behavioural tests. Here, we will summarize the results of generation 2. In generation 2, it was found that hens selected for increased survival showed less fear-related behaviour than hens from the ISA line (Bolhuis et al., 2009). This was confirmed both in young (before cannibalism develops) and in adult birds using sibs of generation 4 (Rodenburg et al., 2009a,b; Nordquist et al., 2011; de Haas et al., 2012). Furthermore, hens of generation 2 selected for increased survival had higher whole-blood serotonin concentrations and a low platelet serotonin uptake, indicating differences in functional activity of the serotonergic system (Bolhuis et al., 2009). Again, these results were confirmed in sibs of generation 4. Furthermore, sibs of generation 4 showed a reduced stress response to manual restraint (Rodenburg et al., 2009a). Based on generation 2 and sibs of generation 4, it can be concluded that selection for increased survival, using selection based on relatives kept in family groups, reduces the fearfulness of the laying hens. However, results of the behavioural tests in generation 6 are needed to confirm this. Furthermore, it would be good to investigate the consequences of selection on social genetics for egg performance and plumage condition.

Based on the results of the selection experiment in laying hens, it can be concluded that applying a selection method that targets both the direct and social effect may be a key factor in improving animal welfare in laying hens kept in small groups. In the future, it will be important to investigate if the selection method has also beneficial effects on the survival of birds kept in large groups.

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A European perspective on meat poultry breeding

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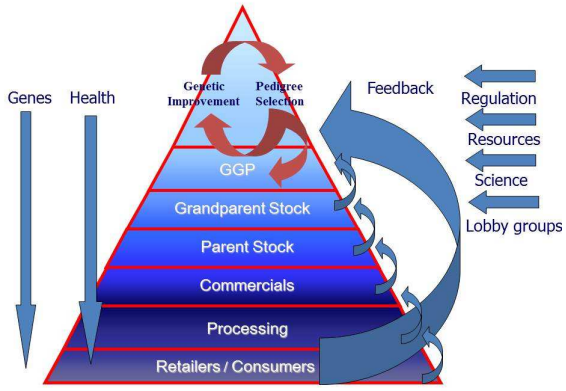
At the European level, various rules and regulations apply and will continue to apply to meat poultry production influencing meat poultry breeding. Poultry breeding companies prepare for the future at least a decade in advance, taking into account customer and society requirements, regulatory developments, demand for resources, and science (Figure 1). It takes approximately six to ten years to develop a novel crossbred product.

Figure 1. Breeding pyramid of meat poultry breeding, feed back mechanisms and travel of genes and health.



The European playing field

Welfare

Welfare is high on the legislative and public opinion agenda. The welfare of poultry for meat production can be described as the bird performing well, in good health, under good conditions and in a way that the animal is able to do easily what it is good at: efficient growth, and excellent reproduction and liveability. It centres around the homeostatic balance of animals between their intrinsic potential and the production environment, which includes nutrition, housing, health, the social environment, and stockmanship (Neeteson et al., 2013b). These are key to achieve animal production under responsible welfare conditions. Managing animals in such a way that all these conditions are met, will lead to good animal welfare.

The public debate

Over time fewer rural people will be producing more food for many more urban people (Neeteson et al., 2013b). This puts animal production more at a distance to citizens. At the same time citizens want to know whether their food is produced responsibly, and as consumers, they wish to be *heard*. However, the animals they are familiar with are pets, horses, or the animals at the city children's farm. This leads to an increasingly anthropomorphic perception of animals, with emotions and a high 'cuddle factor'.

The ethical and societal values are the *welfare perception* part of the debate on animal welfare. Ideally, there should be a 100% match between the welfare of the animal, and the perception of welfare by the consumer and the citizen. That will serve the welfare of the animal *itself* best. In contrast, the agriculture industry has been historically poor at conveying a positive case for poultry production and its welfare (Neeteson et al., 2013a). However, we see more and more proof of breeding companies

and/or their associations engaging in dialogue and improving on transparency. As part of this process, breeding companies publish more often in peer reviewed journals (e.g., Fleming et al., 2007; Ask, 2010; Katanbaf and Hardiman, 2010; Siegel et al., 2011; Tolkamp et al., 2011; Kapell et al., 2012a,b). Recently, the German poultry association has involved German stakeholders to update turkey welfare guidelines, including the turkey sector, science, administrations and welfare organisations (ZDG, 2013). The European poultry association a.v.e.c. has adopted turkey welfare guidelines at the European level in 2012 (a.v.e.c., 2012). Such co-operations are important, and in line with the recommendation of the Welfare Quality Advisory Committee for a holistic approach towards welfare assessments (Welfare Quality AC, 2007).

Sustainability

In order to show in which way breeding organisations contribute to sustainable breeding, the animal breeding sector has developed, in cooperation with a wide range of societal stakeholders, a Code of Good Practice, Code-EFABAR® (EFFAB, 2003). Sustainability is then defined via the areas where breeding companies can make a difference: food quality and safety, efficiency, environment, health and welfare, and genetic diversity. In addition, to account for the other corporate responsibility – on top of the transparency and dialogue - poultry breeding companies often engage in the development of the latest appropriate management tools and information for the relevant breeding stock. This is important, as welfare organisations have also indicated that they expect that breeding organisations do not develop breeding stock that can perform well under very bad conditions (personal communication, SEFABAR project 2000-2003). Thus, the balance needs to be struck to develop robust healthy animals for appropriate management and farming conditions. This also means that appropriate management and management skills are recognised as integrative elements of respectful farming. At the same time, the farmer needs to be able to transfer his farm to the next generation, i.e., being able to sustain in an economically feasible way, and the environmental impact of poultry production needs to be sound and - in order to answer global demand - it should be improving as time develops. Breeding organisations need to provide the breeding stock that enables this long-term process.

The requirements of a breeding company

Healthy breeding stock

In order to be able to supply the European farmers with meat poultry breeding stock in a sustainable way, breeding companies spend much care on the health status of their breeding stock. The aim of breeding companies is to continue to deliver breeding stock free of Salmonella, Leucosis, Mycoplasma and various other diseases and to contribute to decreasing use of prophylactic antibiotics, not only at the selection level, which is already antibiotics free, but increasingly also at commercial level (Hiemstra and ten Napel, 2013).

Genetic diversity

Then, the basis of any breeding programme is its genetic diversity, this is the major asset of any breeding company. Breeding companies maintain lines with various characteristics, and sometimes control lines, to ensure they can supply any foreseeable future and keep rate of inbreeding below 1% per annum (Hiemstra and ten Napel, 2013). Muir et al. (2008) indicated that the modern farming system has contributed less than 5% to the level of inbreeding of 14-15%, despite intense levels of selection, closed populations and industry consolidation since 1950, indicating that the breeding companies maintain their genetic resources in a sustainable manner.

In Europe, the interest for speciality products, e.g., specific taste, growth rate or colour of the bird or the meat, may be of interest for certain market segments, especially in affluent countries or areas with specific eating cultures and related product(ion) specifications. Meat poultry breeding companies offer products to satisfy these markets.

Sustainable balanced breeding

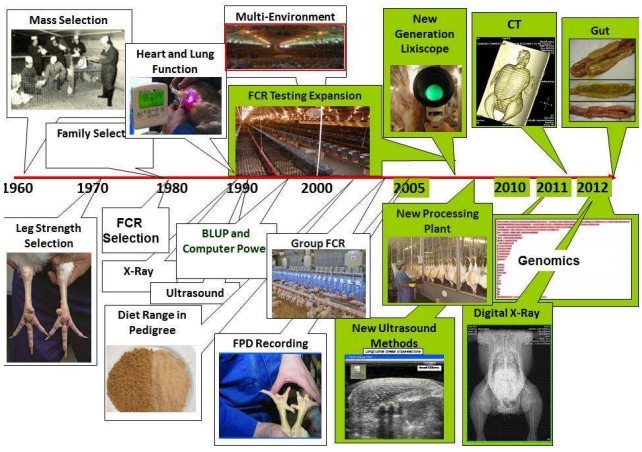
Poultry breeding companies have encountered criticism due to the perceived use of narrow breeding strategies. However, during at least the last two decades they have carefully worked towards the improvement of various partly antagonistic characteristics simultaneously. Now, breeding companies have addressed many of the criticisms levelled in the past (FAWC, 2012), and many of the EFSA recommendations are already common practice in broiler breeding (Hiemstra and ten Napel, 2013). In the past decade, the science which underpins animal breeding (and associated technologies) has been used to identify the trade-offs required for more robust selection strategies. But as long ago as the 1970s some breeding companies recognised the benefits of including welfare (e.g., leg health) in tandem with production traits in their selection programmes (FAWC, 2012). This started with the systematic assessment of leg health in the form of skeletal leg deformities for every bird in the early 1970s (McAdam, 2013). Since then, many other welfare and sustainability traits, including tibial dyschondroplasia, contact dermatitis (footpad dermatitis and hock burn), heart and lung function and liveability have been introduced into broiler and turkey breeding programmes.

In poultry breeding programmes, the source of the genetic progress comes from a wide range of pedigree lines where a high selection pressure is applied for a broad range of traits. The individual lines, each with clearly defined selection objectives, are then combined to give parents and finally commercial birds.

A large amount of data is gathered on a variety of traits for each animal, including information on welfare, health, fitness, reproduction and production efficiency. For antagonistic traits, the breeding goal can be made more sustainable by including both types of traits and to select all into the desirable direction. Therefore, in the presence of an antagonistic correlation remaining significant over time, both traits can show genetic improvement in the desired direction. Kapell et al. (2012) showed that antagonistic correlations between body weight and a range of leg deformities are mild to low, while Neeteson et al. (2013b) illustrated the result of effective simultaneous selection for broiler leg strength and growth rate (1996-2012). This principle can be applied across the whole breeding goal which has typically between 30 to 40 traits, all of which are under selection simultaneously. The desired balance can be maintained with large enough breeding populations, high selection intensities, proper statistical methodology and accurate data recording infrastructure.

The continuous investments in research and development have enabled the introduction and refinement of a variety of methods for the selection of new traits in the breeding programme (Figure 2) and will continue to do so in the future.

Figure 2. Development of Aviagen Poultry Welfare Breeding Programme (1950-2012).



When common resources and knowledge can be shared, extra progress can be achieved across species, e.g., from 2006, directly following the acquisition of BUT by Aviagen, the methods developed in

broilers have also been implemented to improve the effectiveness of selection for welfare and sustainability traits in turkeys.

Breeding for robustness

An important aspect to consider is that long term selection on traits in one environment will lead to increased productivity under those circumstances. On the other hand, the presence of genotype by environment interaction may cause problems when animals are placed in a production environment different from the selection environment. Breeding organisations can use combined crossbred and purebred selection (CCPS) or commercial sibling testing to account for this. To improve the robustness of the birds, Aviagen introduced a commercial sibling test in 2000 in broilers, and in 2010 in turkeys, in which brothers and sisters of selection candidates are grown in a non-biosecure commercial environment assessing gut health, digestive and immune function along with liveability, growth and uniformity, as well as leg health in the form of footpad dermatitis. High genetic correlations between footpad dermatitis in a biosecure pedigree environment and the commercial sibling test environment suggest that a reduction of the disposition to develop footpad dermatitis under commercial conditions can be achieved through direct selection in the first environment and through a correlated response from recording in the second environment (Kapell et al., 2012a). The individuals selected using extra sibling testing data to produce the next generation have shown improved family performance in both environments. This multi-environment strategy has made current generations of birds better able to adapt to the wider range of management circumstances they may encounter in the field. This testing of siblings has led to more robust animal populations with higher liveability and better uniformity.

Breeding for feeding and drinking behaviour

The behaviour of birds is considered important by citizens. Suitable behaviour of the domesticated birds improves their welfare, productivity and ease of management for the farmer. Aviagen selects birds that have been raised and selected under group circumstances from over forty years using accurate individual identification allowing full family and pedigree tracking. In this way, group behaviour was integrated into the Aviagen lines in a natural way. In the last ten years, various new scientific approaches on social interactions have been developed to measure and identify traits related to the behaviour of the animal. These studies also help answering questions or issues brought up by society. For example the measurement of individual feeding events in group circumstances and under competition, has provided large amounts of individual feed-intake and behavioural data, a unique source of information which helps studying feeding behaviour and improving the efficiency of poultry meat production in a responsible way. The data have shown that there is a significant genetic component in feeding behaviour (Howie et al., 2011). In addition, common patterns of feeding behaviour are conserved across chicken lines with widely varying rates of selection for growth (Howie et al., 2009) and even across chickens, turkeys and ducks (Howie et al., 2010). Water intake behaviour in the group is another new trait, currently only applied in turkeys (Swalander, 2012), enabling breeding companies to find the ‘water players’ and the birds causing the wet litter, instead of just looking for birds with higher risk of developing footpad dermatitis when standing in wet litter.

Environmental impact

Poultry production is an important candidate to provide for the demand for protein in a sustainable manner - 35% of animal protein stems from poultry, and this amount is growing – much of that is due to the genetic improvements in food conversion rate during the last decades. Greenhouse gas emissions from beef, sheep, pork and poultry meat in EU27 in 2004, calculated with a cradle-to-gate life-cycle analysis are 22, 20, 15, and 5 kg CO2-eq/kg product, respectively (OECD-FAO, 2011). Fleming et al. (2007) found that selected broilers (from 2005) need 1 kg less feed to produce 1 kg of meat (eviscerated yield) than an unselected control line (1972), i.e., 2.41 and 3.42 kg feed per kg product, respectively. Since 2005 broilers have decreased further environmental load as part of

sustainable balanced breeding. Free range and organic broiler systems have a higher environmental burden compared to the regular system (Leinonen et al., 2012).

The genomics contribution

Since June 2012, Aviagen includes genomics information in the routine selection of its elite lines. Genomics information can be used to improve all traits in the breeding programme, including live performance, critically feed conversion rate, health, disease resistance and welfare. In addition to the observed and measured performance of birds in a range of environments, it is now possible to see at the genetic sequence level the unique qualities of each bird. This is especially important for attributes for which there is a limited amount of individual record of performance at the time of selection, like sex-limited traits. For instance, in the past it was possible to make a prediction of the genetic potential for egg production or hatchability of a male selection candidate based on the qualities of its family, but without individual records it is not possible to differentiate birds from the same male and female parent. With genomics it is now known what is the genetic configuration of each bird, and what has been inherited from its parents. By utilizing this unique insight from the birds, even more accurate selection decisions can be made in order to improve all aspects of the bird's performance at every generation.

Conclusions

Considerable decreases in the prevalence of leg disorders have been achieved by accurately scoring selection candidates and a stringent culling policy of discarding selection candidates that show (sub) clinical leg disorders, combined with identification of families that are prone to develop leg issues. Similarly, liveability and heart and lung function have been improved significantly through accurate measurement and identification of above average families. In addition, selection for welfare traits such as liveability and footpad dermatitis in a highly bio-secure environment are improving the robustness of birds under commercial conditions. Simultaneously, improvement of the environmental impact and economic efficiency of poultry production are key.

Balanced breeding simultaneously improves productivity, efficiency, environmental impact, animal health and welfare, food quality and safety. Broad breeding goals and genetic diversity are essential to achieve a balanced progress in pedigree broiler lines. Investments in science and breeding will continue. This approach has had and will continue to have benefits for sustainable meat production and consumption in Europe and globally.

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Impact of current and future European welfare regulations on animal breeding

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Welfare of animals has become of increasing importance to society, policy makers and legislators across Europe. Regardless of the reasons, this is a fact of life that all players in the animal production chain must recognise and react to. Even where there are marked differences between European countries, this general trend can be seen across all Member States and is reflected in legislation instituted by the European Union. Poultry is no exception to this general trend. Some developments occur first in poultry, possibly reflecting the type of production sector, which is predominantly large scale and standardized at one end, compared with very different, smaller scale, niche production systems at the other.

Animal breeding, especially in poultry, has been perceived by some observers as a contributor to compromised animal welfare, due to the response by breeders to market demands for increased genetic potential for productivity and efficiency. This may be a heavily biased or even totally wrong assumption, but it does clearly influence the opinions of some NGO's, politicians and legislators.

(Poultry) breeding companies are impacted by national and EU policies directly and indirectly. Direct impact can be through legislation, for example on housing systems such as the Welfare of Laying Hens Directive, which makes individual housing for purposes of pedigree-ing or individual evaluations impossible, or controls on invasive treatments of chickens which hinder the execution of breeding programs and preparation of breeding stock for export. Exemptions from the general rule for breeding establishments are at the discretion of national or local authorities and may not be easily obtained. Indirect effects may be via standards and operating procedures for poultry production systems, e.g., maximum tolerable levels of mortality or growth rates in broilers.

Two recent developments indicate important trends for the future:

1. The European Commission is considering legislation which may directly set rules for animal breeding.

   The European Commission has recently completed a survey among a range of stakeholders in broiler meat production to evaluate the impact of breeding programs on broiler welfare and to investigate how regulation could best "optimize" this impact. Regardless of the conclusions, the very commissioning of the report shows the line of thinking among authorities.
   The European Commission is now drafting wide-ranging proposals for a new Animal Welfare Law, which is likely to include measurement of outcome-based welfare indicators, risk assessments and clauses directly related to animal breeding.

2. Covenants are being agreed between producer and buyer organisations to set higher voluntary production standards which go beyond formal legislation.

   Retailers and producers in The Netherlands have agreed that, from 2015 onwards, only broiler meat which is derived from birds which did not grow faster than 50 grams per day will be available in Dutch supermarkets. It may well be that other countries, like Germany, will follow similar lines of action for broilers and turkeys.

The above trends have, obviously, not gone unnoticed by the breeding companies in the EU. All major breeding programs have paid significantly increased attention to breeding goals which support societal concerns and aim to improve the welfare of production animals. In addition, all major breeding

companies, united in EFFAB at the European level, have adopted a Code of Good Practice (Code-EFABAR) which sets down the principles for good care of the animals kept by the companies and also demonstrates their efforts to maintain and improve animal welfare related traits in their breeding programs.

Most importantly, EFFAB represents breeding organisations at the European level providing a dialogue with policymakers, politicians and other stakeholders, promoting factual information about breeding programs and the impact on animal welfare in the European poultry sector. This is the most effective way to ensure that any changes to current practices for the benefit of animal welfare can be implemented in an effective and practicable manner.

It is vitally important that all poultry geneticists are aware of, and actively engage in, the on-going public debate about animal welfare; contributing to it by clearly explaining the potential for and limitations of genetic selection programmes to improve the welfare of production animals across Europe.

Transgenesis in hens with DNA microinjection into zygote

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P-01. Transgenesis in poultry gives the possibility of reaching the selection objective rapidly. Gene constructs containing the β-galactozidase gene with the cytomegalovirus promoter was used in the experiment. DNA injections were carried out with a glass pipette into the centre of the egg cell blastodisks when the egg cells were in the infundibulum accessed surgically. Eggs laid on the next day were incubated. The embryos were dissected at the 4th to 6th days of incubation. The embryos were stained for β-galactozidase and examined in a stereo microscope where the tissue blue colour was developed by β-galactozidase reaction with X Gal substrate demonstrating integration of the β-galactozidase gene. Embryonic development was detected in 16 eggs of 21 injected eggs. Transgenic embryos were identified in 4 of 16 embryos. One embryo of these 4 was fully dyed indicating that the β-galactozidase gene was in all tissues. Three embryos were partly dyed. Dyed chorda and internal organs, including the germ cells, were also observed. These results suggest that transgene integration is rather frequent using this method.

Chicken genome modification with β-interferon gene

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P-02. Chicken lines created with genetic resistance to virus diseases may be one use of transgenesis in practical poultry breeding. For example, it may be that an increased interferon level in the body may be desirable to protect birds from disease. A mouse metallothionein promotor (MT1), being activated by hard metal ions, may be such a regulator gene. Its natural concentration in the body is normally enough for the gene expression to be regulated by MT1 and promoter induction can be increased with zinc salts added to the feed, if necessary. A gene construct, pMT-hIFNb1, containing the human gene for β-interferon under the control of a mouse MT1 was injected surgically into hen egg cells at the zygote stage. A total of 29 egg cells were subjected to injections; 25 externally normal eggs were laid and embryonic development has been found in all of them. A total of 10 chicks were obtained including 2 cockerels and 5 hens and were reared under normal husbandry procedures. Blood DNA analysis with PCR showed that the injected gene construct sequences were present in both cockerels. The offspring of these cockerels after artificial insemination of hens with these cockerels' semen produced 46 offspring. PCR-analysis has shown that none of 46 offspring were transgenic.

Detection of QTL controlling digestive efficiency and anatomy of the digestive tract in chicken

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P-03. Improving digestive efficiency (DE) is a major challenge in poultry production to reduce production cost, through the use of alternative feedstuffs and to decrease the animal manure. Since measuring DE is rather difficult, identifying molecular markers would alleviate the need for extensive measures. Detection of QTL were undertaken on 820 meat-type chickens genotyped for 6000 SNPs in an F2 cross between D- and D+ lines divergently selected for lower or higher metabolizable energy, respectively. The anatomy of the digestive tract, DE and body weight at 23 days was measured on all birds. Using linkage analyses by interval mapping, we detected nine QTLs controlling DE traits, 11 QTLs controlling anatomy-related traits and two QTLs controlling body weight. Two QTLs were genome-wide significant on chromosome 20 at the same position for starch and dry matter digestibility. Two QTLs controlling both DE and the relative intestine length were found on chromosomes 16 and 26. Further studies are needed to identify the genes underlying these effects, and to validate them in commercial populations and breeding environments.

Detection of QTL influencing egg production in layers receiving various diets

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P-04. Egg production in layers is affected by quantitative trait loci (QTL) and by environmental factors, such as the diet composition. The aim of the research was to identify QTL involved in egg production and to estimate interaction effects between QTL and diet. A population of 440 sires was genotyped using a high-density SNP *Affimetrix* chip (600K). A total of 31,539 crossbred daughters were phenotyped in 3 hatches in collective cages of 12 hens. One half of the birds were fed on a high energy diet (2881 Kcal) and the other half on a low energy diet (2455 Kcal). Egg number per cage was recorded every day from 18 to 75 weeks of age and eggs were collected at two ages, at 50 and 70 weeks, for quality measurements. Two "phenotypes", one per diet, were calculated for each sire as the mean of his daughters' phenotypes. Several methods were used to search for QTL involved in egg production and quality traits. The QTL stability according to diet was variable. These results highlight the importance of taking into account genetic x environment interactions in the genetic evaluation of layers.

Genome-wide transcriptomic analysis of liver from chicken lines selected for residual feed consumption

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P-05. The improvement of chicken feed efficiency is an important goal in the poultry industry to increase profitability and to limit the environmental impact of production. Such improvements have largely been achieved through quantitative genetic selection, but the causal genes or biological processes underlying feed efficiency and other related traits are still poorly known. Two lines from a Rhode Island Red base population have been divergently selected for high (R+) and low (R-) residual feed intake (RFI). After 37 generations of selection, retaining the same body weight and egg production, the two lines differ by 5 phenotypic standard deviations for RFI, and the R+ line has a feed intake 75% higher than the R- line. We used these lines as models to study the genetic basis of feed efficiency in layer chickens. We performed a genome-wide transcriptomic analysis of liver using a 8x60K custom Agilent microarray on 16 adult birds, 8 for each line. Differential expression analysis was performed using the function "treat" of the bioconductor package Limma, setting a fold-change threshold of at least ±20% and an adjusted *p*-value less than 0.01. In total 78 differentially expressed (DE) genes were identified, 57 of which had a fold-change >2. Biological process analyses of RFI-induced DE genes indicated an alteration of defense/immune processes, cell cycle, lipid metabolism and hormone activity.

On the analysis of pooled data of 3 different chicken breeds

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P-06. The analysis was conducted on a next-generation sequencing data set of pooled genotype data of three different chicken breeds. We demonstrated that it is possible to analyse pooled data using the ratios of genotype information. The analysis focused on 4 chromosomal locations that were assumed to comprise SNP variants and candidate genes for egg shell quality and colour traits, based on a previous study (Tuiskula-Haavisto et al., unpublished). Some advantages and disadvantages of our pooling approach were identified and we obtained the first phenotype-independent results. Specifically, we identified variant hotspots that differed between the chicken breeds and were able to interpret them with respect to close-by genes. As the read-depth of next-generation data is crucial, we applied an exhaustive analysis to check for peculiarities between the breeds. This was done by applying a novel in-house R-package for the visualization of the data. Possible technical abnormalities of the read-depth with respect to manufacture specifications are: systematic differences between the average read-depth of the breeds and loci with multifold larger (or smaller) read-depth than expected. The read-depth was then considered as one possible quality measure of the data.

Genomic prediction in laying hens

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P-07. Genomic prediction has revolutionized the field of animal breeding. In laying hens, availability of genomic breeding values may allow more accurate selection at an earlier age and better differentiation of full sib males regarding their genetic potential. This study focuses on assessing the accuracy of genomic prediction in a sample of around 850 individuals from a Rhode Island Red line. All individuals were genotyped with the Affymetrix Axiom® Genome-Wide Chicken Genotyping Array which contains ~580,000 SNPs. After quality control (call rate >95%, MAF >0.5%) around 308,000 SNPs were available for further analyses. Conventional breeding values (EBVs) and de-regressed proofs (DRPs) of economically important traits were used as quasi-phenotypes for genomic breeding value prediction. Predictive ability was studied using a genomic BLUP model in a random five-fold cross-validation as well as in a stratified analysis where genomic breeding values of the youngest individuals were predicted. In the five-fold cross-validation, correlations between EBVs (DRPs) and genomic breeding values were 0.58 (0.45) for laying rate in the early stage and 0.70 (0.62) for egg shell strength. When predicting the youngest individuals, accuracies of prediction were 8 to 16% lower than in a comparably unstratified analysis.

Studies on QTL effects on some performance traits in ducks

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P-08. The goal of this study was to examine the chromosomal regions influencing meat production traits in ducks. The research was based on a crossbreeding experiment conducted on two populations of Peking ducks (A55 x GL-30) to obtain the first generation. After mating unrelated individuals, 388 crossbreed ducks (both sexes) of the F2 generation were recorded. All experimental birds (449) were genotyped whereas only individuals (from F2) were recorded. A total of 38 traits (body weight, carcass, and technological and sensory evaluation characters) were recorded. Sex of birds (in F2 generation) was verified by molecular test. For the association study, 29 microsatellite markers located on chromosome 1 were employed. The analysis was done on a model that included the sex effects and putative additive effects of QTL. The computations were performed using the GRID QTL package. Statistically significant effects of QTL were estimated for skin weight with subcutaneous fat and some traits of the leg muscles and were confirmed by the use of alternative statistical models.

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Comparison between the protein egg yolk content of six local hen breeds: a relative quantitative proteomic study

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P-09. Hen egg plays an important role in human nutrition both as a source of components with relevant biological activities, and as responsible for one of the most common allergies in infants. The importance of eggs in human health has both clinical and industrial implications. Poultry breeding has been massively implemented in the last decades, selecting of more productive hybrids as opposed to local breeds, which were, on the other hand, pressed to extinction. The present work aims to characterize the proteome of the soluble protein fraction of egg yolk belonging to five local breeds (Pepoi, Padovana, Ermellinata di Rovigo, Polverara and Robusta Lionata) and a commercial layer line (Golden Comet), used as reference. To this aim a relative quantitative proteomic study has been carried out using on-column stable isotope dimethyl labeling of primary ammine groups (Boersema et al., 2009. *Nature Protocols* 4:484-494) coupled to mass spectrometry analyses. Experiments were conducted by analyzing pools of 2-3 eggs coming from different hens of the same breed, and carried out in two biological replicates. Results reveal differences in the relative protein composition among the six considered yolk fractions. Further bio-statistical analyses will define breed specific characteristic, to improve the knowledge about these genotypes, and hopefully provide a tool to describe breed specific protein markers, useful for product traceability.

Successful identification of candidate gens for muscle quality

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P-10. The objective of the research was to identify genes and mutations underlying variation in meat quality. We hypothesised that heat stress would amplify genetic differences between layer and broiler lines and enhance the ability to detect candidate genes. Two male-line broiler and 2 White Leghorn males in 16 climate chambers were subjected to a Control (21°C, 50% RH) or Heat stress (32°C, 75% RH) experiment for 2 hours (32 birds in total). Breast muscle RNA was reverse transcribed into cDNA, pooled within line and chamber and hybridised to 32 Affymetrix slides. Following ANOVA, 2213 differentially expressed genes were identified (breed x treatment $p<0.05$) and subjected to bioinformatics analyses in BioLayout Express and Ingenuity Pathway Analysis. A total of 25 candidate genes and 4-5 SNPs per gene were selected for SNP genotyping on 134 DNA samples from 8 week-old chickens representing 34 breeds that had been phenotyped for 9 meat quality traits (meat colour, pH and creatine kinase activity). Significant SNPs collectively accounted for 15-55% of the phenotypic variation within type (layer, traditional or broiler). The results may lead to genetic selection to improve muscle and meat quality and reduced susceptibility to heat stress in broiler chickens.

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Bone development in embryos from commercial and slow-growing broiler strains

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P-11. Modern commercial broilers often suffer from leg problems associated with their rapid growth and high body weight. This study compares leg bone properties during incubation of embryos of a commercial strain (Cobb 500) and an experimental population representing commercial broilers in 1986. One-hundred eggs per strain (Cobb and "1986") were incubated. Eggs (n=10) were randomly selected on embryonic days 17 (E17), 19 (E19) and 21 (E21; day-of-hatch); the tibia and its muscles were dissected from each embryo and weighed separately. Each tibia was subjected to biomechanical testing, cortical structure analysis, histology staining and fluorochrome labeling. Cobb broilers had a 12% higher embryo and muscle weight on E21, whereas bone weight was similar in both strains. Compared to Cobb, the "1986" showed 9-20% higher tibial-stiffness between E17 and E21, 33% higher cortical area on E21, 100% higher moment-of-inertia on E21, 35% higher osteocyte (load-sensing cell) concentration on E21 (visualized and measured by H&E staining), and lower calcium incorporation between E19 and E21 (visualized by fluorochrome labeling). In conclusion, compared to the relatively slow-growing broilers of the 1980's, the bones of the modern commercial broiler are inferior in terms of lower load-sensing ability (lower osteocyte concentration), reduced structural parameters (cortical area and moment-of-inertia), reduced mineralization (calcium incorporation) rate and lower mechanical capabilities (stiffness).

Robustness to chronic heat stress in laying hens: a meta-analysis

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P-12. Heat represents a major stress factor in laying hens, but it is difficult to compare published studies, done under very different conditions. A meta-analysis enabled us to make a quantitative review of the results from 100 published papers. Relative effects of four factors (genotype, age, group size and amplitude of temperature variation) were analyzed on feed intake, egg production, egg weight, egg mass and FCR. The GLMselect procedure in SAS was used to select the best model for each trait and to estimate the main effects and their interactions. Daily feed intake, egg mass and hen-day egg production were more sensitive to heat stress as they varied from -16.2% to -19.8% between 20 and 35°C while egg weight and FCR showed less variation (-3.8 to -6.4% between 20 and 35°C). Interactions were most often significant, which reinforces the interest of meta-analysis to summarize literature data. This study highlighted that the impacts of heat stress in laying hens depends on the genotype, age and the amplitude of temperature.

Genetic relationships in two meat type slow growing chicken (*Gallus gallus domesticus*) breeds using microsatellite markers associated with productive and health related traits

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P-13. Different chicken populations are traditionally present in Italy. Genetic information on molecular basis can supply useful tools in selection strategies to maximize genetic diversity. Furthermore, a marker assisted selection for productive and health related traits can be considered an effective support in breed conservation and diffusion success. In this study 8 microsatellites, associated with productive and health related traits, were utilized. The aim was to investigate the genetic relationships between two traditional Italian breeds for low input production. A total of 100 blood samples were analysed (Bianca di Saluzzo=BISA, n=50; Valdarnese Bianca=VABI, n=50) with these 8 microsatellite loci (ADL102, ADL158, ADL176, ADL181, ADL210, ADL267, ADL136, ADL171). PCR amplification products were separated on ABI Prism DNA Sequencer (Genescan and Genotyper softwares; Applied Biosystems) and the molecular data was analysed using MolKin 3.0 software. Number of alleles, observed heterozygosity and PIC for each analysed marker, and the proportion of diversity between birds were calculated. The heterozygotic deficiency within the breeds (F_{IS}) was 0.05 for BISA and 0.27 for VABI, and among the breeds (F_{ST}) was 0.03. Kinship distance showed very similar results in the two breeds (BISA=0.40, VABI=0.48). Molecular co-ancestry, measuring the similarity within birds of the same breed, was almost the same for BISA (0.24) and VABI (0.23). Results underline the importance of monitoring the genetic variation of traditional breeds focusing on productive and health related traits to improve the efficacy of selection strategies.

Improving immune competence: screening for new parameters describing the innate immune status in chicken

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P-14. The intense selection conducted for chicken production-related traits has led to deleterious consequences for health and welfare, so that it is now necessary to include parameters related to robustness in selection programs, like the immune competence, i.e., the ability to mount an effective immune response against one or several pathogens. We intend to identify both new parameters describing the innate immune status and the genes controlling their variations, in order to predict the bird's immune competence and eventually improve it by genetic selection. To reach this goal, we are currently assessing the use of measuring the blood expression level of genes involved in the innate immune response and the levels of indirect parameters potentially related to animal health. We are also interested in the relations between gut microbiota composition and the host immune response. Once new and relevant parameters describing immune status will be determined, we shall identify their genetic architecture in animals confronted to pathogens in order to find innovative ways of improving the chicken immune competence.

Investigation of immune response to *Eimeria maxima* in broilers

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P-15. Coccidia are mainly intestinal parasites affecting animals and leading to substantial economic losses in poultry production. A genetic variability for resistance to coccidiosis in chicken has been evidenced in different studies and suggests that applying a selection for this natural resistance could limit the effects of the disease. In this study we focused on the impact of *Eimeria maxima* on different traits (e.g., weight gain, body temperature, blood composition, intestinal lesions scores, oocysts count) in two challenge experiments on commercial broilers (pilot then larger experiment with 200 and 2000 animals challenged, respectively). We demonstrated that it was possible to replicate the challenge effects observed across the two experiments. As some traits are difficult to measure on large scale, we tried to predict those from traits easier to phenotype using partial least squares models, with moderate success. Finally, we demonstrated that the challenged population presents a genetic variability for resistance to *Eimeria maxima*. The next step will be to identify genomic regions involved in the resistance determinism, for further selection use.

Avian populations in Algeria (Ghardaya): phenotypic characterization of local breeds

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P-16. In Algeria, local chickens (*Gallus gallus domesticus*) are part of the traditional breeding; they occupy the first place without competition among avian species. They are characterized by extremely varying phenotypes, such as coloration of plumage, types of peak, crest, feathered legs, and other features which are hardly recorded. Many of these features are monogenic traits controlled by a single gene and/or, in some cases, by two or three genes. In order to characterize the local chicken population of the region of Ghardaya, more than thirty traits were r over four months in the majority of the villages which belonged to Ladira, Lachboure, Oasis, Ougba, Street daya, Malika, Ben yesgen, El atteuf, Berriane, and Guerrara. The exploration of the database concerned various characters: coloration of the beak, the peak and the plumage; the presence of the crest, feathered lugs, the presence of ergot in females and their absence in males, and naked neck. The recording of different phenotypes allowed deduction of the allele and genotypic frequencies of this population which is considered as a natural breed, at least the presence of several allelic forms showed that this population conceals a high genetic variability.

Breeding system and laying performance analysis of local poultry population in the region of Batna

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P-17. Since independence of Algeria (1962) up to (1970), breeding units were essentially farms without any particular organization. Food of animal origin, particularly poultry, occupied a very modest place in the ration of Algerian diet structure. Between 1980 and 1990 the industrial poultry sector has evolved and white meat production has tripled while egg production has multiplied by nine. This development has improved the average human food ration. Furthermore, as a consequence of industrial poultry development, breeding of local chickens decreased. The current work was done in the region of Batna and was based on studying laying performance and breeding analysis system observed at various locations (Tazoulet, Timgade, Oued Taga Ichemoule, Thniat el Abed Bouzina, Merouna, Seriana Fesdis, Zarma and Aine yagoute), to permit characterizing the different types of breeding schemes and prospects for improvement.

Assessing genetic diversity and phylogenetic relationship of sixteen Mediterranean chicken breeds

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P-18. Genetic diversity and relationship among 16 Mediterranean chicken populations from five different countries were assessed by sequencing mitochondrial DNA and genotyping 27 microsatellite markers. In addition, six commercial populations were used as reference. A 506 bp fragment of the mtDNA control region was sequenced in 160 DNA individual samples. Twenty-five variable sites were observed. As a result, 21 haplotypes were identified and assigned to three clades. Haplotype E1, which might have its roots in the Indian subcontinent, was most frequent. Other sequences were included in the haplogroups A and B. For the microsatellite analysis, 465 individual blood samples were randomly collected from the 16 local breeds. A total of 242 alleles was found with a mean number of 8.96 alleles per locus. In some populations reduced heterozygosity was observed. This points to the need for appropriate measures to be taken in order to prevent negative effects of inbreeding. Structure analysis exhibited extensive genetic admixture in many of the studied populations. The study revealed that Mediterranean chicken populations, which may originate from three maternal lineages, retain moderate levels of genetic diversity.

Genetic diversity of a large range of domestic chicken breeds and its use in genome-wide association studies

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P-19. Domestic chickens show massive phenotypic variation reflecting their genetic make-up. This can be used to detect relationships between breeds and genomic regions influencing trait divergence. This study aims at (1) evaluating the relationships of chickens from an extensive spectrum of breeds mainly according to the fancy chicken breed standard of Germany, and (2) applying a novel genome wide association approach to detect chromosomal regions causing trait differentiation. 2000 chicken blood samples from 111 breeds and colour variants were genotyped with the 600k Axiom-Genome-Wide Chicken Genotyping Array, and phenotypic trait information was collected for each bird within the framework of the SYNBREED project. Filtering excluded SNPs with call-rate <99% and animals with call-rate <95%. A principal component analysis visualized a strong association of individuals with their respective breed. Groups of breeds differing in trait variants were contrasted genotypically in a comprehensive genome-wide association study, establishing different test-statistics and sliding window strategies. As a proof of concept, this approach mapped known mutations for yellow skin colour and rose-comb mutation to the correct position. Methods established will be used to search for selection signatures and so far unknown genomic regions associated with phenotypic trait variations.

A global assessment of population structure and genetic diversity in chicken populations from Africa, Asia, and Europe

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P-20. Genetic diversity and population structure of 114 chicken populations from Africa, Asia, and Europe were studied using 29 microsatellite markers. These populations included three wild chicken populations (RJF), nine commercial purebred lines, and one inbred line with four sublines to be used for comparison. Allele frequencies, mean number of alleles, heterozygosity, and Wright's fixation indices were estimated to investigate the extent of genetic variability between and within chicken populations from different geographical regions. Geographic population structure was determined by using Bayesian model-based clustering. High heterozygosity and lower genetic differentiation were observed in African and Asian chickens relative to European and Commercial breeds. European chicken breeds showed higher range of variability in heterozygosity, while the majority of Asian and African chicken populations had heterozygosity levels above the mean of all populations. The cluster analysis revealed high admixture in African and Asian chicken populations whereas European breeds partitioned into distinct groups with minimum sharing of genetic material.

CRB-Anim: a project to support the preservation and management of biological resources for poultry

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P-21. Breeding programmes and research projects on genome diversity are depending upon the possibility to access to documented biological samples. The network of Biological Resources Centres 'CRB-Anim' has been set up to store and characterize reproductive and genomic material for domestic animals in France, as a component of the national strategy for biodiversity. The network currently holds around 530,000 biological samples (semen, embryos, cells, tissues, DNA, RNA). Several poultry species are involved: new cryopreservation methods will be developed for semen as well as for somatic cells in order to improve the recovery rate of a genotype stored in the Cryobank. Blood and/or nucleic acids as well as reproductive cells will be stored in parallel for 1220 different male donors sampled across chicken breeds, turkey lines and experimental lines (chicken, quail). Genotyping will be performed with a high-density SNP chip to characterize the French chicken breeds stored in the National Cryobank. A Web portal will be developed to facilitate the access to data and samples for researchers and breeders. Common rules and methods for cost calculation will be set up. The training strategy will cover all levels from undergraduate to PhD and continuous training.

Conservation and valorisation of avian local Italian breeds: the CoVAL project

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P-22. Genetic resources have to be optimized in order to preserve endangered animal genomes in a world of decreasing biodiversity. CoVAL is a three year project, funded by Regione Lombardia. It aims to develop a conservation programme of four local poultry breeds of two species, chicken and turkey including both *in situ* and the complementary *ex situ* measures. All breeds are from the Italian region Lombardia. The chicken breeds considered are *Mericanel della Brianza* and *Milanino* and the turkey breeds are *Brianzolo* and *Nero d'Italia*. The project includes many activities for a detailed characterization of the breeds and for their use in niche production. The main goals of CoVAL are:

- genetic variability analysis using DNA markers;
- morphological, reproductive, productive and behavioural characterization of the breeds;
- reference procedure for semen cryopreservation;
- selection of a breeding nucleus for each breed following FAO guidelines;
- standard management guide for rearing chicken breeds in extensive farming system;
- cost analysis and market investigation;
- dissemination and technical support.

The future of CoVAL local avian breeds is based on their genetic conservation, use for niche production and diffusion for the economic development of rural marginal areas.

Genetic variation of two local chicken populations: 'Bianca di Saluzzo' and 'Bionda Piemontese'

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P-23. In Piemonte region two autochthonous chicken breeds exist: 'Bianca di Saluzzo' and 'Bionda Piemontese'. These slow-growing meat breeds are reared in farms mainly located in Cuneo and Asti provinces. In the past decades both breeds were subjected to a strong reduction in population size and were frequently replaced by fast-growing lines. Only recently, conservation plans were undertaken. The main objective of this work was to determine the genetic structure of these breeds. We used 26 microsatellites which have been suggested in the literature. Allelic richness was 5.9 and 6.4 for the 'Bianca' and the 'Bionda' respectively, gene diversity was 0.665 in both breeds. Differentiation was measured by Fst (0.056) and was highly significant ($p<0.001$). Using a Bayesian analysis (Structure software) under the hypothesis of two clusters, all runs split the dataset into samples from the 'Bianca' and from the 'Bionda' and most individuals were correctly assigned with a membership coefficient >0.9. With three or more clusters the 'Bionda' breed splits into two different subclusters whereas the cluster including most of the 'Bianca' was very robust, showing that this breed was most different but also most homogeneous within the dataset.

The BIODAVIT research group: an Italian network for conservation of avian biodiversity

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P-24. The genetic structure and diversity of the breeds are crucial points in decision making for animal genetic conservation. The maintenance of genetic variability is widely shared as a strategy to carry out genetic improvement and to allow a response to changes of farming systems, for better adaptation to environmental changes and even more important for the understanding of the phenotypic variability. The global use of highly productive animals has led to the gradual erosion of genetic variability in most species, and among them the poultry are the most threatened. Objectives of the BIODAVIT research group, which is of national interest, are to study the biodiversity of Italian avian breeds with a multidisciplinary approach by analyzing genomic, transcriptomic, and proteomic data, and to investigate new phenotypes related to adaptability, well-being and quality of poultry production species. The project focus on the study of two avian species: *Gallus gallus*, with 16 different breeds of chicken originating from 7 different regions of Italy, and *Meleagris gallopavo*, with 5 different breeds of turkey originating from 3 different Italian regions. A link with other regional avian conservation projects will be guaranteed. The actions of the BIODAVIT group are supported by the Italian branch of the World's Poultry Science Association.

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Local poultry breed assessment in Piedmont (north-west Italy)

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P-25. A research project founded by the University of Torino (Italy) aims at improving the poultry production chain of two local breeds ("Bionda Piemontese" and "Bianca di Saluzzo") by the characterization, conservation and enhancement of these breeds. These breeds are included in the list of the Slow Food Presidia. Preliminary data about census of these local breeds are given. The size of population results showed that they were composed of around 16,000 birds for "Bionda Piemontese" and 4000 birds for "Bianca di Saluzzo". It is estimated that, in 2012, 45,000 chicks of "Bionda Piemontese" and 12,000 chicks of "Bianca di Saluzzo" were produced. Animal density is 0.25-1.7 m^2/bird for indoor space, and 1.5-25.0 m^2/bird for outdoor paddock; the slaughter age (days±s.d.) is 223±69 for hens, 202±46 for cocks and 268±8 for capons. The slaughter weight (kg±s.d.) is 1.8±0.2 for hens, 2.2±0.2 for cocks and 2.9±0.3 for capons. The 56% of farmers are men (average age: 41 years) and 44% women (average age: 51 years). Farming is the main activity in 66% of cases, followed by agritourism (17%) and other activities (17%). The preservation of these local populations contributes to biodiversity and conservation of local farming systems.

Development of avian reproductive biotechnologies for the management of genetic diversity

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P-26. The general goal of the project was to develop complementary reproductive biotechnologies focused on avian germ cell storage and use that must be considerably improved and standardize for the management of avian genetic resources. The specific goal of the project was to include in a unique strategy the development and use of the haploid male/female gametes and diploid reproductive cells. This was first carried out by improving the actual methods of storage and use of semen, blastodermal and primordial germ cells, developing new and innovative approaches tuned to these cell types and also to gonadal tissues, and developing quality tests of the cell and tissue cryopreserved to enlarge the capacity of use of these germ cell resources. Main results: elaboration of procedures of storage of semen, blastodermal and primordial germ cells; elaboration of gonadal tissues storage; standardization of quality measurements for the cryopreserved cells and tissues; application of these reproductive biotechnologies to the strategy of management of genetic diversity of local and experimental genotypes; and beginning the first international network of avian germ plasm cryobank.

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The BIONET project: an Italian regional network for conservation of poultry biodiversity

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P-27. Over the past 10 years, in the Veneto region several conservation and characterization activities of poultry species were conducted. The main goal was to develop plans to safeguard indigenous Veneto poultry breeds using EU and regional agricultural funding as well as national projects. The breeds involved in this conservation programme belong to four different species: chicken, guinea fowl, duck and turkey, and they are reared in 5 different conservation centers. From a genetic point of view, it is essential to maintain an effective population size (Ne) of 50, as shown in the literature, through proper selection of males and females for breeding purposes. In 2012, the BIONET project was approved by the regional government (PSR2007-2013, 214H) with the main objective of providing genetic information useful for the production and preservation of poultry local breeds. Aims of the BIONET projects are:

1) to monitor the genetic variability of the groups of animals under conservation by DNA analysis with molecular markers of the latest generation;
2) to characterize production traits, which will cover data analysis of reproductive (data related to incubation, and candling) and production (live weight, age at maturity and average daily gain of animals) performances, as well as the qualitative aspects of carcasses and meat of turkey, duck and guinea fowl.

For some of these species it is planned to set up a survey for recording information of carcass quality and meat traits in order to detect possible peculiarities and characteristics to be exploited in the market. A link with other regional and national avian conservation projects will be guaranteed.

Selection and correlated response for egg weight in Japanese quail

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P-28. The present study was conducted at the Poultry Research Center, Poultry Production Department, Faculty of Agriculture, Alexandria University. The experiment aimed to measure the direct response of selection for high egg weight of the first 10 eggs laid at the beginning of egg production period (EW10) and its correlated responses with other growth, egg production, egg quality and reproduction traits in Japanese quail through three generations of selection. The birds were randomly assigned to two mating groups, the first group was selected for high egg weight of the first 10 eggs laid at the beginning of egg production period and the second group was maintained for contemporary comparison (control line). The data were statistically analyzed, discussed and the results showed that the EW10, as direct response, increased in the selected population when compared with control population from 10.43 g (100.19%) in the base generation to 12.52 g (107.01%) in the second generation after selection with a rate of 0.69 g per generation. In conclusion, the comparison between selected and control populations for the whole selection period indicated that this type of selection has positive effect on EN45, EW45, BW6, DBG2-6, GR2-6 and improved FCR. However, it had a reduction effect on ASM, BWSM and DU10. Also, a slight effect was shown on reproductive and egg quality traits. Finally, the present study confirmed that quails are alternative birds able to develop for both meat and egg production and should be supported by more or/and continuous studies.

Polymorphism of TLR4 in European breeds of the domestic fowl

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P-29. Toll-like receptors (TLRs) represent cornerstone components of vertebrate pathogen-recognition apparatus. They belong among the first elements able to detect danger and initiate immune response. It is well established that TLR genes are variable on interspecific as well as intraspecific levels and that TLR genetic polymorphism may have significant effect on receptor function and immune responsiveness. Unfortunately, we have only limited information about TLR polymorphism in birds. Although TLR genetic variability has been investigated in Asian chickens, nothing is currently known about their variability in the breeds maintained in Europe. In this study we focused on genetic polymorphism in the endotoxin (lipopolysaccharide, LPS) -sensing TLR4 in European breeds of the domestic fowl. In a sample of 96 individuals representing 24 breeds of the domestic fowl maintained in Europe we have described 74 TLR alleles. Most of these alleles occurred in a single individual while only 7 were detected in more than 5 copies in our dataset. These alleles varied at 29 positions (SNPs), all except one synonymous SNP were found repeatedly in our samples. We have identified 35 non-synonymous haplotypes varying at 13 aa positions. We discuss the possible impact of the detected polymorphism on the TLR4 receptor structure and binding features.

Identification of QTL of transcripts (eQTL) and of proteins (pQTL) on foie gras of mule ducks

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P-30. In France, 97% of the foie gras production comes from mule ducks, an inter-specific hybrid obtained by crossing a female common duck (*Anas Platyrhyncos*) and a Muscovy drake (*Cairina Moschata*). As mule ducks are infertile, the genetic improvement of the fatty liver quality has to be done through the genetic selection of parental lines. Thus, knowing the impact of genes on the quality traits could be useful for selection purposes. The project is based on a QTL (Quantitative Trait Loci) experimental design with more than 1500 phenotyped mule ducks. In 2013, Kileh-Wais et al. found 11 significant QTL related to liver quality traits, with QTL clusters on chromosomes 2 and 9. Focusing on these QTL, proteomic and transcriptomic analyzes were performed on fatty livers of a subset of 300 mule ducks. Proteins were quantified using the bi-dimensional gel electrophoresis method and transcripts were quantified by quantitative PCR (Fluidigm® technology). The regulatory networks implicated in the fatty liver quality are presently under investigation at both levels through the identification of pQTL and of eQTL, i.e., QTL controlling protein and transcript levels, respectively.

Characterization and improvement of Pollo Brianzolo chicken breed in four generations: phenotypic results

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P-31. *Brianpollo* is a research project funded by Regione Lombardia, Italy (2011-2013) aiming to standardise and value Pollo Brianzolo (PB) local breed (*Gallus gallus domesticus*) selected for non-conventional rearing systems. This breed historically originated (1930s) from the cross between White Leghorn (WH) and New Hampshire (NH). The present research involves a foundation group of 6 pure breed families (M/F = 1/3 = WL/NH) and the following four generations; F1 to F4 reared to obtain a standardised PB. The aim of this study was to characterize F1 and F4 generations for phenotype, performance and eggs laying characteristics. Bird size and body proportions, together with plumage colour and comb shape have been the selected traits to standardize the birds' morphology. Each bird was phenotypically screened at the age of 16 weeks, prior to breeder selection. Animals had been weighted at hatching and at 4, 8, 16 and 24 weeks of age. Qualitative and quantitative parameters of hatching eggs were recorded weekly. Data have been analysed with SPSS statistic package. Differences have been observed for growth rate, with higher live body weight for F4 males (1275±256 g) than for F1 birds (1010±221 g) at 16 weeks of age. Preliminary results considering production performance, correspond to productive goals typically requested for slow growing breeds in order to be adapted to alternative low input production systems and specific markets.

Genetic parameters along the near infrared spectra to predict melting rate of the duck fatty liver

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P-32. In the framework of a duck genetic design, we carried out a genetic analysis along the near infrared (NIR) spectra of fatty livers in relation to their melting rate (lipid exudation during cooking). We used 1418 livers with weight ranging from 300 to 830 g. NIR spectra were collected with 2 spectrometers (FOSS NIRSystems6500 on grinded livers or ASD LabSpecPro on liver surfaces) in order to predict the liver melting rate. NIR spectra were represented by absorbance values at 400 wavelengths (one datapoint every 4 nm). As the mule duck is a hybrid duck, the progeny of a Muscovy drake with a common duck female, genetic parameters were estimated on both parental lines by Gibbs sampling using the software gibbsf90, for each of the 400 absorbances and for the liver melting rate. Heritabilities of the absorbances along the NIR spectra varied between 0.05 and 0.19 with values significantly higher on common ducks versus Muscovy ducks. FOSS and ASD spectra have very different heritability patterns. Genetic correlations between melting rate and absorbances are similar between FOSS and ASD spectrometers. Moreover, there is a great similarity of the genetic correlations in the two parental populations, except for two discrepancy areas specific to spectrometers.

Effects of genetic by nutrition interaction on poultry production sustainability

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P-33. Selection is a powerful tool to improve broiler performance under optimal environments. As the cost of the diet increases, birds' capacity to digest alternative feedstuffs is of interest. By selecting broilers for high (D+) or low (D-) digestive efficiency, we showed that selection could improve this capacity. However, before introducing this trait in selection schemes, it is necessary to check whether this selection could negatively impact performance, environment, health and welfare and therefore poultry production sustainability. We studied the interaction between genotype (D+/D-) and diet (easy/difficult) to evaluate how it affects sustainability criteria through economic (growth, body composition, meat quality), environmental (litter, air quality) and welfare (behavior, pododermatitis, resistance/susceptibility to colibacillosis and coccidiosis) parameters. A multifactorial correspondence analysis showed that D+ birds were much less susceptible to diet change than D- birds, whatever the trait recorded. Moreover, except for air quality, D+ birds showed equal or better performances than D- birds. Including digestive efficiency in selection would thus not deteriorate any of the main components of poultry production sustainability.

Genetic selection on home pen locomotor activity in chickens

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P-34. It has been suggested that feather pecking in laying hens might be genetically linked to their activity level (Kjaer, 2009). If feather pecking and activity are genetically correlated, selection for increased activity should lead to higher levels of feather pecking. Before this hypothesis can be tested, lines genetically selected on locomotor activity need to be developed. Hitherto such selection was done in experimental test cages but here, for the first time, selection was performed on undisturbed locomotor behaviour in the home pen. Two selection lines were developed from a New Hampshire control line. They were selected on low (LA) or high (HA) levels of activity respectively. In each generation and line, 10 sires and 20 dams produced an average of 200 offspring for selection. In S4, 850 chickens of all three lines were phenotyped for comparison. Activity was recorded over 5 days in the home pen at 5 weeks of age, using a transponder system (Kjaer, 2009). Response to selection was good and fairly symmetric, with activity in LA and HA being 58% and 28%, respectively, relative to the control line. Contrary to the expectation, the low activity chickens had lower (GLM, $F_{2,850}$=96, $p<0.001$) BW at 5 weeks of age compared to the C and HA chickens, which did not differ significantly (368 g, 413 g and 407 g, respectively).

Development of a new colour feathered broiler for free range farming systems

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P-35. The growing ability of a new hybrid, Tetra HB Color was tested. In trials 1 and 2, 2868 birds in total were reared intensively. In part 3, 120 birds were placed outdoors at 7 weeks of age, while the rest remained indoors. In trial 1 and 2, parent and crossed lines (EE, HH and EH), in trial 3, the new hybrid and two controls (Tetra-H, Shaver Farm) were used. The pure sire line (EE) had the highest carcass and slaughter yield, but alsothe highest abdominal fat in both sexes ($p<0.05$). The dam line (HH) showed poorer meat producing ability. Slaughter yield was highest in HB Color in trial 3 compared to Shaver Farm at 7 weeks of age (71.06 and 69.63%, 69.93 and 69.36% in males and females, respectively), but not significantly ($p>0.05$). Abdominal fat was lower in birds kept outside at both slaughter age, however, other slaughter parameters were not effected by the keeping method. We concluded that market weight (1700 g alive) can be reached at 7 weeks of age for Tetra HB Color and Shaver Farm, while up to 12 weeks was needed for slow growing genotypes. Mortality was also lower in free range birds and they generally looked healthier than their counterparts left indoors.

New shell color evaluation method for more accurate selection in brown layer lines

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P-36. Colour is a very important aspect for egg consumers, as it is often related to its quality. Breeding companies all make egg quality measurements, using various methods to obtain data. For egg shell colour, CIELab system is the most commonly used. The data collected is routinely transformed to a so called shell color index (SCI). However, by using SCI, individual differences in L*, a*, b* values could remain hidden. Freshly laid eggs of 7692 hens from 3 pedigree lines (A, B and C) were used for examination. L*, a*, b* coordinates were determined and upper and lower tolerance limits (TL) and standards for each lines were generated and compared to the SCI values. We have found significant differences in the variance of L* and a* values between the three lines. No differences were determined for b* values. Upper limits of L* and a* values were higher ($p<0.05$) in line C. Lower L* and a* limits determined for line B differed significantly from the other lines. Fewer hens were measured up to the standard with the TL method, although it was not significant. It was determined that, while SCI is widely used in practice, the TL method is probably a more accurate way for measure egg shell colour during pedigree selection programs.

Cannibalistic hens are not less “friendly”

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P-37. Beak trimming is painful (sense organs and nerve cells are removed). After trimming neuroma's may develop that cause lifelong phantom pains, touch stimuli may also be experienced lifelong as pain. Selection against feather pecking and cannibalism has therefore urgency. Feather pecking is disturbed feeding behaviour performed by particular individuals. These individuals must be selected out. A possible procedure is to put a hen of a line under selection (selection hen) in a cage with a hen of a line known for little feather pecking (standard hen). By scoring the standard hens that did not survive it is possible to select out the feather pecking selection hens. Eventually a short fast may be introduced to induce feather pecking. When white and brown hens are used simultaneous selection on production traits is possible. This procedure does not imply time consuming behavioral observations. Therefore it may be used under commercial breeding conditions. In the Netherlands (Bijma et al., 2007. *Genetics* 175:289-299) hens are selected against feather pecking and cannibalism on the basis of the myth that these are social behaviours, aggressive behaviours connected with the pecking order, that feather peckers are “less friendly.” However, aggression is always directed towards the head, feather pecking to other parts of the body. Flocks with much aggression do not necessarily have much feather pecking. This last approach is inevitably inefficient.

"This is not a chicken"

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The Belgian artist Koen Vanmechelen (1965) is an internationally renowned, conceptual artist who has presented his work on almost all continents, from the U.S. to China and Iceland to Senegal. He is mostly known for his Cosmopolitan Chicken Project. An artistic-scientific breeding project, which he started in 2000. In this millennium year he made his first crossbreeding between two chicken species; the Mechelse Koekoek (BE) and the Poulet de Bresse. Since then, 17 generations of "Mechelse" chickens came into existence, the Mechelse Styrian being the latest one. The ultimate result of this project is a truly cosmopolitan chicken that carries the genes of all of the world's breeds.

With the CCP, Vanmechelen positions art categorically where it belongs: in the middle of society, between people, and always committed. His oeuvre is as diverse and hybrid as the cosmopolitan chicken itself: a unique mix of painting, drawing, photography, video, installations and wooden sculptures, whose unifying theme is always the chicken and the egg. But nothing is what it seems. The core of the project is neither the chicken nor the egg, but crossbreeding and the diversity that comes from it. In this way, the artist uses the animal and his offspring as important symbols that allow him to make links between scientific, political, philosophical and ethical issues. The intrinsic philosophical system he thus developed is not only translated in his work but is also often the subject of debates, discussions and lectures by the artist.

During these lectures, Koen Vanmechelen explains his Cosmopolitan Chicken Project thereby introducing the listener to a world characterized by a symbiosis between art, science, philosophy, politics and ethics. A new way of reflecting on existential questions and on contemporary issues that relate to, among others, diversity, globalization, racism, genetic modification and cloning. In 2008, for example, he was invited at the World Economic Forum in Davos to share his perspective on diversity and in 2010 he spoke at the TEDxConference. But he also presented his project and vision at the World Expo in Shanghai, the Biennial of Venice, the Climate Change Congress and the Creativity World Forum. "Because cross-breeding is the one thing. We need to cross breed across the borders if we do not want the world to perish. We need to think cosmopolitan. Nothing is as beautiful as joining with other cultures and taking energy from it."

2013

- The Cosmopolitan Chicken Project, Galerija Kapelica, Ljubljana (SI)
- When happiness happens, BOZAR (in cooperation with United Nations), Brussels (BE)
- The Cosmopolitan Chicken Project, University of Kortrijk, Kortrijk (BE)
- The Cosmopolitan Chicken Project, Community Center De Markthallen, Herk-De-Stad (BE)
- The Cosmogolem Foundation, JOC De Kouter, Poperinge (BE)
- The Cosmopolitan Chicken Project, University of Mechelen, Mechelen (BE)
- The Cosmopolitan Chicken Project, Het Paleis, Antwerp (BE)
- The Cosmopolitan Chicken Project, EuropeN, European Economic and Social Committee, Brussels (BE)
- The Cosmopolitan Chicken Project, Cranbrook University, Detroit (US)
- The Cosmopolitan Chicken Project, Wayne State University, School of Medicine, Detroit (US)
- The Cosmopolitan Chicken Project, Museum of Contemporary Art, Detroit (US)
- The Cosmopolitan Chicken Project, Day for Cultural Education (by the Flemish Government, Ghent (BE)

2012

- The Cosmopolitan Chicken Project, Tori Oso (SR)
- The Cosmopolitan Chicken Project, COMBAT Alden Biesen, Bilzen (BE)
- The Open University of Diversity, Z33, Hasselt (BE)
- The Open University of Diversity, TEDxYouth Flanders, Antwerp (BE)
- The Open Univeristy of Diversity, Nanjing Agricultural University, Nanjing (CN)
- This is not a Chicken, World Appreciative Inquiry Conference, Ghent (BE)
- The Cosmopolitan Chicken Project, Genetic Freedom, Europe (to the power of N), Berlin (DE)
- The Cosmopolitan Chicken Project, Bioethics Congress, Rotterdam (NL)
- The Walking egg, Unite for Sight Global Health & Innovation, Yale University (US)
- Artist talk met Marcel Pinas, VUB university, Brussels (BE)

2011

- This is not a chicken, Galerie Für Zeit Genössische Kunst, Leipzig (DE)
- Modified Spaces –C.C.P., with Peter Noever, Guanzhou Museum of Art, Guangzhou (CN)
- In-Vetro – C.C.P., Symposium Transparent vision – the art and science of glas, Kijkduin Biënnale (NL)
- The Open University of Diversity, Creativity World Forum, Hasselt (BE)
- The Chicken's Appeal, Pecha kucha, Brussels (BE)

2010

- The Chicken's Appeal, TedxFlanders, Antwerp (BE)
- The Cosmopolitan Chicken Project, European Conference On Computational Biology, Ghent (BE)
- The Cosmopolitan Chicken Project, Belgian pavilion World Expo, Shanghai (CN)
- Arts meets Science, Doctor Honoris Causa, Faculty of Medicine UHasselt, Hasselt (BE)
- The Accident, Debate with Professor J.-J. Cassiman, Dr. Mike Philips, Dr. Luc Vrielinck and Peter Adriaenssens, moderator: Indra Dewitte, Museum M, Leuven (BE)
- The Cosmopolitan Chicken Project, PULSE New York (US)

2009

- 3rd Moscow Biennale of Contemporary Art, Moscow (RU): "The Chickens appeal"
- 53e Biennale di Venezia, Venezia (IT): "The Cosmopolitan Chicken Project"
- Janssen-Cilag Pharmaceutical company, series of ten lectures: "The Cosmopolitan Chicken Project", art and science

2008

- World Economic Forum, Davos (CH), debate: "The Cosmopolitan Chicken Project"
- Climate Change Congress, Copenhagen (DK): "The Cosmopolitan Chicken Project", Culture and Nature Balance
- 24th Los Angeles Art Show, Los Angeles: debate with Dr. Mike Phillips
- Day of Hope, Mumbai (IN), Jeanne Devos Foundation, in presence of Princess Mathilde: Cosmogolem
- Conner Contemporary Art, Washington (US): "The Cosmopolitan Chicken Project"
- Creativity world forum, Lotto Arena, Antwerpen: "The Cosmopolitan Chicken Project", art and innovation
- Rijksuniversiteit Groningen, Groningen (NL): "Social Control"
- University of Greenwich, Londen (UK): "The Chickens appeal"
- Vlaams Wetenschappelijk Economisch Congres, K.U.Leuven – faculteit economie, Leuven
- Kabinet Patricia Ceysens, Brussel: "The Cosmopolitan Chicken", DNA-research
- 30th Arts & Buisiness Awards, Londen (UK): "The Cosmopolitan Chicken Project"
- Victoria and Albert Museum, Londen (UK): "The Cosmopolitan Chicken Project"
- Kunst Palast, Düsseldof (DE), "The Cosmopolitan Chicken Project"

2007

- Expert meeting "Fertility in Developing Countries", Arusha (TA): "The Walking Egg"

2005

- "Child abuse neglecting the facts", K.U.Leuven with Peter Adriaenssens, Leuven: CosmoGolem

2004

- The Jacobs Foundation, Zurich: "CosmoGolem and Cosmopolitan Chicken Project"

2002

- Natural History Museum, Londen (GB), art and antropology

1998

- World Congress of Philosopy, University of Island: "The Cosmopolitan Chicken Project"