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26 – 28, June 2017

Saint-Malo, France

WPSA France

Proceedings of the
10Th European Symposium on Poultry Genetics

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SAINT-MALO, FRANCE

Edited by

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WPSA France

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Foreword

Dear friends and colleagues,

The French branch of WPSA and the Working Group 3 "Breeding and Genetics" of the European Federation of branches are happy to welcome you at the conference center of Saint-Malo for the 10th edition of the European Symposium on Poultry Genetics. Whereas this symposium takes place for the first time in France, it inherits from a long tradition of meetings gathering scientists and industry, from the British Poultry Breeders Roundtable, followed by the European Poultry Breeders Roundtable, and the Aviagen meeting, which merged in 1997 under the umbrella of WPSA.

We would like to express our thanks to the European Federation of branches and to our sponsors for their support.

We have kept the format used in previous symposiums. The program features 14 invited talks presenting the state of the art and the perspectives on current issues in poultry breeding, 3 talks on hot topics for the industry session, 26 posters with a viewing session introduced with a one-slide oral presentation for each poster, and a PhD fellowship session giving the opportunity to PhD students to present their work.

The scientific committee has set up a session on animal welfare and health, to make a thematic transition with the European Symposium on Poultry Welfare which takes place the preceeding week, also in the Brittany region. Why two WPSA symposiums in the same region? Brittany is a region with a strong poultry sector and it also harbors major companies in poultry breeding. Brittany also harbors beautiful coastal landscapes and the city of Saint-Malo is both a historical city and a holiday place.

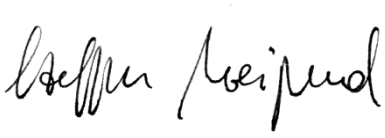
We hope you will enjoy both the scientific program and the beautiful surroundings.

Michèle Tixier-Boichard

Vice-President of the French WPSA branch

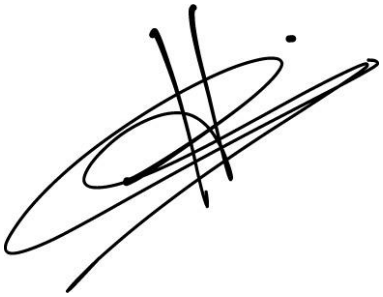
Steffen Weigend

Chair of WPSA Working Group 3



Christophe Bostvironnois

President of the French WPSA branch



Scientific programme committee

Chairs	Michèle Tixier-Boichard - INRA, France, vice-president of the French WPSA branch Steffen Weigend Friedrich-Loeffler-Institut, Germany, chair of WPSA WG3,

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Martino Cassandro	University of Padova, Italy
David Cavero	Lohmann Tierzucht GmbH, Germany
Olivier Demeure	Grimaud Frères, France, co-chair of the organizing committee
Daniel Guéméné	SYSAAF– INRA, France, member of the scientific committee of the Poultry Welfare Symposium
Paul Hocking	Roslin Institute, University of Edinburgh, United Kingdom
Yves Jego	Hubbard SAS, France

Local organizing committee

Christophe Bostvironnois	Elanco, President of the French WPSA branch
Olivier Demeure	Grimaud Frères, co-chair of the organizing committee
Catherine Hamelin	CCPA, Treasurer of the French WPSA branch
Christine Lessire	Editorial assistant, French WPSA branch
Michèle Tixier-Boichard	INRA, chair of the organizing committee

Certificate of attendance

Upon request a ‘Certificate of Attendance’ will be handed out to participants at the Registration Desk.

INFORMATION about the VENUE

The City of Saint-Malo (map)

Thanks to their rich heritage and the beautiful landscapes of the Emerald Coast, Saint-Malo and its region are a destination which remains engraved on the memory and the imagination of the visitor.
A town of artistic interest, Saint-Malo has a turbulent maritime history.

Chateaubriand, Surcouf, Jacques Cartier… Whether they were writers, privateers or sailors, Saint-Malo was the cradle of many brave hearts. Just like them, proud and independent, the Corsair city erects its forts and its ramparts in full view of the sea, and appeals both by its character and its exceptional setting.

To visitors and conference delegates alike, it offers the beauty of its seascapes, the wealth of its historical heritage and the friendliness of its inhabitants, proud of their town and happy to welcome large conferences.

A town of 49,000 inhabitants lively all year long, it lives to the rhythm of the large events that it welcomes, internationally famous Water Festivals or Races and, in particular, “Etonnants Voyageurs” and the “Route du Rhum”.

The Conference Centre : Palais du Grand Large

The Grand Large makes sure your event takes place right at the heart of this bustling town. When you get here, put away your luggage and forget the logistical problems. Located in the immediate vicinity of the historic walled town, the train station, hotels and restaurants, the Conference Centre is an ideal starting point for many activities: a few minutes’ escape to the beach, a stroll on the ramparts, a seafood meal, shopping, etc.

Internet access

Internet is available via WiFi on **WPSA** terminal and password **ESPG2017**

Lost and found

Please visit the Registration Desk for lost and found

Smoking areas

Smoking is prohibited in all facilities

Meeting policies

Photographing and audio recording without permission are prohibited.

Please set your cell phones into silent mode or turn off during the sessions.

PhD awards

Since 2015, the policy of Working Group 3 is to offer free registration to a few PhD students submitting an abstract.

The aim is to support scientists in the early stage of their career and promote their participation to conferences in order to meet the scientific community as well as the poultry breeding companies, which may be interested in recruiting young talents.

PhD students or young Post-docs were eligible. The deadline was March 15, 2017. Candidates were invited to declare themselves on the registration form.

All participating abstracts were evaluated by the international scientific committee. It was quite difficult to separate the candidates so that 5 fellowships were attributed, however the 2 French candidates were allocated a half-fellowship, considering the low cost of traveling for them. In addition, a candidate from Finland was supported by the LUKE institute, which organised the previous ESPG in 2015 and used the left-over to cover one registration fee.

The winners will give a 10 min oral presentation of their work on June 27, 11:20-12:30.

Recipients of the PhD fellowship (by alphabetical order of family name)

Olubunmi DUDUYEMI
A review of performance differences between the Nigerian indigenous and the commercial layers in sub-optimal conditions

Marja HEIKKINEN, supported by the organisers of the 9th ESPG
Genomic analysis reveals a spectrum of hybrid background in European domestic geese and their wild progenitor (Anser anser)

Florian HERRY
Design of a low density SNP chip for genomic selection in layer chicken

Dorcus Kholofelo MALOMANE
The effectiveness of different filtering strategies to reduce the effects of ascertainment bias when using SNP panels in a chicken diversity study

Eva PAMPOUILLE
Mapping QTL for white striping in relation to breast muscle yield and meat quality traits in broiler chicken

Angus REID
Selection for low cholecystokinin A receptor (CCKAR) expression in fast-growing broiler chickens

PROGRAMME

Monday June 26

12:30 Lunch at the Conference Center for all participants

13:50 **Opening of the symposium**
Michèle Tixier-Boichard and Steffen Weigend

14:00-16:40 **Session 'One health'**

Part 1 Genetics and welfare Chair Michèle Tixier-Boichard

14:00 -14.40

Werner Bessei: *Institute of Animal Husbandry and Breeding, University of Hohenheim, Germany*
Genetics of welfare: a comprehensive study of feather pecking in laying hens

14:40-15:20

Bertrand Bed'hom: *GABI, INRA, AgroParisTech, University Paris-Saclay, Jouy-en-Josas, France*
An overview of the trade-off in resource allocation between production and immunity traits in chicken

Part 2 Biotic and abiotic stresses Chair Olivier Demeure

15:20-16:00

Androniki Psifidi *The Roslin Institute and Royal Dick School of Veterinary Studies,*
University of Edinburgh, Roslin, and Royal Veterinary College, University of London, Hatfield, UK.
Mapping disease resistance in poultry

16:00-16:40

Susan Lamont *Department of Animal Science, Iowa State University, Ames, USA*
Genomics of response to abiotic and biotic stressors in chickens

16:40-17:00 coffee break

17:00-18:00 : Poster Session **Chair Steffen Weigend**
Each poster will be presented with one slide for 2 minutes.

18:00-21:00 : Poster viewing
and **cocktail diner in the Conference Center for all participants.**

Tuesday June 27 – morning

9:00-11:00 Session 'New Breeding Technologies' chair Paul Hocking

9:00-9:40
Benjamin Schusser: *Reproductive Biotechnologie, TU Munich, Freising, Germany*
Genome editing in birds - from concept to reality

9:40-10:20
Mike Mc Grew: *The Roslin Institute and Royal Dick School of Veterinary Studies,*
University of Edinburgh, Roslin, UK
Biobanking of poultry breeds using primordial germ cells

10:20-11:00
John Hickey: *The Roslin Institute and Royal Dick School of Veterinary Studies,*
University of Edinburgh, Roslin, UK
Potential of genome editing and gene drive technologies to increase genetic gain in livestock breeding programs

11:00-11:20 coffee break

11:20-12:30- PhD Awards session chair Michèle Tixier-Boichard

Olubunmi Duduyemi: A review of performance differences between the Nigerian ndigenous and the commercial layers in sub-optimal conditions

Marja Heikkinen: Supported by the organisers of the 9th ESPG
Genomic analysis reveals a spectrum of hybrid background in Europeandomestic geese and their wild progenitor (Anser anser)

Florian Herry: Design of a low density SNP chip for genomic selection in layer Chicken

Dorcus Kholofelo Malomane: The effectiveness of different filtering strategies to reduce the effects of ascertainment bias when using SNP panels in a chicken diversity study

Eva Pampouille: Mapping QTL for white striping in relation to breast muscle yield and meat quality traits in broiler chicken

Angus Reid: Selection for low cholecystokinin A receptor (CCKAR) expression in fast-growing broiler chickens

12:30 Lunch for all participants in the Conference center

Tuesday June 27 – afternoon

14.00-15:20 Session 'Genomics **chair Steffen Weigend**

14.00-14:40
Mahendra Mariadassou : *MAIAGE, INRA, Université Paris-Saclay, Jouy-en-Josas, France*
Genomics of chicken domestication

14.40-15:20 *Hy-Line International*
Janet Fulton: *, Dallas Center IA, USA*
MHC-B diversity of domestic chickens

15:20-16:00 coffee break and last chance for poster viewing

16:00-17:30 Industry session **co-chairs Pieter van As and David Cavero**

16:00-16:30
Grégoire Leroy: *Animal Production and Health Division, FAO, Rome, Italy*
New issues for dual-purpose chicken breeding for small producers

16:30-17:00
Rudolf Preisinger*:* *EW Group GmbH, Visbek, Germany*
Solutions from the industry: dual purpose breeds or sex determination in the egg

17:00-17:30
Frédéric Fagnoul: *Hubbard, Chateaubourg, France*
Breeding for Premium broiler markets: Chicken of Tomorrow case study

17:30-18:30 Business meeting of Working Group 3 **chair Steffen Weigend**

As usual, the meeting is open to all congress participants and will take place in the auditorium.

We will welcome colleagues from the Czech branch who will present their invitation to host the 11th ESPG.

19:00 – 24:00
Gala dinner in the Conference Center

Wednesday June 28

9:00-10:20 Session 'Genetics of product quality' chair Martino Cassandro

9:00-9:40
Elisabeth Le Bihan-Duval: *URA, INRA, Nouzilly, France*
Genetics of meat quality defects in broilers

9:40-10:20
Ian Dunn: *The Roslin Institute, University of Edinburgh, Edinburgh, Scotland, UK.*
Improving egg quality: win-win traits

10:20-10:40 : coffee break

10:40-12:30 Session 'Breeding issues for other species' chair Daniel Guémené

10:40-11:20
John Ralph: *Aviagen Turkeys Ltd, Chowley Five, Chowley Oak Business Park,Tattenhall, Cheshire, UK*
Current and future challenges for European turkey breeding

11:20-12:00
Alain Vignal : *GenPhySE, Université de Toulouse, INRA, INPT, INP-ENVT, Castanet Tolosan, France*
Guinea fowl whole genome assembly and application for genetic
diversity in African and European populations

12:00-12:30
Guillaume Le Mignon: *Grimaud Frères Sélection, Sèvremoine, France*
Genetic parameters of electronically recorded feeding behavior traits
in meat Pekin ducks

12:30 Closing remarks Steffen Weigend

Lunch at the conference center for all participants

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Posters Abstracts

Session - One Health

Genetics and Welfare

Genetics of welfare: a comprehensive study of feather pecking in laying hens

Bessei, Werner,* Joergen B. Kjaer,† Vanessa Lutz,* Michael A. Grashorn* and Joern Bennewitz*

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Abstract

Feather pecking is a serious problem in poultry production. It causes high economical losses and suffering in the affected birds. In spite of intensive research, the causes of this damaging behaviour are not fully understood. Genetic studies revealed sufficient genetic variation for selection against the damaging behaviour. However, feather pecking is a complex behaviour and more detailed information on the genetic background of the motivation is required to successfully implement this trait in breeding programmes. The prevailing hypothesis explains feather pecking as misdirected foraging behaviour, but other motivations, such as feather eating, aggression, fear and general locomotor activity may be involved. The interrelationships of feather pecking and the above mentioned behaviours have been studied using more than 900 birds of a F2-cross of two lines which have been selected for high and low feather pecking. Heritability, genetic and phenotypic correlations between the traits have been estimated using standard statistical models. In addition, Structural Equation Models (SEM) were applied to estimate putative causal relationships between feather pecking and other traits. Genetic correlation and Lambda coefficients showed a strong causal effect of feather eating and feather pecking. This supports the hypothesis that feather eating represents a primary cause of feather pecking. There was a substantial causal influence of aggression and general locomotor activity on feather pecking. Open-field activity (fear) and foraging in contrast did not show clear effects on feather pecking. Significant SNPs were identified for feather pecking and aggressive pecking in a genome-wide association study, using data of 817 birds of the F2-cross. Some of SNP clusters indicate relationships with physiological effects of monoamines, which are involved in the development of feather pecking and aggression. This information will be helpful in selection against the damaging behaviours in commercial layer lines.

Key words: Laying hens, feather pecking, aggression, fear, activity, foraging

Introduction

Welfare has become a major welfare issue in poultry production in industrialised countries. Intensive management systems and selection for production traits are assumed to compromise animal welfare. In the past high performance has been considered as an indirect criterion of welfare. There is however, increasing awareness of the fact that selection for growth or laying rate may lead to welfare problems, and the responsibility of scientists for the wellbeing of farm animals has been expressed in particular by the Precision Animal Framework concept of Flint and Woolliams (2007)**. Economic and welfare-related criteria are not necessarily in contrast. Damaging feather pecking and cannibalism for instance, represent severe welfare problems in poultry and have important economic implications. The problem will become more important when beak trimming is banned for welfare reason. Recent experiments with intact-beak birds have shown that management procedures may**

attenuate feather pecking but do not prevent it. Since feather pecking has a genetic component, methods are sought to include this behaviour in selection programmes. However, feather pecking is a complex behaviour and indirect genetic effects have been found to play an important role in its expression (Ellen et al., 2008). Understanding the causes of feather pecking is a prerequisite to solve the problem. In the following we present quantitative genetic analyses of feather pecking and causal relationships between feather pecking and selected behavioural criteria using structural equation models as introduced by Gianola and Soerensen (2004) in animal breeding. We will further present results from genome-wide association studies for feather pecking and aggression which may be used to support genetic selection against feather pecking in breeding programmes.

Hypotheses on feather pecking. Despite extensive research during the last decades the underlying causes of damaging feather pecking are not known. There exist however various hypotheses on its motivation. The most widespread theory relates feather pecking with feeding and feed searching (foraging). According to the foraging theory it is assumed that foraging behaviour is redirected towards the feathers of group mates (Blokhuis, 1986). The foraging theory however, has failed to explain feather pecking in various studies (Hocking et al., 2004; Newberry et al., 2007). Bessei and Kjaer (2015) proposed feather eating as primary underlying motivation. Other motivations for feather pecking which are under discussion are aggression (Bessei et al., 2013), fear (de Haas et al, 2010) and spontaneous locomotor activity (Kjaer, 2009). Observations of feather pecking (FP), feather eating (FE), aggressive pecking (AP), open-field activity (OFA), general locomotor activity (GLA) and foraging (FOR) have been observed in a large F2-cross population (> 900 birds) originating from two lines selected for high and low feather pecking (Bennewitz et al., 2014). Standard multi-trait models (SMS) and structural equation systems (SMS) have been used to estimate genetic parameters and to discover causal relationships between the above mentioned behaviours. Sets of three behaviour traits each were used. Only behaviours with assumed causal relationships have been chosen.

Feather pecking, feather eating and aggressive pecking. Previous studies have shown that feather pecking and feather eating are closely related. On the basis of previous information, we hypothesised that FE influences FP and AP, and that AP influences FP. The results of quantitative genetic analyses are shown in table 1. The heritability estimate for FP varied between 0.11 and 0.20, depending on the statistical model. FE showed considerably higher heritability (0.36 – 0.57). There were very high genetic correlations of FP with FE and AP (0.73 and 0.83 resp.). All phenotypic correlations were positive but on a lower level than the genetic correlations. The estimated causal effect of FE on FP, λ(FP,FE) was 5.94. For the influence of feather eating on aggressive pecking λ(AP,FE) and of aggressive pecking on feather pecking λ(FP,AP), the λ- values were positive (0.11 and 0.23) but considerably lower than for the effect of feather eating on feather pecking. The results confirmed our hypothesis that FE is a primary motivation for FP. The effect of FE on AP was also low. The high genetic correlation between feather pecking and aggressive pecking and the positive λ(FP, AP) show that the influence of aggression has been underestimated in the past. Though aggression and feather pecking are different in their motoric pattern and the underlying motivation, aggression may reinforce the expression of FP. Parts of the results are described in detail by Grams et al. (2015).

Feather pecking, feather eating and general locomotor activity. Locomotor activity is usually activated through numerous different motivations, such as exploration, aggression, egg production, flight. Locomotor activity can also be hampered by fear. Kjaer (2009) hypothesised that feather pecking may be the result of a hyperactivity disorder. Recording of locomotor activity in pullets of the above mentioned F2-cross were used to test this hypothesis (Lutz et al., 2016). The heritability of GLA was 0.29 and both, phenotypic and genetic correlations were positive (0.16 and 0.47) (table 1).

Table 1 Heritabilities (h^2), phenotypic (r_p) and genetic (r_g) correlations (standard error in brackets) of feather pecking (FP) with other behavioural traits and λ estimates of related traits on feather pecking in a F2-cross of lines selected for high and low feather pecking. Estimates from different sources vary in response to different statistical models.

Trait	h^2	r_p	r_g	λ
Feather pecking (FP)	0.11 [1)] 0.14 (0.06) [1)] 0.20 (0.07) [2)]			
Aggressive pecking (AP)	0.11 [1)] 0.27 (0.07) [4)]	0.81 [1)]	0.34 (0.00) [1)]	0.23 [6)]
Feather eating (FE)	0.57 [1)] 0.37 (0.09) [5)]	0.36 (0.00) [1)] 0.37 (0.09) [5)]	0.73 [1)] 0.13 (0.27) [5)]	5.71 [5)] 5.94 [6)]
General locomotor activity (GLA)	0.29(0.08) [5)]	0.09 (0.04) [5)]	0.12 (0.27) [5)]	1.20 [5)]
Foraging (FOR)	0.00 [3)]	0.07 (0.03) [3)]	No estimate [3)]	0.004 [3)]
Open-field activity (OFA)	0.14 (0.06) [4)] 0.21 (0.09) [3)]	-0.03 (0.04) [4)] -0.02 (0.03) [3)]	0.03 (0.32) [4)] 0.26 (1.48) [3)]	0.02 [3)]

[1)]Bennewitz et al. (2014) [2)]Lutz et al. (2016) [3)]Bessei et al., in prep. [4)]Grams et al. (2014) [5)]Grams et al (2015) [6)] unpublished results

Regarding the causal associations between the traits we hypothesized that GLA influences FP and that FE influences FP and GLA. Structure coefficients supported a causal influence of GLA on FP (λ = 1.20) and of FE on FP (λ= 5.71). The results support the hypothesis that GLA influences FP.

Feather pecking, foraging and fear. Foraging (FOR) and fear play an important role in the surviving strategies of feral animals. High fear levels inhibit exploration and, thus, exploitation of unknown feed resources. However, fear is essential for survival under high pressure of predators. Hence the foraging activity observed under natural conditions is considered a compromise of exploration and fear. The open-field test has frequently been used in laboratory animals and in the domestic fowl. This test was considered suitable for the present study because high OFA in young chicks was reported to be predictive for low incidence of feather pecking (Rodenburg et al., 2004). We hypothesised that OFA and FOR influence FP. The heritability of FOR was zero (table 1). Therefore, no genetic correlations could be estimated for this trait. The heritability of OFA was 0.21 in this study and 0.14 in an earlier study. Both phenotypic correlations between FP and OFA and one of the genetic correlations were close to zero. Another genetic correlation was relatively high (0.26). This value was however, not reliable for an extremely high standard error (1.48; not shown in table). All SEM-λ-coefficients were close to zero (data not shown). The results show that the assumed role of foraging and fear on feather pecking as influencing factors for feather pecking have been over-estimated. In contrast to the prevailing hypotheses neither FOR nor OFA showed a relevant causal effect on FP in our large data set.

Genomic analysis of feather pecking and aggressive pecking. A total of 817 hens of the F2-cross was genotyped using the Illumina 60k Infinium iSelect chip. After filtering (exclusion of SNPs on the sex chromosomes, SNPs not allocated to a particular chromosome or linkage group, monomorphic loci, SNPs with call rates below 0.95) 29376 SNPs remained in the data set. Single marker association analysis was conducted using a generalized linear model (i.e. a Poisson model to account for the count-type data structure). In a second step a meta-analysis was performed merging data from a GWAS study with those obtained from a selection signature experiment using the divergent selected lines (Grams et al. 2015). Clusters of SNPs were built with a minimum of two significant SNPs ($p <= 5x19^{-5}$) with maximum distance of 3 Mb. Nine genome-wide significant SNPs were identified for feather pecking and four for aggressive

pecking (Lutz et al. (2017). Most significant SNPs were located in clusters. One of the clusters contained a potential candidate gene which is related with the monoamine function of the monoamines serotonin and dopamine, which have been reported to be involved in the development of feather pecking and aggression. Further analyses of the genomic data will reveal other functional gene associated with feather pecking and other influencing behaviours.

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An overview of the trade-off in resource allocation between production and immunity traits in chicken

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Keywords: trade-off, production, immunity, chicken

Trade-offs in evolutionary biology

In biology, trade-offs are ubiquitous because every biological function has a cost associated. They represent the equilibrium achieved by the organism in the allocation of the available resources (nutrients, energy) among consuming processes (i.e. growth, reproduction, immune response etc.), which cannot be simultaneously optimal because the improvement of one function is detrimental to others (Stearns, 1989).
In all organisms natural selection has been acting to maximize fitness (i.e. the efficiency of gene transmission to the next generation) since the beginning of life on earth several billion years ago (Dawkins, 1976). In wild species, the constantly changing environment requires a permanent and dynamic evolution of individuals to improve their fitness (Red Queen hypothesis, Van Valen, 1976). As a consequence, the biological mechanisms, including genetic polymorphisms and trade-offs, which affect fitness variability, have gone through a long period of selection, and consequently, of evolution. In natural conditions, trade-offs are selected toward intermediate values, as selection for extreme traits could compromise the survival or the fitness when the environment is limiting (Beilharz et al., 1993).
These trade-offs between different physiological compartments have been largely studied in wild bird species (Lochmiller et al., 2000). By measuring the consequences of a strong immune challenge on life-history traits these studies demonstrated the existence of trade-offs between immune response and growth rate or reproduction (Nilsson 2003, Mauck et al., 2005).

Trade-offs in chicken

For some species, as the chicken, the domestication process that occurred several thousand years ago, determined that the animal's rearing environment and their individual fitness are strongly influenced (when not determined) by human choice (selective breeding). In this new and more controlled environment, the balance between processes involved in trade-offs has shifted toward a new equilibrium favouring domestication-related traits, characterized by changes in animal's size/morphology, reproduction (precocity, prolificacy, less or no seasonality), behaviour (fearness, sociality), increased production, etc.
The strong selection for intensive, efficient and specialized production has exacerbated the trade-offs among competing animal functions. Genetic selection has been extremely powerful in improving growth rate, meat or egg production and feed efficiency over the last 50 years. The study of Havenstein et al. (2003) showed that modern broilers, compared to a chicken strain from the fifties, require approximately one-third of the time to reach the weight of 1.8 kg (32 vs. 101 day), while having a threefold lower feed conversion ratio (1.4 vs. 4.4). However, this selection toward highly improved performances led to many correlated responses in farm animals, some of them probably detrimental to other processes, like immune function (Bayyari et al. 1997, Cheema et al., 2003).

Van der Most et al. (2011) in their recent meta-analysis, based on data from 14 studies on three different poultry lines, showed that the selection for growth decreased immune function of an average effect size (ES) of -0.8. In turkey they reported a detrimental effect of selection for body mass for both the humoral immune response (ES=-0.65 P=0.022) and cellular immunity (ES =-0.81, P= 0.09), leading to the ineluctable conclusion that selection for accelerated growth has a large negative effect on resistance/immune functions.

Is selection for immune response detrimental to production?

In the same study, Van der Most et al. (2011) looked if the improvement of immune function had a consequence on production. The results showed no significant overall effect, but within the selection lines, the results were heterogeneous. Some supported a reduced growth when selecting for increased immune functions, and other showed a significant effect in the opposite direction.

Several studies have reported that the cost of immune responses have a negative long-term effect on growth. In an ongoing project in our group, we compared growth and egg production between a line selected, since 1996, for antibody response 3 weeks after vaccination against Newcastle disease virus (ND3) and its control line (Pinard-van der Laan, 2002). We found significant body weight reduction at 10 weeks of age (-7%) for the NDV line, while no differences were observed between the two lines on egg production and age of first egg (in prep.). Similarly, Gross et al. (2003) showed that selection of a Leghorn population for high SRBC antibody response resulted in a reduced body weight, and Warner et al. (1987) described that selection for resistance to Marek's disease in chickens induced a reduction in adult body weight and in egg weight as compared to unselected lines.

These results support the idea that selection for improved immune functions is possible but it has to be done with caution in order to minimize the negative effect on production. First of all, "immune function" cannot be treated as one trait because the different arms of the immune system are not genetically correlated (Pinard-van der Laan, 2002). This means that the increased response of one immune compartment (innate, humoral or cellular) does not reflect necessarily the overall immune function improvement. Secondly, broilers and layers do not seem to employ the same immunity responses, which is not so surprising considering that time scale of the production investments differs between the two types of chicken.

A study investigating broiler and layer immunological differences suggested that broilers are more specialized in short-term humoral immune responses, while layers are more specialized in long-term humoral and cellular immune responses (Koenen et al. 2002). The same study also showed that broiler chickens have dysfunctional cellular and humoral immune systems, which lead to an increased sensitivity to diseases.

Considering the trade-offs in chicken selection

Selection to enhance immune-competence in poultry is needed to improve animal welfare, production system sustainability and to reduce the large-scale (ab)use of antibiotics, which represents a major healthcare issue also for humans (Landers et al. 2012). However, more comprehensive measures of immune function are required in breeding programs, to avoid that the selection of a single immune trait (i.e. antibody production, improved cellular immunity response etc.) leads to the weakening of other arms of the immune system. Although a large body of knowledge already exists, more studies are necessary to estimate the nutrient / energy cost for the animal of improving the different immune strategies and the final benefit on health of selection for enhanced immune functions.

It is also necessary to measure the possible cost of improved immune response on production traits to consider the economical balance between improved health and production yield in selection schemes. The estimation of the metabolic cost of antibody response and its consequence on animal production are among the goals of several ongoing projects. They intend to measure how high or low antibody responses influence production, and what proportion of dietary protein sources is allocated to production or to immune response. It is also important to decipher the relation existing between negatively correlated traits and the balance in resource allocation. Is it related to a central regulatory mechanism (through hormones) or is it a direct pleiotropic effect of the same genomic variant?

For the last case, a nice example has been recently demonstrated in sheep, where a non-synonymous SNP in the SOCS2 gene has been associated to an impaired immune function (increased susceptibility to mastitis because of uncontrolled inflammation) and to a higher weight gain and milk production (Rupp et al., 2015). This example illustrates a direct negative correlation between the two traits mediated by one SNP in SOCS2: the selection for improved production has increased the allelic frequency of the variant affecting ewes' susceptibility to mastitis.

A follow-up project will study how to manage this polymorphism in the sheep breeding scheme, i.e. to favor production yield or resistance to mastitis. This is also a trade-off to consider at the scale of the production system.

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Session - One Health

Biotic and abiotic stresses

Mapping disease resistance in poultry

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Keywords: GWAS, disease resistance, immune responses, breeding programmes, chickens

Introduction

Industrial scale production of eggs and meat from chickens provides an environment where pathogens can rapidly transmit and lead to severe disease outbreaks with concomitant economic losses. In low to middle income countries, backyard poultry production is critically important to small-holder farmers, where losses to disease are a major constraint on productivity. Moreover, poultry are a key reservoir of zoonotic pathogens such as *Campylobacter* and *Salmonella*. With the use of antimicrobial agents being reduced due to legislation and lack of efficacy, and the absence of sustainable cost-effective vaccines for many diseases, an attractive strategy to control infectious disease is to focus on host genetics. The aim is to optimise inherent resistance to infection, or resilience to disease, and enhance responses to existing vaccines. This requires a thorough understanding of the genetic mechanisms underpinning heritable resistance to pathogens. The objective of this article is to summarise four recent studies on the genetic architecture of disease resistance in different chicken populations.

Materials and Methods

Populations and Data collection

Campylobacter resistance in commercial broilers. *Campylobacter* spp, are the leading bacterial cause of foodborne diarrhoeal illness in humans. A pedigree line of broilers was used to map *Campylobacter* gut colonisation resistance. Caecal samples were collected from 3,000 birds raised under commercial farm conditions. The birds were naturally colonised with *Campylobacter* and the *Campylobacter* load in CFU/gram of caecal content was estimated as described before (for more details see Psifidi et al., 2016a). Body weight was also measured. All birds were genotyped with a high-density genome-wide 580K single nucleotide polymorphism (SNP) array (Affymetrix® Axiom® HD). The phenotypes (*Campylobacter* load) were log-transformed to normalise their distribution.

***Salmonella resistance in commercial layers*.** *Salmonella enterica* serovar Gallinarum, causes fowl typhoid in chickens. Commercial layers from two *Salmonella* Gallinarum (SG) outbreaks in 2007 and 2011 in Norther Ireland were used to map resistance to SG. From the first natural disease outbreak tissue samples from 150 layers (resistant and susceptible) were collected, DNA was extracted and the animals were genotyped using microsatellites located in a previously identified quantitative trait locus (QTL) (*Sal1* region) on chromosome 5 (Fife et al., 2009). The phenotypes were analysed as a binary trait. From the second natural disease outbreak liver samples were collected from 300 (susceptible and resistance) layers belonging to a different line and the SG load in liver was estimated in CFU/gram as described previously (Fife

et al., 2009). All the birds were genotyped with the 580K array. The phenotypes were log transformed and the trait was analysed as continuous as well as binary (survival) phenotypes.
***Eimeria resistance in commercial broilers*:** The costliest infectious disease to the poultry industry is coccidiosis caused by *Eimeria* spp apicoplexan parasites. Commercial broilers (final crosses) (n=1,000) were used to map *Eimeria tenella* (*E. tenella*) resistance. A challenge experiment was performed; percentage body weight gain, caecal lesion score (scale 0-4) and serum interleukin-10 (IL10) titre measured by Captured ELISA were the phenotypes analysed. IL10 was log-transformed. The birds were genotyped with a 62K genome-wide SNP array.
Disease resistance in indigenous Ethiopian chickens: Two populations of indigenous Ethiopian village chickens located in Horro (n=384) and Jarso (n=376), two geographical regions about 800 km from each other were used in this study. Information on village, farm, shed, month of sampling, sex, age, weight and body condition score were recorded. Serological data for fowl typhoid (SG), fowl cholera (PM), infectious bursal disease (IBDV), Mareks' disease (MDV) and parasitic (*Eimeria*, cestodes, ascarides, lice spp) egg counts, body weight and body condition were collected as described before (Psifidi et al., 2016b). The phenotypes were log-transformed. All the birds were genotyped with the 580K array.

Statistical analyses

In all cases a multidimensional scaling analysis (MDS) was performed first using an IBS distance matrix to investigate population substructure. Data were pre-corrected for fixed effects and MDS cluster (when applicable); the residuals were used as phenotypes in the ensuing genome-wide association studies (GWAS). The quality control criteria included: call rates above 0.95, individuals with missing genotypes 0.05, Hardy-Weinberg equilibrium $P<10^{-6}$, minor allele frequency 0.05%. The software GEMMA v0.94 was used to run the GWAS analyses based on a mixed model that included the genomic relationship matrix among individuals as a random effect. The significance threshold was set at $P<0.05$ and a Bonferroni correction for multiple testing was performed. Individual SNP found significant in GWAS were further tested using mixed model analyses to verify their significance and assess the magnitude of their effect. The same models were used to estimate the heritability of each trait. Bivariate analyses were conducted to estimate genetic correlations between health and production traits. Genomic breeding values were estimated for the indigenous Ethiopian chickens; results were validated and accuracy was estimated by subdividing the data in 5 sets and running permutation analyses. The ASReml v3.0 software was used in all cases. The genes located 200 kb upstream and downstream of the significant SNPs identified by the GWAS were annotated using the BioMart data mining tool (http://www.ensembl.org/biomart/martview/) within the Ensembl database and the Gal-gal5 assembly. Identification of potential canonical pathways and networks underlying the candidate genomic regions associated with the resistance to each of the diseases were performed using the Ingenuity Pathway Analysis (IPA) programme (www.ingenuity.com).

Results and Discussion

***Campylobacter* gut colonisation resistance in commercial broilers.** The GWAS revealed two quantitative trait loci (QTL) on chromosomes 16 and 26 with a genome-wide significant association and a QTL on chromosome 14 with suggestive genome-wide significance. Interestingly, associations on chromosomes 16 and 14 were previously identified using a back-cross and a 9^{th} generation advanced intercross experiments between two inbred lines (6_1 and N) with known differences in resistance to *Campylobacter* gut colonisation susceptibility (Psifidi et al., 2016a). The markers on chromosome 16 were located in the Major Histocompatibility Complex (MHC) region. Significant additive effects were estimated for the putative QTLs on

chromosomes 16 and 14. There was not significant genetic correlation between *Campylobacter* colonisation resistance and body weight. The heritability of the trait was relatively low (h^2=0.11), implying that environmental factors contribute significantly to *Campylobacter* gut colonisation resistance.

***Salmonella* gut colonisation resistance in commercial layers.** For the first *Salmonella* outbreak significant associations with markers on chromosome 5 located close to RAC-alpha serine/threonine protein kinase homolog, *AKT1* (protein kinase B, PKB) gene were found. For the second *Salmonella* outbreak the GWAS revealed one QTL on chromosome 3 with a genome-wide significant association and several others with suggestive genome-wide significance. On the same chromosomes and in some cases in close proximity, QTLs for SG resistance have been previously reported in other chicken lines (Calenge et al. (2010). Although, significant associations with SNPs located in the *Sal1* locus were not identified by the GWAS, in a mixed model analysis using SNPs located in this region significant (P<0.05) associations with 4 markers spanning *AKT1* gene were identified, implying that this gene is a good candidate for SG resistance. Significant additive effects were estimated for all the putative QTLs. A moderate heritability of the trait was estimated in both populations (h^2=0.21 and 0.33 for the layers in the first and second outbreak, respectively).

***Eimeria* resistance in commercial broilers.** Body weight gain, caecal lesion score and circulating IL10 titre had high phenotypic and genetic correlation, implying that IL10 may be a good biomarker for *E. tenella* resistance. The GWAS revealed several suggestive significant genome-wide SNPs for *Eimeria* resistance with the stronger association identified on chromosome 24 close to the IL10 receptor (*IL10R2*), indicating an interesting candidate gene affecting *E. tenella* resistance.

Disease resistance in Ethiopian indigenous chickens. MDS analysis revealed that the two indigenous Ethiopian populations are genetically distinct. Therefore, initially each population was analysed separately. GWAS revealed several significant associations between SNP markers and the disease and immune traits for Horro and Jarso chickens (Psifidi et al, 2016b). A subsequent joint analysis of the two populations was performed to increase the power of the test and revealed certain common genomic regions affecting the disease and immune traits. The Manhattan plots from the joint GWAS analysis are presented in Figure 1. The heritability of the traits was low to moderate (Table 1). No significant genetic correlations between health and production traits were found, implying that concomitant breeding programmes to improve both set of traits are plausible. An across-breed genomic selection programme seems also to be feasible, since the accuracy of the genomic predictions were improved when we joined the two populations.

Conclusion: The chicken diseases studied were heritable and polygenic traits which could be modified through selective breeding. Their correlation with production and other important traits needs to be estimated before incorporation in poultry breeding programmes.

Figure 1. Manhattan plots displaying the genome-wide association analysis results in Ethiopian chickens.

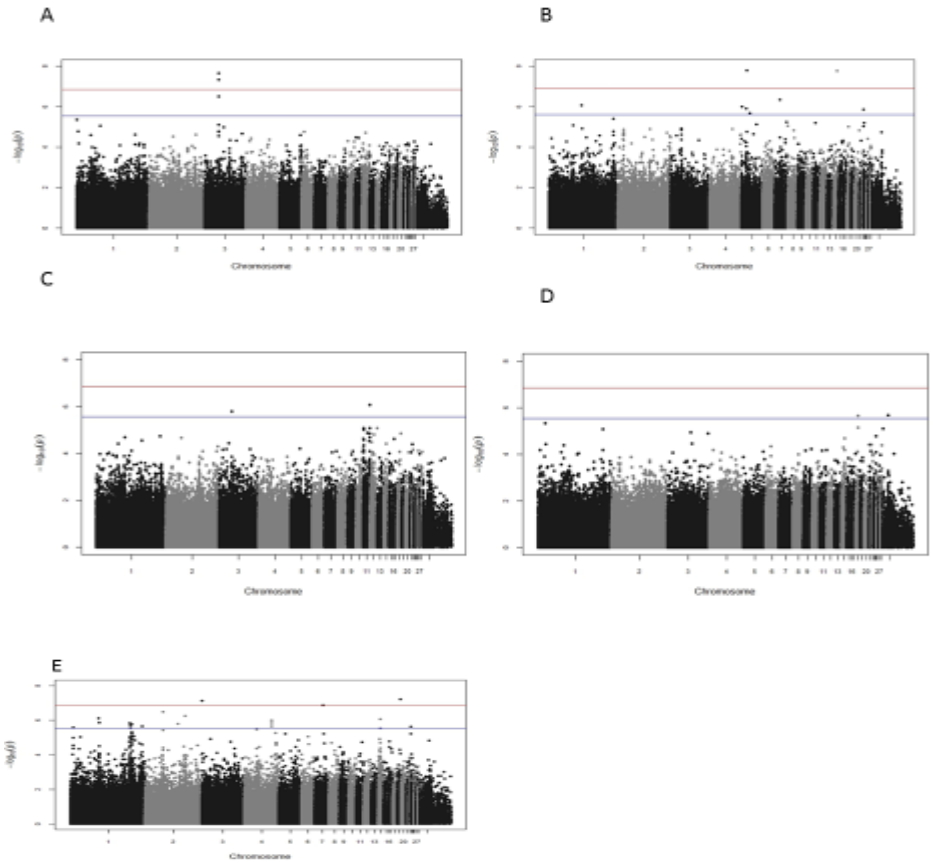

Table 1. Heritability estimates for all the traits studied in Ethiopian chickens

Trait	Heritability estimate(h^2)
PM	0.30
IBDV	0.46
MDV	0.42
Eimeria	0.22
Cestodes	0.31
SG	0.08
Survival	0.70
BCS	0.16
Body weight	0.43

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Genomics of response to abiotic and biotic stressors in chickens

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The ability of poultry to perform at their true genetic potential is reduced by challenges with stressors such as infectious disease and thermal stress. In real life, biotic and abiotic stressors often occur in combination, while research studies often focus on only a single factor, ignoring the interaction of multiple factors. To more fully understand the complex physiology and genetics of responses to combined stressors, we characterized responses to high environmental temperature and inflammatory stimuli, both singly and in combination. We profiled gene expression of tissues from diverse lines of chickens exposed to stressors *in vivo*, and also isolated chicken cells exposed to stressors *in vitro*. The results shed light on the genes and pathways involved in response to these stressors.

Key-words: heat-stress; endotoxemia; immune-response; genomics

Introduction

Biotic stressors, such as infectious diseases, have a significant negative impact on the poultry industry. In developed countries, 10-15% of the potential profit in commercial poultry production has been estimated to be lost because of disease (Biggs, 1982). In developing countries, the impact is even greater on the livelihood of the rural poor, where up to 25% of income may be lost due to disease (Rist et al., 2015). The additional increment of loss in developing countries may result from greater disease burden or from the more challenging environmental conditions, which add abiotic stressors to the bird. Disease can cause mortality, leave birds immunosuppressed and therefore susceptible to secondary infections, or reduce their production efficiency because fighting infections diverts resources from production traits (Siegel et al., 2008). The biological impact of a microbial burden reduces reproduction and growth (Klasing and Korver, 1997). As a corollary, after long-term selection for growth and reproduction, some populations seem to be at greater risk for immunological disorders (Rauw et al., 1998). This negative consequence of selection may arise from selective sweeps of deleterious alleles nearby the genes influencing the commercial traits (Hocking, 2014).

Abiotic stressors, such as heat, can reduce the ability of birds to live up to their genetic potential. Much of the world's poultry production takes place in areas with hot climate. Heat stress causes multi-million dollar annual losses in poultry (St. Pierre et al., 2003). Exposure to temperatures above the thermoneutral zone can result in production losses from mortality, reduced body weight, egg production, and feed intake, and poorer feed conversion (Lin et al., 2006; Sohail et al, 2012). The negative biological impact of heat stress may be mediated by a phenomenon in which the normal gut barrier becomes more permeable and allows pathogens and inflammatory substances, such as lipopolysaccharide (LPS) from bacteria, to reach the blood-stream, causing endotoxemia and systemic inflammation (Quinteiro-Filho et al., 2012). This is known as "leaky gut" syndrome. Heat stress has also been reported to modulate immune function, usually by decreasing immunocompetence. Thus, it is important to understand the interactions of multiple stressors on the performance of poultry to develop strategies to breed birds that perform well under a wide range of environmental challenges.

Materials and Methods

To more fully understand the complex physiology and genetics of responses to combined biotic and abiotic stressors, we characterized gene expression in response to high environmental temperature and inflammatory stimuli, both singly and in combination, using heterogeneous tissues from diverse lines of chickens exposed to stressors *in vivo*, and also using chicken cells exposed to stressors *in vitro*.

CHALLENGES OF DIVERSE GENETIC LINES OF CHICKENS

Chickens of the Fayoumi (heat and disease resistant) and broiler (heat and disease susceptible) lines were stimulated at 22 days of age, using a 2x2x2 full factorial design including: chicken line, inflammatory stimulus [lipopolysaccharide (LPS) or saline], and temperature (35C or 25C). The LPS injection was given 3.5 hours after the initiation of the heat treatment. Body (clocal) temperature was recorded at 3.5 hours after LPS injection, then birds were humanely euthanized and tissues harvested. Transcriptional changes in spleen were analyzed using RNA-sequencing on the Illumina HiSeq 2500. Four individual library cDNA libraries were used for each of the 8 treatment groups, for 32 total birds (Van Goor et al, 2017).

CHALLENGES OF CHICKEN CELLS AND CELL LINES

To gain insight into the response of individual cell types, and to explore the possibility of using *in vitro* systems to avoid the expense and welfare issues of challenging live birds, we conducted studies of the response of two different chicken cell types to heat and LPS. These cell types are involved in immune function, and were chosen to help determine interactions of stressors that may be mediated through the immune system.

Bone-marrow derived dendritic cells (BMDC) were isolated from day 18 embryos of the same Fayoumi and broiler lines used in the live-bird studies. Bone marrow was collected from the tibias and femurs, pooled with line, and then cultured with GM-CSF and IL-4 to support dendritic cell growth and maturation (Van Goor et al., 2016). A full factorial design of chicken line and treatment (control, heat, LPS, heat+LPS) was used.

The HD11 cell line is a macrophage-derived, immortalized chicken cell line, widely used in immunologic studies. Four treatments (control, heat, LPS, and heat+LPS) were used (Slawinska et al, 2016).

Results and Discussion

CHALLENGES OF DIVERSE GENETIC LINES OF CHICKENS

In response to the single treatments of heat or LPS, the body (cloacal) temperature increased significantly ($P < 0.05$) in both breeds of chickens and the increases were of similar magnitude for each treatment. The combined treatment of heat+LPS generated a body temperature that was significantly ($p < 0.05$) higher than that of either single treatment. This suggests that each single treatment generates a fever response and that the two challenges (heat and LPS) combined interact to increase the body temperature above that of either single treatment (Van Goor et al, 2017).

Differentially expressed genes (DEG) in the spleen were detected using EdgeR software. Stimulation with LPS induced more DEG in the broiler than the Fayoumi, whereas heat induced fewer DEG in the broiler than the Fayoumi. The double stimulus of heat and LPS induced 2- to 5-times more DEG than either single-stressor by genetic line combination, and 399 of the DEG for the combined stimulus were shared between lines, indicating a consensus response in

different genetic lines. Analysis of pathways revealed Remodelling of Epithelial Adherens Junctions due to heat stress, Granulocyte Adhesion and Diapedesis due to LPS, and Hepatic Fibrosis/Hepatic Stellate Cell Activation due to LPS+heat. These results may point the way to mechanisms of response of chickens to stressors at the whole-organism level.

CHALLENGES OF CHICKEN CELLS AND CELL LINES

Differences were identified in responses of BMDC to *in vitro* treatment with heat, LPS, or both stressors (Van Goor et al. 2016). Nitric Oxide (NO) was induced in BMDC from both lines in response to LPS and LPS + heat stimulation. The Fayoumi BMDC produced more NO with LPS treatment. Fayoumi had higher phagocytic ability and MHC II surface expression. Gene expression for the heat-related genes BAG3, HSP25, HSPA2, and HSPH1 was strongly induced with heat, with few line differences. Expression for the immune-related genes CCL4, CCL5, CD40, GM-CSF, IFN-g, IL-10, IL-12b, IL-1b, IL-6, IL-8, and iNOS was highly induced in response to LPS and different between lines. These results highlight the specificity of stress response by genetic line and by gene family in the BMDC.

Expression of heat shock proteins, stress-related genes, signaling molecules and immune response genes were analysed in the HD11 cell line (Slawinska et al. 2016). HD11 cell line responded to heat stress with higher expression of the *HSP25*, *HSPA2* and *HSPH1* chaperones and the co-chaperones *DNAJA4* and *DNAJB6*. The anti-apoptotic gene *BAG3* was highly up-regulated, suggesting that the cells were using genetically regulated, pro-survival mechanisms. The immune response to LPS of the HD11 cells was higher under heat stress than in thermoneutral conditions, as determined by higher expression of *CCL4*, *CCL5*, *IL1B*, *IL8* and *iNOS*. The results suggest that the induced extracellular heat shock proteins may not only mitigate the effects of heat stress in the HD11 cell line, but also in trigger a higher level of the immune responses.

SUMMARY

The combination of biotic (LPS) and abiotic (heat) stressors interact to generate a transcriptional response in chickens that is distinct from the response to either single stressor, although some genes and pathways are shared. Understanding the stress responses of relatively resistant and susceptible lines of chickens may provide insight into genomic mechanisms that can be exploited to improve resiliency in commercial poultry.

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Session - New Breeding Technologies

Genome editing in birds - from concept to reality

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Keywords: chicken, CRISPR/Cas9, knockout, immune system

Introduction:

The chicken has a long history as a biomedical animal model and contributed significantly to research areas such as phylogeny, microbiology and immunology. In addition, the chicken embryo became a leading model organism in developmental biology because of its easy accessibility(Stern, 2004, Stern, 2005). Several seminal contributions to basic immunology came from studies in birds, for example the discovery of the graft versus host response(Murphy, 1916), the identification of B (bursa derived) lymphocytes as the antibody producing cells(Glick et al., 1956) and the development of the first vaccine against a natural tumor (the Marek's Disease vaccine(Okazaki et al., 1970)). Beside its importance as a research model chickens supply more than one-third of the animal protein in human diets globally and chicken meat is suspected to replace porcine meat by 2020 as the number one source. This is largely due to the high feed conversion rates in poultry and the absence of religious barriers for chicken meat. However, poultry production is severely threatened by infectious diseases including avian specific and zoonotic diseases with high risks for humans. Well known examples are avian influenza, Salmonella and Campylobacter infections. Sustainable poultry production therefore critically depends on progress made in infectious medicine, immunology and vaccinology which will also have an impact on other important issues in poultry medicine such as antibiotic usage.

The availability of entire animal genome sequences and efficient gene targeting technologies have revolutionized biomedical research in particular in mouse models and more recently in porcine disease models in translational medicine(Hauschild et al., 2011, Petersen et al., 2011). Progress in avian transgene technology has been hampered by the unique reproduction biology, a problem which was solved by the introduction of primordial germ cell (PGC) cultures for genetic manipulations(van de Lavoir et al., 2006). By demonstrating that targeted gene knockouts can be achieved in chickens(Schusser et al., 2013) exciting new avenues have been opened in avian research in general and new opportunities have been provided to address long standing questions in avian immunology in particular.

The first gene knockout chicken (the immunoglobulin (Ig) JH segment knockout (JH-KO)) is a valuable tool to gain a better understanding of the B lymphocyte development. In addition infection studies using the antibody deficient JH-KO birds will enhance our knowledge about the role of antibodies in the control of various infections.

Beside the humoral immune response the cell mediated immune response plays a crucial role in controlling infections. T cells represent an important effector of the adaptive immune system. The chicken T cell repertoire has many characteristics similar to mammals. Both T cell receptors (TCR) γδ and αβ have been described in the chicken(Sowder et al., 1988, Lahti et al., 1991).

Even though γδ T cells represent the major T cell population in chickens(Paul et al., 2014), their role in the immune response is still poorly understood. Most of the γδ T cells home to several

tissues such as epithelia of the intestine and spleen(Itohara et al., 1990). It is assumed that this lymphocyte-population participates in tissue homeostasis and resistance against infections(Hayday and Tigelaar, 2003), but their precise functions are largely unknown.
Our aim is to establish a knockout of the γδ T-cells in chicken in order to uncover the functions of γδ T-cells in γδ T-cell high species.

Material and Methods:

Homologous recombination in primordial germ cells (PGC) was previously used to generate light chain knockout chickens(Schusser et al., 2016). In our study, depletion of γδ T-cells was achieved by designing a targeting vector to knock out the single known gene for the constant region of the T-cell receptor γ - chain.
The targeting was performed in PGCs via homologous recombination in combination with the CRISPR/CAS9 system to increase the targeting efficiency. Germline chimeras were generated and crossed with wild type hens to obtain the first generation of heterozygous chickens with a deletion of the ɣ- chain constant region of the T-cell receptor (γ-TCR+/-). By crossing heterozygous γ-TCR+/- hens and roosters, homozygous offspring (γ-TCR-/-) with a deletion of the y-TCR constant region on both alleles were generated.
Genomic DNA was isolated for genotyping by PCR. Genotype and the integration locus were determined. The copy number of the integrated marker cassette in the offspring´s genome was determined by digital droplet PCR. In order to confirm the intended phenotype, blood was taken to analyze the lymphocyte composition. Peripheral blood mononuclear cells (PBMCs) were obtained by density gradient centrifugation and cell analysis were performed by FACS.

Results and Discussion

The successful targeting was confirmed by PCR spanning the homology regions. Furthermore, the correct integration of the targeting vector at the intended locus was confirmed by PCR. Copy number was determined by digital droplet PCR and additional integration beside the desired locus were excluded.
FACS analysis of the PBMCs showed a depletion of γδ T cells in homozygous knockout birds while the other lymphocyte populations including αβ T cells were not effected by the knockout.
By targeting the constant region of T cell receptor γ locus, it was possible to generate a chicken line deficient for γδ T cells. The established chicken line may provide a valid tool to understand the function of γδ T cells in birds. Further experiments will provide a more detailed characterization of this new chicken knockout line. The established knockout chicken will be used for the investigation on the role of γδ T cells in the immune response towards infectious diseases in challenge experiments

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Biobanking of poultry breeds using primordial germ cells

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Key words: biobank, primordial germ cells, activin, FGF, cryopreservation

Introduction

Biosecurity and sustainability for poultry flocks would be vastly increased if a reliable methodology existed to regenerate chicken flocks from frozen cells (Glover, 2012). Avian species pose a particular difficulty in comparison to other farm animals as the early chicken zygote cannot be cryopreserved due to the large amount of yolk present in the egg.

Primordial germ cells are a type of diploid stem cell found in the very early embryo. They give rise to germ cells in the gonad which produce semen and eggs. These cells are present in very low numbers in the early embryo. To expand the number of cells we developed a culture medium to increase the number of cells *in vitro* and applied this medium to the biobanking of several breeds of inbred chickens.

Material and Methods

For medium condition experiments, 1 µl of blood isolated from stage 15–16 (H&H) embryos Blood was isolated from single ISA brown embryos (stage 15-16HH) and cultured for three weeks in serum freem medium (Whyte, 2015). PGCs were counted and cultures containing >50,000 cells were scored as positive. FAOT medium and derivatives of this medium contained Avian KO-DMEM basal medium DMEM (250 mosmol/kg, 12.0 mM glucose, 0.15 mM calcium chloride) plus 1x B-27 supplement, 2.0 mM GlutaMax, 1× NEAA, 0.1 mM β-mercaptoethanol, 1× nucleosides, 1.2 mM pyruvate, 0.2% ovalbumin (Sigma), 0.02% sodium heparin, 25 ng/ml Human Activin A, 25 ng/ml, 4 ng/ml human FGF2, 10 µg/ml ovotransferrin. 25 ng/ml human BMP4 was added to produce FABot and FBot medium. Cells from a male or a female PGC line derived in FABot were diluted to 1 cell / 2 µl in Fot, and single cells were plated and cultured for three weeks in FABot, FAot or FBot. PGCs were counted and cultures containing >50,000 cells were scored as positive. Inbred PGC lines were derived by placing 1.0 µl of blood isolated from stage 15–16 (H&H) embryos in 300µl FAot medum in a 48 well plate as described (Whyte et al, 2016). Cells were counted at three weeks and at five weeks and then frozen.

Results and Discussion

Using defined medium conditions we investigated the relationship between Activin and BMP growth factor ligands in chicken PGC proliferation. We asked whether there was a quantitative difference in cell proliferation in the presence of both Activin A and BMP4. We found no significant differences in cell proliferation between PGCs cultured in either Activin A or BMP4

but cells did have increased proliferation with both growth factors present (Table 1). To determine if Activin A and BMP4 were both sufficient for the derivation of PGC lines, embryonic blood from single chicken embryos was placed in single wells and the resulting PGCs were counted after three weeks. We found that both male and female PGC lines could be derived in these three medium conditions. PGC lines could be derived in medium containing BMP4 alone, but the derivation rate was significantly lower than the derivation rate in medium containing Activin A (Table 1). We finally asked whether Activin A and BMP4 could both support PGC propagation in clonal growth conditions. A single PGC was plated into a single well and cultured for 21 days. Under clonal growth conditions, PGCs could be propagated in medium supplemented with Activin A, but not in medium supplemented with BMP4 alone (Table 1). No difference in clonal growth was observed between medium containing Activin A alone and medium containing both Activin A and BMP4.

Media	Derivations/Cultures (%)	Clonal Growth (%)
FABot	21/32 (65.6)	10/15 (60.0)
FAot	32/62 (51.6)	10/15 (60.0)
FBot	7/48 (14.6)	8/15 (50.0)

Table 1 Derivation rates and clonal growth in different medium conditions (from Whyte, 2015)

As a test case we used eggs from several lines of inbred chicken currently maintained at the National Avian Research Facility, UK. These lines were backcrossed for several generations to generate lines with single MHC haplotypes. Fertile eggs were incubated for 2.5 days (stage 16 Hamburger & Hamilton (HH)) and 1 µl of blood was aspirated from the dorsal aorta and transferred to culture medium. The blood sample was cultured in suspension for two to three weeks. At the end of three weeks, the cultures containing more than 100,000 cells were scored as a positive derivation of a cell 'line' and these cells were cultured for an additional week in increasing volume (100,000 cells per 0.5ml medium) and then frozen in aliquots of 50-100,000 cells per vial.

The data in Table 2 show the results from these experiments using six lines of inbred chicken. A total of 200 cultures were started from single embryos. For 200 cultures initiated from single embryos, 137 independent cell lines (genotypes) were expanded and frozen in a total of 494 vials. The overall derivation rate was 69%, which is consistent with past cell line derivation results (Whyte, 2015).

Inbred chicken line	Male embryos sampled	Male lines frozen	Male PGC derivation rates (%)	Total male vials	Female embryos sampled	Female lines frozen	Female derivation rates (%)	Total female vials	**Total** embryos sampled	Total frozen lines	Total derivation rates (%)	Total vials
6	15	12	80	45	18	15	83	52	33	27	82	97
15	3	1	33	4	4	3	75	12	7	4	57	16
N	32	13	41	52	27	16	59	62	59	29	49	114
O	17	13	76	52	25	17	68	68	42	30	71	120
P	19	18	95	58	24	14	58	44	43	32	74	102
W	9	5	56	15	17	10	59	30	26	15	58	45
All lines	95	62		226	115	75		268	210	137		494

Table 2 Derivation of PGC cultures from inbred chicken lines (based on (Nandi, 2016)).

These results show that we can easily biobank a sufficient number (over 100) of primordial germ cell lines to reconstitute a breed of chickens. The development of a sterile host bird will aid these efforts in flock reconstitution (Taylor, 2017). These results are major step in developing and demonstrating that frozen stem cells could be used for the biobanking of specialised flocks of birds used in research.

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Potential of genome editing and gene drive technologies to increase genetic gain in livestock breeding programs

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Genome editing represents a set of molecular technologies for modifying genomes, while gene drive is a molecular technologies that skews inheritance of segregating alleles. Both sets of technologies are rapidly developing and attracting attention from academia, industry, and society. Although application of these technologies is still uncertain and several practical issues need to be solved, we thought it timely to perform a simulation to quantify their potential on increasing genetic gain in livestock breeding programs.

We simulated five different breeding and editing scenarios with a common overall structure. Each scenario began with 21 generations of genomic selection, followed by 20 generations of genomic selection based on true breeding values where the breeder used genomic selection alone, genomic selection in combination with genome editing, or genomic selection with genome editing and gene drives. For each scenario, we evaluated genetic gain, rate of increase in favourable allele frequencies of edited quantitative trait nucleotides (QTN), rate of change in inbreeding and the efficiency of converting genetic variation into genetic gain. Genome editing in livestock breeding results in short-, medium- and long-term increases in genetic gain. The increase in genetic gain occurs because editing increases the frequency of favourable alleles in the population.

Gene drives accelerate the increase in allele frequency caused by editing, which results in even higher genetic gain over a shorter period of time with no impact on inbreeding. Gene drives enhanced the benefits of genome editing in seven ways: (1) they amplified the increase in genetic gain brought about by genome editing; (2) they amplified the rate of increase in the frequency of favourable alleles and reduced the time it took to fix them; (3) they enabled more rapid targeting of QTN with lesser effect for genome editing; (4) they distributed fixed editing resources across a larger number of distinct QTN across generations; (5) they focussed editing on a smaller number of QTN within a given generation; (6) they reduced the level of inbreeding when editing a subset of the sires; and (7) they increased the efficiency of converting genetic variation into genetic gain. These results show large potential of genome editing and gene drive techniques for livestock breeding.

To enable genome editing and gene drives to be discovered in sufficient numbers to enable the benefits that we observed in our simulation strategies to undertake such discovery are needed. To data this has been too great an obstacle to overcome. However, there has been progress in this area in recent years that gives us reasons to be optimistic. We now have access to data sets and technology with unprecedented power including: data sets that will have whole genome sequence level information on hundreds of thousands of individuals; large expression data sets;

functionally annotated animal genomes; and established cell lines and animal populations (cloned and traditional) for *in vitro* and *in vivo* testing of edited loci. Instead of using progeny testing schemes to "prove the causality" of sires, as rolling animal breeding programs have in the past, we could develop allele testing schemes to "prove the causality" of variants underlying polygenic traits. Perhaps the following cascade of technologies could be used in such testing schemes:

- all genomic variants could be initially assigned an equal probability of being causal,
- genome-wide association studies using data sets with hundreds of thousands or even millions of individuals could be used to increase the probability of a much smaller subset of the variants being causal,
- functional annotation information and expression data could be used to further increase the probability of an even smaller subset,
- there may be opportunity to study subsets using genome editing in cell lines to further increase the probability of an even smaller subset,
- the resulting subset could then be edited (perhaps into a heterozygote state) into sires within the breeding program and have the effects analyzed in the descendants as part of rolling breeding activities,
- variants shown to have positive effects could be edited in all subsequent sires, those with negative effects could be reversed using editing, and those that are neutral could be ignored,
- in the extreme case, variants with well-understood effects could be edited alongside synthetic gene drive mechanisms.

Genome editing and gene drives have the potential to transform animal breeding. Its effectiveness will depend on societal acceptance and our ability as a community to overcome current technological hurdles. Ultimate success also depends on the biology of quantitative traits, which is still largely unknown, being amenable to allele changes independently of the closely linked alleles. This requires alleles to act additively. Finally, just as genomic selection decoupled phenotyping from selection, use of genome editing could decouple selection from genetic gain. Will we need a new breeders equation?

Session - PhD Awards

A review of performance differences between the Nigerian indigenous and the commercial layers in sub-optimal conditions.

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In Nigeria, as in most developing countries, there are two parallel poultry industries: one using high-performing commercial layers; and the other based on lower-performing, dual-purpose indigenous breeds. The proportions vary widely among countries, but in lower-income countries, indigenous stock comprises as much as 80 percent of the poultry population. The distinction between the two forms of production relates to breed and management: commercial stocks are generally reared in confinement and housed in flocks ranging from 1000-2000 birds (small) to more than 10 000 birds (large). The birds are usually given compounded feeds, and the larger facilities are normally located close to urban areas. Indigenous stocks are typically kept by families in rural and sometimes peri-urban areas, in small semi-scavenging flocks of 10 to 100 birds, which are fed with household scraps and small amounts of other feeds. Women and children are usually responsible for managing family flocks. The large difference in genetic potential to produce eggs is the time that a broody indigenous hen spends hatching a clutch of eggs and rearing the chicks to about seven weeks of age. Quantity and quality of feed is another significant factor in the disparity in annual egg production between the two genotypes. Commercial layers are normally provided with carefully compounded feeds, which include nutrients in the correct proportions for maximizing egg production. They are usually also fed ad libitum. The energy and protein intake of indigenous birds in scavenging flocks is determined by the scavenging feed resource base, and is usually quite limited, particularly in the dry season. In commercial flocks, egg number, size, shell and internal quality, and layer livability, persistency of production and feed efficiency continue to improve, owing to ongoing selection for these and correlated traits. In conclusion, the review showed that both genotypes are affected by these factors. Apart from the efforts to improve their environment, possible genetic approach should include selection in commercial layers for improved tolerance to prevailing conditions; cross-breeding between commercial and indigenous layers; or a selection for improved performance of indigenous layers in a sub-optimal condition.

Keywords: Performance; local indigenous; commercial layers; sub-optimal conditions

Genomic analysis reveals a spectrum of hybrid background in European domestic geese and their wild progenitor (Anser anser)

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There are two types of domestic geese: European and Chinese. The domestic goose in Europe is derived from the greylag goose (Anser anser) and the Chinese type from the swan goose (Anser cygnoid) but the genetic basis for domestication remains largely unknown for both types. Most of the current knowledge on details of goose domestication process comes from archaeological findings and historic writings. Genetic studies on goose domestication so far have been based on mitochondrial data with limited resolution or genome wide analyses with limited intra-specific sampling. Here we shed more light on the genetic signatures of domestication of the European type of domestic geese. We used GBS (genotyping-by-sequencing) to investigate genetic differences between wild greylag geese and domestic geese on genome wide level. Our dataset consisted of 58 wild greylags and 75 domestic geese genotyped for 33 527 SNP (single nucleotide polymorphism) loci. Our results confirm clear genetic divergence between greylag goose and domestic goose but also past or still ongoing hybridization between these two types. Bayesian clustering analysis showed that all the greylag goose populations had (3.2–58%) admixture proportions with the domestic geese and the greylag goose populations that were most notably admixed (15.8–58%) with the domestic geese were those in the Netherlands and Turkey. The results also revealed that many breeds of European domestic goose share considerable (> 10%) ancestry with Chinese domestic geese. We also found that expected heterozygosity is 34.2% lower in domestic geese than in greylag geese, an expected result reflecting either selective breeding or domestication bottleneck. Several hundred SNPs showed allele frequency differences between greylag and domestic geese, an indication of selection acting on nearby region.

Keywords: admixture; Anser anser; Chinese domestic goose; domestication; European domestic goose; genotyping-by-sequencing; selection; SNP

Design of a low density SNP chip for genomic selection in layer chicken

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The main goal of selection is to choose breeders of the next generation among a set of selection candidates. In genomic selection, the choice of breeders rests on the use of information on DNA polymorphisms, in particular SNP, in addition of performance measures. Since 2013, a commercial high density genotyping chip (600 000 markers) for chicken allowed the implementation of genomic selection in layer and broiler breeding. However, genotyping costs with this chip still remain high for a routine use on a large number of selection candidates. Consequently, the current aim of genomic selection is to develop, at a lower cost, low density genotyping chips (LD chips). To do so, a set of SNP markers has to be selected to enable an imputation (prediction) of missing genotypes on a high density chip (HD chip).
In this perspective, various simulation studies were conducted to choose the best strategy for low density genotyping of laying hen lines. Different genotyping densities, several population scenarii and two imputation programs (FImpute V2.2 and Beagle V4.1) were tested. Low density genotyping chips were designed according to two strategies: a choice of SNP depending on a clustering based on linkage disequilibrium threshold or a choice of SNP at regular intervals. Three criteria of imputation accuracy were compared: genotyping error rate, allelic error rate and correlation. It appears that genotyping error rate as a criterion is more discriminating than the two others. FImpute software is preferred to Beagle software because FImpute is a better compromise between imputation accuracy and calculation time. The study of different low density genotyping chips shows that imputations improve with SNP density, when the LD threshold increases and when SNP with low MAF (Minor Allele Frequency) are considered. Population studies show that imputations are better when the size of the reference population increases. The influence of kinship degree between reference population and target population, and the impact on accuracy of genomic evaluations still remain to be deepened.
These studies will be extended to a second laying hens line to see if a shared low density genotyping chip can be created for both lines.

Keywords: Imputation accuracy ; layer chickens ; SNP density ; reference population, genomic evaluation

The effectiveness of different filtering strategies to reduce the effects of ascertainment bias when using SNP panels in a chicken diversity study

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Single nucleotide polymorphism (SNP) panels have been widely used in studies of genomic variation within and between populations. The methods of SNP discovery have been a matter of debate for their potential of introducing ascertainment bias, and genetic diversity results obtained from the SNP genotype data can be misleading.
We used a total of 42 chicken populations from the SYNBREED Chicken Diversity Panel with pooled whole genome resequencing (WGS) data and individual genotype data obtained with 580K SNP Affymetrix® Axiom® Genome-Wide Chicken Genotyping Array. We compared genetic diversity measures (expected heterozygosity (H_e), fixation index (F_ST) values and principal components analysis (PCA)) between the two data types. With the array data, we applied different filtering options (SNPs polymorphic to the Gallus gallus (Gg; two wild populations), linkage disequilibrium (LD) based pruning and minor allele frequency (MAF) filtering) to assess option/s that could account for the ascertainment bias effects and are therefore viable to improve the accuracy of subsequent studies.
The H_e estimated from WGS data ranged from 0.042 to 0.129. The raw array data overestimated H_e (ranging from 0.098 – 0.295). Filtering of SNPs that are polymorphic to Gg had H_e ranging from 0.106 to 0.341. LD based pruning of SNPs improved the H_e estimates only a bit (0.086 – 0.270), however with ranking of breeds (from one with low to high H_e in the WGS data) more consistent to the WGS (rank correlation of 0.97). The raw array data and that with polymorphic SNPs to Gg underestimated pairwise F_ST values between breeds which had low F_ST (<0.25) in the WGS, and overestimated F_ST (>0.25) for high WGS F_ST. LD based pruned SNP data underestimated F_ST, however in a consistent manner easily comparable to the WGS. MAF filtering didn't improve the results. PCA was less affected in all array versions; the populations' structure on the PCA plot was well captured in comparison to the WGS data.
Among the different tested filtering strategies, LD based pruning was found to account for the effects of ascertainment bias in the relatively best way, producing results which are most comparable to those obtained from WGS data and therefore is recommended for practical use.

Keywords: chicken diversity; ascertain bias; SNP panels

Mapping QTL for white striping in relation to breast muscle yield and meat quality traits in broiler chicken

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White striping (WS) is an emerging muscular defect occurring on breast and thigh muscles of broiler chickens and characterized by the presence of white striations parallel to the muscle fibers. This defect has significant consequences on meat quality, altering both its appearance, nutritional values and technological properties. The etiology of WS remains unknown but previous studies demonstrated that the prevalence of this defect is directly related to broiler growth and muscle yield. Moreover, recent studies showed moderate to high heritability values of WS, which emphasized the role of genetic in its determinism.
The aim of this study was to identify the first QTLs for WS as well as breast muscle yield and meat quality traits by taking advantage of two divergent lines of chickens selected for meat quality through Pectoralis major ultimate pH (pHu) and concerned by WS defect. A total of 558 birds (278 pHu +, 280 pHu-) were genotyped using the Illumina chicken SNP 60K Beadchip and phenotyped for WS, breast yield and meat quality traits evaluated through the measurements of color parameters (L*, a*, b*), drip and cooking loss. Genome Wide Association Studies (GWAS) were performed using a univariate linear mixed model implemented in GEMMA software. Then, an eQTL detection was performed for 15 candidate genes identified either by the GWAS analysis or based on their function. Their expressions were quantified on 281 out of the 558 birds.
Forty-two SNPs associated with WS, breast yield and meat quality traits were identified. They defined 18 QTL regions located on 13 chromosomes. These results were in favor of a polygenic determinism of the studied traits and suggested a few pleiotropic regions. Identified candidate genes were involved in muscle structure, extracellular matrix and sarcolemma composition, protein and lipid metabolism and muscular dystrophies. A total of 128 SNPs was associated with molecular phenotypes and defined 19 eQTL regions located on 15 chromosomes. Interestingly, several co-localizations between QTL and eQTL regions were observed (on GGA4, GGA5, GGGA12 and GGA17) which could suggest causative genes and gene networks involved in the variability of meat quality traits and breast meat yield.

Keywords: White striping; Meat quality; QTL; Candidates genes; eQTL; Broilers

Selection for low cholecystokinin A receptor (CCKAR) expression in fast-growing broiler chickens

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The most significant QTL for growth in chickens is underlain by the cholecystokinin A-receptor (CCKAR) gene. Cholecystokinin (CCK), is a peptide hormone involved in regulating energy homeostasis and growth. Generation of an advanced intercross line (AIL) established from a single broiler-layer mating has allowed study of offspring segregating for high-growth (HG) or low-growth (LG) haplotypes at the CCKAR locus but otherwise effectively homogeneous. In the AIL F20, CCKAR haplotype predicted a sex-independent difference in AIL homozygote bodyweight of ~15-20% at age 10 weeks (♂HG=1902±261g, ♂LG=1508±238g, ♀HG=1419±136g, ♀LG=1240±98g, p=0.007).
The encoded protein products of each CCKAR haplotype locus are identical, however HG individuals exhibit lower CCKAR expression than LG individuals. To pinpoint and characterise the causative variation(s), the CCKAR genomic locus (~20kb) was sequenced to define the broiler- and layer-associated alleles. Over 300 novel variations were identified and categorised based on association with the broiler or layer alleles when aligned to the red junglefowl reference genome (galGal4).
Forty broiler-specific variations were selected and used to genotype a diverse population including commercial broiler and layer strains and traditional breeds. Using genotypes at each variation as explanatory factors in ANOVAs for bodyweight highlighted one SNP in CCKAR intron 3 as significant. This SNP (AR24_4_72822482) predicted a within-strain difference in bodyweight of 14%, approximately consistent with data from the AIL.
AR24_4_72822482 is within close proximity (15bp) to another SNP which disrupts consensus binding sites of three transcription factors. Work is ongoing to elucidate whether reinstatement of these transcription factor binding sites leads to increased expression of CCKAR.
These data demonstrate a marked difference in bodyweight control explained by a single genomic locus. Because lower CCKAR expression predicts a greater bodyweight, CCKAR likely plays a key role in determination of the bodyweight set point. Further characterisation of the genetic control of growth offers an avenue for optimisation of broiler management, especially in ameliorating welfare concerns surrounding the broiler-breeder paradox.

Keywords: broiler-breeder; feeding behaviour; genetic selection; energy homeostasis

Session - Genomic

Genomics of Chicken Domestication

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Keywords: Chicken Genomics, Hybridization, Introgression, Domestication

Introduction

Among all birds, the domestic chicken, *Gallus gallus domesticus*, is the most common on earth. Some studies indicate that it has a complex ancestry not restricted to a single species of junglefowl. However, little is known on the genetic relationships between the four species of the genus Gallus whose adjacent geographical ranges span a large part of Southeast Asia and conflicting phylogenies have been reported (Kimball and Braun 2014, Meiklejohn *et al.* (2014), Hosner *et al.* (2015)). The red junglefowl (*G. gallus*) species contains five sub-species (gallus, spadiceus, bankiva, murghi and jaboullei) and has 3 sister species in the genus: the green junglefowl (*G. varius*), the Lafayette's junglefowl (*G. lafayetii*) and the grey junglefowl (*G. sonneratii*).

Using whole genome resequencing of 43 specimens (18 domestic ones, 16 from wild *G. gallus* subspecies and 9 from other Gallus species), we identified a set of 33,552,454 high quality autosomal biallelic SNPs and used it to reconstruct the genetic relationship of these birds and establish the background on which to map the ancestry of the domestic chickens.

Material and Methods

The 43 specimens were sequenced using paired-end sequencing (2x100 bp for a first wave of 36 specimens and 2x150 for the remaining 7 ones), targeting a mean coverage of 25~30x (~400 millions reads per sample). The specimens were chosen to (i) maximize the diversity of domestic chickens and (ii) include as many other junglefowls as possible at the time of collection.

SNP calling was performed following *GATK Best Practices* (DePristo *et al.* 2011) from the Broad Institute. Briefly, reads were aligned to the *G. gallus* reference genome (Galgal5 assembly) and filtered to remove low quality reads. Variants were then detected using a pair-HMM model and a validation set of 600,000 high quality snps from the 600K SNP chip were used to train a learning model and classify detected variants into true and false positives. Filtering out false positives and keeping only biallelic SNPs resulted in a total of 35,600,322 SNPs (including 33,552,454 from autosomes, 8,625 from Chromosome W and 810 from the mitochondrial genome).

The SNP density is quite high (~3.64/100 bps for autosomes and 4.83/100 bps for the mitochondry) and we performed all analysis using 20kbp sliding windows. Each window

contains an average of 728 (± 203) SNPs and 99% of the windows have at least 93 SNPs, justifying the use of local statistics.

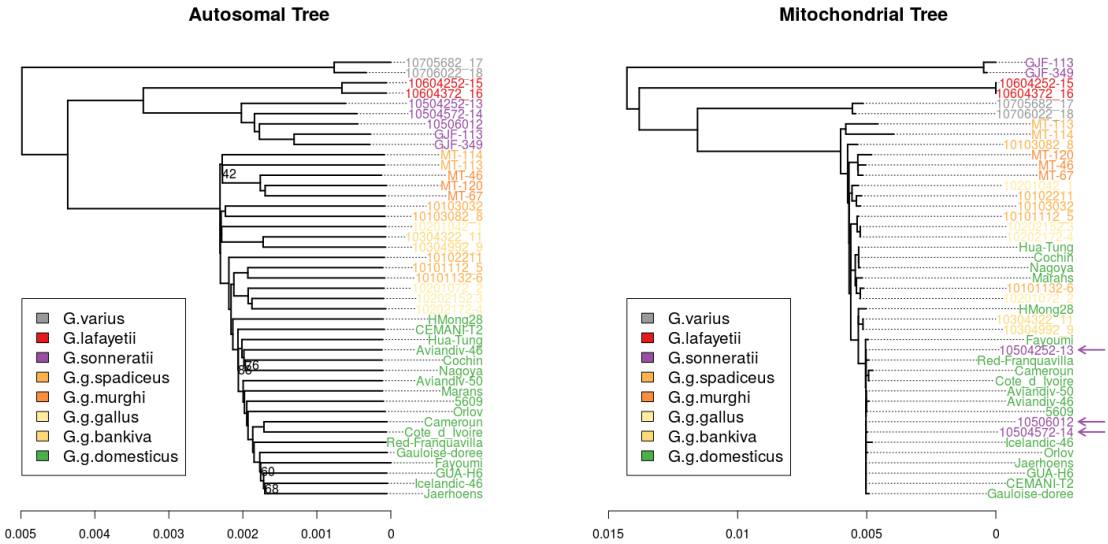
Genetic distances, from one bird A to either bird B or population B (*e.g. G. varius*), were computed as the probability that A and B (or a member of B) have the same allele at a random locus. Phylogenetic trees were reconstructed using BioNJ (Gascuel 1997) on the pairwise genetic distance matrice. Bootstrap values were computed over 100 replicates using either standard bootstrap, for the mitochondrial tree, or block bootstrap, with 20kb blocks, for the autosomal tree.

The model used to reconstruct the local ancestry of each domestic specimen is based on a Markov-Chain model *à la* Structure (Falush *et al.* 2003). Like Structure, the genome of a domestic chicken is modeled as a mosaic, each portion of which comes from one of several donor populations. Each donor population is defined by its own allelic frequencies. Unlike Structure, our model assumes 4 donor populations (the four species from the genus Gallus) and we estimates allelic frequencies in the donor populations using our resequenced specimens rather than during the algorithm.

Results and discussion

Evidence of Hybridization in the Genus Gallus

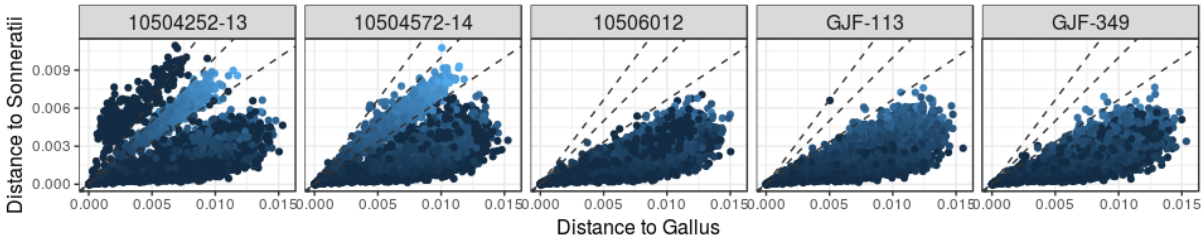
The phylogenetic autosomal tree of our 43 birds suggests an history of peripatric and parapatric speciations. However, the maternal genalogy reconstructed from 810 mitochondrial SNPs and 8625 from chromosome W suggests that hybridization occured in the ancestry of grey junglefowls specimens (*G. sonneratii*) conserved in zoological parks, whereas specimens captured in the wild are unaffected, in line with reports from Nishibori *et al.* (2005).



Only bootstrap values lower than 95 are shown. Inconsistencies are shown with an arrow.

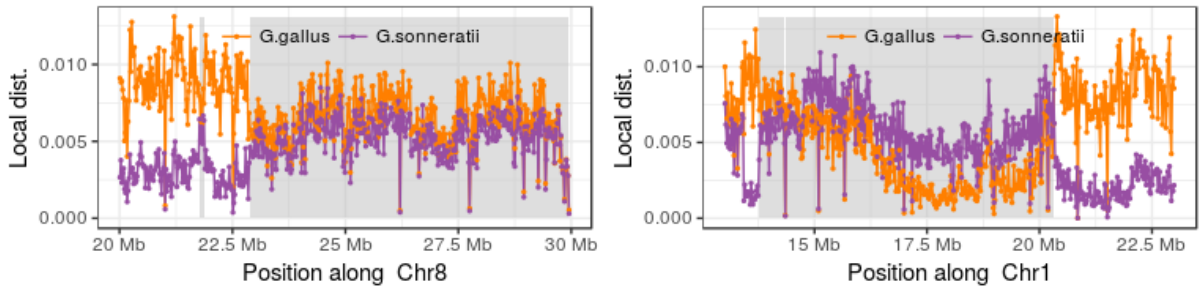
A close examination of the genomic content of 20kb windows within each *G. sonneratii* specimen reveals 3 classes of windows: (1) normal ones, (2) hybrid ones, which are equidistant from the *G. gallus* and *G. sonneratii* population, and finally (3) introgressed ones, which are closer to the *G. gallus* population. Hybrid windows are furthermore enriched, and introgressed ones depleted, in heterozygote positions suggesting that (i) hybrid windows correspond to 1 *G.*

sonneratii and 1 *G. gallus* local haplotype and (ii) introgressed one to 2, potentially identical, *G. gallus* haplotypes.



Local distance (over 20kb windows in Chromosome 1) of each Sonneratii specimen to the G. sonneratii (x-axis) and G. gallus (y-axis) populations. Local heterozygosity is color coded from dark blue (low level) to light blue (high-level).

This hybridization has a limited impact on the autosomal genome of specimens from zoological parks (ranging from 6.47 to 13.35% of the genome) suggesting asymmetry between males and females in interspecies crossings. Hybridization is not limited to small regions: among the 780 hybrid or introgressed regions, 155 are longer than 500kb and account together for 83% of the cumulated length of introgressed or hybrid regions. The longest region, located on chromosome 8, is 7.06Mb long (out of 30Mb for the complete chromosome). It corresponds to a hybrid region with one Sonneratii haplotype and one Gallus haplotype.

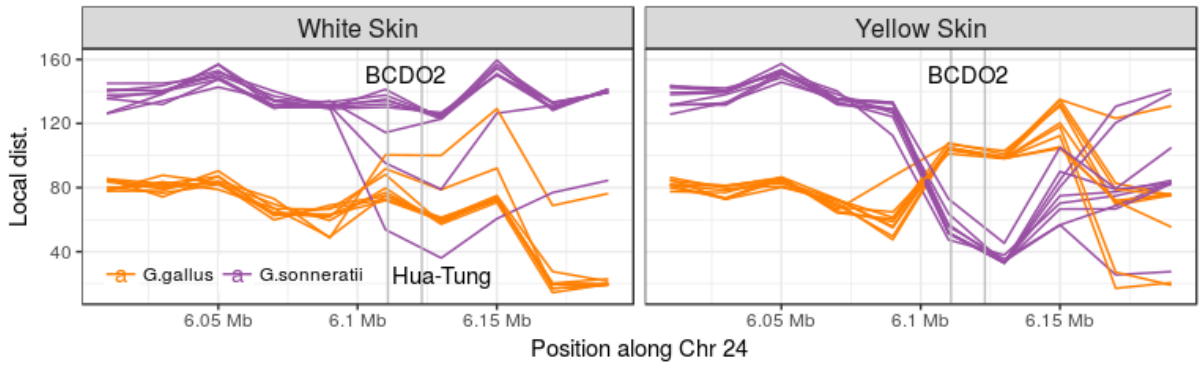


Left: Example of an introgressed region with two G. gallus haplotypes. Right: Longest hybrid region, with one haplotype from G. gallus and one from G. sonneratii. Distance to G. gallus population in orange, and to G. sonneratii population in purple.

It is important to identify and remove such regions from the autosomal genome as they can lead to false positives when scanning domestic genomes to identify genetic material with a *G. sonneratii* origin, as opposed to the expected *G. varius* origin. Note that similar analyses do not reveal the same extent of hybridization in *G. lafayetii* and *G. varius* (less than 0.08% for all specimens). False positive should therefore not be a problem for those two species.

Genetic flow to domestic lines of *G. gallus*

A previous study on the yellow skin gene BCDO2 (Eriksson *et al.* 2008) reported evidence for a hybrid origin of the domestic chicken. Our sliding window approach confirms that, around the BCDO2 gene, yellow skinned specimens exhibit two haplotypes likely to originate from *G. sonneratii*. The yellow skin phenotype allows for convenient experimental manipulation but may obscure the origin of some material due to its recessive nature. For example, although the Hua-Tung specimen is white skinned, and thus does not have two Sonneratii haplotypes at the BCDO2 locus, it appears to have inherited at least one *G. sonneratii* haplotype in that region.



Example of contribution of G. sonneratii to the genetic make-up of domestic chickens around the BCDO2 gene. Two lines (one orange, one purple) per specimen.

The genome-wide scan based on a Stucture-like (Falush *et al.* 2003) model revealed that more than 99% of the domestic chicken genome has the expected *G. gallus* origin. The *G. sonneratii* contribution, although small, is quite significant and ranges from 0.1 to 1.2% according to the specimen. By contrast, *G. lafayetii* and *G. varius* contributions are quite small, ranging from 0.05 to 0.1%, and indistinguishable to expected level of statistical artifacts.

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MHC-B diversity of domestic chickens

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Introduction

The major histocompatibility complex (MHC) is a cluster of polymorphic genes involved in immune response. The chicken MHC is composed of two gene clusters, MHC-B and MHC-Y which are both located on chromosome 16 but segregate independently. The chicken MHC has a very strong association with resistance to disease and specific haplotypes have been shown to influence response to various pathogens, including viral, bacterial, as well as internal and external parasites. MHC haplotype influences have also been implicated in differential response to vaccinations (see review by Miller and Taylor, 2016). With the decline in availability of antibiotics and antiparastics for treatment of poultry, disease resistance is of particular relevance for poultry production.

Variation in the chicken MHC was initially identified by the use of serological reagents. Comparative studies amongst several laboratories defined a set of 27 haplotypes (Briles and Briles, 1982). The majority of these haplotypes were identified within the White Leghorn breed, thus little MHC information is known for other chicken breeds, including those used for meat production. With the advent of DNA-based detection methods such as Southern blots, restriction fragment length polymorphisms and sequence, the study of MHC variation was extended to include other lines, including meat type birds (Iglesias et al. 2003; Uni et al. 1993). However, these methods are time consuming, costly and not suitable for testing large numbers of samples as would be needed for commercial selection.

The complex variable number tandem repeat (VNTR) identified as LEI0258 is located within the MHC. This VNTR has been shown to be useful as a marker of MHC haplotypes (Fulton et al., 2006) because the allele size differences are generally large (12-13 bp) allowing the variation to be visualized on agarose gels. This highly variable marker has been used to examine MHC variation in numerous random bred indigenous chickens world-wide (Chazara et al., 2013). LEI0258 does have some limitations; serologically different haplotypes can have the same allele size and variability in the allele size within the same MHC haplotypes can occur due to mutation of the repeat number (Fulton et al., 2006).

SNP Panel

A high density SNP panel was recently developed, composed of 101 SNP that encompassed 230.000 bp region of the MHC-B. This panel was derived from a student PhD thesis (Chazara et al., 2010), but with modifications to the detection chemistry to utilize a competitive allele-specific PCR (KASP^{TM}, LGC, Middlesex, UK) and to extend the region covered to include the BG region of the MHC. The final SNP panel included 45 MHC-B genes, extending from BG2 through CD1A1. However, the SNP within the first 20.000 bp often show evidence of gene duplication or deletion. Genetic distance analysis, and haplotype comparisons are done using only the 90 SNP that cover the region encompassing LOC425771 through CD1A1 (210.000 bp).

This MHC SNP panel was applied to DNA from multiple sources, including serologically-defined university laboratory lines, university heritage breeds and lines used for commercial egg production. The SNP panel genotyping of serologically MHC-defined lines provided information that established SNP-based references for defined MHC haplotypes. A total of 78

haplotypes were identified from all sources. A topological network, typically applied to compare gene sequences for phylogenetic studies was used to examine the degree to which the multiple haplotypes were similar. The haplotypes clustered into 22 families (labeled as A through V), based on SNP identity, with many families having multiple members.
A novel nomenclature was developed to identify each haplotype. The name BSNP was used to indicate that the haplotype was based on SNP within the B region. When available, B haplotype information and LEI0258 allele size for that haplotype was also added. Thus BSNP-A04(357;B21) describes the B21 haplotype which has the LEI0258 allele size of 357 bp (Fulton et al 2016a,b).

MHC Recombination

Several MHC recombinants have been identified with alloantisera and histocompatibility testing, and the recombination rate was estimated to be 0.05%. MHC SNP panel genotypes of known recombinants confirmed that the genetic composition was consistent with that of the known parental haplotypes. Application of the SNP based panel to a pedigreed population of 1200 birds resulted in a 10-fold higher estimate of recombination (Fulton et al., 2016b). This higher rate was likely due to the higher resolution of variant detection for the SNP panel compared to serological detection. Within the university held serologically-defined laboratory lines, university heritage breeds and lines used for commercial egg production, 33 novel recombinant haplotypes were identified.

MHC Variation in other populations

The MHC SNP panel was used to examine MHC variation in other populations. Samples of wild, Vietnamese Red Junglefowl were highly heterogenous for their MHC. From 199 samples, a total of 313 haplotypes were identified, only 3 of which were shared between the four locations sampled. Furthermore, none of these haplotypes had been previously identified within any of the domestic chickens sampled (Nguyen-Phuc et al., 2016). The Finnish Landrace breed is a historical breed found within that country and is currently held in a genetic conservation program. A total of 195 samples were tested from 12 distinct populations. From these samples, 36 haplotypes were found, 16 of these had been previously reported within the university-held and commercial egg lines (‘original’ haplotypes), but a surprising 20 (56%) were novel (Fulton et al., 2017 in press).
The SNP panel has been applied to two diverse sets of samples; 34 traditional breeds from Germany (courtesy of Stephen Weigend) and 18 rare breeds within the US (courtesy of The Livestock Conservancy). Haplotype analysis was done within each breed, and then averaged across all breeds within each country source. The data is summarized in Table 1. The mean number of haplotypes per population is greater in the German vs. the US breeds (5.1 vs. 3.3), which may be a reflection of the greater population sample size for the German breeds. The haplotypes found within each population were compared with the 78 ‘original’ haplotypes reported by Fulton et al., 2016b. Amongst all populations tested, close to 50% of the haplotypes were novel as averaged for each country source, consistent with what was seen for the Finnish Landrace breed.

	# Breeds	Mean Pop. Size	Mean No. Haps	% Novel
German Traditional	34	18.1	5.1	57%
US Rare Breeds	18	12.8	3.3	48%

Table 1. MHC variation within German Traditional and US Rare breeds.

Many of the 'original' haplotypes were seen in multiple populations. Table 2 summarizes the most frequently encountered 'original' haplotypes found within the multiple breeds from Germany and the US, and the proportion of the respective populations with those haplotypes (i.e 26.5% of the German Traditional breeds contained at least one example of haplotpe BSNP-K03(B2)). There is commonality of most frequently identified haplotypes with both BSNP-K03(B2) and BSNP-O03(B26) being found in a high proportion of breeds from both countries.

	Haploype	% of Pops
German Traditional	BSNP-K03(B2)	26.5%
	BSNP-O03(B26)	23.5%
	BSNP-A09(BQ)	20.6%
	BSNP-B03(B22)	20.6%
	BSNP-K05	20.6%
US Rare Breeds	BSNP-O03 (B26)	27.8%
	BSNP-J03	27.8%
	BSNP-K03(B2)	22.2%
	BSNP-K02	16.7%

Table 2. The most commonly found haplotypes within the German Traditional and US Rare Breeds.

Furthermore, multiple novel MHC recombinants were detected in many of the populations (data not presented). In depth study of these recombinants could provide information on the significance of the specific genes or gene regions within the MHC that confer resistance to different pathogens.

Conclusion

Application of the MHC SNP panel to samples from German Traditional and US Rare breeds shows the presence of extensive amounts of MHC diversity. There is very little known about the disease resistance status of these multiple novel haplotypes. The SNP panel allows for rapid and relatively inexpensive detection of novel MHC haplotypes, and can potentially be used as a selection tool. With the decreasing availability of antibiotics and antiparasitic drugs for therapeutic use in commercial chickens, further investigations on the impact of MHC variation, and the genes within this important region of the chicken genome and resistance to disease are greatly needed.

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Industry session

New issues for dual-purpose chicken breeding for small producers

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With the world population projected to reach 8.5 billion people in 2030, more poultry meat and eggs will be needed from chickens reared in a broad range of environments and production systems. In developing countries, a large part of produced chicken meat and eggs come from small scale production systems raising indigenous poultry. For instance, in sub-Saharan African countries, 64% of the chicken population are raised in backyard systems, which provide 37% of the egg production (GLEAM 2016).

As an affordable source of animal protein, poultry contributes to the reduction of malnutrition. For many rural and peri-urban households, keeping dual purpose local chickens provides a punctual or regular source of income and contributes to the empowerment of women raising those chickens.

In 2015, the Second Report on the State of the World's Animal Genetic Resources for Food and Agriculture (FAO 2015) identified demand (in quantity and quality), changes in marketing infrastructure, retailing and trade, issues related to policies, technologies and environment as the main drivers of change on animal genetic resources and their management. In this regard, the future use and development of dual-purpose chickens will depend on the ability of the production systems relying on those resources to connect to markets, and to have access to necessary inputs.

Sustainable food value chain approaches aim at investigating how the full range of stakeholders and their coordinated activities allow to produce, transform and provide food products to consumers in a manner that is profitable throughout, has broad-based benefits for society and does not permanently deplete natural resources. Such approach is used to identify the challenges related to dual-purpose chicken production.

Dual-purpose chicken value chain analysis

As a preliminary step, it is important to define the extent of the demand and need for dual-purpose chickens. It is conceivable that dual-purpose chickens, which have been identified as an "integral component of the livelihood of poor rural households" (FAO 2008), will be suitable for the evolution of a sustainable and viable family poultry sector (Thieme et al, 2014).

Apart from household consumption, meat and eggs from chickens raised in small-scale production systems are distributed to final consumers through low value rural and urban markets, high value urban markets, formal and informal fast food outlets and restaurants.

In urban areas, meat from indigenous chickens raised in small-scale production systems fetch a higher price than broiler/layer equivalents as it is perceived to be tastier (Kyarisiima et al. 2011). Urban consumers are, therefore, ready to pay more for meat and eggs from indigenous chicken (Bett et al. 2013, Bwalya and Kalinda 2014). This supports the feasibility of developing a niche market in urban areas, with increased added-value for small-scale chicken producers. Dual-purpose chickens will fit nicely into this niche.

It has yet to be underlined that dual-purpose chicken value chains involving small-scale producers are generally undermined by a number of challenges, as illustrated in figure 1. Considering support functions, a large part of the constraints are directly or indirectly related to the provision of inputs. For instance, low productivity can be due to a combination of poor or no supply of feed and/or breeding stock.

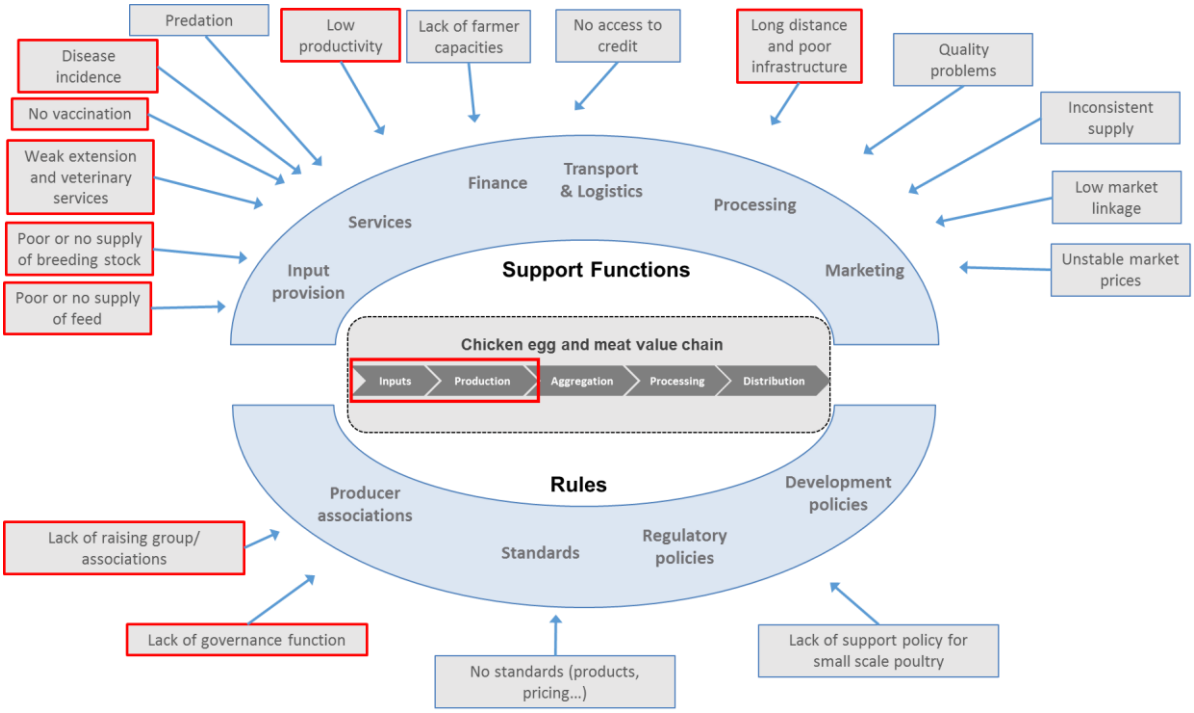

Figure 1. Market system analysis of dual-purpose chicken value chain involving small-scale producers (constraints related to input provision are in red frames,)

Genetic resources options

Diverse options have been considered to improve chicken genetic resources raised by small-scale producers. Those options roughly resulted in co-existence four types of chickens: i) indigenous ecotypes, which presently dominate small-scale production system, ii) improved indigenous chickens through structured breeding programs involving straight and/or cross-breeding, iii) F1 crosses between indigenous and commercial hybrids, and iv) commercial hybrids. The performances of these different genotypes in the field, retrieved from a preliminary meta-analysis of 26 studies are briefly summarized in Table 1. The results show that performances are largely impacted by the variability of production environment conditions and genetic background. For instance, the number of eggs produced per hen per year ranged from 23 for local chicken in Nigeria, to 248 for commercial Harco in Bolivia (Sorensen, 2010) with

a median value around 62 eggs, indicating that production environments may range from highly restrictive ones, to conditions close to those observed in modern farming facilities. The lack of comparative studies considering the different Genetic x Environment conditions make it difficult to provide recommendations on specific genotypes to be used in specific conditions.

Table 1. Range of performance of chicken raised in backyard conditions, based on 26 publications

			Genotypes			
Traits	N. obs.	Median (range)	Local	Local improved	Crossbred	Exotic
Number of eggs per year	42	61.8 (23-248)	--	=	+	++
Age at first egg (days)	24	173.6.7 (143-253)	=	=	=	=
Egg weight (g)	12	48.7 (37-80)	=		++	+
Hatchability (%)	14	82.3 (63-90)	=	+	-	-
Adult Body weight Male (g)	10	1769 (1099-3000)	-	+	+	+
Adult Body weight Female (g)	10	1429 (1049-2500)	-	+	+	+
Survival at 6 month (%)	9	68 (25-89)	+	+	=	-

Genotype performance are qualitatively assessed from -- (very poor) to ++ (very high), with = meaning average level

The above table does not consider several elements. Traits like scavenging ability, broodiness and aggression required for the protection of chicks from predation are important features for the small-scale production system and determinants of breed development (Olori, 2008), but are rarely measured, and hence not included in table 1. Indigenous chickens require less input (feed, drugs), a critical feature in backyard systems, which may result in higher cost-benefit ratio despite a lower net return, when compared with more demanding genotypes (Khan et al. 2010). This depends however on the physical or financial availability of input within the system. Development and routine supply of improved chickens (either improved indigenous, F1 or commercial) will require specific infrastructural and policy development. The merits and constraints of using either a specialized or a dual-purpose chicken have to be assessed in terms of their economic and social (food security) sustainability. Consumers' preference for a specific product should also be considered. These elements illustrate the importance of aligning the breeding strategies with the specific context of the whole value chain.

Logistical and governance challenges

Genetic improvement is then a key component of value chain input, which has to be imbedded within a scheme that is sustainable and profitable for all stakeholders involved. The inability to sustain a continuous supply of improved genetic material to small producers has been reported as major cause of failure of improvement projects in developing countries. Likewise, the involvement of the private sector in the organization of breeding schemes tailored specifically to the needs of small-scale poultry production constitutes a major challenge for poultry production (Thieme et al., 2014). For example, the breeding scheme of the 'Kuroiler', a dual purpose chicken specifically designed for small-scale poultry production, involves an

integrated distribution channel of vaccinated three-week old chicks through a network of mother units run by local entrepreneurs. It constitutes an interesting case study of how to handle different input-related challenges (Ahuja et al. 2008).
A good governance at each step of the value chain is critical to ensure equitable sharing of the benefits between all actors. If the development of improved dual purpose chicken is a viable solution, agreements between the different stakeholders on ownership, access right, distribution channels and support services, as well as respective responsibilities has to be worked out to ensure a sustainable supply.

Conclusion

Value chain approaches are interesting tools to investigate the relevance of dual-purpose chicken production in developing countries, especially in relation to market and demand-led drivers. Other factors has yet to be considered in parallel, such as inclusiveness, especially for women, and potential impact of changing environment on the production systems.
Diversification of production systems and chicken genetic resources could be key to the sustainable supply of poultry products to meet the anticipated high demand fueled by future population growth and income increase. Development of improved dual purpose chickens, in addition to existing commercial meat and egg genotypes, will offer new options, in particular for small-scale production in rural areas. The specific genetic material to be used and the modalities for its development depend on the local production environment and the broader value chain context.

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Solutions from the industry: Dual purpose breeds or sex determination in the egg

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As highly negative correlations harm genetic progress in both laying performance and meat yield at the same time, specialised chicken lines have been bred over time which feature either an efficient production of high quality eggs, or high growth rates. This results in the culling of the day-old male chicks in the hatchery that poses problems both in terms of animal welfare as well as ethics.

The layer industry in Germany has always been clear about their commitment to implement different solutions to the culling of day-old layer chicks as early as possible.
Different research projects including sex determination in the egg, growing layer males, and breeding dual purpose breeds, have been established. The research group coordinated at the University in Dresden plans to construct a prototype for gender determination with a near-infrared fluorescence spectroscopy in summer 2017. After 3 days of incubation, a portion of the shell (a CO_2 lasered hole with 12mm) is removed and a non-invasive spectral analysis is applied. The Raman spectrum is unique for each molecule and often called a 'molecular fingerprint'. Small negative effects on hatchability are recorded and more than 95% accuracy in sex determination can be achieved.

Growing day-old commercial layer males for up to 10 weeks (1 to 1.1kg live weight), or up to 1.5kg at 13 to 15 weeks of age, are currently being tested in Austria and Germany.
The cost of growing these males (€4 - €6) is supported by an egg price increase of 2 to 3 cents per egg from the sister bird. The meat of these males can only be used as ingredients for sausages or similar products. In 2016 and 2017, some retailers have already started to market these products with a special brand name in the retail and discount segments.

Dual purpose breeds are offered by different breeding companies in Europe and the U.S.A. Traditional breeds like New Hampshire and Sussex are not commercially viable solutions.
Different crosses between broiler and layer lines can be used to generate new versions of dual purpose breeds. Using a dwarf male line has the advantage of having dwarf layers for egg production with lower nutrient requirements and regular growing males.
Dual purpose breed males need a two to three week longer growing period with a significantly lower yield of breast meat. Layers produce 40 to 60 eggs lesser and eggs that are smaller sized eggs as compared to regular commercial layers.

Due to these significant disadvantages on the side of the females and males, dual purpose breeds have not been able to gain any significant market shares. The layer industry is focusing on sex determination in the egg as a potential solution on a worldwide scale where animal welfare and ethic-related problems of culling day-old males are being intensively discussed.

Breeding for Premium broiler markets: Chicken of Tomorrow case study

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Key words: Premium chickens, breeding, market developments, the Netherlands, Chicken of Tomorrow

What market are we producing for?

It is very important to realise for which consumers or markets we are producing for. The actual demand depends on spending power and level of development, with more or less focus on animal health and welfare.
In (North) Western Europe concerns on use of antibiotics in animal production and animal welfare have grown. Retailers operating in this market also play an important role as they 'translate' consumer demand and the influence of animal welfare associations can affect retailers and consumer behaviour.

History and development in Europe

Taste and 'cuisine' were the basis of the French 'Label Rouge' chicken. In the early nineties French integration 'Duc' developed a new segment called 'Certified' chickens grown indoor to 56 days with lower stocking density. Later on the EU developed its own official marketing terms laid down in each European language.

The Netherlands: a special case

In 2006 the first 'extensive indoor' chicken (56-days + 'wintergarden') was introduced in the Netherlands. Sales really took off with full support of the Dutch Animal Welfare Association and when supermarket 'Albert Heijn' started to promote it. Almost all other supermarkets in the Netherlands followed their example.

In 2013 the 'Chicken of Tomorrow' was born in the Netherlands as a reaction to the huge pressure of the extreme animal welfare association 'Wakker Dier' (Awoken Animal). This new segment was positioned between conventional broilers and the 'extensive indoor' chickens focussing on slower growth (max. 50 g/d ADG) and enriched environment. By the beginning of 2016 the two leading supermarkets (AH and Jumbo) already replaced all standard chicken (fresh) meat with their own 'Chicken of Tomorrow' concept and started to use it for other chicken products, like deli meat, salads, pizza's as well. The retailers also introduced many new chicken products to make much better use of the whole carcass. End of 2016 Premium chickens (organic, extensive indoor and 'Chicken of Tomorrow') represented about 90% of all chicken meat sold in supermarkets in the Netherlands. Really a unique development in the world. Use of antibiotics for all these kind of products is close to zero and meat quality defects are not seen at all.

Hubbard Premium R&D

Hubbard has already 50 years' experience in the selection and marketing of Premium products. To cover the different demands around the world we have a large product range with a choice of different Premium females and males. Several years ago Hubbard intensified it Premium research program and introduced several new techniques to ensure the quality and efficiency of these products. We continue to focus on welfare, robustness, breeder and broiler efficiency and meat quality.

Session - Genetics of product quality

Genetics of meat quality defects in broilers

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Keywords: genetics, meat quality, meat defects, selection, chicken.

Context: Face to a growing demand, poultry meat should soon become the first meat produced and consumed in the world, with 37% of the total in 2023 (OCDE-FAO, 2014). Poultry meat is now mostly consumed as cut up parts or processed products, which has emphasized the importance of breast meat yield in addition to body weight and feed efficiency. Genetic selection, together with progress in nutrition and veterinary medicine, has led to an incredible increase of the efficiency of birds to produce meat in a reduced rearing time. While 8 weeks were needed to produce a bird of around 2 kg in the sixties, chickens are now reaching 2.2 kg at 5 weeks, with a proportion of breast meat higher than one fifth of live body weight (Petracci et al., 2015). Higher muscle growth has been accompanied by changes in histological and metabolic characteristics of breast muscle. Thus, muscle hypertrophy resulted in an increased fiber size as well as lower glycogen reserve and blood supply (Berri et al., 2001; Berri et al., 2007; Le Bihan-Duval et al., 2008). With the increased use of meat as cut-up parts and processed products, meat quality defects were also revealed. Metabolic defects due to abnormal post-mortem pH fall were first reported. In poultry as in pigs, rapid post-mortem decline (evidenced by low pH value measured 15 min post-slaughter) is responsible for PSE (pale, soft, exudative) meat that exhibits a pale aspect and reduced water-holding capacity (Owens et al., 2000). Variations in the extent of decrease in pH are also responsible for variations in meat quality. Low ultimate pH (measured 24h post-slaughter) results in "acid meat" which is often qualified as PSE-like since it presents similar defects (Barbut, 1997), while high ultimate pH leads to DFD (dark, firm, dry) meat with dark color and poor storage quality (Allen et al., 1997). More recently, a global attention was paid to the increasing incidence of structural defects described as myopathies. The two main defects are White Striping and Wooden Breast that are both characterized by myodegeneration, and regenerative events along with fibrosis and lipidosis (Kuttappan et al., 2016). As for metabolic disorders, they largely affect the sensorial and technological quality of the meat. As it was the case for meat quantity, genetics could be an efficient way to improve meat quality by reducing the occurrence of meat defects. This however depends on the role of genetics in the control of meat quality as well as on the possibility to develop tools such as biological or genetic markers that could improve the applicability and efficiency of selection against meat defects.

Genetic determinism of meat quality traits and defects: Genetic analyses conducted on different chicken lines, experimental or commercial, slow-growing or fast-growing, reared in controlled or commercial conditions, have revealed a significant role of genetics in the control of meat quality traits. Thus, the heritability of breast meat ultimate pH (pHu) ranged between 0.31 and 0.49, that of meat lightness (L*) between 0.29 and 0.75 (Le Bihan-Duval, 1999, 2001, 2008; Chabault et al., 2012; Gaya et al., 2011). Two independent experiments proved the feasibility of a divergent selection on the two latter parameters and allowed to create useful

resource populations for the study of PSE- and DFD-like meat (Harford et al., 2014; Alnahhas et al., 2014). After six generations of divergent selection on breast meat ultimate pH, mean pHu was estimated at 5.67 in the pHu- line while it was equal to 6.16 in the pHu+ line. This implied that 61% of the breast meat in the pHu- line could be classified as acid or PSE-like (pHu < 5.7) and 63% of breast meat in the pHu+ line as DFD (pHu > 6.1). Divergent selection resulted in a higher glycolytic potential in the *Pectoralis Major* muscle of the pHu- line compared to the pHu+ line. Metabolic changes were also observed in the thigh with a difference of more than 0.3 pHu unit in the *Sartorius* muscle. Regarding meat quality, the divergence of pHu led to breast fillets less pale, less red and less yellow in the pHu+ than in the pHu- line. The reported divergence was also associated with higher curing-cooking yield and lower drip and cooking loss in the pHu+ compared to the pHu- line. Regarding the sensorial quality, the breast fillets of the pHu+ were tenderer and had a less pronounced acidic taste than those of the pHu- line (Alnahhas et al., 2015). After 8 generations of divergent selection on L*, this criterion was increased by 7 points in the High Muscle Color (HMC) line by comparison to the Low Muscle Color (LMC) line. Selection resulted in correlated responses on drip loss and post-mortem pH fall associated to PSE and DFD-like meat. As shown by these experiments, criteria such as lightness or ultimate pH can be efficiently selected. Although we know that genetic variability is partly dependent on the line, these results indicate that these two measurements could be used to monitor meat quality in the selected lines and to limit or prevent the incidence of PSE- and DFD- like conditions.

While divergent selection on breast meat pHu had no impact on abdominal fat or body weight, a favorable effect on thigh and breast meat yield was observed in the pHu+ line. The cross-sectional area of muscle fibers in the pectoralis major was not modified, but significant reduction of the number of capillaries per muscle fiber was observed in the pHu+ by comparison to the pHu- line. The structural and metabolic changes observed in the pHu+ line appeared to be predisposing factors to the development of White Striping (WS) whose incidence of moderate (MOD) and severe (SEV) cases was higher than in the pHu- line (Alnahhas et al., 2016). Regardless of the line, the MOD and SEV cases of WS were phenotypically characterized by increased body weight and breast meat yield compared to normal muscles, confirming the unfavorable relationship between muscle growth and susceptibility to myopathies (Kuttappan et al., 2012; Petracci et al., 2013). WS was found to be highly heritable (h^2=0.65), and highly positively genetically correlated with breast meat yield and body weight that appeared to be the major determinants of this defect (rg = 0.68 and 0.33, respectively) in the pHu lines. The intramuscular fat content of the *Pectoralis major* was also strongly correlated with the defect (rg = 0.64), which was consistent with the presence of lipidosis described in affected muscles. Bailey et al. (2015) reported lower WS heritability in two commercial lines of broiler chickens characterized by high (h^2 = 0.34) or moderate (h^2 = 0.18) breast meat yield. Quite low heritability was also obtained for wooden breast (h^2<0.10). In this second study, the genetic correlations between myopathies and breast muscle yield were null to moderate (0 to 0.25). These differences in magnitude (for heritability and genetic correlations) may be related to differences of scoring, methods of genetic parameters estimation but also history of selection that affects the within line genetic variability and possibility of genetic progress.

New tools for breeding on meat quality and against defects: Current research programs are aiming to identify biological and genetic markers that could be useful for a more efficient selection on meat quality traits and against defects. High-resolution NMR was used to characterize the metabolic signature of the muscle and serum of the pHu+ and pHu- lines and

to look for predictive biomarkers (Beauclercq et al., 2016). Lowering or increasing muscle glycogen by selection affected several muscle and serum metabolites. Because of their high ability to store glycogen in muscle, pHu- birds were using carbohydrates as main source of energy. By contrast, pHu+ birds that are depleted in muscle glycogen, used amino-acid catabolism and lipid oxidation leading to oxidative stress and to an adaptative response of protection by releasing antioxidant molecules. Statistical analyses allowed identifying sets of muscle or serum markers able to discriminate clearly groups of birds with high (DFD) or low (PSE-like) ultimate pH. Serum biomarkers are of special interest since they require a less invasive sampling technique than muscle biopsy. The more parsimonious model established with serum metabolites still ensured a good level of discrimination. It included a set of 7 biomarkers among which xanthine and hypoxanthine were the most discriminant. It is worthwhile to note that the metabolomics analysis of muscle affected by wooden breast revealed some common metabolic characteristics and biomarkers with those evidenced in the pHu+ line (Abasht et al., 2016). Glycogen content was considerably lower in samples taken from Wooden Breast affected birds when compared with samples from unaffected birds. Affected tissues exhibited biomarkers related to increased oxidative stress, elevated protein levels, muscle degradation, and altered glucose utilization. Affected muscle also showed elevated levels of hypoxanthine, xanthine, and urate molecules (the generation of which can contribute to altered redox homeostasis). This suggested again a possible link between the deficit in muscle energy and the susceptibility to myopathies. The use of genetic markers as an alternative to sib-selection is of course a promising way of progress. Few studies have been conducted in order to look for QTL of meat quality and, among the 6633 QTLs reported in Animal QTL database, only 105 concern meat quality traits. The divergent lines selected for meat quality traits constitute relevant genetic materials to identify new genetic markers. The pHu+ and pHu- lines were recently used in order to look for selection signatures and pHu QTLs. Several regions of interest have been revealed and will be further investigated in order to look for candidate genes and mutations.

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Improving egg quality; win-win traits

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It is a cliché that you do not get something for nothing. That can seem apposite when it comes to improving egg quality traits. Many egg shell quality traits are intrinsically involved with the formation of the shell. The formation of the shell requires a large amount of energy and a large amount of calcium, which if not carefully managed will result in reduced mineralisation of the skeleton. In this presentation, I want to look beyond the shell at some of the issues and needs in the field of egg quality research. These are traits that have application to both layer and broiler type chickens and, potentially, to other species of poultry. These are relatively neglected structures in the egg which have potential beneficial influences on the poultry industry. I have in mind the vitelline membrane and the cuticle. Improvements in both of these structures should not be correlated with negative effects on hen welfare, but yet they promise improvements in bio-security, processing, and the potential to improve hatchability. These are all potentially positive messages for the industry.

I will look at some potential solutions to measuring and then improving these aspects of egg quality by using genetics. I hope, through this, to try and encourage work on the vitelline membrane and I will present new results on the trait of cuticle deposition where we have made some progress.

Firstly the vitelline membrane, it is a structure for which we have very little of a positive nature to report about its measurement. The vitelline membrane consists of three layers; the innermost layer is produced by the granulosa cells in the ovary, and contains many of the proteins which are involved in sperm recognition and fertilisation. The other two layers are elaborated in the infundibulum and include a few dominant proteins, some with antimicrobial properties, and may be considered as a specialised part of the egg white (Mann, 2008). The function of the vitelline membrane is, therefore, to mediate fertilisation of the ovum, to maintain separation of yolk and white, and to offer a final barrier to micro-organisms reaching the yolk where there is potential for rapid multiplication. However, the vitelline membrane is important for egg processors and for the hatchability of the egg. Clean separation of the yolk and the white is critical in the egg processing industry as there is demand to produce products from the yolk and white separately. The strength of the vitelline layer is critical in this process and poorer quality vitelline membranes are prone to rupture; this produces problems for the respective product. The properties of albumen are particularly compromised by contamination with yolk, because of the reduction in its foaming properties. As little as 0.02% yolk can have an undesirable effect on egg albumen (Wang and Wang, 2009). Given the role of the vitelline membrane in sperm recognition, it is also likely that the quality of the vitelline membrane would influence fertility. Certainly treatments such as the nicarbazin cocciodiostat, which is reported to damage the

vitelline membrane, has effects on fertility (Cunningham, 1977, Cunningham and Ylander, 1980).

The challenge for the improvement of this trait has been to find a suitable measurement that could be realistically made for selection, in other words with high throughput. A number of papers have been published on the topic of measurement, the earliest applied pressure to the egg (Moran, 1936) to measure vitelline membrane pressure. More recently methods using force to determine either rupture strength or deformation have been applied (Kirunda and McKee, 2000, Fromm and Matrone, 1962, Marzec et al., 2016). However this approach, although relatively simple, is heavily influenced by the yolk properties, in particular its viscosity, and so the measurement is unlikely to be specific to the vitelline membrane strength. When we used this approach we found the repeatability at the level of the bird to be only 19% (Lund et al., 2011), in other words 80% of the variance was attributable to sources other than bird, such as the measurement, which did not suggest it would be a method suitable for use in selection. We believe a practical solution will require an approach that minimises the influence of the yolk and ideally allows more than one measurement per egg. This will most likely require the application of negative pressure, or vacuum, to satisfy that requirement. Even then it may not be completely without influence of the yolk. Papers using this approach were published in the 1960s (Fromm and Matrone, 1962) and appear to offer some promise, although there was significant variation with different membrane areas. A 'Diplomarbeit' of Birgit Waterloh (personal communication) demonstrated its potential for genetic studies but still its practical limitations prevented adoption. Whilst we have given this some thought, there is a need to combine engineering skills with biology and genetics to get a practical tool to make the measurements rapid enough required for genetic improvement. That said, the measurement using negative pressure were well correlated with membrane weight and in fact the relatively simple measure of yolk index, these might provide a suitable alternative to direct measures of strength. Researchers should, of course, be mindful that traits involved in fertility often have an optimal value rather than a linear response, egg traits of course fall in to this category. Moving away from the optimum in any direction will reduce fertility; however, in this case, it seems likely that the optimal structure will suit both reproduction and processing. For this reason this topic should provide a fertile ground for researchers wanting to improve egg quality.

In contrast, in the case of the cuticle we have made more progress towards delivering usable methods for the breeding industry. The cuticle is an invisible layer deposited on the outside of avian eggs (Gilbert, 1971), and, when intact, fills the gas-exchange pores and prevents micro-organisms entering the egg (Bain et al., 2013, Vadehra et al., 1970). Although better anatomically separated than many people imagine, both the egg and faeces exit from the avian cloaca, which, along with potentially dirty nest sites, can result in eggs having high bacterial loads (Barrow, 1994). The evolution of an aqueous and antimicrobial barrier around the egg, such as the cuticle, would seem a suitable response to these challenges. However, despite scientists being aware of its function and importance for at least 130 years (Tyler, 1964, von Nathusius, 1882), there is a remarkable degree of confusion about its formation, how it is related to the pigmentation of eggs and, perhaps most remarkably, exactly where and when it is deposited on the egg. Building on our original studies which alerted us to the potential of this structure for genetic improvement (Bain et al., 2013) we have further investigated both the genetic parameters for cuticle measurement and the functional and physiological properties of the cuticle. Our most recent estimates of heritability in the same line for the trait of cuticle deposition are 0.49±0.12 and 0.43±0.12 at 32 and 50 weeks of age, respectively, and are higher than our previous estimates of 0.27±0.13 for a Rhode Island Red line (Bain et al., 2013) . Importantly, it has been possible to replicate the observations in genetically distinct genetic lines of poultry. Most notably in a line of broilers we have been able to demonstrate a respectable heritability value of 0.24±0.04 or higher, and an estimate of 0.31±0.10 was made

for a white leghorn line. As with many egg traits, the repeatability of the measurement is relatively high when measuring eggs from the same hen. Estimates of variance explained by bird come in around 50% for a broiler line. This is reflected in a large genetic correlation, with an estimate of 0.96±0.04, between the measurement of cuticle deposition taken from the same Rhode Island Red hens at 32 and 50 weeks of age. To strengthen our belief in the value of improving the cuticle, we have extended observations on the effect of the natural variation in cuticle coverage on the penetration of bacteria through the shell, from laying-type hens to a broiler line. We have also extended the types of microbes studied, from *E. coli* to *Salmonella*, a relatively more motile bacterium. Categorisation of the eggs from broiler dams by whether they were not penetrated by *E. coli*, or were heavily penetrated, demonstrated a significant effect of the amount of cuticle deposited on eggs ($P<0.02$). Around 25% more cuticle was found to be present on eggs that were not penetrated by *E. coli*. Categorising the penetration of two eggs from the same hen, by whether none, one or both eggs were penetrated by *Salmonella*, showed a highly significant effect of the amount of cuticle on the respective eggs ($P<0.001$). Eggs in the 'none' category had a cuticle deposition value of ~50% more than the category where both eggs were penetrated.

In our efforts to understand more about the cuticle we have determined that a stressor, such as relocation, contributes to the non-genetic component of the variation in cuticle deposition and acts to reduce its deposition. We have also established that the cuticle is deposited on the eggs in the shell gland, and not the vagina of the oviduct. Cuticle deposition occurs immediately prior to oviposition. The factors that induce the deposition of the cuticle remain unknown but the proximal factors inducing final oviposition, arginine vasotocin and prostaglandin, do not appear to stimulate its release, since eggs prematurely induced this way have no cuticle. However, early oviposition using gonadotrophin-releasing hormone, which induces a premature but normal ovulatory surge, does result in the appearance of an egg with a normal cuticle. There is some debate in the literature regarding the relationship between pigment and cuticle, with some sources suggesting that the pigment in brown eggs is found in the cuticle. It might, therefore, follow that hens selected for having brown shells might have more cuticle. Whilst we did not set out to compare lines of hen and the amount of cuticle, there is certainly no suggestion from our observation that white or tinted shells have any poorer cuticle deposition. Nor did we observe any significant genetic correlations between the colour of eggs and the amounts of cuticle, with estimates ranging from -0.33 to +0.37 with errors near the size of the estimate in 5 different lines or generations. Experimentally, when we manipulated the amount of pigment in the shell, or compared eggs from hens laying eggs with high or low amounts of cuticle, the results actually supported a small negative relationship between the amount of cuticle and that of the pigment. This may represent competition for resources in the secretory apparatus of the shell gland at the end of shell formation. It, however, does not seem to support the existence of any genetic association between the shell colour and cuticle deposition.

In conclusion, we have identified two traits that offer potential improvements in egg quality without dramatically increasing the metabolic demands on the hen. They have the potential to improve fertility and hatchability. The estimates we have reported for the cuticle are on populations of between 1000 and 1500 hens, it will probably need larger populations to demonstrate effects on fertility. In the case of the cuticle, from our trial challenges we are confident it should reduce the incidence of contaminated eggs, reduce the vertical transmission of potentially pathogenic organisms, and help improve biosecurity. For the vitelline membrane we will need to work harder to find a practical solution to measure the trait in an easy and reproducible manner, in order to deliver improvement.

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Session - Breeding issues for other species

Current and future challenges for European turkey breeding

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Introduction

The turkey industry supply chain flows from the breeders through a number of multiplication phases which culminate in commercial birds being grown and processed and finally sold to the consumer. Primary breeders are at the start of this process. Delivery of genetic progress from the breeding programme to the end-consumer typically takes around 4 years. This means the challenges of the industry today had to be anticipated by the primary breeders 4 years ago and the challenges for the future consumers need to be anticipated now.

This paper will review the processes driving change in turkey breeding. It will then turn to look at how the primary breeders are integrating these changes into the genetic improvement programme, and finally consider the future challenges for turkey breeding.

The turkey industry structure

European turkey production is going through dramatic shifts in the production bases. In general, there is static or declining placements in the long established producing countries of France, Germany and the UK. Increasing investment in production is seen in the growing markets of Russia, Poland and Spain. There are a variety of reasons for this: government led desire for self-sufficiency in protein production, low cost of production and access to export markets, innovation in processed turkey products available for the consumer.

The turkey industry is served by two major primary breeding companies: Aviagen Turkeys and Hybrid Turkeys. They provide breeding stock free of a number of major diseases. The market requirement for breed types is principally divided into two segments: the heavy sector where males are typically grown to 19.5kg – 22kg and the medium sector where birds are grown up to 18.5kg. As an example of the heavy breeds used, Aviagen Turkeys supplies the BUT6 and the TP7 (a product being tested). In the medium sector, the BUT Premium is supplied. There are a number of smaller, speciality segments across Europe which includes organic producers and the farm fresh sector in the UK which supplies high value whole birds and other products specifically for Christmas. A combination of the mainstream breeds and slower growing or coloured breeds are used in this segment and supplied by businesses like Aviagen Turkeys' Hockenhull subsidiary.

Determinants of breeding goals

Turkey breeders receive direction from many sources directly and indirectly through a complex web of communication. The job of the breeders is to disentangle these messages and distil them into practical breeding goals. The consumer, as the end purchaser, is the key driver, forming their opinions from a range of sources and these are typically reflected in their purchasing habits which in turn affect the retailers, producer and ultimately the breeders.

Consumer preference research indicates that, alongside the traditional drivers such as price, there is an increasing emphasis on evolving drivers such as health and wellness, safety and social impact (Ringquist *et al.*, 2015). Transparency is increasing in importance as consumers want to know more about their food and how it is produced.

Within the primary breeding operations, many of the consumer preferences have been incorporated into the breeding objectives for their genetic lines, as breeding companies have moved from a focus on productivity related traits towards multi-dimensional breeding goals (Neeteson-van Nieuwenhoven *et al.*, 2013). Whilst economic efficiency remains hugely important, there is increasing emphasis on traits related to welfare, sustainability and robustness.

Historically, primary breeders were cautious about releasing details of their breeding operations as this is their source of competitive advantage. However, this is changing as breeders recognise the role they have in informing society of the improvements being made through selective breeding. Examples of activities now undertaken by the primary breeders include open days for industry stakeholders, publication of results of breeding research, sharing information at conferences and participation in a range of research programmes and working groups developing policies on turkey production.

Integrating evolving needs into the breeding programme

Integrating expanding consumer needs into the breeding programme requires the primary breeders to continuously search for more accurate ways to collect data, and analyse data for different purposes and for new traits. Some selected examples of these challenges are: progress in sustainability, improving gut health and robustness and application of genomics.

Sustainability is an evolving need of increasing importance to the consumer. Sustainable resource utilisation is a by-product of growth, FCR and liveability traits which have been incorporated into the breeding programme for many years and whose selection has evolved with the adaptation of new technology and analysis techniques. As an example, the turkey of today compared to the bird of 1993 is 3.9kg heavier at 20 weeks and 48 points more efficient in FCR to 21kg (source: BUT6 performance objectives). These improvements have direct effects on sustainability as less feed is required to produce the world's annual requirement of 5.4 million tonnes of turkey meat. The reduction in feed means fewer road trips to collect raw materials and deliver feed and the valuable land resource requirement to grow cereal crops is reduced. Further Leinonen et al., (2016), through the use of life cycle analysis modelling, highlighted the importance of feed efficiency in reducing the environmental impact of turkey production.

Use of animal medicines in agriculture have come under increased scrutiny due to concerns about antimicrobial resistance and the lack of new antibiotics being approved for human use. Turkey producers are being required by government intervention to dramatically reduce their usage of these treatments, much of which can be done through improved management. The German turkey industry antibiotic use has declined by 40% since an antibiotic use and improvement system was introduced in 2014 by the Federal Office of Consumer Protection and Food Safety. Breeders contribute to reduced antibiotic use through the selection of birds which are generally more robust i.e. birds which have good overall fitness and vitality across a wide range of production environments. Gut health, skeletal strength and immune function are key components.

Water consumption is an indicator of gut health and functionality and has the benefit of improving litter conditions and footpad health. Birds are responsible for most of the moisture found in the barn. Whilst most birds consume on average around 1.65 litres/kg liveweight at

market age, some birds consume considerably more. Around 20% of water consumed is assimilated; the rest is exhaled or excreted. Selection against excessive water consuming birds has resulted in around a 5 litre per bird reduction in consumption since we began the work in 2012. This reduces the amount of water consumed by an average 8,000 bird flock by around 41 tonnes with consequential benefits on footpad health and litter use.

Leg health is assessed through a combination of traits such as gait scoring, x-raying with the lixiscope to identify clinical and subclinical tibial dyschondroplasia, scoring of angular leg deformities and footpad health (Kapell *et al.*, 2017).

Multi-environment selection is used to overcome the breeder's dilemma of needing to ensure biosecurity of pedigree birds whilst addressing the need to identify birds which underperform in more challenging environments. This is done by placing pedigree siblings in a separate farming system where they are grown in conditions seen in commercial farming environments. Measurements are made in both environments and the data is used in the genetic evaluation and selection. This allows the identification of birds with the capability to do well, regardless of environment.

The development of genomic selection in turkeys has progressed rapidly since the publication of the turkey genome in 2010. Lessons from the commercial application of genomic selection in broiler breeding have paved the way for rapid implementation in turkey breeding. In 2016-17, the era of genomic selection in commercial turkey breeding began (Kranis et al., 2016). With genomic selection, phenotypic measurements are combined with information at the DNA level to improve the accuracy of predicting the birds with the best genetic potential. In a proof of concept, genomics results in a 40% improvement in accuracy for feed efficiency through a better prediction for non-phenotyped individuals (Kranis et al., 2016).

Future challenges for turkey breeding

In many ways, future challenges will likely be an extension of what we have seen in recent years. An increasing global population will put further pressure on natural resources and so efficient production will remain key (Neeteson and Avendano, 2016). The drive for reduced medicine use will continue as will the preference for improved animal welfare. Particularly challenging areas may emerge for novel traits required to overcome alterations to production systems as a result of legislative changes, for example the banning of infra-red beak treatment.

Novel traits and recording technologies present new breeding opportunities. One example is the use of 3D imaging technology to predict breast meat yield and product quality. This technology has been implemented for broiler breeding and its feasibility in turkeys is being evaluated.

Novel behavioural traits are under evaluation. For example, a by-product of individual feed and water intake evaluations has been the collection of associated behaviour measurements (Howie et al., 2010, Rusakovica et al., 2016). Whilst these behavioural traits have shown useful heritabilities, their utility needs further exploration.

Commercial birds are routinely grown to near-sexual maturity and expression of aggression and pecking is a natural behaviour. Currently, damage by pecking is typically controlled through beak treatment which reduces the potential of pecking damage. Whilst this is seen as a welfare benefit in many countries, the industry is being challenged to find methods to grow birds without beak treatment. In Germany, there is a proposal to ban beak treatment in 2018. There are differences in the aggressiveness of turkey breeds and there are likely to be differences within lines which could be exploited through selection. However, this requires identification and recording of aggressive individuals through behavioural monitoring and has

been successfully demonstrated in layer chickens (Kjaer and Sørensenb 2001). The genetic basis of aggressive behaviour and pecking in turkeys still needs to be elucidated, and methods to accurately identify aggressive individuals need to be developed.

Volatility and uncertainty in the marketplace is becoming the new norm. Climate change, feed price volatility, health challenges and political unpredictability etc. have knock-on effects in trade and investment and also on consumer confidence and attitudes. As breeders, altering breeding direction to meet changed needs takes a long time due to the lag between the breeding operations impacting the commercial birds. Breeders therefore need to be geared to deal with the uncertain times the future holds. This is handled in a number of ways: product choice to cater for different needs, broad breeding goals to cover many traits simultaneously, strong emphasis on building robustness to deal with current and emerging production systems, carrying a wide gene pool of lines, many of which have no utility at present but are our security should new requirements arise. Finally, geographically separated breeding operations allow breeders to select for local requirements and offers security against health issues.

Conclusion

Turkey meat is in direct competition with other protein sources. Future success will rely upon adapting the breeding goals to meet the developing needs of the consumer. Through increased transparency and factual communication, the breeder also has a role in shaping consumer preferences.

The future will likely be driven by an expansion of the general challenge to deliver more progress in more traits. These will principally be economic drivers and evolving demands such as sustainability, welfare and robustness. Some of the future challenges will require investment in innovative breeding solutions. Breeders will also need to adopt breeding strategies to cater for rapid changes in market needs. To meet the future challenges for a successful turkey industry, there will be an ongoing need to increase investment in turkey breeding and a requirement to recoup part of the additional value generated from the supply chain.

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Guinea fowl whole genome assembly and application for genetic diversity in African and European populations

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Context

Nowadays, a whole genome sequence assembly is considered a pre-requisite for any large-scale work involving genomics. Chicken and other major poultry species, such as turkey and the common duck, have had such a resource available since several years and in the case of chicken, several updates of the reference genome were released, making it one of the best available for vertebrates and a focal point for birds (Warren *et al.* 2017). Nowadays, the so-called NGS (Next Generation Sequencing) technologies allow the automated production of very large datasets at low cost and allowed to raise the number of bird whole genome sequencing up to forty-eight species, chosen to represent all bird orders. These were used for analyses of bird evolution (Jarvis *et al.* 2014, Zhang *et al.* 2014). However, the species in these two studies were chosen so as to represent at best all bird species, whose number is estimated between 9,000 and 1,000. As a consequence, no new galloanserae species, from which the major poultry species broadly used in breeding are from, were included. To initiate genomics analyses in Guinea fowl, a poultry species widely used in Africa and Europe, we produced a first generation whole genome sequence draft assembly and used it as a reference genome for a diversity study.

Guinea fowl genome assembly

The whole genome assembly of the helmeted guineafowl *Numida meleagris* was produced by Illumina paired-end sequencing at 100X depth and assembled with AllPaths. As result, 2728 scaffolds were obtained and the N50 contig and scaffolds were 232 kb and 7.8 Mb respectively.

To assemble the scaffolds at the chromosome level, we took a reference-guided assembly approach using the closely related chicken genome and the high quality Gallus_gallus-5.0 genome as a reference. The comparison of cytogenetic and genetic maps from several gallo-anseriformes showed to date very limited number of inter-chromosomal rearrangements for macrochromosomes and concerning microchromosomes, no inter-chromosomal rearrangements between ducks and chicken, were detected to date (Fillon *et al.* 2007). Based on this, guinea fowl scaffolds were aligned to the Gallus_gallus-5.0 reference genome with LASTZ and ordered relative to one another. Chromosome assignments of scaffolds were then

done and chromosome nomenclature of the resulting assembly was done according to known cytogenetic data (Shibusawa *et al.* 2002).

Diversity study

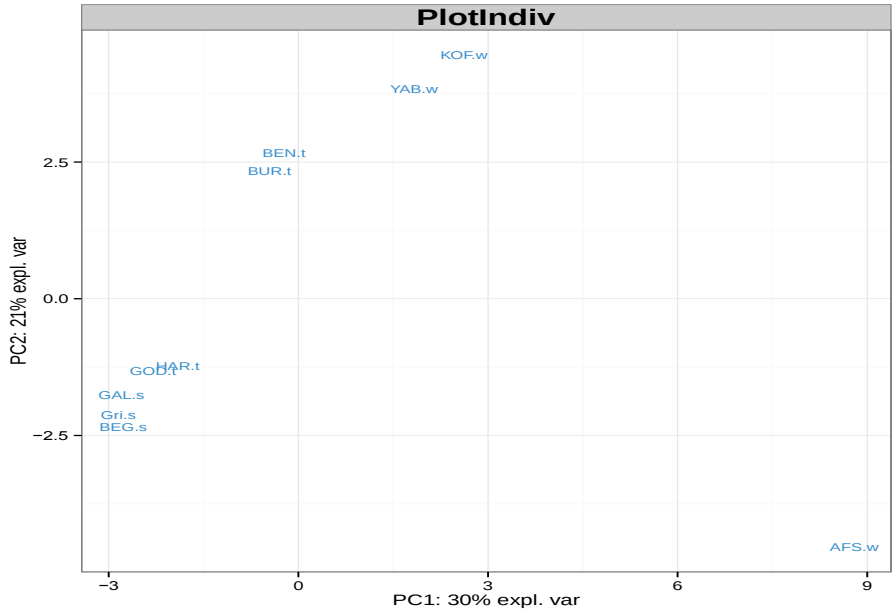
In order to evaluate genetic diversity in guinea fowl, we studied individuals from 10 African and European samples and sequenced (Illumina, paired-end) a single DNA pool from each sample. Samples originated from 3 geographic areas for wild African guinea-fowls (1 from South Africa, 2 from Burkina Faso), from 2 countries for domestic African samples (1 from Burkina-Faso and 1 from Bénin), 2 conservation flocks from Hungary and 3 French breeding companies for domestic European samples.

Table 1 : Samples used for Pool-seq analyses and sequencing depths.

Population	Nb. individuals	Sequencing depth
AFS-w: South Africa, wild	3	14.47
KOF-w: Burkina Faso, Koflandé, wild	8	17.64
YAB-w: Burkina Faso, Yabé, wild	8	20.08
BUR-t: Burkina Faso, traditional	15	29.90
BEN-t: Benin, traditional	15	15.69
GOD-t: Hungary, traditional	30	16.76
HAR-t: Hungary, traditional	30	18.04
BEG-s: French breeder, selected	12	15.06
GAL-s: French breeder, selected	29	37.01
GRI-s: French breeder, selected	20	38.71

To capture the overall structure of the genetic diversity in our samples, we first performed a principal component analysis (PCA) of population allele frequencies (Figure 1). The first axis of this analysis, which explained 30 % of the variance, opposed the wild population from South Africa, on one hand, and all domestic populations, on the other hand, with the two wild populations from West Africa in an intermediate position. This axis outlines the major effect of domestication on genetic diversity, and shows the close relationship between domestic and wild guinea-fowls from West Africa. European domestic populations appear more distant from South-African wild population than from the West-African ones but are not as close as the domestic from West Africa. The possible contribution of other wild populations to the European gene pool remains open. The second axis, which explained 21 % of the variance, opposed European populations and West African populations (either wild or domestic). This axis reflects the importation of domestic guinea fowl into Europe. PC2 also outlines the difference between traditional populations from Hungary and intensively selected populations from France.

Figure 1 PCA of allele frequencies



We next investigated if the serial founder events associated to domestication, importation to Europe and intensive breeding, as outlined above, were associated to adaptation at some specific loci. We looked for such loci using two different approaches.

First, we detected selective sweep signatures within each population following the approach of Boitard et al (Boitard *et al.* 2012). This approach looks for genomic regions with reduced genetic diversity, while accounting for specificities of Pool-seq data. Combining the results from the 10 analysed populations, thousands of candidate genomic regions under selection were detected. Among these, 5 were potentially related to domestication, as they were detected in at least 6 (out of 7) domestic populations, while showing no significant signal in any of the 3 wild populations. Similarly, 31 regions were potentially related to the importation into Europe, and 64 were potentially related to recent intensive selection for production traits.

Second, we detected genomic regions with outstanding genetic differentiation between populations, following the approach of Fariello et al (Fariello *et al.* 2017). This approach computes a p-value measuring the evidence for selection for all observed variants, and looks for genomic regions with an excess of low p-values, based on the statistical local score theory. This allows accounting for Linkage Disequilibrium (LD) between markers when detecting selection, despite the fact that individual genotypes cannot be observed from Pool-seq experiments. Eight regions were detected with this approach. Two of these regions overlap with domestication signatures detected by the within-population approach, and one overlaps with a European signature. Local population trees in the 5 other regions suggest that 3 of them are also related to domestication or importation into Europe.

Conclusion

As a result of the reference-guided assembly of the guinea fowl genome, some inter-chromosome rearrangements between chicken and guinea fowl may be missed in regions composed of small scaffolds, but knowledge of galliformes karyotype evolution suggests that at least the chromosomal assignment of the sequences is correct. This reference genome was successfully used for a diversity study based on a pooled sequencing approach, recapitulating the domestication process of this species and highlighting genome regions having been selected throughout the domestication and selection processes.

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Genetic Parameters of Electronically Recorded Feeding Behavior Traits in Meat Pekin Ducks

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Feed Intake Recorder Equipment, Pekin duck, Feed efficiency, Feeding behavior

Introduction

Nowadays feed cost represents up to two third of poultry production cost. For Pekin ducks, feed efficiency has thus now become one of the most important traits to improve, while still making sure to maintain the performance level of other production traits.

To meet this expectation, Grimaud Frères Sélection (GFS) has developed an automated feeding system equipped with Radio Frequency Identification (RFID), enabling a very effective control of the feed intake of its Pekin lines. Another important advantage of this technology is that it allows access to numerous criteria indicating the feeding behavior of the subjects. This study aimed at estimating the genetic parameters of these new traits in order to validate the interest of incorporating them into the breeding programs.

Material and methods

Data were collected on two GFS Pekin duck lines during a period of 28 days between 3 to 7 weeks of age. Animals of both sexes were measured. The number of subjects was N = 5121 in line A (female line) and N = 8940 in line B (male line). Ducklings were placed in pens equipped with RFID feeders from day 1 in order to become familiar with the material, and then raised *ad libitum* until 49 days. At the end of the test, raw data were processed by successive algorithms in order to:

- identify outliers according to some methods described by Casey *et al.* (2005).
- determine an appropriate time unit or "meal criteria" more relevant to analyze the temporal structure of feeding behavior (Tolkamp *et al.*, 2000; Howie *et al.*, 2009)
- compute the feeding behavior traits for each subject according to this meal criteria.

The 8 feeding behavior traits used in this study were obtained by averaging data between 3 to 7 weeks of age:

- **NM**: total **N**umber of **M**eals
- **MD**: **M**eal **D**uration
- **FIM**: **F**eed **I**ntake per **M**eal,
- **FRV**: **F**eeding **R**ate per **V**isit
- **ADFI**: **A**verage **D**aily **F**eed **I**ntake
- **VM**: number of **V**isits per **M**eal
- **TDSF**: **T**ime per **D**ays **S**pent **F**eeding

- **PFTM**: **P**roportion of **F**eeding **T**ime per **M**eal

The 3 performance traits measured were the following:

- **BW7**: **B**ody **W**eight at **7** weeks
- **FI**: total **F**eed **I**ntake between 3 to 7 weeks
- **FCR**: **F**eed **C**onversion **R**atio between 3 to 7 weeks

The phenotypic description of the analyzed traits is given in Table 1.
The 11 traits were analyzed simultaneously with a linear mixed model including the fixed effects of sex and batch, and the additive genetic effect of the animal. We used the gibbs2f90 software from the blupf90 package (Misztal *et al*., 2002). The Gibbs Sampling methodology gives access to the distribution of the parameters which are summarized in Table 2 by their mean and their 95% confidence interval. A total chain length of 450,000 iterations was run, and 100,000 samples were discarded as burn-in.
To take in account the body size of each animal, the 8 feeding behavior traits were pre-adjusted for BW7.

Results

Comparison between lines

Table 1 shows differences in feeding behavior between the two lines in addition to their difference in body weight: for female line A, the number of meals (NM) is greater, but their duration (MD) and the feed intake per meal (FIM) is lower than in male line B. Therefore, the time per days spent feeding (TDSF) and the total of feed intake during the test (FI) are higher in the male line.

Table 1: Mean and Standard Deviation for each trait in both lines

	Female Line A		**Male Line B**		**Line effect**
	mean	sd	mean	sd	
NM	256.8	72.2	231.9	61.5	***
MD (min/meal)	2.37	1.6	2.49	1.2	***
FIM (g/meal)	24.4	7	30.9	8.9	***
FRV (g/min)	25.2	6.7	27.2	7.1	*
ADFI (g/day)	212.6	26.2	246.1	32	***
VM	1.272	0.18	1.33	0.21	***
TDSF (min/jour)	11.9	5.4	12.71	4.6	*
PFTM	0.64	0.2	0.67	0.2	***
BW7 (g)	3465	315	3835	390	**
FI (g)	5706	998	6722	1184	*
FCR	2.47	0.25	2.54	0.27	-

Feeding behavior traits

The eight feeding behavior traits are moderately to strongly heritable: h^2=0.32 for the proportion of feeding time per meal (PFTM) in male line to h^2=0.69 for the number of meals in female line (NM). The average daily feed intake (ADFI) is less heritable (h^2 = 0.2 in female line to h^2 = 0.3 in male line). Comparison of heritabilities between lines (Table 2) reveals great similarities for the genetic variability of feeding behavior traits, with the exception of the feeding rate per visit (FRV), which is much more heritable in the female line. BW7 and FI heritabilities are similar in both lines.

Table 2: Heritabilities and 95% confidence interval for each trait in both lines

Trait	Female Line A		Male Line B	
	h^2	$IC_{95\%}$ (min ; max)	h^2	$IC_{95\%}$ (min ; max)
NM	0.69	0.62 ; 0.75	0.67	0.59 ; 0.74
MD (min/meal)	0.44	0.36 ; 0.51	0.44	0.36 ; 0.51
FIM (g/meal)	0.67	0.60 ; 0.73	0.66	0.59 ; 0.72
FRV (g/min)	0.60	0.53 ; 0.68	0.45	0.39 ; 0.52
ADFI (g/day)	0.20	0.15 ; 0.26	0.30	0.24 ; 0.37
VM	0.37	0.30 ; 0.44	0.42	0.36 ; 0.50
TDSF (min/day)	0.55	0.47 ; 0.63	0.46	0.38 ; 0.54
PFTM	0.40	0.33 ; 0.47	0.32	0.26 ; 0.38
BW7 (g)	0.68	0.62 ; 0.75	0.68	0.63 ; 0.73
FI (g)	0.47	0.41 ; 0.54	0.50	0.44 ; 0.57
FCR	0.24	0.18 ; 0.30	0.31	0.25 ; 0.38

Relationship between feeding behavior traits and performances traits

With the exception of ADFI, FCR shows weak to moderate genetic correlation with feeding behavior traits in line A and slightly larger in line B. More analyses are necessary to estimate the interest of adding these feeding behavior traits in order to improve accuracy of the evaluated breeding values of classical traits used. Given the small amplitude of these correlations, the expected gain is likely to be moderate, but can be significant, due to the relative independence between the feeding behavior traits, which makes it possible to cumulate the information provided by each of them.

Table 3: Genetic correlation for each trait in both lines

	NM	MD	FIM	FRV	ADFI	VM	TDSF	PFTM	BW7	FI	FCR
NM		-0.13	-0.97	-0.02	0.09	-0.30	0.05	-0.62	-0.16	-0.08	-0.05
MD	-0.38		0.15	-0.76	0.00	0.39	0.89	-0.30	-0.09	-0.07	0.07
FIM	-0.97	0.41		0.01	0.07	0.36	-0.05	0.59	0.19	0.17	0.18
FRV	0.06	-0.70	-0.06		0.06	-0.25	-0.87	0.05	0.14	0.14	0.00
ADFI	-0.02	0.18	0.20	-0.01		0.08	0.10	0.06	0.18	0.62	0.84
VM	-0.44	0.69	0.46	-0.36	0.13		0.20	-0.23	0.02	0.05	0.13
TDSF	-0.01	0.83	0.06	-0.89	0.26	0.41		-0.13	-0.13	-0.05	0.15
PFTM	-0.61	-0.05	0.63	-0.21	0.07	-0.08	0.00		0.24	0.21	0.08
BW7	-0.02	-0.05	0.06	-0.09	0.16	-0.05	0.14	0.34		0.88	0.02
FI	-0.03	0.07	0.16	-0.08	0.69	0.05	0.26	0.28	0.82		0.42
FCR	-0.16	0.27	0.33	0.05	0.86	0.22	0.19	0.00	-0.05	0.46	

Conclusion

Genetic parameters obtained in this study are very similar of those estimated by Howie *et al.* (2011) in broiler. The feeding behavior traits appear moderate to strongly heritable and therefore easily to control by selection especially since the correlations with performance traits are weak. As observed in chicken broilers, we found only marginal differences of genetic parameters for feeding behavior traits between duck lines selected with different breeding goals.

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Posters

Impact of breeding population size and selection method on effective population size (Ne): A simulation study

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In poultry populations of limited size, such as local poultry breeds and Label Rouge genetic lines delivering less than 1 million end products per year, reconciling genetic gain and management of genetic variability is often a complicated task. This is especially true in light of increased costs of both selection and production.Under such conditions, breeders of these populations often tend to reduce the number of sires, dams and selection candidates per generation in order to reduce costs. With this in mind, the objective of this work was to study both the effects of population size and of selection method on genetic variability in small populations.In our simulations, six different population sizes were combined with four selection strategies, resulting in 24 scenarios and two factors of variation.The strategies were 1): Increased genetic gain conditional on the conservation of parental origins ; 2): Minimization of co-ancestry of selected individuals ; 3) Same as 2, conditional on the achievement of a genetic gain equal to (1) ; and 4): Maximization of genetic gain, conditional on a controlled increase of co-ancestry. Strategies 2 to 4 were based on a Simulated Annealing (SA) algorithm to meet the constraints. Simulated phenotypes included two antagonist traits, body weight and laying rate. At each cycle, several variability indicators were computed.These included inbreeding, co-ancestry, and several derivations of the effective population size (Ne) based on sex-ratio, average inbreeding or average co-ancestry.Data were then analyzed by performing a series of linear regression models. In each model, the variability indicators were regressed on the year of birth (i.e. generation) then, regression coefficients and their confidence intervals were extracted. The effect of simulated scenarios was analyzed by comparing confidence intervals of regression slopes. As expected, decreasing population size reduced the initial level (i.e. regression intercepts) of variability whatever the selection strategy. However, strategies based on SA proved to be more effective in terms of conserving genetic variability in the long-term. In fact, regression slopes were more stable when methods based on SA were used compared to the method based on conservation of parental origins.

Keywords: Effective population size; genetic variability; selection; small populations

Monitoring small poultry populations using simulated annealing algorithms

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When dealing with small populations, such as local breeds or experimental lines, combining immediate genetic gain and preservation of genetic variability is a well-known issue, which requires relevant methods. Simulated Annealing algorithms are notoriously flexible and provide a wide range of solutions to select breeders and/or to design mating plans, especially when the objective function to optimize is subject to constraints. Using stochastic simulation, we compared various methods of selection and design of mating plans in a population obtained from 15 sires, each mated with 3 dams, representative of French poultry local breeds. Each full-sib family consisted of 3 male and 3 female offsprings. Up to 30 successive selection cycles were simulated. Two antagonist traits were considered. Selection aimed at improving the target trait ($h_1^2=0.4$) measured on the whole population, without impairing the ancillary trait ($h_2^2=0.2$), measured on females only. A constraint of a null evolution was therefore set on the second trait. Genetic variability indicators included average inbreeding and coancestry, as well as various estimators of effective population size. Reference selection method consisted in sorting the male and female candidates on the main objective, within sire and dam families respectively, after truncation on the ancillary trait. It actually led to an acceptable trade-off between genetic gain and inbreeding rate. Optimized methods, based on Simulated Annealing algorithm, improved both genetic gain and genetic variability indicators. Only these latter methods could meet the constraint of a null evolution of the ancillary trait. Optimization of mating plan did not significantly affect the inbreeding rate, but delayed the onset of inbreeding by four generations. Preventing mating of related females to the same sire did not significantly reduce the inbreeding rate. Nevertheless, this practice should be perpetuated to avoid situations not taken into account by the modelling approach such as infertility problems in the sire. Interestingly enough, present results suggested that pedigree depth should exceed 10 generations to ensure the reliability of pedigree-based effective population size.

Keywords : Small populations; simulated annealing; poultry

Different methodologies to genetically improve the robustness of bones in layers

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The presence of fractures and bone deformities in layers is a current animal welfare issue. An effect on these bone changes has been shown by the genetics, which might allow selecting birds with better bones. The aim of the current study was to evaluate the suitability of keel bone palpation, ultrasound measurement of the humerus and breaking strength test of the humerus and tibiotarsus in two white pure lines. The palpation was conducted twice on 5,869 layers with a four graded scoring system at 46th and 70th weeks of life. While 75 % of the birds from line-A showed modified keel bones, 85 % of the birds from the line-D had no deformation or fracture in the keel bones. The intraobserver- reliability for the keel bone palpation was 0.82. The estimated heritability for the palpation varied between 0.30 in line-A and 0.15 in line-D. The phenotypic correlation between the two palpations was 0.8. The ultrasound examination at 64 weeks of life showed no significant differences between the two groups ‘top class’ and ‘tail class’ based on the calculated palpation breeding value (100 birds of each group and line were tested). Within both groups the coefficient of variation was below 6 %. The repeatability for the two lines was above 0.8. The ultrasound measurement showed a heritability of 0.23. The breaking strength test, conducted on the 50 birds with highest and lowest ultrasound value of the line-A, showed no significant differences by the ultrasound ranking. Significant differences in breaking strength were found between the ‘top’ and ‘tail’ groups based on the palpation breeding values. The average breaking strength of the tibiotarsus was 181 N in the ‘top class’ and 153 N in the ‘tail class’ of palpation breeding value. Considering the moderate heritability of the palpation evaluation, its simplicity and the relatively low labour input for data collection, we conclude that the palpation is the most readily implemented method to determine bone changes in layers. Moreover, the palpation had a good agreement with breaking strength. The ultrasound measurement in this study showed no relationship to breaking strength and should be further investigated.

Keywords :"keel bone";"bone strength";"keel bone palpation";"ultrasound";"breaking strength"

Associations between gene variants and shell and other egg quality traits in White Leghorn lines

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Improving the quality and safety of eggs are among the main goals in layer genetics. This requires precise measurements, in particular for eggshell quality. In addition to traditional quantitative methods, molecular techniques allow identification of specific genes and their proteins involved in shell structure, composition and mineralization. For example the role of the ovocalyxin-32 (OCX-32) gene and of the SIBLING complex (cluster of 5 genes, which play key roles in bone mineralization and remodeling) can be explored. The SIBLING's equivalent region of the chicken genome identifies a cluster containing four of these genes, whose expression has been found within the oviduct, suggesting significant roles for this gene complex in eggshell formation. Variants of OCX-32 and SIBLING have been associated with shell quality traits. The SIBLING gene cluster is found on chromosome 4, which contains a high proportion of QTL influencing egg weight and relative size of various egg components.
Data for two White Leghorn lines (1, 2) from a single generation were used to test egg quality trait association with OCX-32 and SIBLING complex. Association analyses were carried out with a linear model including hatch effect, and both the genotypic model and the allele substitution effect for variants within OCX-32 and SIBLING complex. Variants within both gene regions showed significant effects on egg (EW) and yolk weight (YW), albumen height (AH), shell color (CO), and shell quality. Of particular interest are the differences for shell quality, which were measured with dynamic stiffness (Kdyn), breaking strength (BS) and micro-crack (MCr) detection. BS was significantly superior for one of the variants of OCX-32 for both lines. Kdyn was superior for one of the variants in line 1. For the SIBLING cluster, haplotypes were fixed in Line 1; for Line 2: Kdyn was significantly superior for one of the variants. Results for EW, YW and CO were significant for both genes. Albumen height was significantly affected by OCX-32 (both lines) but not by SIBLING variants.
Improvement in egg quality through standard quantitative selection will continue; however, gene assisted selection can also be implemented for candidate genes to improve specific aspects of egg quality.

Keywords: Egg quality; shell quality; gene variants; gene asociation

Advances in the management of poultry genetic diversity within CRB-Anim

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The National French Infrastructure Network CRB-Anim aims at joining Genomic and Reproductive Biobanks of cryopreserved cells in a unique strategy. These joined cryobanks provide DNA samples to study genetic diversity and reproductive cells in order to re-introduce genetic diversity when needed. Since 2013, CRB-Anim has supported the improvement of semen cryopreservation methods in several species of birds and has resulted in a marked increase of the number of lines currently preserved in the French National Cryobank which contains 59 lines and breeds of 5 different poultry species and more than 40000 semen straws plus blood cells of the same males. A first collection of Primordial Germ Cells of a local line (Noire du Berry) has also been included. The possibility of restoring a whole line with frozen semen or re-using chosen sires based on pedigree criteria has been tested in several instances. Resulting fertility highly differ (from 5% to 95%) depending on the inbreeding line level, on the individual fertility potential of males, on breeders' management and technical skills. As an example, a recent trial aimed at restoring 6 family origins in a highly subfertile inbred experimental line resulted in one family fully restored, and 2 partially restored. The males which had progeny tended to show better sperm quality traits. Consequently, the CRB-Anim infrastructure has also supported the development of new criteria to predict individual male fertility. Currently, a proteomic method is being developed to rank males that are candidates for storing frozen semen, in order to enhance the efficiency and reliability of using frozen semen in birds (Labas et al., 2014; Soler et al., 2016 a, b). By separating the profiles of sperm proteins ranging from 1000 to 20000 Da, the ICM-MS (Intact Cell Maldi tof Mass Spectrometry) method coupled with adapted modeling allows the screening of the males on the fertilizing ability of their semen. Since bird semen technology needs specific skills, it also became obvious that specific training sessions for bird insemination with frozen semen would be needed. All these results show that birds reproductive specificities induce specific cryobanking strategies and practices.

Key words: cryobanking, fertility, frozen semen, germinal cells, genetic diversity

Serum IL10 – A novel prognostic biomarker for tolerance to Eimeria tenella induced coccidiosis in the domestic chicken.

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A major contributor to economic loss in commercial poultry production is coccidiosis, a disease caused by species of the protozoan parasite, Eimeria. Control using prophylactic in-feed coccidiostats has been essential, but widespread drug resistance and consumer concerns about drug use in food production are driving the search for alternatives. Consequently, a considerable incentive to breed chickens with greater resistance and/or resilience to the disease has arisen. In a large-scale study (n = 1200) seeking evidence of selectable variance in response to infection with E. tenella, correlations between a novel prognostic biomarker, serum interleukin-10 (IL10, an anti-inflammatory cytokine), and caecal lesion damage were found. Further, using Eigenanalysis to dissect the relationships between these and an additional trait, percentage body weight gain, three distinct vectors revealed that susceptibility is not simply the inverse of resistance or tolerance. Moreover, unlike the resistant and susceptible vectors, IL10 carried negative Eigenvalues. A GWAS based on a proprietary 62K SNP array (Cobb-Vantress) produced suggestive genome-wide significant SNPs for each of the measured traits. Specifically, those relating to IL10 were located on chr1 in the region of IL10R2 on the chicken genome. Upregulation of this gene is essential in the production of IL10 in innate immune response to infection. Relevance of this work to industry is that tolerance to Eimeria spp. may be a characteristic that commercial breeders would preferentially select against, due to possibilities of unreliable bird health and super-shedding of oocysts by tolerant birds increasing environmental contamination.

Keywords: body weight gain; caecal lesion score; between-trait correlations; Eigen analysis

Deterministic modeling of a poultry selection scheme

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Deterministic modeling of selection schemes consists of calculating the expected genetic gain after a selection process. Consequences of selection on the genetic trend are calculated using equations based on estimated breeding values and on parameters describing the population and the selection process. Professionals could use this tool to compare different strategies for the improvement of their selection schemes. In this study, the model is based on a layer chicken breeding scheme. Breeding goal includes numerous traits such as laying performances, egg quality and body weight. This is a two steps model combining a familial selection process and an individual one. Within selected families, candidates from the good families are undergoing a mass selection whereas candidates from medium families are undergoing a within family selection of a fixed number of candidates. The model requires a large number of parameters: number of candidates, proportions of selected families and selected candidates per family, accuracy of breeding values, genetic correlations between traits, weights of traits in the selection index. The variability of the parameters composing the model enables comparing a large diversity of scenarios, including the use of genomic evaluations. Two analyses are presented to illustrate the usefulness of the model. The first analysis compares scenarios where the family selection rate varies (i.e. the proportion of maternal origins kept for the first step of selection). Results show a slight increase of the predicted genetic gain when the proportion of selected families was increased from 30% to 40%. A plateau was observed in the predicted genetic gain when this proportion was increased beyond 40%. In the second analysis, two scenarios based on classic breeding values or genomic breeding values were compared. The model included two traits C1 ($h^2 = 0.13$) and C2 ($h^2 = 0.33$), with a genetic correlation varying between 0 and 0.70 and a weight in the index of 40% and 60% respectively. Results show an increase of predicted gain for both traits with genomic evaluation. Improvement is constant for C2, whereas for C1 the increase of predicted gain is much better when genetic correlation between C1 and C2 is high.

Keywords: deterministic modeling; genetic gain; genomic selection; poultry; selection scheme

Identification of genomic regions associated with caecal microbiota composition in a broiler line.

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Recent studies in Human as well as in animal livestock species have shown that intestinal microbiota composition is related to variations of many traits of interest, including health and production traits. Variations in intestinal microbiota composition probably contribute, together with host genetics, to the expression of chicken phenotypes of interest.
We report the study of 200 F8 animals derived from an advanced intercross between two lines genetically divergent for their digestive efficiency, namely D+ and D-. Previous studies have demonstrated that in addition to digestive efficiency, gut microbiota composition in these lines is partly under genetic control, with the detection of several QTL controlling the abundance of specific bacterial species, major bacterial groups or ratios of major bacterial groups measured using a targeted, specific Q-PCR approach. Here we used a more exhaustive approach by sequencing the V3-V4 region of the 16S rRNA bacterial gene to characterize the caecal microbiota composition. The composition in OTU (operational taxonomic units) was determined by using a QIIME pipeline. In parallel, animals were genotyped using a 580k SNP chip (Affymetrix) and a genome wide association analyses (GWAS) was performed using the GenAbel package of R, with OTU relative abundances as phenotypic traits.
We identified several genomic regions controlling the variations of specific OTU abundances, such as that of Butyricicoccus pullicaecorum, at the species level and higher taxonomic ranks. Some of these regions co-localised with previous QTL controlling either digestive efficiency (on chromosomes 2, 8 and 11) or abundance of bacterial groups previously identified. These results confirm that gut microbiota composition in the D+/D- line is partly under genetic control. Co-localisations with loci controlling other traits allow us to formulate some hypotheses on the putative candidate genes being involved in digestive efficiency or immunity. This study improved our understanding of the genetic relations between digestibility, immunity and microbiota composition.

Keywords: Gallus gallus; gut microbiota; GWAS; 16S approach; digestibility

Benefits of testing birds in both bio-secure and production environment in genomic selection breeding programs for commercial broiler chicken

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A breeding program carried out under a strict biosecurity condition might show reduced genetic gain due to genotype by environment interactions (GxE) between bio-secure (B) and production (P) environments, since only gains obtained in P have economic value. Classical methods of bird testing in P using pedigree-based BLUP have low accuracy of prediction for EBVs of the birds in B. Genomic prediction based on dense genotypes might improve the accuracy. Stochastic simulation was used to explore the benefits of genomic selection breeding schemes for broiler chicken with birds tested in both B and P. Given a limited testing capacity, different proportions of offspring from breeding birds were allocated to be selection candidates and tested in B or tested birds in P where birds cannot be selection candidates. We investigated proportions of 0, 15, 30 and 45% of offspring tested in P and the remaining birds were selection candidates and tested in B. Different magnitudes of GxE were explored, in which genomic correlations (rg) between records in B and P were 0.5, 0.7 and 0.9. Heritability of records in B was 0.28 while it was 0.15, 0.25 or 0.35 for records in P. All animals in both environments were genotyped and GBLUP was used to estimate breeding values. Economic value in the breeding goal was assigned to trait expressions in P only. Results showed that with rg of 0.5 and 0.7, transferring birds for testing in P significantly increased genetic gain in genomic selection broiler breeding programmes and the improvement was largest with 30% of birds transferred to P. With rg of 0.9, genetic gains among scenarios with 0, 15 and 30% of birds tested in P were not significantly different. The increase in genetic gains by transferring birds to P increased with increasing heritability of records in P. This study implies if there is a strong GxE, a genomic selection scheme that uses a considerable proportion (30%) of birds to be tested in P has larger genetic gain than if all birds are tested in B environment only.

Keywords: GxE; bio-secure environment; production environment; genomic selection; broiler

Evaluation of an ongoing breeding program to develop a dual purpose chicken breed for villages of Ethiopia

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ACGG is an initiative working to test, deseminate and continuously improve tropically adaptive strains of chicken. A candidate strain, an indigenous chicken from Ethiopia, Horro has been under selective breeding program for the last 9 generations. The breeding program aimed to improve body weight at 16 weeks of age (BW-16) and cumulative egg number in 24 weeks after start of egg laying (EN-24) to develop a dual purpose indigenous chicken suitable for harsh environments. Each generation 50 males and 300 females were selected to produce the next generation. This represents selcetcted proportaions of approximately 10-20 % in the males and 50-60% in females. The aim of this paper is therefore to evaluate the phenotypic trends of the earlier generations (4-6) with (7-9). Body weigh at 16 weeks of age (BW16) and cumulative egg number in 24 weeks after start of egg laying (EN-24) for generations 7, 8 and 9 were 1235 grams and 84 eggs, 1267 grams and 87 eggs, and 1290 grams and 94 eggs, respectively. Rates of phenotypic changes per year in response to mass selection between generations (4-6) and (7-9) were also compared. Changes in body weight at 16 weeks of age during generations (4-6) was 174 grams/year while (7-9) was 19 grams. Changes in cumulative egg number in 24 weeks after start of egg laying was 1.4 eggs during earlier generations (4-6) and 3.33 eggs in later generations (7-9). This evaluation indicates positive changes in the selective breeding program which appears to exploit the growth potential faster than egg in the eariler generations (4-6) than later (7-9). The result supports an earlier study indicating that the Horro strain subjected to mass selection is showing more improvements in cumulative egg number in 24 weeks after start of egg than body weight at 16 weeks of age (BW16). Egg production of the Horro can further be improved for some more generations.

Keywords: "ACGG"; "Horro"; "Breeding Program"; and "Ethiopia

From Genebank into Breeding Line – An animal model for introgression of blue egg shell color into a White Leghorn line

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Under the umbrella of Horizon 2020 and as part of the EU project IMAGE (Innovative Management of Genetic Resources), the objective of this study is to demonstrate the efficient transfer of a specific trait maintained in gene bank, here blue egg shell color, into a contemporary high performing white egg layer chicken line by means of marker-assisted introgression.
Blue egg shell color is inherited as a dominant trait (Wang et al. 2013, Wragg et al., 2013). The causal mutation is a 4,2 kb retroviral insertion on chromosome 1 upstream of SLCO1B3 at 65,22 Mb (Wang et al. 2013, Wragg et al., 2013). The insertion induces overexpression of SLCO1B3 in the oviduct which results in increased biliverdin concentrations (Wang et al. 2013, Wragg et al., 2013). The insertion is present in the breeds Araucana, Donxiang and Lushi, while the insertion sites are different (Wang et al. 2013, Wragg et al., 2013).
In 2016, six Araucana cocks were mated with twelve White Leghorn hens. For the Araucana cocks whole genome sequencing was performed on the Illumina HiSeq2500 (Illumina, San Diego, CA) in paired-end mode with a mean coverage of 30x. The White Leghorn hens were genotyped using the 600K Axiom® Genome-Wide Chicken Genotyping Array (Affymetrix, Santa Clara, CA). All F1 cocks were genotyped on a 60K SNP Chip (Affymetrix). Based on genotype data of the Araucana and White Leghorn, we identified 24 informative SNPs on chromosome 1 surrounding the insertion site. By combining them with 12 SNPs from the 60K SNP chip we constructed Araucana and White Leghorn specific haplotypes from 60 to 71 Mb on chromosome 1. Haplotype information in combination with 60K SNP genotypes will be used to select animals with a recombination site closest to the insertion, highest proportion of recipient genome and highest degree of diversity for breeding. Based on this, two marker-assisted backcross generations followed by an intercross-generation will be generated, aiming at a high performing White Leghorn-like line which is homozygous for blue egg shell color.

Keywords : "chicken";"blue egg shell";"marker-assisted introgression"

Genome specific expression in the liver of mule and hinny duck hybrids

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Among different duck genetic types, Muscovy and especially mule ducks are the only ones involved in fatty liver production. Mule ducks are hybrids from male Muscovy ducks (Cairina moschata, Cm) and female common ducks (Anas platyrhynchos, Ap). They benefit from a heterosis effect on feed ingestion capacity and fatty liver weight and thus they account for 95% of fatty liver production. Conversely, common ducks and hinny hybrids (male common duck X female Muscovy duck) are not used to produce fatty liver.
In order to better characterize these reciprocal hybrids, genome specific expression was analyzed. RNA sequencing was conducted in the liver of common, Muscovy, mule and hinny ducks fed ad libitum or overfed (n=10). SNPs with genome specific alleles were selected in common and Muscovy duck RNA sequences by discosnp++, a de novo assembly method that does not require a reference genome. Sequence reads corresponding to the two alleles of genome specific SNPs were then counted in RNA sequences from mule and hinny hybrids. These counts were considered as expression levels.
Only three loci were found to be expressed in a strict genome specific manner. They were localized in the mitochondrial genome and, as expected, the maternal allele of the hybrids was found to be expressed.
Interestingly, some genes were also found to be expressed with allelic imbalance, i.e. the ratio of Ap allele expression on Cm allele expression being < 1 in at libitum fed and > 1 in overfed ducks or conversely > 1 in at libitum fed and < 1 in overfed ducks, pointing out orthologue genes with and without regulation by (over)feeding.
In conclusion, genome specific expression and allelic imbalance could be observed in duck hybrids, strongly suggesting regulatory divergence between parental alleles.

Keywords: SNP; duck; hybrids; genome specific expression

A simulation approach to optimize breeding programs with application to the introgression of the blue egg color into a high performing layer line

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Predicting and evaluating the genetic progress of a breeding program is essential to make optimal selection decisions. The variety of approaches for the deterministic assessment of genetic progress ranges from simple gene-flow approaches to statistically complex methodologies. These approaches, however, are limited with respect to certain model assumptions and only applicable to scenarios using either no or very simplistic selection strategies. This makes a realistic and precise evaluation of a complex breeding program difficult. Therefore, stochastic simulation has become a fundamental tool for this evaluation. To this end, we developed a highly flexible and computational efficient simulation tool based on the gene-dropping method that can incorporate real genetic information, such as genotype data and recombination maps, to account for the true genetic architecture of the actual population.
We applied our simulation tool for a selection program, which aims at introducing the blue egg shell color as single monogenic dominant trait from a gene bank population into a high performing, white egg layer chicken line. Initial crossing of gene bank population individuals carrying the blue egg shell genotype and white layer chickens will be followed by two generations of marker-assisted backcrossing, followed by an intercrossing to generate individuals that are homozygous for the blue egg color gene. Using the simulation tool, we aim to optimize each breeding step to construct a population, which is homozygous for the introgressed locus and at the same time shows maximum similarity to the recipient white egg layer line – especially in the proximity of the target locus – while maintaining a high genetic diversity. The results indicate the superiority of the simulation-based protocol compared to a non-optimized procedure.

Keywords: breeding program; simulation; blue egg shell color; introgression

A comparison study of keel bone deformities in four lines of laying hens kept in two husbandry systems

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Keel bone deformities (KBD) are an important welfare problem in laying hens. The aim of the study was to assess the extent of KBD in two brown (BLA, L68) and two white (WLA, R11) egg layer lines, differing in performance and phylogenetic origin. A total of 384 hens were housed in floor pens with two compartment sizes. Another 288 hens were kept in single cages equipped with perches. Keel bone status was recorded by palpation, in the 15th and 21st week of age, followed by an 8-week-interval, with the last palpation in week 69. A three-scale scoring system (Habig & Distl, 2013) was used for keel bone assessment (4 = no deformity, 3 = mild deformity, 2 = moderate to severe deformity). Based on Chi-square tests at each time point, a significantly higher frequency of KBD was detected in white-egg layers kept in cages (46% to 97%) compared to those housed in floor pens (1% to 46%). In the brown-egg layers similar differences were observed between the housing systems which were significant from weeks 29 (BLA) and 57 (L68) onwards, respectively. The incidence of KBD increased with advancing age in all lines. In both housing systems KBD occurred significantly more often and more severe in white- than in brown-egg layers, although the brown-egg layers had a significantly higher body weight from the 2nd week of age onwards. This is in contrast to previous studies, indicating a high body weight as one factor negatively affecting keel bone status. While no significant difference was shown between R11 and WLA, neither in floor pens nor in cages, in the cage system keel bones of L68 hens were significantly less deformed (3% to 29%) than those of BLA (13% to 84%) at any time of examination. The results of the present study confirm that the husbandry system is one of the main factors affecting keel bone damage. Furthermore, the high number of keel bone deformities in white- compared to brown-egg layers may suggest a phylogenetic effect, while differences according to performance level seem less important and were only shown for brown-egg layers.

Keywords: keel bone status; phylogeny; performance; husbandry system

Functional genomics of the digestive function in broilers

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While poultry breeding strategies have so far favored highly performing animals, the sustainability of poultry farming relies on the development of production systems incorporating more various feedstuffs. This implies to integrate adaptation to feed in breeding programs, such as digestive efficiency of feedstuffs with lower values. The aim of the ADIGEN project was to improve the understanding of genes involved in digestive function by characterizing the transcriptome of different compartments of the gastrointestinal tract: the junction between proventriculus and gizzard, the gizzard, the gastroduodenal junction, and the jejunum.

Total RNA from the 4 tissues were sequenced on HiSeq2500 for six individuals from a F2 cross between two broiler lines divergently selected for digestive efficiency (D+ / D-) on a Rialto wheat based-diet. Reads were aligned against the chicken genome (Galgal4 version) with TopHat and then quantified with featureCounts. Biostatistical analysis was performed with EdgeR and a total of 11216 differentially expressed transcripts (representing 9155 genes) between at least two tissues was characterized. In total, eight groups of genes with markedly different expression profiles were identified. The greatest differences in expression were related to the expression in the jejunum, which is a particular tissue compared to the other three, as its main function is the absorption of nutrients. Nevertheless, we also detected a cluster of genes overexpressed in the two junctions (proventriculus to gizzard and gizzard to duodenum), which included among others, different genes involved in muscle contraction. The functional analysis of the expression clusters by "Gene Ontology" showed a significant enrichment of the immune function and the catabolism of small molecules in the jejunum, which is in agreement with the literature. In a more original way, we observed an enrichment for genes involved in protein synthesis in the gizzard and the gastro-duodenal junction, compared to the other compartments.

This analysis allows us to draw the first molecular portrait of the different compartments of the digestive tract, which will serve as a basis for future studies on the genetic and physiological control of the animal response to feed variations.

Keywords: digestive efficiency; broiler; transcriptomics

Assessment of inter and intra genetic diversity of Turkish dying out Denizli chicken breed using SSR markers

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Assessment of inter and intra genetic diversity of Turkish dying out Denizli chicken breed using SSR markers
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The aim of this study was to characterize genetic diversity, genetic relationship and population structure within dying out Turkish local Denizli chicken subpopulations using 19 microsatellite markers. A total of 105 alleles were found across 19 microsatellite loci with a mean number of 5.53 alleles per locus. Considering all subpopulations and loci genetic differentiation based on global FST was low with a value of 0.030. Global FIS values (0.200) indicated that non-random mating occurred in all subpopulations of Denizli fowl and all subpopulations deviated significantly ($P<0.01$) from Hardy Weinberg equilibrium. Over all subpopulations, the mean observed heterozygosity was 0.473, ranging from 0.399 to 0.562. The subpopulations derived from rural area showed higher heterozygosity and lower inbreeding coefficient values than the subpopulations that derived from conservation flocks. Genetic differentiations between pairs of subpopulations based on proportion of shared alleles (DPS) ranged from 0.140 to 0.297. The Neighbour-Net network tree, based on DPS, constructed at subpopulation level revealed two main clusters. Based on the results of the STRUCTURE analysis, the most probable structure clustering of the six Denizli fowl subpopulations was at K=4. Moreover, substructing according to geographic location could not be observed which might be due to some gene flow across the subpopulations of Denizli fowl. The results of this current study can be used as baseline genetic information for implementing breeders' flocks into Denizli fowl conservation program to control inbreeding and safeguard the genetic variability of the populations.

Keywords: Denizli fowl; microsatellites; population structure; genetic diversity; subpopulations

Transgenerational effects of modifications of the embryonic environment in quail

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Environmental modifications, for instance exposures to chemicals, are known to impact phenotypes on the long term, sometimes across several generations. Such transgenerational phenotypes may be promoted by epigenetic alterations such as DNA methylation, an epigenetic mark involved in the regulation of gene expression. However, it is yet unknown whether transgenerational epigenetic inheritance of altered phenotypes takes place in birds. The purpose of this study was to investigate whether changes to the embryonic environment had a transgenerational effect that could alter the phenotypes of third-generation offspring.

We observed phenotypic differences in the third generation between two quail “epilines”, which were obtained from genistein-injected eggs (Epi+) or from untreated eggs (Epi−) from the same founders. We used genistein as it is known to interfere with the epigenome, especially with DNA methylation. A “mirrored” crossing strategy was used, with parallel genealogies in each line, to minimize the effect of mendelian inheritance on trait variability. After 3 generations without any further treatment, a significant difference in sexual maturity was observed between the lines, with the Epi+ G3 birds starting to lay eggs later. A significant interaction between line and sex was observed for 3-week body weight and for eye temperature traits. Two behavioral traits were also significantly affected in the G3 by the initial treatment. Global methylation analyses revealed no significant difference between the epilines, however.

These observations demonstrate the impact of a modification of the founders' embryonic environment on the phenotype of quails, three generations later. While between lines genetic variability cannot be ruled out, the mirrored animal design should have minimized its effects, and observed differences in the G3 may be attributed, at least partly, to transgenerational epigenetic phenomena. Preliminary results from a further study, using several genetic lines and testing several methyl-modifiers, will be presented.

Keywords : transgenerational; epigenetics; quail; genistein

Genetic characterization of French local chicken breeds

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The small population sizes of French local breeds raise the question of their in- or ex-situ conservation. For that goal the characterization of their genetic diversity with molecular tools appears as a preliminary key step. A total of 22 local breeds and 4 commercial lines with an average of 60 individuals per breed were genotyped using a 57K DNA chip leading to a total sample of 26 breeds and 1517 individuals. The commercial lines used as control populations included 2 broilers lines from the AvianDiv collection, one French 'label' slow-growing line and one brown-egg line. This project named BiodivA was supported by the CASDAR programme of the French ministry of agriculture and the CRB-Anim infrastructure covered part of the genotyping costs.

Within breed genetic diversity was good but variable among them (mean F comprised between 3 and 28%), inbreeding coefficients being related to the population sizes. Among breed diversity was large (Fst=0.25) allowing for a clear genetic identification of breeds. There was no evidence for admixture with commercial broilers but admixture could not be ruled out in the case of one local breed and the brown-egg commercial line.

Relationships among breeds were consistent with their history (origin, breeders) or usage (broilers, layers...). Finally, combining these genetic analyses with morphological data could help in detecting genomic regions of interest in a selection perspective. To conclude, French local breeds appeared to be largely diversified genetically and morphologically making them a good example of a successful management by both breeders and selection centers. Nevertheless attention should be paid on them for long term conservation.

Keywords: Diversity ; conservation ; management ; SNP ; Chicken ; Genetics

Genomic inbreeding level in commercial chicken population estimated by two approaches

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One of the potential consequences of long term selection is an increase of homozygosity. There are several approaches to estimate the inbreeding level. By contrast to pedigree analysis, molecular data allows more reliable estimates of inbreeding coefficients. The main objectives of this study were to: estimate inbreeding coefficients based on runs of homozygosity (FROH) and inbreeding coefficients based on genomic relationship matrix (FGRM) as well as to evaluate inbreeding effects on production traits.

Data contains information about 862 male broiler chickens (from commercial population) genotyped using the 60K Illumina iSelect chicken beadchip comprising 57 636 SNPs. Analysis covered 31 chromosomes, including sex chromosomes. Following production traits were recorded per single birds: body weight at day 36, body weight at day 39, body weight changes between 39 and 46 days, feed intake between 39 and 46 days and feed conversion ratio. Data were provided by Cobb-Vantress Inc. The estimation of inbreeding coefficients was based on runs of homozygosity (McQuillan R. et al. 2008. Am. J. Hum. Genet. 83, 359–372) and the genomic relationship matrix (VanRaden P. 2008. J. Dairy Sci. 91: 4414-4423).

Generally, the FROH approach leads to more reliable estimates of inbreeding coefficients compared to FGRM. Hence, the FROH estimates were used in further analysis. FROH is highly dependent on number of SNPs and settings used for the runs of homozygosity identification process. These settings should be optimized for each species or even population. Estimated coefficients of inbreeding ranged from 0.67% to 12.43%. For the analyzed population, the estimated genomic inbreeding coefficient was 5.18% based on runs of homozygosity. Inbreeding effects on performance traits are examined by the use of one-way analysis of variance (parametric and non-parametric) and linear regression.

It should be stressed that no inbreeding effects were observed for all studied traits. Hence, it indicates an effectiveness of the genetic improvement program applied to the studied population.

The study was supported by the European Union Seventh Framework Programme (FP7/2007-2013) as part of the ECO-FCE project.

Keywords: genomic relationship matrix; runs of homozygosity; chicken; feed conversion;

Genetic characterization of nine Italian local chicken breeds using high-throughput technologies for SNP genotyping

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Over the past 10 years, in Italy, several conservation actions have been conducted to safeguard local avian genetic resources and thus preserve biodiversity. The aim of this study was to compare inter- and intra-genetic variation and population structure of nine local chicken breeds of Veneto region (north-east Italy), involved in a conservation program, using a high-throughput technology for SNP genotyping. A custom array of 64 SNP was designed using a QuantStudioTM 12K Flex system (Life Technologies), with OpenArray technology. A total of 763 blood samples from the following native chicken breeds were collected and analyzed: Ermellinata di Rovigo, Millefiori di Lonigo, Polverara Bianca and Nera, Pepòi, Robusta Lionata and Maculata, Padovana Dorata and Camosciata. Also, a commercial line (Bresse) was included in the study and considered as reference population. Number of alleles, observed heterozygosity, PIC and Wright's F-statistics were calculated using MolKin 3.0 and Genetix 4.05 software. A total of 329 alleles were detected across the marker loci (5.14 alleles per locus). Global inbreeding coefficient (FIS) value (0.116) indicated that non-random mating occurred in all chicken populations. Molecular co-ancestry (Fij), measuring the similarity between individuals of the same breed, compared to the reference was lower for the two Padovana and Ermellinata di Rovigo breeds, and it was similar for the other populations. Breeds were highly differentiated with a mean value of genetic differentiation index (FST) of 0.167. Most differentiations were reported between the groups formed by the Padovana and Polverara, and by the Robusta, Millefiori Lonigo and Ermellinata di Rovigo breeds. Structure analysis was performed under the hypothesis of six clusters (K = 6). The affiliation was successful in all Veneto chicken breeds except for Millefiori di Lonigo, which was assigned a distinct cluster at later stages. Our results demonstrated the utility of the proposed custom array of 64 SNP using a high-throughput technology for monitoring and preserving the genetic variability of native chicken breeds involved in conservation programs.

Keywords: local chicken breed; genetic diversity; QuantStudio platform; SNP genotyping; population structure

Assessment of surface temperature variation under chronic high ambient temperature in four experimental layer chicken lines differing for heat resistance and feed efficiency traits.

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In this study we used infrared thermography to quantify the effect of ambient temperature (AT) on surface temperature (ST) variations of the comb, the shank and the eye in contrasted chicken genotypes at thermo-neutrality (22°C) and at high AT (32°C). Four INRA experimental chicken lines, representing a unique range of genetic variation for traits known to be important for heat tolerance and feed efficiency, were measured: the Fayoumi breed, known to be resistant to high AT and to parasitic infections; a naked neck dwarf (LS) line of brown-egg layers selected for longer clutch periods; and two lines of Rhode Island Red layers divergently selected for high (R+) and low (R-) residual feed intake. In total 189 hens (50 Fayoumi, 46 LS, 49 R+ and 44 R-) were reared under thermo-neutrality (22°C). At 28 wk of age the AT was increased to 32°C until 33 wk of age. Infrared thermographic pictures were recorded on all animals at 3 time points to estimate ST: at thermal neutrality (T1), 1 week after the increase of the AT (T2) and 4 weeks later (T3). Rectal temperature was also recorded at the same time points. At 22°C a wide variability in body ST was observed, both between and within genotypes, particularly for comb and shank temperatures, while rectal and eye temperatures were more homogeneous. At 32°C, the opposite trend was observed, with differences among lines existing only for rectal and eye temperatures. At 22°C the Fayoumi had the lowest and the R+ the highest rectal and eye temperatures. The same ranking was observed at 32°C. On the contrary, at 22°C the Fayoumi and R+ had the highest comb and shank temperatures, respectively, and no significant differences were observed at 32°C among lines. Significant genotype × temperature interactions were observed for the comb and shank ST alone. Although large correlations were observed among ST (r between 0.75 and 0.84, $p<0.0001$), correlations between surface and rectal temperatures were generally low, with the eye being slightly more correlated to the rectal temperature (r= 0.30) than the comb or the shank (r= 0.24 and 0.25, respectively).

Keywords : Heat stress; Surface body temperature, Infrared thermography; chicken

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